A Thesis

entitled

"Analytical and Synthetic Studies of Boronic Acids and Their Derivatives"

Submitted in part fulfilment of the requirements for admittance to the degree of Doctor of Philosophy

by

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- For my family -

CONTENTS

ABBREVIATIONS

Aqueous	aq.
Atomic weight	At.wt.
Boiling point	b.p.
Centigrade degree	°C
Compound	compd.
Concentrated	conced.
Concentration	concn.
Dilute	dil.
Equation	eq.
Exempli gratia (for example)	e.g.
Experimental	Exptl.
Injection	injn.
Melting point	m.p.
Methyl	Me
Millimoles	mmole
Molecular weight	mol.wt.
Phenyl	Ph
Polymerisation	polyn.
Precipitation	pptn.
Reaction	ren.
Reduction	redn.
Room temperature	R.T.
Round bottom flask	R.b.f.
Saturated	satu.
Solution	soln.
Tetrahydrofuran	THF
p-Toluene sulphonic acid	p.TSA
Vacuum distillation	vacu.distiln.
Vacuum sublimation	vacu.subln.
Volume	vol.

SUMMARY

The discovery in 1954 of the selective reactions of boronic acids with proximal diols, and subsequent studies of other bi-functional substrates, affording cyclic derivatives, opened a new field in studying the chemical properties of these polyfunctional compounds. A wide range of specific boronates has been prepared, and many workers have shown how useful they can be for example, as crystalline derivatives, reagents for structural analysis, intermediates in synthesis, derivatives for use in a range of separatory techniques, and reaction catalysts. Boronic acids and their derivatives are also of potential value in cancer therapy and possibly as hormone analogues.

There are two widely applicable procedures for the synthesis of these boronic acids: Grignard and hydroboration reactions. In the work reported in this thesis, a hydroboration procedure has been applied for the attempted preparation of the boronic acids from alkenes (Table 3, Chap. 3 , p.96) and the Grignard procedure for the preparations starting from 5β-cholan-24-ol (Chap.3 , p.103).

The addition of "catecholborane" to appropriate alkenes yielded the cyclic catechol esters of octadecane-, cyclohexane-, norbornane-2-, β-pinane-10ξ-, 3-methyl-5α-cholestane-2ξ-, and 5β-cholane-24-boronic acid. These all proved to be stable under vacuum or under nitrogen, when kept below 10°C, in the absence of light. The esters were, however, not stable in air, in organic solvents (not containing dissolved air) or in water: under these conditions they gradually changed from colourless to black liquids within

a very short period. This effect may be due to aerial oxidation and subsequent hydrolysis. Unsuccessful attempts were made to obtain the free boronic acids by direct hydrolysis of the above catechol boronates, except in the case of catechol cyclohexaneboronate which was hydrolysed in cold water and readily yielded cyclohexaneboronic acid which crystallised out directly. As a result of these difficulties, an indirect method was adopted for the preparation and characterisation of the boronic acids. This depended on the sequential displacement of catechol by other diols (e.g. 2,3-butanediols, cis-indane diol, pinacol). 2,3-Butanediols displaced catechol from its cyclic boronates, and were themselves displaced from their esters by pinacol and cis-indane diol. The 'stable' cyclic boronates of these latter diols were resistant to hydrolysis or displacement by other diols. These displacement and hydrolysis experiments were monitored by TLC and GLC. The boron-containing compounds were detected in TLC with diphenylcarbazone. The boronic acids studied differed markedly in their solubility in water; a hexane-water extraction technique was adopted for the attempted purification, along with other chromatographic techniques. These methods were not successful in obtaining pure boronic acids, owing to the instability of the compounds studied. The boronic acids were analysed by GLC and GC-MS, indirectly as their diol boronate derivatives; IR and ^{1}H-NMR data were also measured.

The selectivity and ease of reaction under mild conditions of boronic acids with vicinal diols attracted our interest in studying certain biologically important catecholamines and catechol estrogens, in parallel with some model compounds. These studies were carried out at microgram levels, mainly by GLC and GC-MS. Where possible, larger samples were examined by IR and ^{1}H-NMR techniques. The corresponding acetate and trimethylsilyl ether derivatives were also studied.

A number of catecholic substrates have been prepared, such as N-acetyldopamine N-acetyltyramine and N-acetyl-4-hydroxy-3-methoxyphenethylamine, (via selective N-acetylations). The corresponding fully acetylated and novel peracetylated amine ($-NAc_2$) were also prepared for comparative studies.

The catechol estrogens, 2-and 4-hydroxyestrone and 2-and 4-hydroxy-17-deoxoestrone, were prepared and characterised.

Crystalline cyclic boronate esters were obtained of various catechol derivatives including 3,4-dihydroxyaryl carboxylic acid methyl esters (benzoate, phenylacetate, dihydrocinnamate and cinnamate); N-acetyldopamine; estra-1,3,5(10)-triene-3,4-diol and 4-methyl-7,8-dihydroxy-coumarin. These new compounds, prepared under neutral conditions in ethyl acetate were stable in organic solvents and toward aerial oxidation and atmospheric moisture. They proved to be unstable on TLC, or dry column chromatography. The GLC, GC-MS, IR and ^{1}H-NMR of these compounds were studied.

In general the cyclic boronate derivatives of catecholic compounds were suitable for characterisation by GLC and

GC-MS; but not for quantitative analysis, by GLC. This limitation resulted from the adsorption of many of the boronates on the chromatographic columns(1% OV-1,OV-17, OV-25), which was especially severe when additional "free" hydroxyl or amino group were present (e.g. in 2-hydroxy-estradiol). It was found that the catechol boronates were largely hydrolysed in aqueous media. In addition to hydrolysis, products were susceptible to decomposition probably via autoxidation of the free catechol group; thus boronates of brazilin and apomorphines appeared to be lost within a few hours of formation. Most of the catechol boronates which have been prepared undergo either total decomposition or displacement reactions after the addition of reagents for trimethylsilylation or acetylation.

The boronate derivatives studied, were divided into three main groups, according to the number of other reactive groups; (e.g. -OH, $-NH_2$, -NHR). The corresponding trimethylsilyl and acetyl derivatives were also studied for these groups of compounds for comparative GLC studies.

The mass spectra recorded by GC-MS for cyclic boronate derivatives of catechol amines and catechol estrogens were recorded along with their corresponding acetate and trimethylsilyl ether derivatives. The general mode of breakdown was found to be the same for methaneboronates and benzeneboronates; examination of these boronate analogues has facilitated the interpretation of mass spectra by a "substituent shift" technique. The ions containing boron are readily detected by the characteristic isotope ratio of $^{11}B:^{10}B$.

Chapter 1

INTRODUCTION

I. BORON ELEMENT

Boron was isolated by Sir Humphry Davy and Gay-Lussac and Thenard in 1808[1]. It belongs to group 3a of the periodic system: boron, $_5B^{10}$; aluminium, $_{13}Al^{27}$; gallium, $_{31}Ga^{69}$; indium, $_{49}In^{115}$; and thallium, $_{81}Tl^{204}$. Boron having the electronic configuration $1s^2 2s^2 2p$, it has 3 valence electrons, and forms planar, tricovalent derivatives, that are electron deficient, and which as Lewis acids, accept two electrons from bases to complete the boron outer-shell octet and give tetrahedral adducts. Boron has a fairly high electronegativity within its group and shows largely non-metallic characteristics. The other elements in the group are metals so that there is a distinct difference in properties between boron and the other elements.

Boron occurs in nature chiefly in the form of boric acid and its salts, and as a constituent of certain complex alumino-silicate minerals[1,2]. Free boron is never found in nature. Large beds occur of such minerals as kernite, $Na_2B_4O_7.4H_2O$, colemanite, $Ca_2B_6O_{11}.5H_2O$, and ulexite, $CaNaB_5O_9.8H_2O$. Boric acid, H_3BO_3, occurs in solution in the water from certain hot springs[1]. It is estimated that boron constitutes 0.001% of the earth's crust.

Pure boron[1] (> 99%) has been made by the thermal decomposition or hydrogen reduction of boron trichloride or

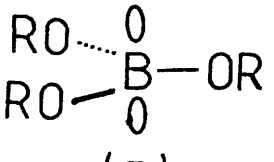
tribromide, and by the electrolytic reduction of solutions of potassium fluoborate in fused potassium chloride.

Boron has a density of about 2.34g per ml. It melts at a temperature between 2100 and 2200°. Boron is a very poor conductor of electricity at room temperature, but its conductivity increases enormously with rise in temperature. Liquid boron boils at about 2550°.

Boron compounds[1,3,4,5] exist as :- oxides (e.g. B_2O_3); acids [e.g. $B(OH)_3$, HBO_2, $H_2B_4O_7$, $RB(OH)_2$]; halides (e.g. BX_3); hydrides B_xH_y (e.g. B_2H_6, $NaBH_4$); boranes (e.g. BH_3, BH_2R, BHR_2, BR_3); diborons R-B-B-R[6]; borates $B(OR)_3$; boronates $RB(OR)_2$; borides, metal-boron (MB, e.g. AlB_2MgB_2); sulphides (e.g. B_2S_3, B_2S_5); borax (sodium pyroborate $Na_2B_4O_7$, $10H_2O$); metaborates [e.g. blue cobalt metaborate, $Co(BO_2)_2$] and many other compounds.

II. CONFIGURATION

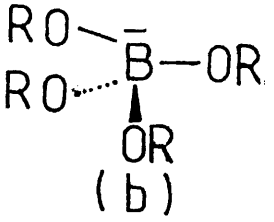
Organoboron compounds, in general, exist in two principal configurations[5,4]. In the majority of cases, the boron atom is trigonal coplanar with sp^2 hybridization. Vacant lobes of a 2p - orbital lie above and below the BO_3 plane (a). Thus most organoboron compounds are electron deficient



(a)

in the sense of Lewis octet theory and much of the recorded organoboron chemistry is related to the resulting electro-

philicity of the boron atom. In the second general configuration, boron assumes tetrahedral character with sp^3 hybridization (b). Clearly, the electron deficient nature



(b)

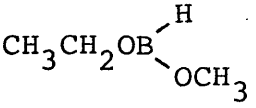
of boron is alleviated in this series of compounds.

III. NOMENCLATURE

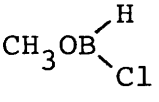
The trigonal coplanar compounds of boron, which comprise the majority of organoboron compounds, are named as derivatives of borane (BH_3), for example:-
[known earlier as borine[7,8], for example:-
$C_6H_5BH_2$, phenylborine and $(CH_3O)_2BH$, dimethoxyborine)].

CH_3OBH_2	methoxyborane
$(CH_3O)_2BH$	dimethoxyborane
$(CH_3O)_3B$	trimethoxyborane
$(CH_3)_3B$	trimethaneborane
$(CH_3COO)_3B$	triacetoxyborane

When two or more different substituents are present, the substituents are named in alphabetical order[5] :



ethoxy (methoxy)borane



chloro (methoxy)borane

In the case of trisubstitution with halogen atoms, the following naming is used:

BCl_3	boron trichloride
BF_3	boron trifluoride

Acidic boron compounds have been named depending on the number of the hydroxy groups attached directly to the boron atom, and on the substitution of these hydroxy groups with one or two groups (e.g. alkyl or aryl), then ending the name with ic acid, as follows:

$B(OH)_3$	boric acid, (orthoboric acid)
$HB(OH)_2$	metaboric acid
$H_2B_4O_7$	pyroboric acid, (tetraboric acid)
$RB(OH)_2$	boronic acid, (R=alkyl;aryl;arenyl, etc) (dihydroxyborono for $-B(OH)_2$ group)
$CH_3B(OH)_2$	methaneboronic acid (formerly methylboronic) *
$C_6H_5B(OH)_2$	benzeneboronic acid (formerly phenylboronic)
p-$CH_3.C_6H_4B(OH)_2$	p-toueneboronic acid
R_2BOH	borinic acid; (R, as above)
$(CH_3)_2BOH$	dimethaneborinic acid
$(C_6H_5)_2BOH$	dibenzeneborinic acid

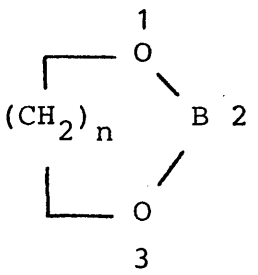
The acyclic ester derivatives of the above acids, are named according to the IUPAC rules for the esters of their corresponding carboxylic acids, by using the name of the acid with ending -ic acid changed to ate. For example:

$(CH_3O)_3B$	trimethyl borate, (methyl borate)
$(C_6H_5O)_3B$	triphenyl borate, (phenyl borate)

* For convenience the earlier style is used to some extent in this Thesis.

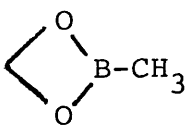
Naming of the cyclic ester derivatives of the above boric or boronic acids may be based on three principles[5,9] :

(a) the central use of the ester hetrocyclic rings. The numbering system starts with the heterocyclic atom other than boron (I); then the following names are given

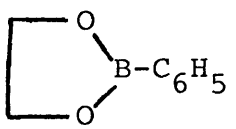


(I)

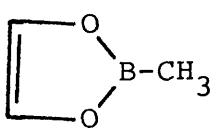
to each ring according to the size and the state of saturation



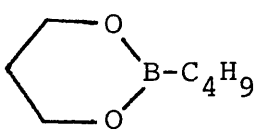
2-methane-1,3,2-dioxaboretane



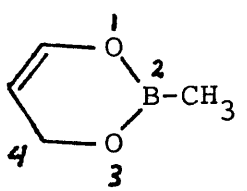
2-benzene-1,3,2-dioxaborolane



2-methane-1,3,2-dioxaborole

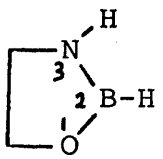


2-n-butane-1,3,2-dioxaborinane

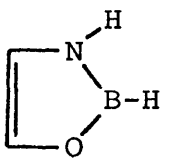


2-methane-4H-1,3,2-dioxaborine

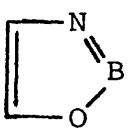
Names of related heterocylic rings are given in the following examples:



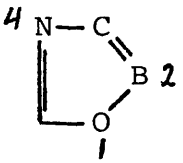
1,3,2-oxazaborolidine



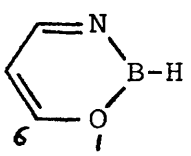
Δ^4-1,3,2-oxazaboroline



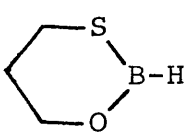
1,3,2-oxazaborole



1,4,2-oxazaborole

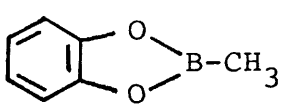


2H-1,3,2-oxazaborine

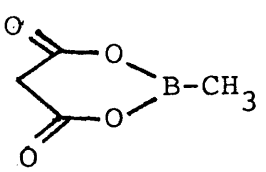


1,3,2-thiaoxaborinane

Applications:



2-methane-1,3,2-benzodioxaborole



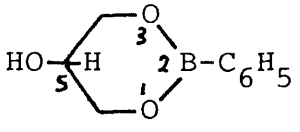
2-methane-1,3,2-dioxaborinane-4,6-dione

(b) the use of radical prefixes:"borylene" or boranediyl;

$H-B\langle$,borylene $CH_3-B\langle$,methaneboranediyl;

(c) the use of ester terminology, (as described for the acyclic derivatives, above).

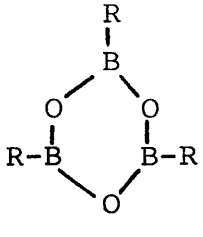
Thus, for example, according to these respective procedures, the glycerol derivative (II) may be named: A. 5-hydroxy-2-benzene-



II

1,3,2-dioxaborinane; B. 1,3-O-(benzeneborylene)glycerol, or 1,3-O-(benzeneboranediyl)glycerol; or C. glycerol 1,3-benzeneboronate. This last style of naming will be used for most of the boronic acid ester derivatives in this Thesis.

The cyclic anhydrides of boronic acids were given the trivial names: boroxine or boroxole (III). Boroxine is more commonly used in the literature, in the manner as shown in the following examples:



(III)

R = methyl, n-butyl, cyclohexyl, phenyl.

trimethane boroxine; tribenzene boroxine, etc.

IV. BORIC AND BORONIC ACIDS

A. Historical

The selective reaction of boric and boronic acids, with compounds containing two or more polar functional groups (OH, NH, SH, COOH), by forming cyclic (sometimes acyclic) derivatives, opened a new field in studying the chemical properties of these polyfunctional compounds. A wide range of specific boronates has been prepared, and many workers have shown how useful they can be for example, as crystalline derivatives, reagents for structural analysis, intermediates in synthesis, derivatives for use in a range of separatory techniques, and reaction catalysts. Organoboron chemistry goes back more than one hundred years to 1846, when Ebelman and Bouquet[10] reported their preparation of the methyl, ethyl and amyl esters of boric acid, from the reaction of the appropriate alcohol and boron trichloride. This work not only describes the first preparation of a boric acid ester but also represents the first recorded synthesis of an organoboron compound.

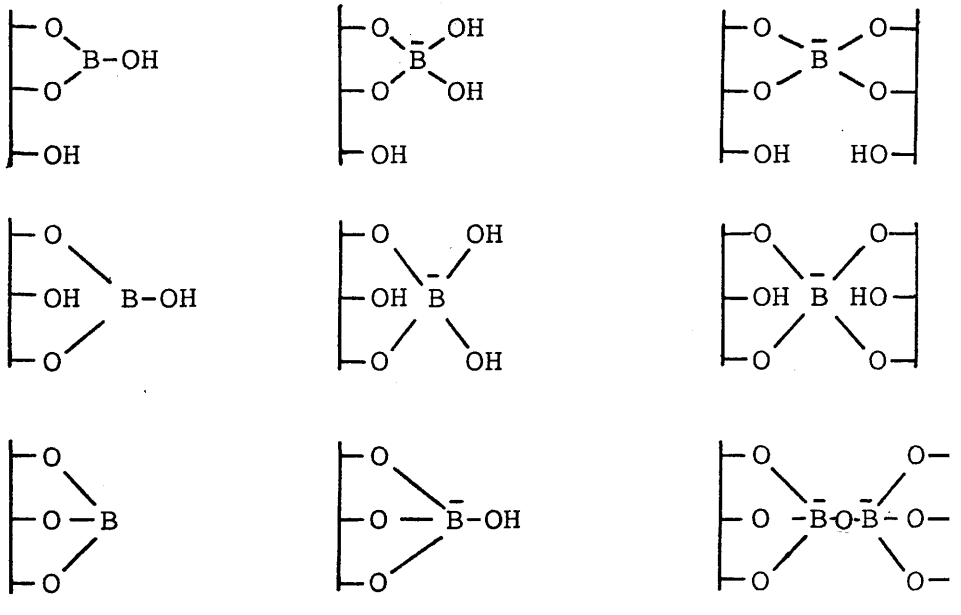
Early studies of boric acid interaction with hydroxylated compounds in aqueous solution were made in 1874, when Vignon[11] observed increases in the acidity of boric acid, by adding various compounds to it, as in the case of glycerol, which was used to determine this weak acid titrimetrically. In the years 1911-1949, Boeseken and other workers[12] used boric acid in the determination of the configuration of the carbohydrates in aqueous solution, after complex formation and conductivity measurements. These studies were among the first basic

foundations of the stereochemical analysis of organic compounds.

Boric acid ionizes, in aqueous solution, not by direct deprotonation, but by hydration and subsequent ionization, to give the symmetrical borate anion[4], (eq.1.1):

$$B(OH)_3 + H_2O \rightleftharpoons \bar{B}(OH)_4 + H^+ \qquad (1.1)$$

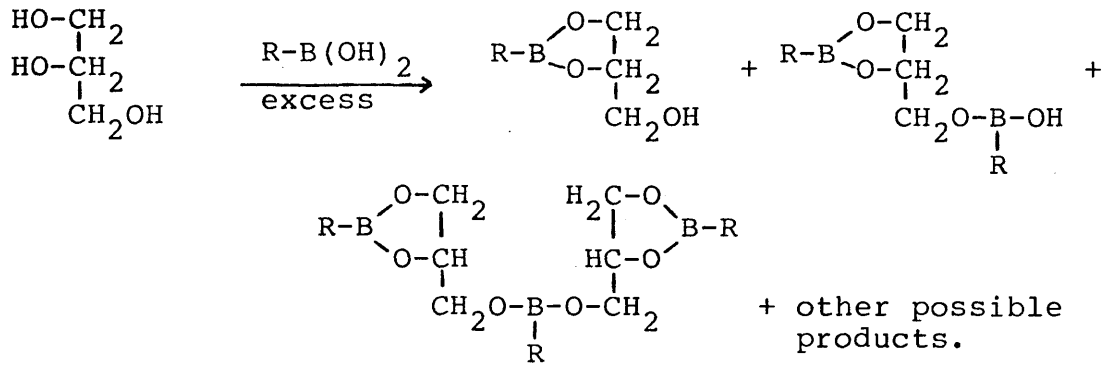
The applications of boric acid in the isolation and synthesis of specific carbohydrate borates are, however, few[9], because of its complexity as an esterifying agent. As well as affording five-or six-membered rings, it may also lead to dimeric or polymeric products containing boron-oxygen-boron linkages. Reaction of a conformationally unrestricted, contiguous triol could, for instance, result in the formation of many species exemplified in Scheme 1.1:



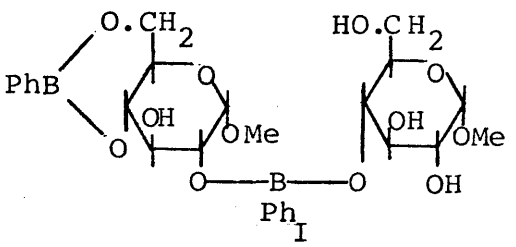
Scheme 1.1

With a view to exploiting the clear potential of boric acid derivatives in preparative, carbohydrate chemistry, but with reagents not subject to such diverse means of reaction, Kuivila, et al.[13] reported, in 1954, a study of the condensation undergone between benzeneboronic acid and diols and polyols in aqueous and aqueous-methanol solutions, and a number of crystalline areneboronates of these polyhydroxy compounds were isolated and characterised. Then several benzeneboronates of free sugars[14,15] were prepared in anhydrous media either by fusion techniques, (the product being isolated with hydrocarbon solvents) or by reaction in anhydrous solvents followed by removal of water azeotropically. In the early 1960's Ferrier had started to develop applications of benzeneboronic acid, for preparation of crystalline esters of several classes of carbohydrate compounds[15,16], as a suitable protecting reagent during esterification and etherification reactions[15,17], and for use in disaccharide syntheses[17] and separations[18,16]. This subject has been reviewed by Ferrier[9]. Within the last two decades cyclic boronates of a variety of compounds including carbohydrates[19,9], macrolide aglycones[20], steroids[21], and catecholic derivatives (see Chap.3) have been prepared in crystalline form by condensation mainly with areneboronic acids in different organic solvents. By virtue of the high molecular weight and rigidity of these acids, the corresponding boronates are in general easier to crystallise, than those derived from aliphatic boronic acids.

With boronic acids, esterifications are less complex than with boric acid, and formation of dimeric[15] (I), seven-membered ring[15] (pyroboronate,II) and acyclic species is more limited[15,9,22,23], this can be illustrated by hypothetical reactionsof a boronic acid and glycerol (Scheme 1.2), but cyclic products having different ring-sizes[24,25]



Scheme 1.2



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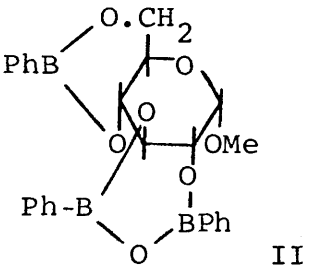
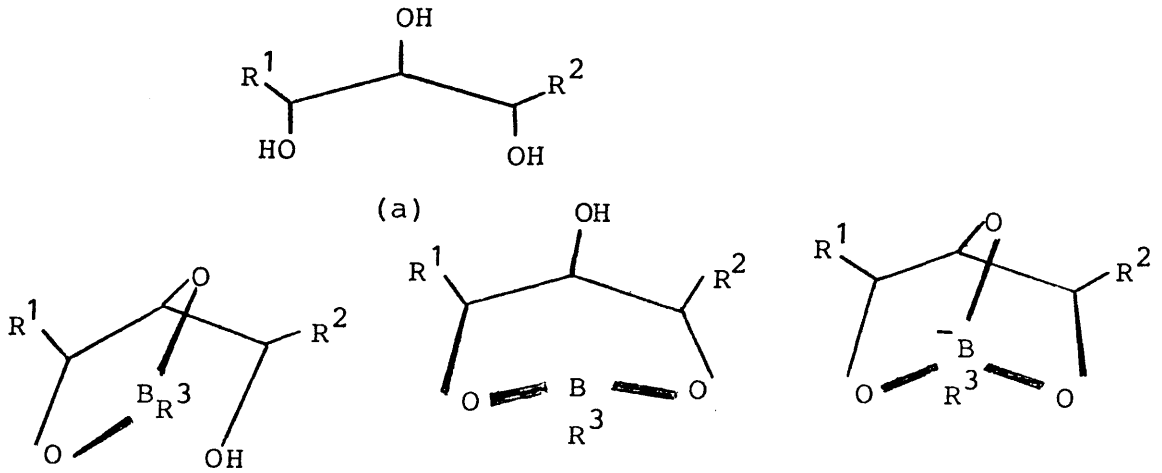


II

and electronic configuration at boron can be formed. Ferrier[9] suggested that isomerisation of certain cyclic boronates may consequently occur much more readily than has been recognised. In the case of 1,2,3 - triols (a)(Scheme 1.3)the five-membered boronates (b) could conceivably re-arrange to the six-membered structures (c) by way of accessible ionic intermediates (d) and thus (b) and (c) may be thought of as tautomers, as well as isomers, bearing the same relation-ship to each other as do furanose and pyranose forms of free sugars[9], and thereby presenting classically difficult



(a) (b) (c) (d)

Scheme 1.3

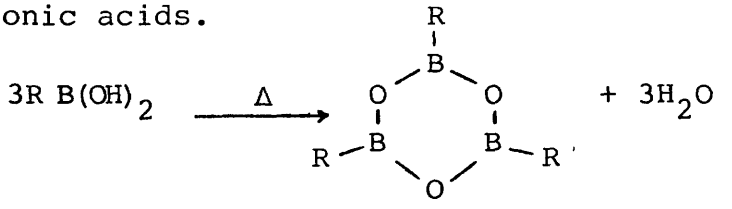
problems of structural analysis. In the chemistry of alditol boronates, this issue is potentially of wide significance[13,9], but boronates of cyclic carbohydrates (with some exceptions[9]), catecholic compounds [26,27,21], α-hydroxy acids[21] and α-and β-hydroxy amines[27,21], and most of the other compounds studied have tricovalent boron - all of which can, however, form tetrahedral complexes with Lewis bases.

Many of the studies concerned with the preparation of boronates by condensation of boronic acids with polar polyfunctional compounds have been carried out on a preparative scale, in solutions, mainly in organic solvents but sometimes in aqueous media. Techniques employed have included IR,UV, optical rotation, NMR (^{1}H or ^{11}B,) electrophoresis, paper or column chromatography, X-ray crystallography , and mass spectrometry. Only a few X-ray crystallographic studies have been made[28,29] . In 1967, Brooks and Watson[30] studied the formation of boronate compounds at microgram levels,

by gas phase chromatography and demonstrated their value in structural studies by combined GC-MS . Alkylboronic acids [e.g. $CH_3B(OH)_2$, $C_4H_9B(OH)_2$],with low molecular weight and polarity, have been generally used for high molecular weight and polar polyfunctional compounds. Arylboronic acids [e.g.$PhB(OH)_2$] remain useful for lower molecular weight polar polyfunctional compounds, and mostly show higher stability under electron impact mass spectroscopy. For comparative GLC and GC-MS, different boronic acids can be conveniently used for one substrate. In general, boronate derivatives of diols are thermally stable and show satisfactory gas chromatographic behaviour, usually superior to that of the free polar polyfunctional compounds (e.g. free diols, discussed in Chap.5).

B. Physical Properties

The melting points given in the literature for particular boronic acids often differ widely from one another. The dihydroxyboron group is easily dehydrated on heating to yield anhydrides or other intermediates[31] (eq.1.2). Therefore the melting point, which is the usual criterion for the purity of a compound, is of minor value in the case of boronic acids.

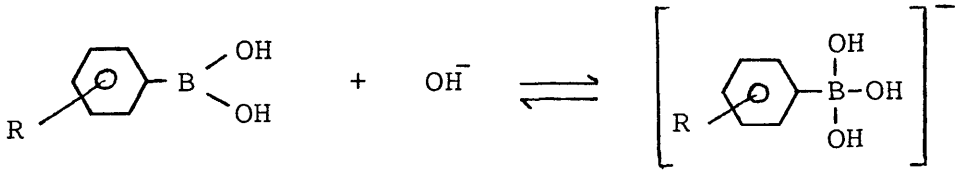


(1.2)

Boronic Acid Boroxine

Cryoscopic molecular weight determinations[32], as well as dipole moment measurements[33], show that boronic acids are not appreciably associated. Molecular weight determinations on boronic anhydrides indicate a trimeric form[34].

Dissociation constants of boronic acids have been measured[35,8]. As mentioned above, they act as acids in the Lewis' sense with formation of a tetravalent boron atom[36,8] (eq. 1·3). For example, the dissociation constants of boric



(1.3)

R, electron releasing group, e.g. NH_2,OH,Ph,CH_3; decreases the acidity

R, electron withdrawing group, e.g. NO_2,F,Cl,Br,COOH; increases the acidity

(65.3), benzeneboronic (137), benzylboronic (75.5), and n-butaneboronic (1.82) acids, were measured in water[35]. These results show the dependence of the acid strength on the group attached directly to the boron atom, (eq.1·3).

Infra-red spectrometry has been useful for determining the existence of the dihydroxyboron groups, and their disappearance upon formation of the corresponding boroxines in certain solvents[37].

Ultraviolet spectrometry of boroxines and boronic acids showed that the spectra of tribenzeneboroxine and its derivatives are different from those of the corresponding boronic acids[38]. These studies suggested stronger interaction

between the boroxine ring and benzene groups, in comparison with the corresponding boronic acids. On addition of water the spectra of boroxines were gradually changed to the corresponding boronic acid spectra; conversely the spectrum of boronic acid was changed to that of the boroxine, in anhydrous organic solvents (except alcoholic solvents, e.g. MeOH)[38].

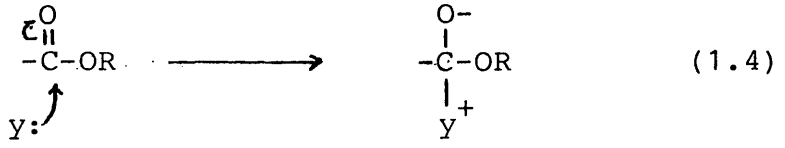
The relationship between nuclear magnetic resonance (proton and ^{11}B) data of substituted benzeneboronic acids, and molecular electronic configurations has been investigated[39]. Particular interest has been shown concerning the effect of substituents on the n.m.r. parameters of aromatic protons of some substituted benzeneboronic acid compounds.

The gas chromatography-mass spectrometry properties of some boroxines (i.e. benzene-,cyclohexane-,tert-butane-, n-butaneboroxine) have been studied[40]. These boroxines were produced in the "flash heater" of a gas chromatograph and carried through the column with an inert gas (nitrogen or helium), then studied directly by GC or GC-MS. On electron impact-MS, the tribenzeneboroxine showed a strong molecular ion as the base peak, compared to the trialkyl-boroxines, which showed weak molecular ions[40]; this stability might reflect some degree of conjugation between the benzene groups and the boroxine ring, as well as the lesser fragmentation of the phenyl as compared with the alkyl groups.

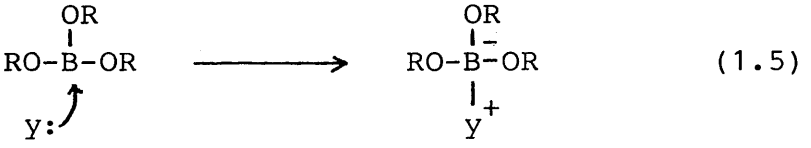
C. Chemical Properties

The electrophilic nature of the boron atom by virtue of its electron deficiency and vacant p orbital permits acceptance of nucleophiles in a manner similar to the

carbonyl groups ($\gtrsim$BOR $\approx$ $-\overset{O}{\overset{\|}{C}}$OR). In the case of the carbonyl group, the mobile electrons of the double bond are shifted to the carbonyl oxygen on entry of a nucleophile (eq.1.4). Boron is capable of tetracovalency and thus accepts a nucleophile by rehybridization from $\underline{sp^2}$ to $\underline{sp^3}$ with the boron atom assuming a formal negative charge[5] (eq.1.5).



(1.4)



(1.5)

1.Acid properties:- The boronic acids have characteristic acid properties. Thus, they dissociate (see p.14) and they readily form anhydrides (see p.13), esters (see p.27), and salts. Benzeneboronic acid formed both a sodium and a calcium salt when treated with the appropriate hydroxide[8]. n-Butaneboronic acid formed a hydrated sodium salt when treated with very concentrated sodium hydroxide[41]. Deboronation of arylboronic acids is a facile process. It may be accomplished by treatment with water at elevated temperatures, or more rapidly with acids, or preferably with bases[35,8,31]* (eq.1.6)

$$C_6H_5B(OH)_2 + H_2O \longrightarrow C_6H_6 + B(OH)_3 \qquad (1.6)$$

* They are in order of relevance.

2. Boron-carbon cleavage reactions (with oxidizing agents):- Many boronic acids tend to undergo aerial oxidation to boric acid, either rapidly or on storage, in contact with air or in solvents containing oxygen[8,31,35]. The conversion of boronic acids to boric acid was thought to involve the following reaction sequence (eq.1.7):

$$RB(OH)_2 + \tfrac{1}{2}O_2 \longrightarrow ROB(OH)_2 \xrightarrow{H_2O} ROH + B(OH)_3 \qquad (1.7)$$

Hydrogen peroxide quantitatively oxidizes alkylboronic acids to boric acid; use has been made of this in estimating the boron content of such compounds[41]. Benzeneboronic acid reacts with hydrogen peroxide, affording boric acid and phenol[8]. The kinetics of this reaction has been studied, and the following mechanism proposed[42]:

$$C_6H_5-B(OH)_2 + HOO^- \rightleftharpoons \left[C_6H_5-B(OH)(O-OH)\right]^- \xrightarrow{\text{rate-determining}}$$

$$\left[C_6H_5-B(OH)(O^+)\right]^- + HO^- \longrightarrow C_6H_5O-B(OH) \xrightarrow{H_2O} C_6H_5OH + B(OH)_3$$

It is reported that electron releasing groups attached to the benzene ring reduced or prevented these types of oxidations[8].

The action of halogens (X=Cl,Br,I) on arylboronic acids afforded boric acid and the appropriate halogenoarene[8,41] (eq. 1.8)

$$ArB(OH)_2 + X_2 + H_2O \longrightarrow Ar\,X + HX + B(OH)_3 \qquad (1.8)$$

3.Boron-carbon cleavage reactions(with metal salts):- The arylboronic acids have been shown to react with aqueous solutions of copper, silver, zinc, cadmium, mercury, and thallium salts [8,43,44] (eqs.1.9 & 1.10).

$$C_6H_5B(OH)_2 + 2CuX_2 + H_2O \longrightarrow C_6H_5X + Cu_2X_2 + HX + B(OH)_3 \qquad (1.9)$$

$$C_6H_5B(OH)_2 + AgNO_3 \longrightarrow C_6H_5\underset{\underset{OAg}{|}}{B}\text{-}OH \longrightarrow C_6H_6 + Ag_2O + B(OH)_3 \qquad (1.10)$$

4.Complex formation:- Benzeneboronic acid was observed to react with nitrogen bases to give complexes (I) which were thought to form by the following reaction sequence[45] (eq.1.11).

$$C_6H_5B(OH)_2 + R_3N \longrightarrow C_6H_5\underset{\underset{\ddot{N}R_3}{\uparrow}}{B}(OH)_2 \xrightarrow{2\ C_6H_5B(OH)_2} C_6H_5B\begin{matrix}\leftarrow:NR_3\\ \\ \end{matrix}\begin{matrix}OH\\ |\\ OBC_6H_5\\ \\ OBC_6H_5\\ |\\ OH\end{matrix} \quad \underline{I}$$

$$+2H_2O \qquad (1.11)$$

D. Preparation of Boronic Acids

The most widely practicable methods for preparation of alkyl and arylboronic acids are Grignard and hydroboration reactions. Other methods will be indicated in brief as follows:

1.Partial oxidation of trialkylboranes,triarylboranes[46,8];

$$R_3B + O_2 \longrightarrow RB(OR)_2 \xrightarrow{H_2O} RB(OH)_2 \qquad (1.12)$$

2.Halogenation of trialkyl and triarylboranes[46];

$$R_3B \xrightarrow[\text{excess}]{Br_2} RBBr_2 + R_2BBr \xrightarrow{H_2O} RB(OH)_2 + R_2BOH \qquad (1.13)$$

R= n-butyl

$$Ar_2BOH + Br_{2(aq.)} \xrightarrow{1:1\,molar} ArB(OH)_2 \qquad (1.14)$$

3.Protonolysis of trialkyl and triarylboranes[46];

$$R_3B \xrightarrow{H_3O^+} RB(OH)_2 \text{ or } R_2BOH \qquad (1.15)$$

The reaction conditions can be chosen so that boronic or borinic acids are the main products[46].

4.Reaction of zinc,mercury, tin and aluminium organic compounds with borates and boron halides[46];

$$BBr_3 + Ar_2Hg \longrightarrow ArHgBr + ArBBr_2 \xrightarrow{H_2O} ArB(OH)_2 \qquad (1.16)$$

5.Grignard reactions, and reactions of organolithium compounds:- The Grignard reactions are usually performed by adding ethereal solutions of trialkylborates (methyl,ethyl, n-propyl,n-butyl, isobutyl) or boron trihalides (X=F,Cl) to the aryl or alkylmagnesium bromides at low temperatures (eq.1.17). The

$$B(OR)_3 \text{ (or } BX_3) + Ar(R)MgX \longrightarrow [ArB(OR)_3]^-MgX^+ \xrightarrow{H_3O^+} ArB(OH)_2 \qquad (1.17)$$

method has been extensively employed in the preparation of aromatic[46,35,8,39,42,43,47,48] as well as aliphatic(primary, secondary, and tertiary alkyl) boronic acids[46,8,41,43,48]. The yields of these reactions varied, depending on the temperature, the nature and the order of addition of the reactants. In some cases higher yields were obtained at temperatures below 0°C[47]. For example a 99% yield of benzene-

boronic acid was recovered at -60°[47] (eqs.1.18-1.21).

$$C_6H_5Br + Mg \xrightarrow{\text{Dry ether}} C_6H_5MgBr \quad (1.18)$$

$$C_6H_5MgBr + B(OCH_3)_3 \xrightarrow[-60°]{\text{Dry ether}} [C_6H_5B(OCH_3)_3]^- MgBr^+ \quad (1.19)$$

$$[C_6H_5B(OCH_3)_3]^- MgBr^+ + 3H_2O \longrightarrow C_6H_5B(OH)_2 + 3CH_3OH + Mg(OH)Br \quad (1.20)$$

$$2Mg(OH)Br + H_2SO_4 \longrightarrow MgBr_2 + MgSO_4 + 2H_2O \quad (1.21)$$

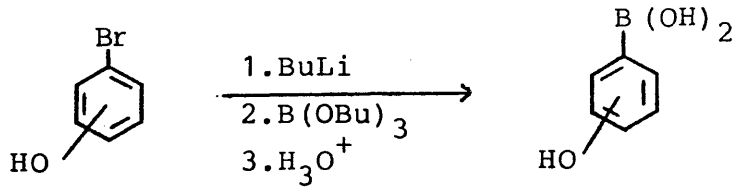
The trialkyl borates in some cases either gave poor yields or did not react at all, with Grignard reagents, but when they were treated instead with organolithium compounds the yields were improved. By halogen-metal interconversion[46,8,38,48,37], (eq.1.22) and by metalation[46] (eq.1.23) a series of organolithium reagents are available, which can react with borates (eq.1.24).

$$RBr + BuLi \longrightarrow RLi + BuBr \quad (1.22)$$

$$RH + BuLi \longrightarrow RLi + BuH \quad (1.23)$$

$$B(OR)_3 + RLi \longrightarrow [RB(OR)_3]^- Li^+ \xrightarrow{H_3O^+} RB(OH)_2 \quad (1.24)$$

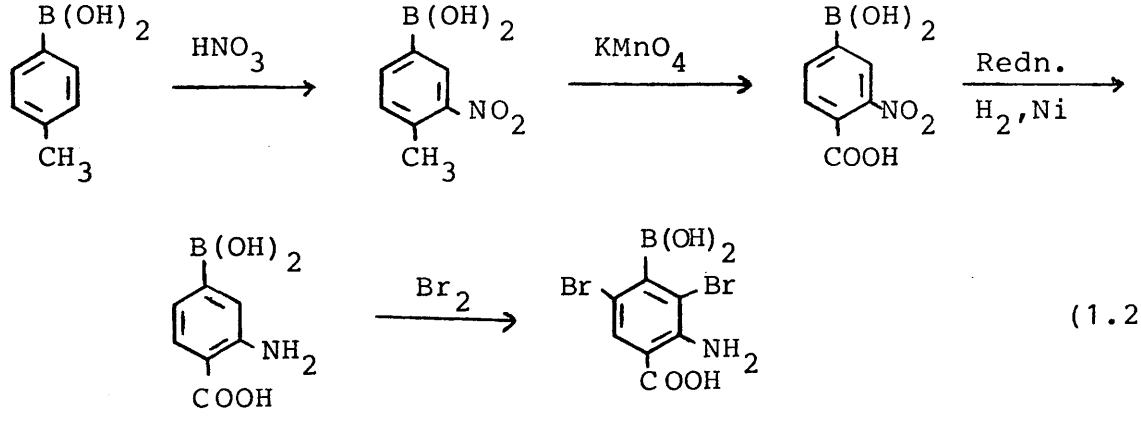
Santucci and Gilman[37] synthesised aromatic boronic acids containing hydroxy, bromine and sulphur groups, for example (eq.1.25):



(1.25)

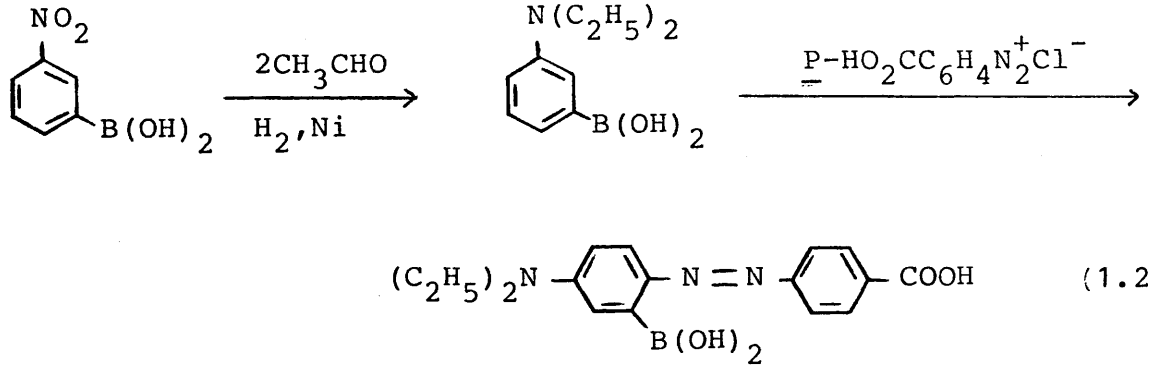
Many of the aromatic boronic acids were prepared by

introducing the dihydroxyborono group into the aromatic nucleus by means of magnesium or lithium compounds. Thus the aryl group must not contain functional groups which could react with the Grignard reagent. These groups,e.g. nitro, amino, carboxy, sulphono, etc., have to be introduced after the dihydroxyborono function. The stability of these aromatic boronic acids during electrophilic aromatic substitution and other reactions (nitration, oxidation, reduction, bromination,sulphonation, etc.) helped in the preparation of a wide range of aromatic boronic acids[46] (eq. 1.26):



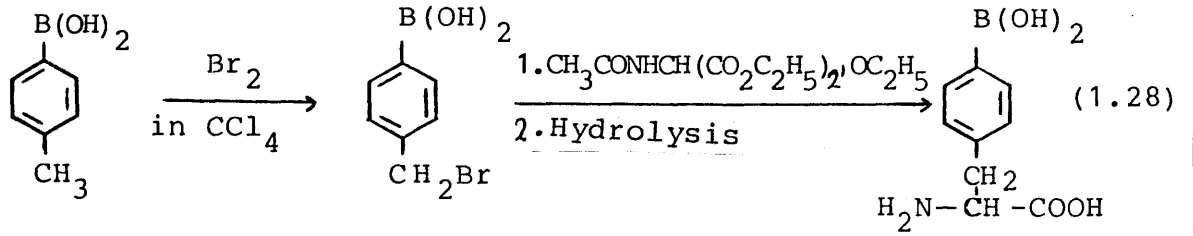
(1.26)

For the synthesis of boron-containing azo dyes desired for use in physiological studies and colour reactions[4,44], diazo coupling has been carried out with certain areneboronic acids[44] (eq.1.27):



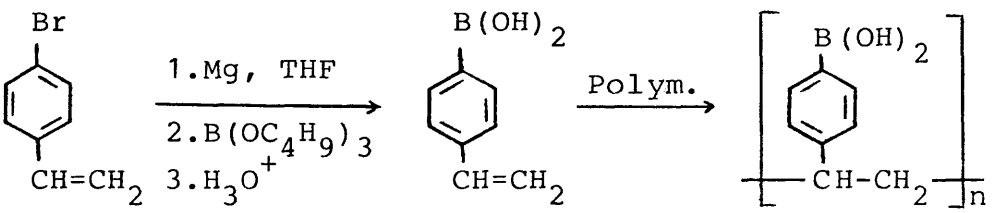
(1.27)

The analogue of the amino acid tyrosine in which the phenolic hydroxyl group is replaced by the weakly acidic boronic acid function has been prepared from p-toluene boronic acid[31] (eq.1.28).



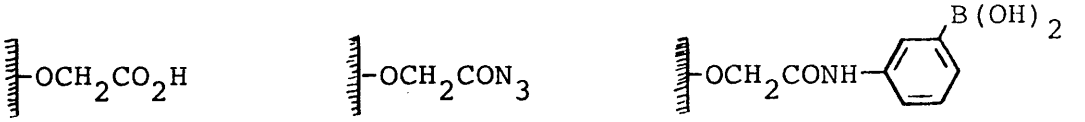
(1.28)

Polymeric boronic acids, which are insoluble in organic solvents but soluble in alkaline solutions, have been used for chromatographic separation of polar polyfunctional compounds, such as sugars[49,9]. One of the methods for preparation of these polymers is by polymerisation of vinyl-benzeneboronic acid, by means of a free radical initiator[46] (eq.1.29).



(1.29)

Another method involves converting O-(carboxymethyl) cellulose (a) into the acyl azide (b), then by treatment with m-aminobenzeneboronic acid, into the borylated form, (c) to give a polymer, which has been used for separation of alditols[9].

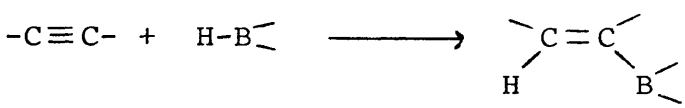


(a) (b) (c)

Recently mixed copolymers prepared by radical-induced copolymerisation of boronic acids have been developed ; these showed higher separation efficiency[49,9].

6.Hydroboration:- Alkane - and alkeneboronic acids have been prepared from the hydrolysis of their corresponding esters, obtained by the hydroboration of alkenes or alkynes, with a disubstituted borane [50,51,52,53,54], (eqs.1.30&1.31).

(1.30)

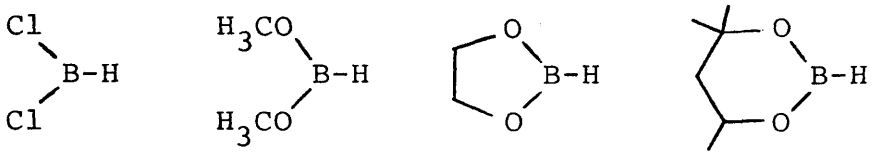


(1.31)

The rapid and quantitative addition of the boron-hydrogen bond to alkenes and alkynes (eqs.1.30&1.31) has made a wide variety of organoboranes readily avaiable and many new reactions of major significance in synthetic organic chemistry have been discovered. This area of chemistry has been extensively reviewed[55,51,52,56,54,57].

Only partial success was achieved in controlling the reaction of borane in THF(BH_3-THF) with olefins, at the monohydroboration stage, with the aim of providing a convenient synthesis of the monoalkylboranes and the corresponding alkaneboronic acids[58,55]. Brown[54,56] and co-workers[51,50] have explored better means of achieving syntheses which will attach only one alkyl substituent to boron. These have been achieved by hydroboration with disubstituted boranes. Many disubstituted borane reagents were used, such as: dichloroborane (I) which reacted sluggishly with olefins[59];

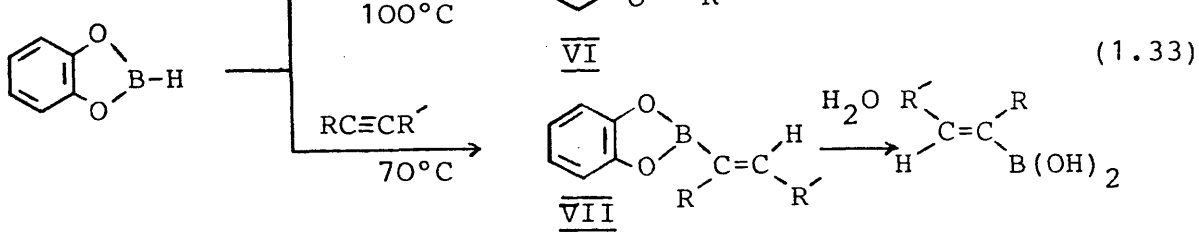
dimethoxyborane[7] (II) and 1,3,2-dioxaborolane[60] (III): these reagents were not spontaneously inflammable in air, but they were rapidly hydrolysed and unstable, II&III undergoing rapid and reversible disproportionation[52]; 4,4,6-trimethyl-1,3,2-dioxaborinane[61] (IV) was more stable, but showed a greatly reduced reactivity relative to BH_3 or to alkylboranes as expected, due to π - bonding between boron and oxygen[61]. Brown and Gupta[50] have



I II III IV

prepared catecholborane(V), from the rapid reaction of borane, within 5 min. at 0°, with catechol in THF(eq.1.32). This disubstituted borane was readily distilled and reacted easily with alkenes at 100° or with alkynes at 70°, giving 2-alkyl-1,3,2-benzodioxaboroles(VI) and 2-alkenyl-1,3,2-benzodioxaboroles(VII) respectively, in nearly quantitative yields[51]. The products were readily hydrolysed to give the corresponding alkane- and alkeneboronic acids[50,51], (eq.1.33). The advantageous properties which catecholborane

(1.32)

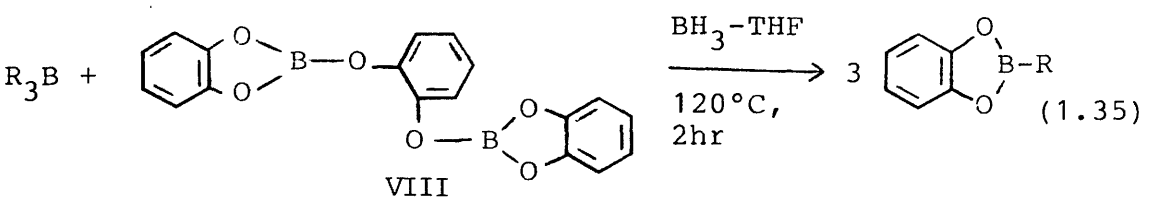


(1.33)

had among the other hydroboration reagents (I - IV) were: it was highly stable on storage, non-inflammable, readily prepared and highly reactive[51,52]. Also it was highly regio- and stereoselective[51,56,54], when it was compared with diborane (B_2H_6). The high reactivity of catecholborane is due to the presence of oxygen atoms bonded directly with the benzene ring, since the oxygen 2p electrons can be delocalised into the benzene ring; consequently, π - bonding between oxygen and boron is less important[52].

In the case of the reaction of catecholborane with low-boiling olefins, a sealed ampoule was required to reach the 100° reaction temperature[50,51]; because of the inherent hazard and the inconvenience involved in this method, Brown and Gupta[50] have developed an alternative preparative route to use with such olefins. They employed a redistribution reaction between a trialkylborane and O-phenylene borate(VIII), which provided alkylbenzodioxaboroles (eqs. 1.34 & 1.35) in essentially quantitative yields.

$$3CH_3CH_2CH{=}CH_2 + BH_3\text{-}THF \longrightarrow R_3B \quad (R{=}C_4H_9) \qquad (1.34)$$

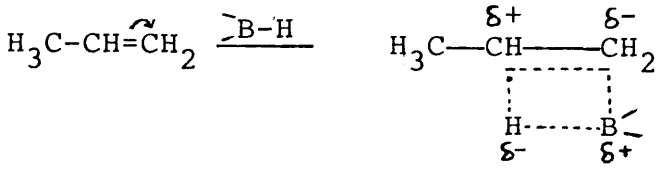


(1.35)

VIII

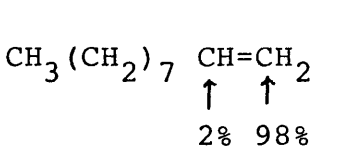
Directive effects in Hydroboration Reactions

The hydroboration reaction involves a simple four-centre transition state, with the direction of addition controlled primarily by the polarization of the boron-hydrogen bond, $B^{\delta+}\text{-}H^{\delta-}$ [55,56,54,57] (eq.1.36).

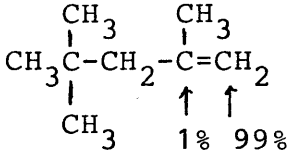


(1.36)

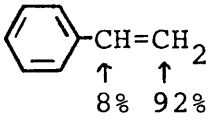
The hydroboration of terminal alkenes proceeds in a highly regioselective manner to place the boron atom preferentially at the terminal position, corresponding to anti-Markownikoff addition to the double bond [51,52,56,54,57]. Directive effects in the hydroboration of alkenes with catecholborane have been studied with selected compounds: 1-decene(IX), 1-diisobutylene(X), styrene(XI), ∝-methyl-styrene(XII) and norbornene(XIII) served as the test series[51,52]. After standard hydroboration reactions, the following results were obtained[51]:



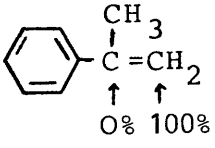
IX



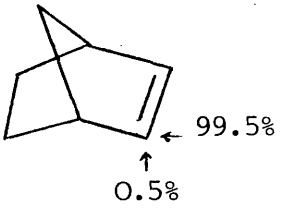
X



XI



XII



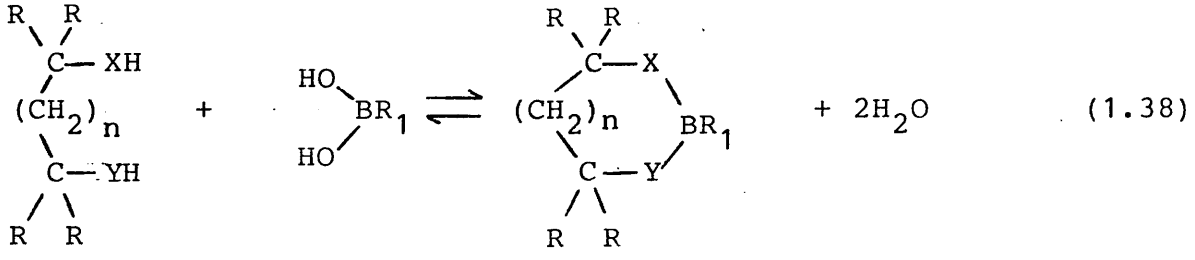
XIII

From the above results, the steric influence of the substituent as in compound(X) had a small effect on the direction of addition of the ≥B-H bond, when compared to compound(IX). The electronic nature of the substituent benzene ring, as in compound(XI), effected a degree of addition of ≥B-H to the carbon atom carrying the benzene ring. In

some compounds, both the steric and the electronic natures of the substituents play major roles in influencing the direction of addition of the >B-H bond, as in compound(XII)[56,54]. In the hydroboration of norbornene(XIII), the >B-H addition was stereoselective, giving predominantly the exo-isomer[51,52,56]. Also the addition of >B-H to a triple bond occurred in a stereospecific cis manner [51,54], (see eq.1.33).

V. CYCLIC BORONATE DERIVATIVES

Bifunctional compounds react selectively with boronic acids in solution to form cyclic boronates[13,15,17,19,20,22,27,62-64]. The reaction can be applied to compounds containing protonic (OH,NH,SH,CO_2H) or enolisable keto groups on proximal (1,2-, 1,3-, or 1,4-)carbon atoms (eq.1.37).



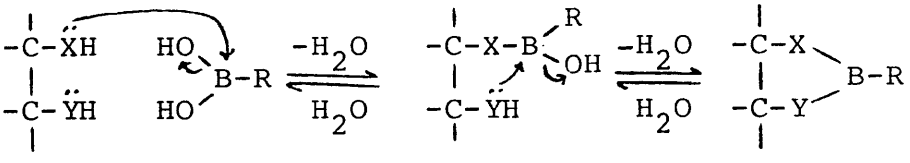
(1.38)

Bifunctional Compound — Boronic Acid — Cyclic Boronate

n=0,1,2

(X & Y) or (X=Y)=O,S,NR

Mechanism



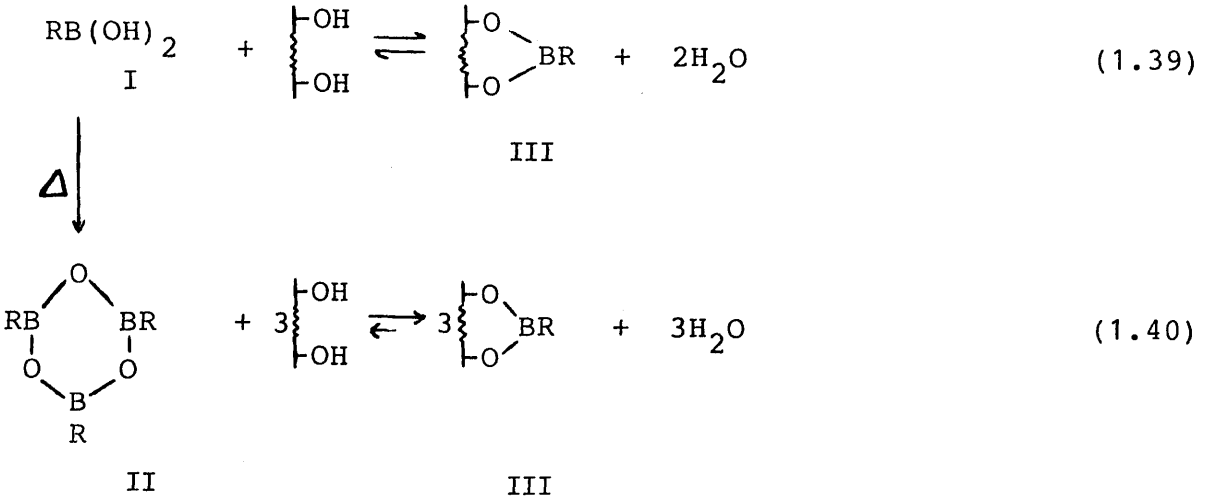
The extent of boronate derivative formation, which is usually an equilibrium process(eq.1.38), depends on the nature

of the solvent and the relative proportions of the bifunctional compound and the boronic acid. In most instances the resulting equilibrium appears to lie strongly towards ester formation: where necessary, the reaction can be aided by azeotropic removal of the water formed as a by-product. Regeneration of the free bifunctional polar compound can often be achieved either by hydrolysis or by displacement with several equivalents of propane-1,3-diol[17,18], or by the use of chromatographic columns, e.g. anionic resins[65], alumina[16]. In many reactions, the cyclic boronate ring was fairly stable during the derivatisation[9,20,66,21] (e.g. esterification, etherification, methoximation) of the remaining functional group(s). Subsequent removal of the boronate group provided a method for protection and selective derivatisation.

The cyclic boronate derivatives of many polar bifunctional naturally occurring organic compounds - carbohydrates, amino acids, catecholamines, fatty acids, prostaglandins, sphingosines, steroids - have facilitated the separation and analytical studies of these substrates.

A. Formation of Boronates

1. Direct condensation of polar bifunctional compounds with boronic acids:- Boronic acids (I) or their corresponding anhydrides (II), react spontaneously, but not necessarily completely, in solution with suitable polar bifunctional compounds (eq.1.38) to give cyclic boronate esters (III), (e.g. eqs.1.39 & 1.40). The boronates formed can, in many cases, easily be isolated by removing the water and solvent,

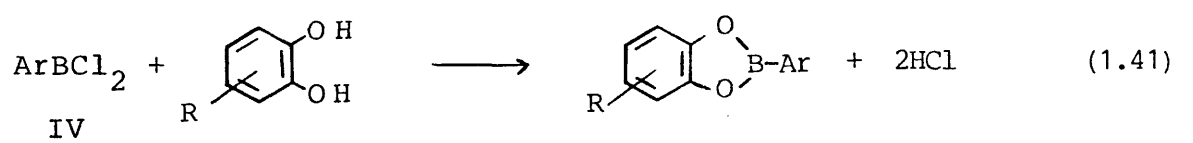


(1.39)

(1.40)

under neutral conditions[17]. The isolated yield of the boronate mainly depends on its stability in water (see p. 31) and the nature of the polar bifunctional compound (see p. 33). In some cases the boronate crystallises out of the solution[13,64]; for example, the benzeneboronates of mannitol and sorbitol were precipitated out of methanol-water solution[13].

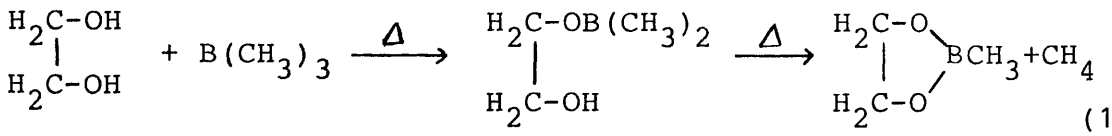
Boronates which were unstable towards moisture have been prepared in boiling dry solvents (e.g. benzene, toluene, acetone, 1,4-dioxan), the water produced being removed by careful fractional distillation of its azeotrope with these solvents[63,64,15,17,20]. Boroxines have been used instead of their corresponding boronic acids (eq.1.40), to minimise the amount of water formed [15,17]. Arylboron dichlorides (IV) were used instead of arylboronic acids, to form boronates[67] (eq.1.41) with the associated production of HCl which could be easily removed.



(1.41)

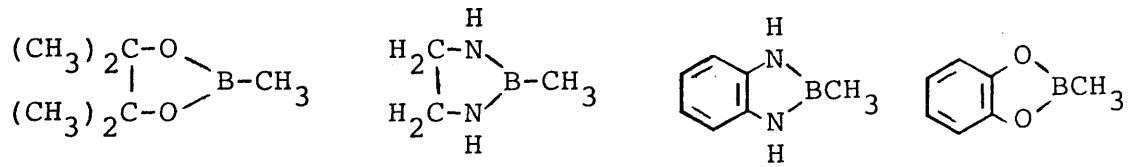
2. Via borinates:- Methaneboronates of dihydroxy and diamino compounds (VII - XI), have been prepared from the

reaction of their corresponding polar bifunctional compounds with trimethaneborane(V) at high temperatures (280-380°)[68], presumably by way of the intermediate dimethaneborinate(VI), (eg.1.42).



(1.42)

V VI VII



VIII IX X XI

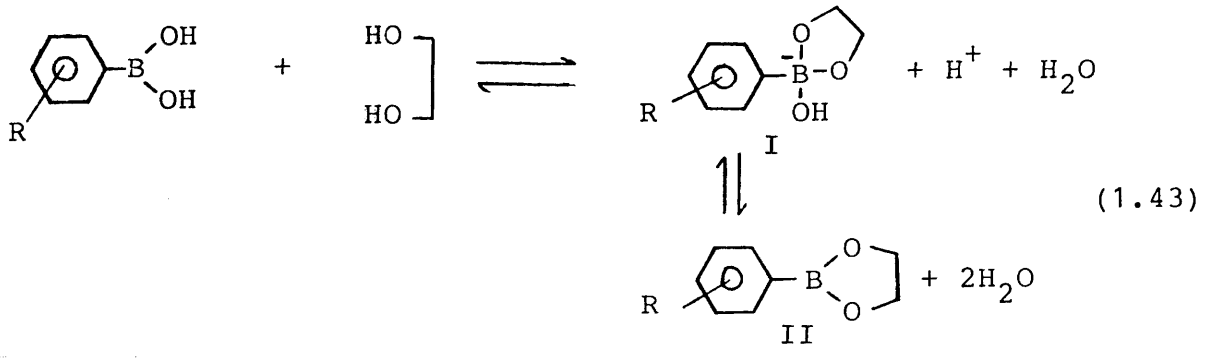
In Ferrier's review on boronates[9], there are several examples cited of carbohydrate methaneboronates, which have been prepared by the above method. The procedure may be used for preparative purposes, and has the advantage of not producing water as the by-product.

B. Boronates in Chemical Reactions

1. Boronates in aqueous systems:-

a. Interaction between polyhydroxy compounds and boronic acids in aqueous media:- The addition of certain polyhydroxy compounds to an aqueous solution of boric[12] or boronic acids[36,69], increases their acidity. Potentiometric titrations have determined formation constants for 1:1 complexes[69,70]. The free boronic acids react with diols to give either the anionic(I) or neutral(II) complex[36,69,9] (eq.1.43). At

equilibrium, however, the two schemes will be indistinguishable



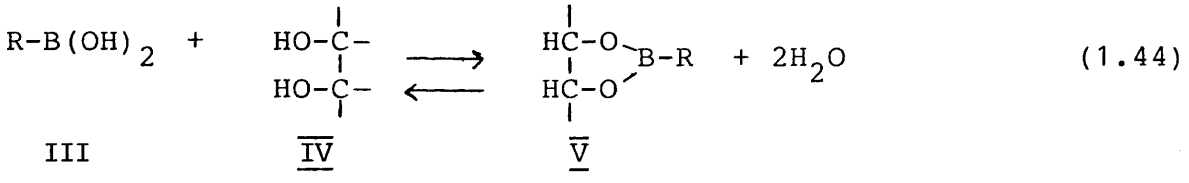
(1.43)

and in both schemes, the formation of the anionic complex(I) is favoured in alkaline solution[69]. The anionic complex(I) should be stabilised by electron-withdrawing groups in the aromatic ring[69,9].

Barker and co-workers[69,71,41] conducted a detailed polarimetric analysis of the complexing undergone between monosaccharides and areneboronic acids. They studied the effect of pH on the optical rotation of equimolar solutions of an acid and sugar. These studies showed that a complex of the sugar has specific optical rotation different from that of the free sugar. As predicted by theory, complexes are not formed at acidic pH values; Barker et al.[69] observed that, at low pH values, the rotation of the cyclic ester corresponds to that of the free sugar; but at high pH an anionic complex was formed, having a different optical rotation value.

b.Hydrolysis of boronates:- Generally, boronic acid esters (boronates) are hydrolysed readily, but some evidence indicates that at least certain esters are relatively stable in aqueous systems: benzeneboronates of sugars and alditols may be isolated by crystallisation from aqueous methanol[13,72]. Boronates of various diols can be precipitated by acidification

of aqueous alkaline solutions[9]. It has been suggested[63] that the first of these points does not establish the stability, but rather the insolubility of the esters in water, and the second point conceivably reflects the insolubility of trigonal esters relative to their tetrahedral, anionic analogues. The addition of water to the reversible equilibrium reaction between boronic acids (III) and polar bifunctional compounds (e.g. diols, IV) (eq. 1.44) will direct this equilibrium considerably to



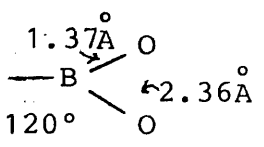
(1.44)

III IV V

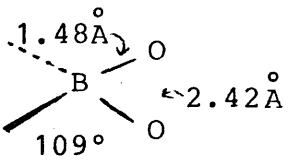
the left, but in many cases[9] the equilibrium could be directed to the right, by extracting continuously the formed boronate (V) with an organic solvent (e.g. chloroform, light petroleum); in this case, the reactants (III and IV) may also be taken separately into the organic solvent only to recondense during the subsequent removal of the organic solvent. It is, therefore, suggested that none of this evidence establishes the stability of any of the esters in aqueous media[9]. The hydrolysis of boronates and their equilibrium constants could be monitored by optical rotation measurements[73,9]; on adding water to solutions of boronates in dry solvents, optical rotational changes in magnitude occur in direction consistent with their being caused by hydrolysis.

Generally, the ring size of cyclic boronates affects their stability towards hydrolysis[24,9]. The relevant bond-lengths and bond angles typically associated with trigonal (VI) and tetrahedral (VII) boron in boronates are as shown[74].

Consequently[74,24], both can be accommodated in strainless,

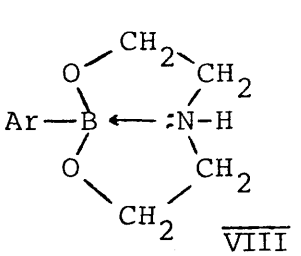


VI



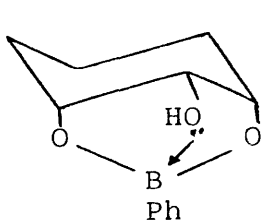
VII

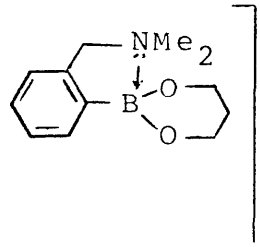
six-membered rings formed from, for example, 1,3-diols, whereas five-membered cyclic boronates (e.g., from 1,2-diols) are free from angle strain only when the boron is tetrahedral. Thus the strained five membered ring exhibits a greater tendency towards hydrolysis, than its six-membered counterpart[75]. The stability of cyclic boronates also depends upon the presence and type of substituent groups on the ester rings and elsewhere in the molecules. Groups that inhibit co-ordination of water with boron stabilise the esters towards hydrolysis, as, especially, do groups which can themselves act as fourth ligands[75,76,72]. For example the arylboronates of diethanolamine[77] (VIII) gave crystalline products which were completely unaffected by atomospheric moisture and which could also be recovered unchanged from aqueous solution. The stability of these esters towards hydrolysis suggests that the electron deficiency of the boron atom is satisfied by donation from the nitrogen[77].



VIII

cf.





2. Removal of boronate groups:- When boronic esters are used as protecting groups in the synthesis of specifically substituted, or modified, derivatives, the cleavage of the esters is normally followed by recovery of the by-products

liberated. The esters can be hydrolysed under neutral[14,72], acidic or alkaline[20,63] conditions. If the hydrolysed substrates are strongly hydrophilic, then the boronic acids can be specifically extracted from aqueous into organic solvents, and in this way, alditols[63] and free sugars[14] have been recovered from their benzeneboronates. In some cases[72], the hydrolysed substrates crystallise out of the solution, and may then be purified by recrystallisation. In other cases the boronic acids have been crystallised out of the solution[50].

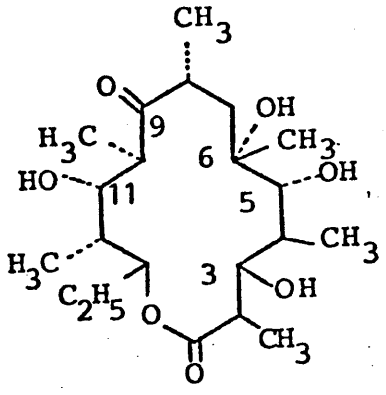
A more widely applicable procedure involves exchanging the boronic acids from the boronate to 1,2-ethanediol[9], or more usually 1,3-propanediol[18,17,78,9], with which they condense to give volatile, cyclic derivatives. Addition of 1,3-propanediol to a solution of boronates in acetone, and removal of the volatile products, usually offers a very efficient method of deboronation.

Column separations of the products of hydrolysis of boronates by use of anionic resins provides an alternative, efficient means of deboronation[65,16,71,49], and other similar separations have used columns of cellulose[79], or alumina[16].

An interesting alternative procedure for removing the benzeneboronic acid involved its conversion by treatment with bromine-water, into bromobenzene and boric acid. After adding methanol, the borate ester was removed by distillation[80].

3. Stability of boronates during chemical reactions:-

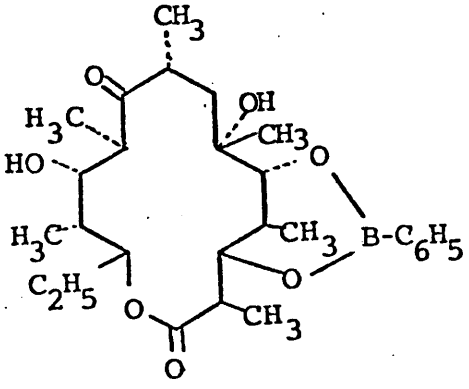
a.Esterification:-Selective acetylations of unsubstituted hydroxy groups in boronate derivatives, without affecting the boronate group, have been reported on several occasions.



Erythronolide B

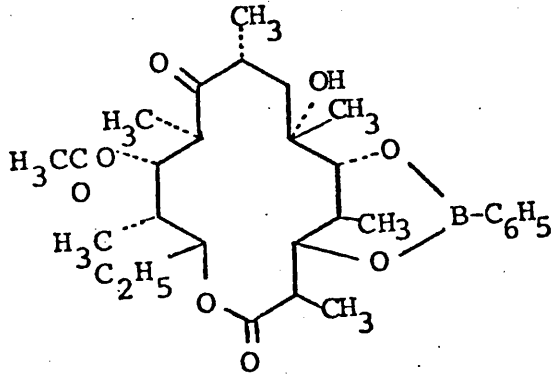
IX

$PhB(OH)_2$ in Anhy. Me_2CO, 3.5hr, reflux



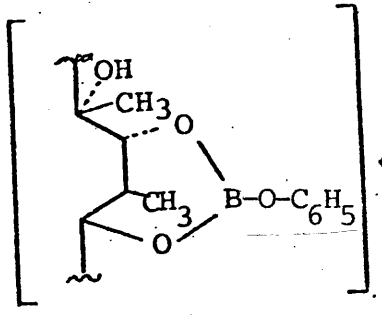
3,5-benzeneboronate

Ac_2O in dry Py. — at R.T. for 2 days.



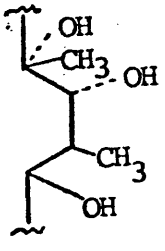
11-acetylerythronolide B-3,5-benzeneboronate

H_2O_2, in dil. EtOH, $NaHCO_3$, as buffer, stirring for 1 day.



X

H_2O

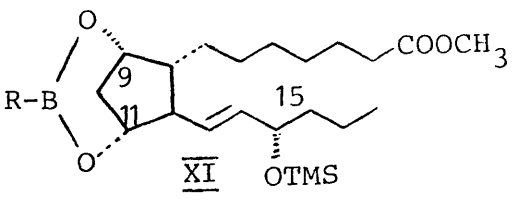


+ H_3BO_3 + C_6H_5OH

Scheme 1.3

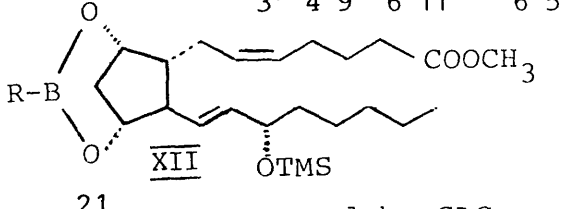
Acetyl chloride (or acetic anhydride) in pyridine has usually been used as the acetylating agent for acetylation of unsubstituted hydroxy group(s) in the boronates of glycosides[15,73,16], alditols[9], and nucleosides[9]. Acetic anhydride in dry pyridine has been used for selective esterification of the C-11 hydroxyl in erythronolide B,(IX), while the C-3 and C-5 hydroxy groups of this molecule have been protected as the cyclic benzeneboronate[20]. The protecting group was removed by treatment with dilute alkaline hydrogen peroxide and hydrolysis of the presumed borate ester intermediate(X), scheme 1.3.

b.Etherification:-The first satisfactory methylation appears to have been effected by Bourne and co-workers[79], who prepared 6-O-methyl-D-glucose in high yield from the 1,2:3,5-bis(benzeneboronate), using diazomethane and boron trifluoride etherate in dichloromethane for methylation, and a final separation on a column of cellulose powder. For volatile derivatives suitable for GLC and GC-MS studies, the unsubstituted C-15 hydroxy groups in the prostaglandin ($F_{1\alpha}$, XI and $F_{2\alpha}$, XII)-9,11-alkaneboronates have been selectively trimethylsilylated with bis(trimethylsilyl) trifluoroacetamide without affecting the boronate group[66].



XI

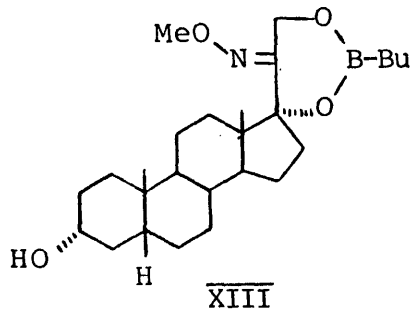
R=CH_3, C_4H_9, C_6H_{11} or C_6H_5



XII

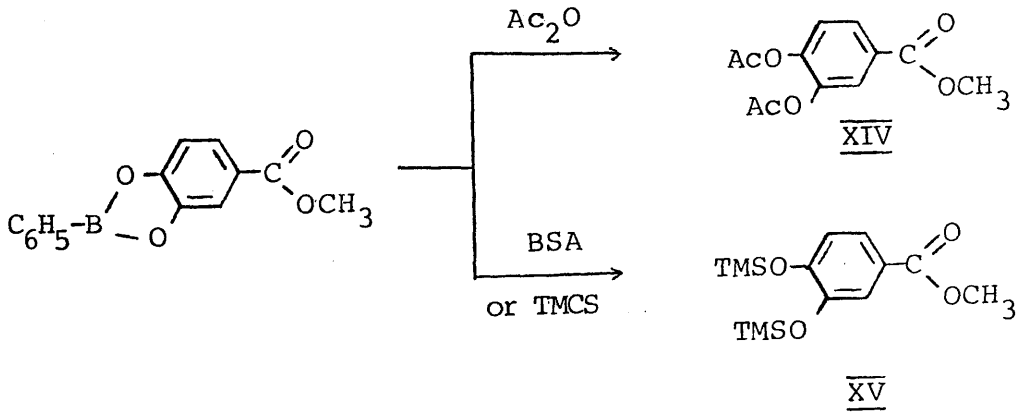
c.Methoximation:- Anthony et al.[21] have proved by GLC, GC-MS and IR analysis, that the 17,20-cyclic boronate ester of the dihydroxyacetone side chain in the steroid"tetrahydro-S", (XIII) was fairly stable during the methoximation of the C-20

carbonyl groups, with methyl-hydroxylamine hydrochloride in dry pyridine.



XIII

d. Displacement of the boronate group with acetyl or trimethylsilyl groups:- Many boronate derivatives of catecholic type of compounds (e.g. dihydroxybenzoic acid esters, estrogens, dopamine, apomorphines), which have been studied in Chapter 5, proved to be unstable towards dilute solutions of acetic anhydride and towards trimethylsilylating reagents(BSA or TMCS). A displacement reaction took place, yielding the corresponding diacetyl(XIV) or di-trimethylsilyl (XV) derivatives, as shown in Fig.2.



XIV

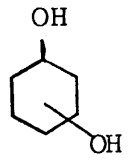
XV

Fig. 2

VI. APPLICATIONS OF BORIC & BORONIC ACIDS & THEIR DERIVATIVES

A. Separation of Polar Bifunctional compounds by use of their Boratesor Boronates

1. (a)The distillation of a reaction mixture of boronic acid or boroxine with an isomeric pair of cis, trans-cycloalkane diols provided a convenient means for the separation of the two isomers. Brown and Zweifel[25] have separated pairs of cis, trans-cyclohexane diol isomers(I), by esterification with butaneboronic acid or butylboroxine. The volatile cyclic

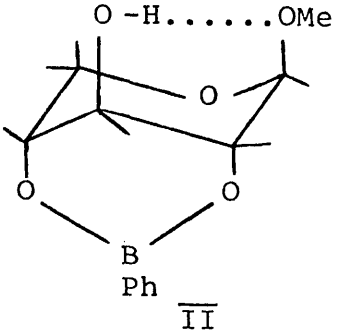


I

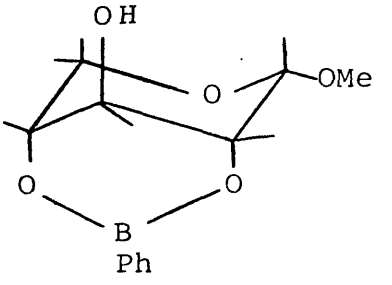
boronates, 1,2-cis and trans,1,3 - cis and 1,4-cis (boat conformation seven-membered ring), were distilled under vacuum, whereas 1,3-and 1,4-trans isomers, yielded nonvolatile polymeric esters.

(b)The differencesin the solubilities of cyclic boronate esters of isomeric carbohydrates greatly facilitated their separation by fractional recrystallisation[16,18,9]. Both methyl D-xylopyranosides reacted under dehydrating conditions with benzeneboronic acid and gave 2,4-cyclic esters, and whereas the 3-hydroxy group in the β-derivative(II) is strongly intramolecularly hydrogen bonded to 1-O, that in the α-compound (III) is free from such bonding. The large difference in the solubilities of these esters (α,0.07g/ml; β, 53g/ml in hot light petroleum), which resulted from this difference

in bonding, provided a simple means of isolating the difficultly obtainable methyl∝-D-xylopyranoside[16].

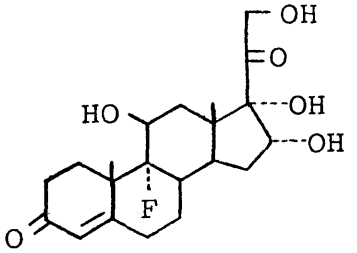


II

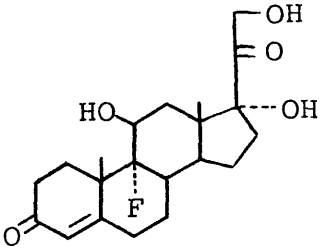


III

The water-soluble borate complex of 9∝-fluoro-16∝-hydroxyhydrocortisone(IV) was separated from the insoluble 9∝-fluorohydrocortisone(V), by ethyl acetate-water extraction[81].



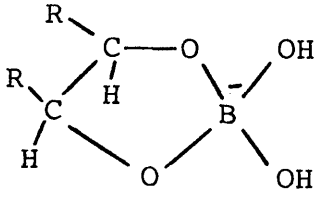
IV



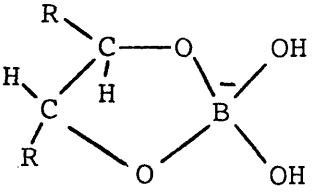
V

A method has been devised for the separation of (IV) and (V), prior to spectrophotometric analysis, and their determination in fermentation broths[81].

The meso and racemic isomers of butane-2,3-diol, pentane-2,4-diol, 1,3-diphenylpropane-1,3-diol, and other related diols, have been separated by means of their borate complexes, by extraction, crystallisation and hydrolysis methods[82]. Also the bis(3-propanol) amines of general formula(VI) [bis (2-nitro-2-hydroxymethyl-alkyl amines)] which exist as two

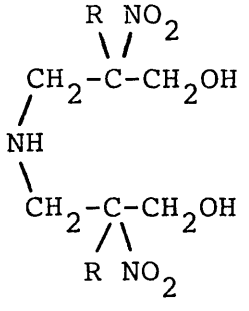


meso

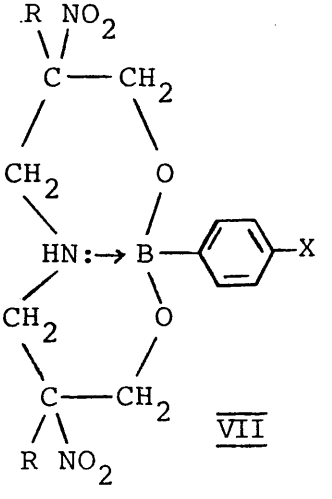


Racemate

diastereoisomers, meso and DL, formed cyclic boronate derivatives (VII) with benzene- or areneboronic acids. These diastereoisomeric boronates showed different solubility in benzene, so that the insoluble cis isomer was separated from the soluble trans isomer[76]. After isolation of the pure isomers they were hydrolysed to yield pure diastereoisomers of the bis (3-propanol)amine.



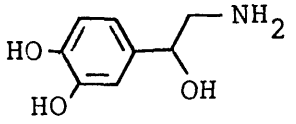
VI



VII

2. Use in paper chromatography:-The mobilities of carbohydrate cis 1,2-,1,3-diols and triols were enhanced on paper chromatography, when benzeneboronic acid was added, and some particularly stable, boronic esters were formed[72,9]. This chromatographic technique has been used in certain cases to detect cis,cis-triols and thereby, to assist in configurational analysis[9].

3. Use in gel chromatography:- The isolation of catecholamines (e.g. norepinephrine, VIII) can be effected by means of boric acid gel, which retains various compounds by two functions; esterification of the .diol moiety and ion exchange with the amino moiety[83].

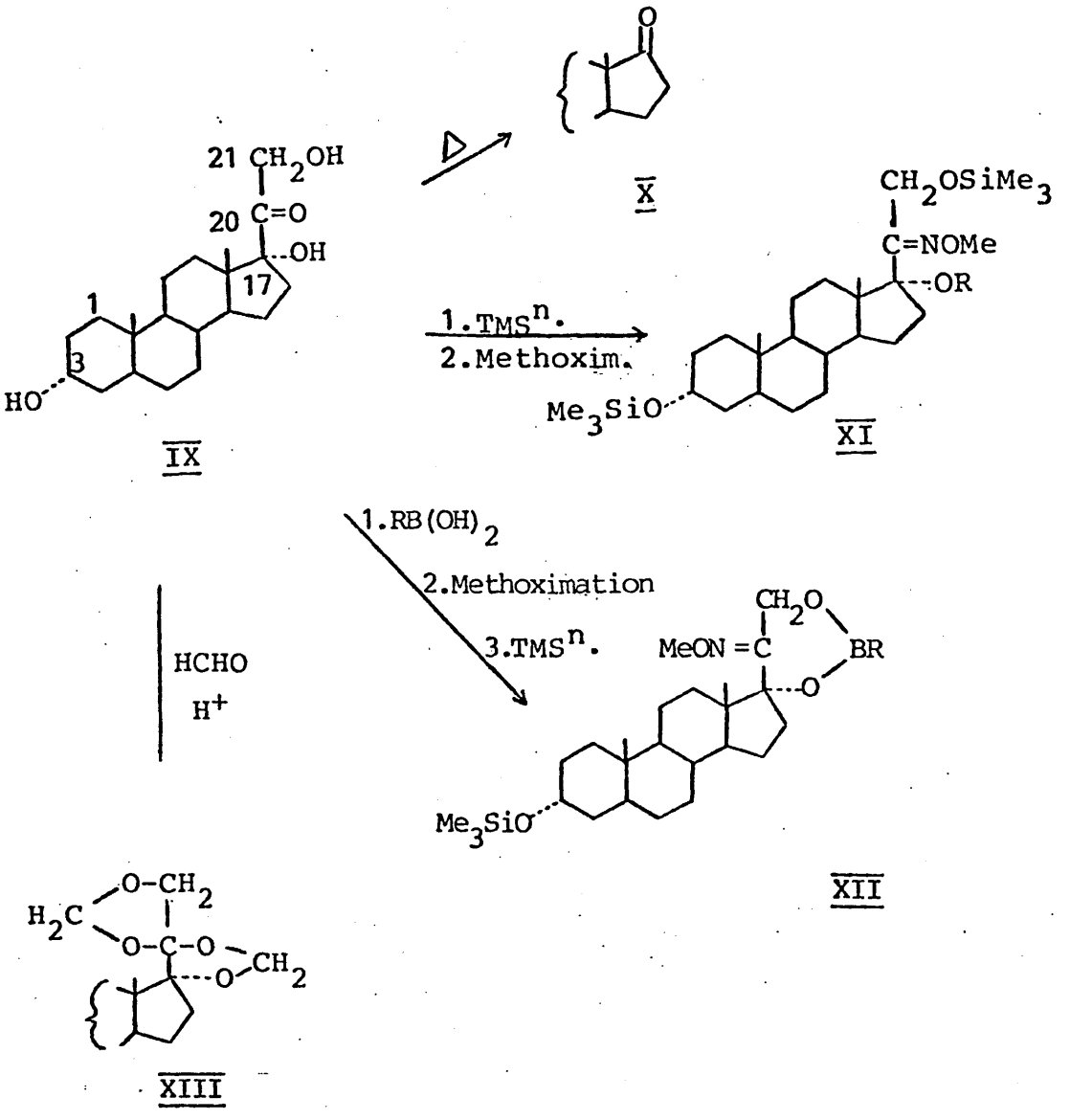


VIII

These properties contribute to the simplicity and high recovery of this method, avoiding deproteinization and pH adjustment of the plasma.

Another good chromatographic separation of carbohydrates[84], catechol amino acids and catecholamines[85] (e.g. dopa,dopamine), on an affinity gel containing immobilised benzenboronic acid, has been extensively used[85]. The advantage of this method lies in separating the catechol compounds without contamination by boron.

4. Use in gas chromatography-mass spectrometry:- Cyclic alkyl-or benzeneboronates are thus valuable and relatively highly volatile derivatives for use in GLC and GC-MS analysis of a great variety of the less volatile polar multifunctional compounds, including many of biological importance. Among these are such species as carbohydrates[23,86-90], catecholamines and their metabolites[21,26,27], ceramides and sphingosines[62,91,92], corticosteroids[30,21,22,93], α and β-hydroxy fatty acids[21,94], monoglycerides[30,91], and prostaglandins[66]. The quantitative determination of these compounds in low concentrations(ng/ml) in biological fluids, could be improved


21 CH2OH
20 C=O
OH
17
1
3
HO
IX
Δ
X
CH2OSiMe3
C=NOMe
OR
1.TMSn.
2.Methoxim.
Me3SiO
XI
1.RB(OH)2
2.Methoximation
3.TMSn.
HCHO
H+
MeON=C
CH2O
BR
O
Me3SiO
XII
O-CH2
H2C
O-C-O
O
CH2
XIII

Scheme 1.4

by employing boronic acids containing electron-capturing groups[95] (e.g. $RB(OH)_2$, R=2,4-dichloro-, 4-bromo-, or 4-iodophenyl); then advantage can be taken of the high sensitivity and selectivity of the electron-capture detector[95,96].

The ease of preparation of these cyclic boronates under mild conditions, their chemical and thermal stability*, compatibility with other derivatisation techniques and good GLC and GC-MS properties recommend them for wide use. For example the underivatised free "dihydroxyacetone" side-chains in corticosteroids (e.g. the steroid "tetrahydro-S", IX, Scheme 1.4) are unstable to GLC[21,93,97], undergoing thermal degradation to a mixture of products including 17-ketones (X). The corticosteroids showed higher stability if the 17,21-dihydroxy groups were trimethylsilylated[93] or acetylated[98], and the 20-keto group was previously converted into a methoxime[21,93], (XI). There is, however, some experimental difficulty in derivatising the sterically hindered 17α-hydroxy group[98]. The 17, 21-cyclic boronate derivatives[21,22], (XII), were easily formed under mild conditions and showed high stability under GLC conditions. On the other hand the preparation of bicyclic bis-methylenedioxy derivatives (XIII) required strong acidic conditions, and the yields were poor[99].

Besides displaying good GLC properties, cyclic alkyl- or benzeneboronates yield useful mass spectra by both electron impact and chemical ionization techniques. In contrast (frequently) with other derivatives (e.g. trimethylsilyl ethers), cyclic boronates usually display a high abundance of molecular ions. Also fragmentation modes can be inferred from a comparison of the shifts in the masses of fragment ions corresponding

*(Some boronates are not stable, see Chap. 1, V,B).

to various substituents such as methyl-, cyclohexyl-, and phenyl-, attached directly to the boron in the boronate ring.

B. Nuclear Magnetic Resonance Spectroscopy of Boronates

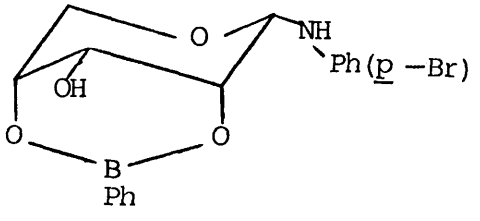
^{1}H-, ^{13}C-, and ^{11}B-NMR studies have been reported, and all provide means for elucidating the structures of boronates. The rigidity of the cyclic boronate derivatives of corresponding 1,2-, or 1,3-diols, requiring relatively fixed conformations, was used in NMR studies of carbohydrate conformations and the position of their functional groups[78,100,9]. The low solubility of certain polyhydroxy compounds, such as carbohydrates and catecholic compounds, in the readily available $CDCl_3$ or CCl_4 solvents for NMR studies, was circumvented by their condensation with alkyl- or benzeneboronic acids. The ^{11}B resonance of a boron ester is a broad line[101,102], ^{11}B-NMR spectra allow distinction between five-and six-membered cyclic and acyclic boronate derivatives[102].

C. X-Ray Crystallography of Boronates

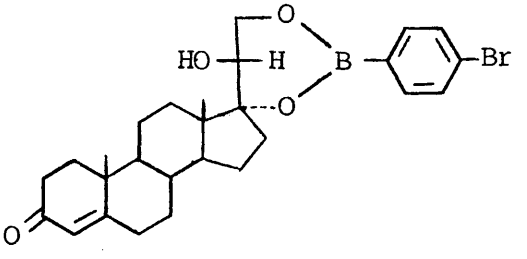
The condensation of areneboronic acids with polar bifunctional compounds in many cases yields crystalline derivatives, which are useful for X-ray diffraction.

This method establishes the conformation of the molecule in the crystalline state, and furthermore gives the bond lengths and angles about the boron atom: thus the absolute configuration and conformatiion of the six-membered, N-(*P*-bromophenyl)-α-D-ribopyranosylamine 2,4-benzeneboronate (XIV), was determined from its crystallographic and physical data[28].

Also the p-bromobenzeneboronate ester of 17∝,20β, 21-trihydroxypregn-4-en-3-one (XV) has been shown by X-ray crystallography to have a six-membered ring structure involving the 17- and 21-hydroxy-groups. The dioxaborinane ring has an envelope-like conformation in which C(20) is the out-of-plane atom. The hydroxy-group at C(20) is not co-ordinated to boron, O...B being 3.05Å[29].



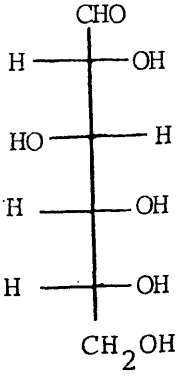
XIV



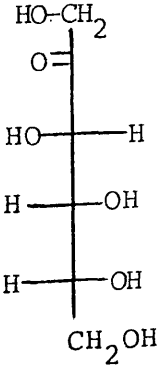
XV

D. Use as Catalysts

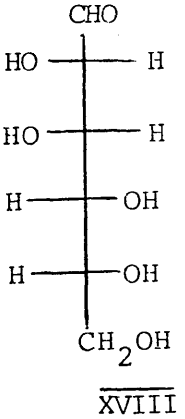
The complexes of areneboronic acids with carbohydrates, formed in aqueous solution in a manner similar to that of borate[12,36], have been used to displace the pseudo-equilibria established in aqueous alkali between D-glucose (XVI), D-fructose (XVII), and D-mannose (XVIII) to give greatly increased yields of D-fructose[49,69,71]. 8-Quinolineboronic acid (XIX) was found



XVI

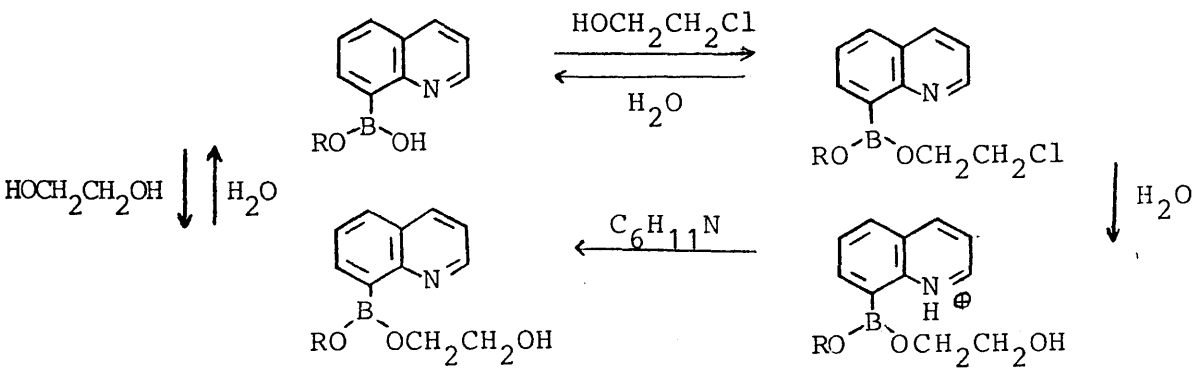


XVII



XVIII

to be a polyfunctional catalyst for hydrolysis of chloroethanol and 3-chloro-1-propanol in dimethylformamide solutions containing water and collidine. In the absence of 8-quinolineboronic acid, the chloroalcohols underwent slow solvolysis in dimethylformamide solution to products that were not glycols[103]. It is proposed that the boronic acid group in 8-quinolineboronic acid functions as a binding site for the chloroalcohol and that the nitrogen participates in the reaction as a basic or nucleophilic transforming site.



E. Boron Compounds in Cancer Therapy

The use of boron compounds in the treatment of cancer has revolved about the unique nuclear property of the nonradioactive ^{10}B isotope to absorb thermal neutrons with the liberation of much energy; these studies have been reviewed by Soloway[104]. The two isotopes, ^{10}B and ^{11}B, differ greatly in this property. Whereas ^{11}B, with a normal abundance of 80.39%, has a cross section for capture of slow neutrons of 0.005 barns (1 barn $\equiv 1 \times 10^{-24} cm^2$), ^{10}B, with an abundance of 19.61%, has a cross section for capture of 4017 barns[105]. It was on this basis that Locher* in 1936 first proposed the use of ^{10}B and other nuclides with high cross

* cited from Reference no. 104.

section capture as a means of selectively destroying or weakening cancerous cells.

The ^{10}B compounds which would be used of necessity must have low toxicity to the mammalian organism in order for the compounds to be tolerated in large doses[104]. The interaction of ^{10}B and slow-moving neutrons, however, results in fission with the fragments sharing an energy of 2.4MeV. This nuclear reaction at the molecular level would be lethal

$$_5B^{10} + _0N^1 \longrightarrow (_5B^{11}) \longrightarrow {}_3Li^7 + {}_2He^4 + 2.4\ MeV \quad (1)$$

to those cells absorbing such an enormous amount of energy. Other main elements (i.e. H=0.32 barns, Ca=0.42,C=0.0045, N=1.7, P=0.15, O=0.001, Cl=32.5) of normal tissue have low cross section values for thermal neutrons compared with ^{10}B. However, with a ^{10}B concentration in the tumour of 50mg/kg of tissue, it has been estimated that 86% of the total radiation dose would result from the ^{10}B-capture reaction. Thus, the destructive radiation would be mainly restricted to those areas having high ^{10}B concentrations[104].

There are three essential factors for the selective destruction of tumours. Firstly, there must be a large concentration of ^{10}B in all areas of the neoplasm. Secondly, a source must be available to irradiate the neoplastic area with a sufficient number of thermal neutrons. Thirdly, a sufficiently large differential of ^{10}B between the neoplasm and adjacent normal tissue must exist to permit the complete eradication of tumour without adversely affecting tissue surrounding the neoplasm[104].

The utility of the great majority of the new organoboron compounds and boron hydrides as medicinal agents and especially for use in cancer therapy remains to be evaluated. For use in this therapy, the boron compounds must meet certain criteria[104]:

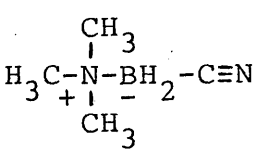
1- They must, of necessity, be highly water-soluble.

2- They should be soluble at the physiological pH of 7.4 to avoid toxic effects.

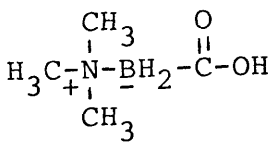
3- High chemical and biological stability is also of prime importance, for many boron compounds are quite susceptible to hydrolysis and oxidation.

4- A suitable boron compound must have not only a low toxicity and high tumour/normal tissue boron ratio but also a lower concentration in the blood and blood vessel walls compared with the tissue to be destroyed.

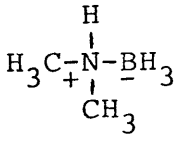
Series of boron-containing analogues of biologically important compounds (e.g. amino acids) that appear to show considerable promise as therapeutic agents in preliminary animal screens have been synthesised[106]. Beneficial effects have been reported from their use in animal models for arthritis (e.g.XX), high blood cholesterol (e.g.XXI), and several types of cancers (e.g.XXII & XXIII),but the mode of action is not clear.



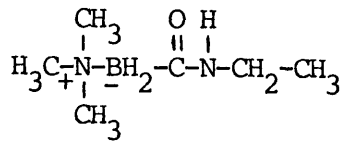
XX



XXI



XXII



XXIII

Syntheses of certain boron compounds have been undertaken because of their close similarity to naturally occurring biologically active compounds. For example, substitution of a boron atom for a carbon atom in the backbone of an amino acid means the final molecule will have one fewer proton and two fewer electrons than its unsubstituted counterpart. Boron can bond tetrahedrally, as carbon does, and with the same stereosymmetry. This close similarity between the boron analogues and naturally occurring amino acids makes them compounds of potential therapeutic value. They should mimic the natural compounds well enough to get into cells and interact with specific enzymes, but they should be different enough from natural amino acids that they may block enzyme activity. This idea could also be applied in respect of steroidal hormones and other biologically active compounds.

Chapter 2

MATERIALS AND METHODS

I. SOLVENTS

The following solvents were obtained from BDH Chemicals Ltd, unless otherwise specified;

Acetic acid glacial "Analar", AcOH, CH_3COOH; Koch-Light.

Acetone "Analar", Me_2CO, CH_3COCH_3.

Acetone-d_6 (deuteroacetone-d_6), Me_2CO-d_6, CD_3COCD_3.

Acetonitrile (Methyl cyanide), CH_3CN; Hopkin and Williams.

Benzene "Analar", C_6H_6.

Benzonitrile (benzene cyanide), C_6H_5CN; Hopkin and Williams.

Carbon tetrachloride "Analar", CCl_4.

Chloroform "Analar", $CHCl_3$.

Chloroform-d (deuterochloroform-d), $CDCl_3$.

Cyclohexane "Analar", C_6H_{12}.

Dichloromethane (Methylene chloride), "Analar", CH_2Cl_2.

Diethyldigol (diethylene glycol diethyl ether), $(C_2H_5O.CH_2CH_2)_2O$.

Diethyl ether (ether), "Analar", Et_2O, $(C_2H_5)_2O$.

Ethanol absolute "Analar", EtOH, C_2H_5OH.

Ethyl acetate "Analar", EtOAc, $CH_3.COOC_2H_5$.

Hexane fraction from petroleum, n-Hexane, C_6H_{14}.

Methanol Analar, MeOH, CH_3OH; May and Baker Ltd.

Methylcyclohexane, $CH_3.C_6H_{11}$.

Petroleum spirit (petroleum ether), boiling range: 30-40° and 60-80°.

Pyridine "Analar", py., C_5H_5N.

Toluene "Analar", $C_6H_5.CH_3$.

II. REAGENTS

The following reagents were obtained from BDH Chemicals Ltd, unless otherwise specified;

Acetic anhydride "Analar", Ac_2O, $(CH_3CO)_2O$, mol.wt.102.09.

Acetyl chloride "Analar", AcCl, CH_3COCl, mol.wt. 78.50.

L-Ascorbic acid "Analar", $C_6H_8O_6$, mol.wt. 176.13, Hopkin and Williams.

Benzene boronic acid, "BB", $C_6H_5B(OH)_2$, mol.wt. 122; Koch-Light Labs. Ltd.

N,O-Bis-(trimethylsilyl)-acetamide, "BSA", $CH_3C\begin{matrix}O\text{-}Si(CH_3)_3\\ N\text{-}Si(CH_3)_3\end{matrix}$ mol.wt. 203.43; Pierce Chem. Co.

Boric acid "Analar", H_3BO_3, mol.wt. 61.83; Hopkin and Williams.

Boron trichloride, BCl_3, mol.wt. 117.17.

Bromine "Analar", Br, at. wt. 79.91; Hopkin and Williams.

2,3-Butanediol,(Meso & DL), (2,3-Butyleneglycol), $C_4H_{10}O_2$, mol.wt. 90.12; Fluka AG, Buchs SG, Switzerland.

D-2,3-Butanediol, ICN. K & K Laboratories Inc.

n-Butaneboronic acid,"n-BuB", $C_4H_9B(OH)_2$, mol.wt. 102; Applied Science Laboratories, Inc.

Caesium fluoride, CsF, mol.wt. 151.9.

Catechol, (1,2-dihydroxybenzene), $C_6H_4(OH)_2$, mol.wt.110.11; Hopkin and Williams.

Catecholborane,95% (1,3,2-benzodioxaborole), $C_6H_5BO_2$, mol.wt. 119.92; Aldrich Chem. Co.

4-Chloro-2-benzenequinazoline, "AM-ex-OL", $C_{14}H_9ClN_2$, mol. wt. 240.5; Aldrich Chem. Co.

Cupric sulphate "Analar", $CuSO4.5H_2O$, mol.wt. 249.68; Hopkin and Williams.

1,5-Diphenylcarbazone, $C_6H_5.N{:}N.CO.NHNH.C_6H_5$ "DPC", mol.wt.240.27

Ferric chloride hexahydrate "Analar", $FeCl_3.6H_2O$, mol.wt. 270.30.

Hexamethyldisilazane, "HMDS", $(CH_3)_3SiNHSi(CH_3)_3$, mol.wt. 161.41; Pierce Chem. Co.

Hydrazine hydrate (98-100%), $NH_2.NH_2.H_2O$, mol.wt. 50.06; Hopkin and Williams.

Hydrobromic acid "Analar", HBr, mol.wt. 80.92; Hopkin and Williams.

Hydrochloric acid "Analar", HCl, mol.wt. 36.46.

Iodine "Analar", I_2, at. wt. 126.90.

(±)-cis-1,2-Indane diol), $C_9H_{10}O_2$, mol.wt. 150; Prof C.J.W. Brooks[107].

Lithium aluminium hydride, $AlLiH_4$, mol.wt. 37.95; Hopkin and Williams.

Magnesium turnings, Mg, at.wt. 24.31.

Methaneboronic acid, "MB", $CH_3B(OH)_2$, mol.wt. 60; Alfa.

Methoxyamine hydrochloride, $CH_3O\overset{+}{N}H_3\overset{-}{Cl}$, mol.wt. 83.52; Eastman.

Methyl iodide, CH_3I, mol.wt. 141.94; Hopkin and Williams.

Molecular sieve type 4A (Crystalline sodium alumino-silicate); Hopkin and Williams.

Perchloric acid (72% w/w), $HClO_4$, mol.wt. 100.46; May and Baker Ltd.

Phosphorus tribromide, PBr_3, mol.wt. 270.70; Hopkin and Williams.

Pinacol (2,3-dimethyl-2,3-butanediol), $C_4H_{14}O_2$, mol.wt. 118.18; Aldrich.

Potassium bromide, KBr, mol.wt. 119.0; Hopkin and Williams.

Potassium carbonate, anhydrous, "Analar", K_2CO_3, mol.wt. 138.21; Hopkin and Williams.

Potassium hydroxide pellets "Analar", KOH, mol.wt. 56.11.

Potassium iodide, KI, mol.wt. 166.0.

Potassium nitrite, KNO_2, mol.wt. 85.10; Thomson Skinner and Hamilton.

Potassium nitrosodisulphonate, FREMY'S SALT, $ON(SO_3K)_2$, mol.wt. 268.0; Alfa.

Propane-1,3-diol, $CH_2(CH_2OH)_2$, mol.wt. 76.10.

Propionic anhydride "Analar", "Pr_2O", $(CH_3CH_2CO)_2O$, mol. wt. 130.15.

Sodium acetate "Analar", NaOAc ,CH_3COONa, mol.wt. 82.08.

Sodium bicarbonate, $NaHCO_3$, mol.wt. 84.01.

Sodium hydroxide pellets "Analar", NaOH, mol.wt. 40.0.

Sodium metabisulphite, $Na_2S_2O_5$, mol.wt. 190.10; Hopkin and Williams.

Sodium sulphate anhydrous "Analar" , Na_2SO_4, mol.wt.142.04.

Sodium thiosulphate, solution, 0.1N, $Na_2S_2O_3 . XH_2O$.

Sulphuric acid "Analar", H_2SO_4, mol.wt. 98.08.

Toluene-4-sulphonic acid, $CH_3.C_6H_4.SO_3H.H_2O$, mol.wt. 190.22.

Trimethylchlorosilane, "TMCS", $(CH_3)_3SiCl$, mol.wt.108.7; Pierce Chemical Co.

Triphenylphosphine (phosphorus triphenyl), $(C_6H_5)_3P$, mol. wt. 262.29; Koch-Light Laboratories Ltd.

III. SUBSTRATES

5-Androstene—3β, 16β ,17β—triol, $C_{19}H_{29}O_3$, mol.wt. 305; Ikapharm.

Apomorphine hydrochloride, $C_{27}H_{27}NO_2HCl$, mol.wt.303; Dr. W.J.A. VandenHeuvel, (Merck Sharp & Dohme Labs. Rahway NJ), and Dr. P.Vouros (Northeastern University, Boston, Mass).

Brazilin, $C_{16}H_{14}O_5$, mol.wt. 286; Fluka.

5β-Chol-23-ene, $C_{24}H_{40}$,mol.wt.328; Dr. R.A. Anderson.

5β-Cholan-24-ol, $C_{24}H_{42}O$, mol.wt. 346; Dr. R.A.Anderson, (University of Glasgow), Ph.D. Thesis 1973.

5α-Cholestan-3-one, $C_{27}H_{46}O$, mol.wt. 386.66; BDH.

Cyclohexaneboronic acid, $C_6H_{12}BO_2$, mol.wt. 128; Alfa Inorganics Inc.

Cyclohexene, C_6H_{10}, mol.wt. 82.15; Hopkin and Williams.

2,3-Dihydroxybenzaldehyde, $C_6H_6O_3$, mol.wt. 138.12; Fluka.

2,3-Dihydroxybenzoic acid (O-pyrocatechuic acid), $C_7H_6O_4$, mol.wt. 154; Koch-Light.

3,4-Dihydroxybenzoic acid (protocatechuic acid), $C_7H_6O_4$, mol.wt. 154; BDH.

3,4-Dihydroxycinnamic acid (caffeic acid), $C_9H_8O_4$, mol. wt. 180; Sigma Chemical Co.

2,3-Dihydroxyestra-1,3,5(10)-trien -17-one, $C_{18}H_{22}O_3$,mol. wt. 286; Prof. D.N. Kirk (MRC Steroid Reference Collection).

3,4-Dihydroxyhydrocinnamic acid,$C_9H_{10}O_4$, mol.wt. 182.12; Aldrich Chem. Co.

3,4-Dihydroxy-α-(isopropylamino) acetophenone hydrochloride, $C_{11}H_{15}NO_3HCl$, mol.wt. 245; Alfred Bader Chemicals.

(±) 3,4-Dihydroxymandelic acid, $C_8H_8O_5$, mol.wt.184; Regis.

2,11-Dihydroxy-10-methoxyaporphine hydrochloride, $C_{18}H_{19}NO_3HCl$, mol.wt. 333; Dr Paul Vouros.

1,2-Dihydroxynaphthalene, $C_{10}H_8O_2$, mol.wt. 160.17; Fluka.

3,4-Dihydroxynomifensine dihydrobromide, $C_{16}H_{18}O_2N\ (HBr)_2$, mol.wt. 432; Hoechst AG.

3,4-Dihydroxyphenylacetic acid, (homoprotocatechuic acid), $C_8H_8O_4$, mol.wt. 168; Fluka.

3,4-Dihydroxyphenethylamine hydrobromide, (3-Hydroxytyramine HBr, dopamine HBr), $C_8H_{11}NO_2HBr$, mol.wt. 234; Aldrich Chem.Co.

3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA), $C_9H_{11}NO_4$, mol.wt. 197.2; Calbiochem.

3,4-Dihydroxyphenylglycol, $C_8H_{10}O_4$, mol.wt.170; Regis.

20β,21-Dihydroxypregn-4-en-3-one, $C_{21}H_{32}O_3$, mol.wt.332; Ikapharm.

3,4-Dimethoxyphenethylamine [homoveratrylamine, β-(3,4-dimethoxyphenyl)ethylamine], $C_{10}H_{15}NO_2$, mol.wt. 181; Koch-Light.

Estra-1,3,5(10)-trien-3-ol (3-hydroxyestra-1,3,5(10)-triene, 17-deoxoestrone), $C_{18}H_{24}O$, mol.wt. 256; Prof D.N. Kirk.

Estradiol-17β (3-hydroxyestra-1,3,5(10)-trien-17β-ol), $C_{18}H_{24}O_2$, mol.wt. 272; BDH.

1,3,5(10)-Estratrien-3-ol-17-one (3-hydroxyestra-1,3,5(10)-trien-17-one, Estrone), $C_{18}H_{22}O_2$, mol.wt. 270.37; BDH.

Gossypol "acetate" (the acetate as a complex from acetic acid), Gossypol, $C_{30}H_{30}O_8$, mol.wt. 518; Makor.

2-Hydroxyestradiol triacetate, $C_{24}H_{30}O_6$, mol.wt.414; Maybridge Chem.Co., Tintagel.

DL-4-Hydroxy-3-methoxymandelic acid, (Vanilmandelic acid), $C_9H_{10}O_5$, mol.wt. 198; Sigma.

4-Hydroxy-3-methoxyphenylacetic acid, (Homovanillic acid, HVA), $C_9H_{10}O_4$, mol.wt. 182; Sigma.

DL-4-Hydroxy-3-methoxyphenylglycol,piperazine salt,[DL-(3-methoxy-4-hydroxyphenyl)ethyleneglycol], $C_9H_{12}O_4 \cdot C_4H_{10}N_2$, mol.wt. 270; Aldrich Chem. Co.

3β-Hydroxy-3α-methyl-5α-cholestane, $C_{28}H_{50}O$, mol.wt.402; Prof. C.J.W. Brooks(sample from Prof.Sir Derek Barton);also prepared in this thesis work see p.89.

DL-Mandelic acid (α-hydroxyphenylacetic acid), $C_8H_8O_3$, mol.wt. 152; Aldrich Chem.Co.

* 3-Methoxy-4-hydroxybenzoic acid (vanillic acid), $C_8H_8O_4$, mol.wt. 168; Prof.C.J.W. Brooks.

3-Methoxy-4-hydroxyphenethylamine hydrochloride (3-methoxy-tyramine), $C_9H_{13}NO_2HCl$, mol.wt. 307; Regis.

3-Methyl-5α-cholest-2-ene, $C_{28}H_{48}$, mol.wt. 384; Prof. C.J.W. Brooks. Also prepared in this thesis work see p.90.

4-Methyl-6,7-dihydroxycoumarin (4-methylesculetin), $C_{10}H_8O_4$, mol.wt. 192; Aldrich Chem.Co.

4-Methyl-7,8-dihydroxycoumarin (4-methyldaphnetin), $C_{10}H_8O_4$, mol.wt. 192; Aldrich Chem.Co.

* The prefixes in common usage for some substances are wrongly positioned e.g.3-methoxy-4-hydroxybenzoic Acid should be written 4-Hydroxy-3-methoxybenzoic acid according to alphabetical rules.

N-Methyl-3,4-dihydroxyphenethylamine hydrochloride (N-methyldopamine, deoxyepinephrine, epinine), $C_9H_{12}NO_2HCl$, mol.wt. 204; Sigma.

Methyl 3,4-dimethoxybenzoate, $C_{10}H_{12}O_4$, mol.wt. 196; Prof. C.J.W. Brooks.

Methyl 3-methoxy-4-hydroxybenzoate (methyl vanillate), $C_9H_{10}O_4$, mol.wt. 182; Prof. C.J.W. Brooks.

Norbornylene (2-norbornene, bicyclo-[2,2,1]hept-2-ene), C_7H_{10}, mol.wt. 94; Ralph N.Emanuel Ltd.

Octadecaneboronic acid, $C_{18}H_{39}BO_2$, mol.wt. 298; ICN. K & K Lab. Inc.

1-Octadecene, $C_{18}H_{36}$, mol.wt. 252.49; Fluka AG, Buchs SG.

1-Phenyl-1,2-ethanediol (Styrene glycol), $C_8H_{10}O_2$, mol. wt. 138; Koch-Light.

(1S)-(-)-β-Pinene, $C_{10}H_{16}$, mol.wt. 136.24; Fluka.

5α-Pregnane-3α,17α,20α-triol, $C_{21}H_{36}O_3$, mol.wt. 336; Ikapharm.

α-Propyldopacetamide, $C_{11}H_{15}NO_3$, mol.wt. 209; Aldrich Chem. Co.

N-n-Propylnorapomorphine hydrochloride, $C_{19}H_{21}NO_2HCl$, mol.wt. 331; Dr Paul Vouros.

Salsolinol hydrobromide (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), $C_{10}H_{13}NO_2HBr$, mol.wt. 260; Aldrich Chem.Co.

Tetrahydroxyterephthalic acid diethyl ester, $C_{12}H_{14}O_8$, mol.wt. 286; Prof. C.J.W. Brooks.

2,3,4-Trihydroxyacetophenone, $C_8H_8O_4$, mol.wt. 168; Prof. C.J.W. Brooks.

2,10,11-Trihydroxyaporphine hydriodide, $C_{17}H_{17}NO_3HI$, mol. wt. 411; Dr. Paul Vouros.

IV. ANALYTICAL TECHNIQUES

A. Thin-Layer Chromatography (TLC)

Glass plates (2.5 x 7.5cm, 5 x 20cm or 20 x 20cm) were precoated with silica gel GF, with (0.25mm) thickness; UNIPLATE, ANALTECH INC.,ANACHEM.

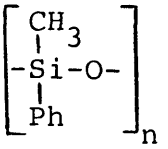
The mobility of the compounds in TLC, relative to that of the solvent front was indicated as R_f.

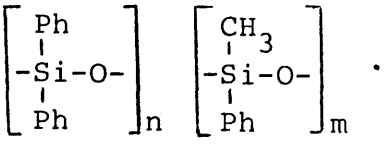
Detection methods; compounds were visualised by any of the following methods: UV lamp; Iodine vapour; spraying with a solution of 5% (w/v) ceric sulphate in 10% aqueous sulphuric acid followed[19] by heating at 80-120° for a few minutes; compounds containing carbonyl groups[108] were detected by spraying with a solution of 0.5%(w/v) 2,4-dinitrophenyl-hydrazine (DNP) in ethanol (95%) containing sulphuric acid (50% v/v) giving yellow or orange spots without heating; compounds containing phenolic or catecholic groups[108,109] gave a green colour on spraying with 1% solution of ferric chloride in ethanol (95%); and compounds containing boron gave a purple colour on spraying with 5% solution of diphenylcarbazone (DPC) in ethanol (95%) without heating[138].

B. Gas Liquid Chromatography (GLC)

The apparatus used was a Packard Model 419 dual column instrument. The following stationary phases were coated on Gas Chrom Q (100-120 mesh):

1% OV-1 (methyl siloxane polymer) $\left[-\overset{CH_3}{\underset{CH_3}{\overset{|}{\underset{|}{Si}}}}-O- \right]_n$;

1% OV-17 (Phenyl methyl siloxane polymer,  and 50% phenyl)

1% OV-25 (Phenyl methyl siloxane polymer,  75% phenyl)

The length and diameter of the coiled glass columns used were; OV-1, 3m x 2mm, i.d.; OV-17 and OV-25, 2m x 2mm, i.d.

Oxygen-free nitrogen was used as carrier gas with a flow rate of 28ml/min (unless otherwise specified). Injection temperature was 280° for both injection port heaters and detector temperature was 300°, and hydrogen flame ionization detectors were used. Oven temperature was varied according to the volatility of the sample.

Samples for GLC were dissolved in appropriate solvent (i.e. EtOAc, hexane, pyridine) and aliquots of 0.5-3ml (1-4mg/ml) were injected using a 10µl Hamilton syringe. Kováts retention indices "I" were measured with respect to standard n-alkanes.

C. Combined Gas Chromatography-Mass Spectrometry (GC-MS)

An LKB 9000 GC-MS instrument was used, this was fitted with a glass column (2m x 4mm,i.d.) of 1% OV-1. The flash heater was at 250°, the molecule separator at 270°, and ion source at 265°. The helium carrier gas flow rate was 30ml/min. The mass spectra were recorded at electron energy 20 eV; the trap current was 60mA, filament current 4A and accelerating voltage 3.5 kV.

D. Mass Spectrometry (MS)

Mass spectra (direct insertion probe) were measured on a VG MICROMASS 2S8 instrument.

E. Infra Red (IR)

IR spectra were measured on a Perkin-Elmer Grating Infra Red Spectrophotometer Model 257.

F. Ultraviolet (UV)

UV spectra were measured on an automatic-recording instrument, the Unicam SP8000 Ultraviolet Spectrophotometer.

G. Nuclear Magnetic Resonance (NMR)

H-NMR spectra were determined on a Perkin-Elmer T 60Mc/S (60MHz) instrument and on an R32 90MHz instrument.

H. Melting point apparatus

Melting points (m.p.) were recorded on a Kofler block with estimated precision of $\pm 2°c$.

V. PURIFICATION TECHNIQUES

A. Column Chromatography

The material used was; Silica gel Woelm, for Dry-Column Chromatography: Activity 111/30mm, contains 0.5% inorganic fluorescent indicator. This silica gel was introduced into columns as a slurry with hexane or EtOAc, then pre-washed with the elution solvent (the eluant) before use. The eluted fractions were received in a pre-weighed flask(s) and

solvent(s) evaporated under vacuum or nitrogen; then most of these fractions were analysed by TLC and GLC.

B. Preparative TLC

TLC plates (20 x 20cm) were pre-coated with silica gel GF with 2mm thickness. Samples dissolved in appropriate solvent(s) were then applied as a band. The developed bands were located under a UV-lamp, then the silica was deactivated with water and each band scraped from the plate, then extracted with appropriate solvent(s). Traces of solid were removed by filtration through a pre-washed cotton wool plug; the filtrate was dried over Na_2SO_4 and evaporated to dryness.

C. Removal of solid particles (impurities) colloidal matter, and polymeric by-products from solution.

The following materials were used as appropriate:

Darco - activated carbon; Serva Feinbiochemica, Heidelberg Germany.

Magnesol - a hydrous magnesium silicate; M. Woelm, Eschwege, Germany.

Celite - a diatomaceous silica; Analytical filter aid; BDH.

"Dry Column" silica gel over a short cotton-wool plug.

All these above materials were pre-washed with the elution solvent(s), then after elution washed several times with the same solvent for maximum recovery of the soluble material(s).

D. Vacuum sublimation (Vacuum subl^n.)

Many crystalline or oily impure compounds were purified by vacuum sublimation/short path distillation using a rotary oil pump. Samples were sublimed at 0.01 torr in a

tube fitted with a cold finger condenser, heated in an aluminium block (or occasionally in an oil bath), provided with a thermometer. Block temperatures were selected according to the m.p. or b.p. of the sublimed materials.

E. Purification of Solvents

The following solvents were purified and dried according to the methods described by Perrin et al.[110].

Acetonitrile

Acetonitrile (boiling range 97% between 79-83°) (200ml) was dried over P_2O_5 for 15 min in a round-bottom flask provided with boiling stick and stones, and a thermometer, then connected to a condenser, provided with a drying tube. A middle colourless fraction (120ml) was collected at 80-81° over 4Å molecular sieve, then used directly.

Chloroform

Chloroform "Analar" contains about 2%(v/v) ethanol as a preservative; to obtain alcohol-free chloroform the following procedure was followed; Chloroform "Analar" was passed through a column of blue anhydrous silica gel (20g), to remove most of the ethanol and moisture, then distilled as above (without P_2O_5); a middle fraction (150ml) was collected at 60-62° over 4Å molecular sieve.

Dichloromethane

Dichloromethane "Analar" was dried over 4Å molecular sieve, overnight, then distilled, and the middle fraction was collected at 38-39°. This fraction was redistilled over P_2O_5, and the middle fraction was collected at 39°, over 4Å molecular sieve.

Methanol

Methanol "Analar" (200ml) was treated with five pieces of metallic sodium, each of a peanut size, in a R.b.f. provided with thermometer, condenser and drying tube. The middle fraction (130ml) was collected at 64-64.5°.

Pyridine

Pyridine "Analar" contains about 0.1% moisture. This was dried over KOH pellets for 2 hours, then refluxed with KOH, followed by fractional distillation. A middle fraction was collected at 114-116° and used directly.

F. Extraction, Washing, Filtration, Drying and Evaporation of Solvents from Organic Extracts

In many reactions, the solvent, excess reagent and volatile byproducts were firstly removed under vacuum by a rotary vacuum system, at temp.30-60° in a water bath (unless otherwise specified). The residue was dissolved in an appropriate solvent (e.g. ether, EtOAc, $CHCl_3$ or CH_2Cl_2, or mixtures of ether-EtOAc or hexane-EtOAc) and washed with water 3-4 times, then each water washing, was extracted 3-4 times with the solvent used. The organic extracts were combined and dried over anhydrous Na_2SO_4, then filtered through a pre-washed cotton-wool plug and solvent(s) evaporated under vacuum as above. In some cases traces of moisture were removed by azeotropic distillation, by adding toluene-MeOH-acetone (1:2:2,v/v), then vacuum evaporation as above.

G. Cleaning and Silane-Treatment of glass GLC Columns[111].

The following steps (from 2) could be carried out for

the treatment of non-siliconised supports-(packings) as well before coating with the phase:

1. Washing with detergent and hot water, to remove dust and grease.

2. Washing with acetone, then with acetone and $CHCl_3$.

3. Washing with conc.HCl to remove traces of iron ions and other ions.
4. Washing with tap water to remove traces of HCl.
5 Rinsing with acetone and drying thoroughly in a clean oven at 120°.
6. Columns were filled completely with a 5%(V/V) solution of dimethyldichlorosilane (Me_2SiCl_2) in toluene, then the ends were plugged with "Teflon", and left overnight at R.T.
7. The Me_2SiCl_2 was removed, then the columns were washed successively with toluene, MeOH, thoroughly to remove HCl and traces of Me_2SiCl_2.
8. Washing with acetone, then toluene.
9. Drying at 100° while N_2 was passed through the column.

Chapter 3

EXPERIMENTAL PROCEDURES

I. METHYL ESTERS OF ACIDS CONTAINING CATECHOL GROUPS

Methyl 3,4-dihydroxydihydrocinnamate (11);

Methanolic HCl was prepared by cautious addition of acetyl chloride (10ml) to dry methanol (60ml). 3,4-Dihydroxy-dihydrocinnamic acid (5) (1.210g:6.17 mmole) was dissolved in this reagent, and the solution refluxed for 4hr : monitoring by TLC showed the reaction to be complete after this time. The solvent, excess reagent and volatile by-products were removed. The residue was basified with a saturated solution of $NaHCO_3$, pH8, to remove any unreacted carboxylic acids, then the product was extracted with ether, and the extract washed with water, dried and evaporated, yielding a yellowish gummy product(1.286g:99%). TLC showed a single spot, which corresponded to the methyl ester derivative:0.785g of this crude oil was crystallised by vacuum subl^{n}., at 120°/0.01 torr; on scratching, the oily sublimed material yielded a white crystalline product (0.663g:83%) of compound(11), m.p. 73.5-75° (Tamura, et al.[112], 74-75°).

The methyl esters prepared by the above procedure are listed in Table 1.

Table 1, Methyl esters of acids containing catecholic groups[a].

Parent acid	No.	Wt. used mg (mmole)	Refluxing period in MeOH/HCl hr	Recovered crude methyl ester, mg (% yield)	No.	m.p.°C	Recovered purified methyl ester (% yield)	m.p.°C of purified ester
2,3-Dihydroxybenzoic acid[b]	1	412 (2.68)	6	408 (91) pure	8	76-77, pure (Lit.[113] 73°)		
3,4-Dihydroxybenzoic acid[c]	2	769 (4.99)	6	832 (99) pure	9	135-135.5 pure (Lit.[113] 134.5°)		
3,4-Dihydroxyphenylacetic acid	3	692 (4.12)	4	736 (98)	10	yellow oil	79	48-50[d] (Lit.[114] 34-36°)
4-Hydroxy-3-methoxy phenylacetic acid	4	46 (0.25)	6	48 (98)	49	yellow oil		
3,4-Dihydroxydihydro-cinnamic acid	5	1210 (6.17)	4	1286 (99)	11	yellow oil	83	73.5-75 (Lit.[112] 74-75°)

3,4-Dihydroxycinnamic acid	6	308 (1.71)	5	313 (94)	12	150-153	83	156-158[e] (Lit.[113] 152-153°&162°)
L-3,4-Dihydroxyphenyl-alanine	7	112 (0.6)	5	17 (14)[f]	50	oil		

a. On TLC analysis, the methyl esters showed single spots, each with a higher Rf value than the corresponding acid. Most of the methyl esters were further characterised by GLC and TLC (Tables 11&25, p.151&182), IR spectrometry (Table 12 ,p.152), and ^{1}H-NMR spectrometry Table 5 ,p.153). Compounds (8) and (9) were also studied by GC-MS (Table II app.,p.218),

b. Crude, m.p.206°; purified *via* vacuum subl^{n}. at 145°/0.05-0.01torr; sublimed crystals, m.p.209° (Lit.[113] 204°).

c. Crude, m.p.204°; purified *via* vacuum subl^{n}. at 182°/0.05-0.01torr; sublimed crystals, m.p.204.5-205.5° (Lit.[113] 199°).

d. 436mg of the crude oil was crystallised by vacuum subl^{n}. at 120°/0.01torr; on scratching the oily sublimed material, yielded white crystalline product (356mg:79%) of compound (10).

e. Crude solid (300mg) was purified *via* vacuum subl^{n}. at 138°/0.01torr; white sublimed crystals were recovered (262mg:83%).

f. This low yield may be due to aerial oxidation of the catechol groups in the presence of base ($NaHCO_3$) during extraction. A deep purple colour appeared during the treatment of the crude product with $NaHCO_3$. This was not observed with any of the other compounds.

II. SELECTIVE ACETYLATION

A. N-Acetyldopamine(20)

Dopamine hydrobromide (2.051g:8.8 mmole) equivalent to 1.341g of free dopamine(19) was dissolved in water (6ml), then with sodium acetate (0.710g:10.8 mmole) in water (2ml), then EtOAc (30ml) was added to this solution. A freshly prepared solution of Ac_2O (0.895ml, 9.5 mmole), in MeOH (30ml), was added dropwise to the dopamine solution, by means of a Pasteur pipette, during 20 min at R.T. with continuous shaking. The water and other solvents were evaporated azeotropically, under vacuum. TLC showed a large spot for the main product and a small spot attributed to oxidized material. The product was purified from NaBr ppt., by extraction with EtOAc, then the NaBr was dissolved in MeOH, and reprecipitated with EtOAc; this step was repeated three times. All the extracts were combined, and the solvents evaporated, then the oily product was dissolved in EtOAc containing 2% MeOH, this solution was passed through a short column of "dry column" silica gel,then the remaining adsorbed products were eluted with EtOAc. Solvents were evaporated. A slightly yellowish-oily product was obtained, and further dried under N_2. The crude product amounted to 1.670g (97.7%), the purity of(20) as judged by ^{1}H-NMR; IR and TLC was about 95%. This product failed to crystallise with acetone-hexane or ether-hexane, even below 0°C: it was further purified, by vacuum subl^n., at 120°/ 0.01 torr, yielding a colourless gummy product, which could not be crystallised and became discoloured in air. Ferric chloride in ethanol showed an

immediate green colour; MS,($M^{+\cdot}$ 195). The corresponding benzeneboronate derivative was crystalline,m.p.159-160°, (Table 2, p. 84). N-Acetyldopamine reported by Mills et al.[173] as yellow oil

The following N-acetyl compounds were prepared according to the above procedure:

B. N-Acetyltyramine(25)

Tyramine hydrochloride (133.4mg:0.768 mmole) equivalent to 105.6mg of free tyramine(24) was treated with Ac_2O (0.079ml:0.838 mmole) by the above procedure. After extraction, the recovered crude oily material was 100mg (73%). This was dissolved in EtOAc and crystallised on cooling in dry ice-acetone bath; slightly yellowish crystals were recovered, m.p.120-130°. The purity of the product(25) was about 98% as judged by GLC and TLC; TLC showed traces of oxidized material. Recrystallisation from acetone-hexane gave, 81mg (59%), m.p.126-128°. Vacuum subl^{n}., at 126°/0.01 torr afforded colourless crystals, m.p.128-129°; (Lit.[173] 129.5-130°).

C. N-Acetyl-3-methoxytyramine(29)

3-Methoxytyramine hydrochloride (31.4mg:0.102 mmole) equivalent to 25.7mg of free 3-methoxytyramine(28) was treated with Ac_2O (0.015ml:0.159 mmole) by the above procedure. After extraction, the recovered crude oily material was 29mg (91%). The purity of compound(28) was about 98% as judged by GLC and TLC. TLC showed traces of oxidized material.

Data for compounds(20),(25) and (29) from TLC; GLC; IR and ^{1}H-NMR are given in Tables 14,15,16,18 respectively.

D. Selective 4-acetylation of DL-(3-methoxy-4-hydroxyphenyl) ethylene-glycol(38)

DL-(4-acetoxy-3-methoxyphenyl)ethyleneglycol(39):

Modified procedure of Biondi[26];

DL-(3-methoxy-4-hydroxyphenyl)ethyleneglycol piperazine salt (4mg:0.015 mmole) equivalent to 2.7mg of compound(38), was dissolved in 12M HCl (0.03ml), to liberate the 4-hydroxy group. This reaction took place in a micro-test tube. This solution was diluted with EtOAc, then transferred into a conical flask (150ml) with stopper. The excess HCl and EtOAc, were evaporated under nitrogen. Water and about 0.5g $NaHCO_3$ were added, then a large excess of Ac_2O (6ml), added in two portions with shaking. More $NaHCO_3$ was added with shaking until saturation, then the reaction mixture shaken for 3hr . $NaHCO_3$ was filtered off and the filtrate extracted three times with CH_2Cl_2; each extract was washed twice with water. The solvent and traces of moisture were removed azeotropically. The total recovered crude product was 3.4mg. (Theoretical yield 3.32mg). TLC (EtOAc-$CHCl_3$,1:3,v/v) showed one major spot; R_f=0.25. The R_f value for the starting material(38) was 0.13, and for its corresponding triacetate, 0.68. By GLC on OV-17 at 180°, the crude product showed only one major peak, I=2095. After treatment with a slight excess of methaneboronic acid, in EtOAc at R.T. for 5min, GLC showed two peaks: I=2045,GC-MS ($M^{+\cdot}$250), which corresponded to compound(39) methaneboronate: and I=2095, GC-MS($M^{+\cdot}$170), as an unknown by-product. The yield of compound(39), as judged from the peak height ratio,after methaneboronate formation was about 45%.

III. ACETYLATION

A. N,O,O-Triacetyldopamine(21)

Dopamine hydrobromide (0.533g:2.277 mmole) equivalent to 0.349g of free dopamine(19) was dissolved in pyridine (3ml), then Ac_2O (3.4ml:31.5 mmole) was added dropwise within 3min , with continuous shaking. The reaction flask was fitted with a drying tube, and the reaction mixture was heated for ½hr at 80°. The solvent and excess reagent were evaporated azeotropically. The organic products were separated from the white ppt. of pyridine-HBr, by extraction with EtOAc with slight warming, then filtered through a small plug of silica gel, the solvent was evaporated and the residue dried in a stream of nitrogen. The crude yield was 635mg(99.8%).

The main product, dopamine triacetate(21), was purified from the other traces of by-products, by chromatography on "dry column" silica gel (60g). Using EtOAc as eluent, nine fractions were collected, and each fraction was monitored by TLC and GLC. Fractions 1(20ml) and 2(25ml) were mainly dopamine tetraacetate(22), (120mg:16%) as a yellowish solid, m.p.69-73°. This was recrystallised from acetone-hexane, yielding fine white crystals, m.p.72-73°:GC-MS($M^{+\cdot}$321). Fractions 3-8 (65ml of eluate) yielded dopamine triacetate(21) as large colourless crystals, m.p.65-67°,(510mg:74%):GC-MS ($M^{+\cdot}$279). As judged by TLC and GLC, this product was highly pure. Fraction 9(5ml) 2.2mg, was mainly dopamine diacetate (23a&b); as judged by GLC, the yield was about 2%.

The following acetylated compounds were prepared according to the above procedure:

B. N,O-Diacetyltyramine(26)

Tyramine hydrochloride(103mg:0.595 mmole) equivalent to 81.6mg of free tyramine(24) was treated with Ac_2O (0.168ml: 1.784 mmole). After extraction and drying, the recovered yellowish solid was 126mg(96.5%) m.p.75-82°, its purity was about 90% as judged by TLC and GLC. The by-products were tyramine mono-and di-acetate(25) and (27). Two re-crystallisations from acetone-hexane gave 70mg(54%), m.p. 85-87°; vacuum subl^n. at 76°/0.01 torr, gave colourless crystals(26); m.p.87-88°.

C. N,O-Diacetyl 3-methoxytyramine(74)

3-methoxytyramine hydrochloride (28mg:0.091 mmole) equivalent to 24.4mg of free 3-methoxytyramine(28) was treated with Ac_2O(0.025ml:0.282 mmole). After extraction and purification *via* preparative TLC (EtOAc); white crystals were recovered, 26mg(81%), of compound(74); m.p.87-90°; highly pure as judged by TLC and GLC.

D. N-Acetyl-3,4-dimethoxyphenethylamine (32)

3,4-Dimethoxytyramine(31) (30mg:0.165 mmole) was treated with Ac_2O (0.047ml:0.51 mmole), by the general procedure. The product formed yellowish crystals (35.5mg:96%) m.p.85-89°. Purification *via* preparative TLC (EtOAc-acetone,1:1,v/v) gave slightly yellow crystals, 22mg(59.5%), of compound(32): m.p.86-89°, highly pure as judged by TLC and GLC.

Data for compounds(26),(74)and(32) from TLC;GLC;GC-MS; IR and ^{1}H-NMR are given in Tables 14,15,III app.,17,19 respectively.

E. Methyl 3,4-diacetoxybenzoate(13)

Methyl 3,4-dihydroxybenzoate(9) (44mg:0.26 mmole) was treated with excess Ac_2O (2ml), by the general procedure. A crude yellowish oil was obtained; when kept at 5°, this gradually crystallised: 64mg(96.9%), m.p.40-43°; recrystallisation from MeOH-water, 1:3 (v/v) gave white crystals, 23.5mg(35.6%) of compound(13); m.p.43-44°. Second crop, 28mg; m.p.41-44°, this was about 90% pure as judged by GLC.

F. Gossypol hexa-acetate(35)

Gossypol(34) (70mg:0.135 mmole) was treated with excess Ac_2O (0.5ml), by the above procedure. The crude product formed orange-yellow crystals, 103mg (98.9%), m.p.168-175° (Lit.[115] for hexa-acetate recrystallised from MeOH as colourless microcrystals m.p.249-250°; 13.4% yield). This product failed to crystallise from MeOH. Purification by chromatography on "dry column" silica gel (10g); gave five fractions, each eluted with $CHCl_3$ (10ml). Fraction 1(8mg:7.6%) crystallised from ether, as white crystals of compound(35), m.p.275-280°, decomposes (Lit.[116] white crystals decomp. 276-279°, re-crystallised from EtOAc-MeOH). Fractions 2-5, (91mg:87.5%) as fine bright yellow crystals of compound(35), m.p.195-200°, (softened at 185°),(Lit.[116]bright yellow microcrystalline mass. m.p.184-186°, soften at 138°, recrystallised from ligroin). For discussion see Chap.5, p.[170].

IV. COMPLETE ACETYLATION

A. N,N,O,O-Tetraacetyldopamine(22)

Dopamine hydrobromide (0.323g:1.38 mmole) equivalent to 0.211g of free dopamine(19) was dissolved in pyridine (2ml), then excess Ac_2O (3ml) was added in one batch. The reaction mixture was fitted with a drying tube and heated at 80°C, overnight. The solvent and excess reagent were evaporated azeotropically. The organic products were separated from the white ppt. of pyridine salt by extraction with EtOAc, with slight warming, then filtered through a small plug of silica gel. Evaporation yielded a dark brownish gum, further purified by washing with water, then extraction with EtOAc. The dried extract on evaporation gave fine yellowish crystals, 420mg(95%), m.p.70-73°.

The main product, dopamine tetraacetate(22) was purified from traces of by-products (dopamine di-and triacetate;(21) and [23a&b]), by chromatography on "dry column" silica gel (40g), using EtOAc as eluent. Three fractions were collected; fraction 1(15ml:10mg); fraction 2(10ml:345mg) and fraction 3 (10ml:15mg). Fraction 2 crystallised upon slow evaporation of an EtOAc solution yielding white crystals(78%), of compound (22); m.p.72-74°; GC-MS ($M^{+\cdot}$321). Anal. calcd. for $C_{16}H_{19}O_6N$ (mol. wt. 321): C,59.81; H,5.91; N,4.36. Found: C,59.84; H,5.80; N,4.80. This was highly pure as judged by TLC and GLC. Fractions 1 and 3 were mainly compound(22) with traces of compounds (21) and (23 a&b).

The following completely acetylated compounds were prepared according to the above procedure. The products;(27), (30), (33) and (13), were highly pure as judged by TLC and GLC.

B. N,N,O-Triacetyltyramine(27)

Tyramine hydrochloride(43mg:0.248 mmole), equivalent to 34mg of free tyramine(24), was treated with excess Ac_2O (2ml), by the above procedure. A yellowish crude oil, 60mg (92.3%) was recovered. This was purified by chromatography on "dry column" silica gel (10g); four fractions each 6ml were collected; fraction 2, 38mg(61%), crystallised on evaporation from ether; as slightly yellowish crystals of compound(27), m.p.78-81°. The other fractions contained product(27) and traces of tyramine diacetate(26).

C. N,N,O-Triacetyl-3-methoxytyramine(30)

3-Methoxytyramine hydrochloride (53mg:0.172 mmole) equivalent to 46mg of free 3-methoxytyramine(28), was treated with excess Ac_2O (1ml),by the above procedure. The crude oily product was purified by preparative TLC (EtOAc). There were obtained 44mg (66%) of compound(30), m.p.62-64°.

D. N,N-diacetyl-3,4-dimethoxyphenethylamine (33)

3,4-Dimethoxytyramine(31) (82mg:0.453 mmole) was treated with excess Ac_2O (1ml), by the above procedure. A crude yellowish oil was obtained, 104.5mg (87%). Purification by preparative TLC (EtOAc-acetone,1:1,v/v); then chromatography on "dry column" silica gel eluted with EtOAc, gave three fractions; each 7ml. The middle fraction; gave 75.5mg(63%) of compound(33), as a colourless oil. This failed to crystallise from ether; acetone-water; methanol-water or ether-hexane.

Data for compounds(22),(27),(30),(33) and (13) from TLC; GLC; GC-MS; IR and ^{1}H-NMR are given in Tables 14,15,III app.,17 & 20 respectively.

V. CATECHOL ESTROGENS

A. Hydrolysis of 2-hydroxyestradiol triacetate(46)

1.Hydrolysis with MeOH and concentrated HCl:- 2-Hydroxyestradiol triacetate (5mg:purified by preparative TLC), was dissolved in MeOH(5ml), and three drops of water were added, then 12M-HCl(0.5ml). The reaction mixture was refluxed overnight. The reaction was monitored by TLC and GLC and worked up by the usual procedure giving 3.3mg(94%) of product. TLC ($CHCl_3$) showed complete hydrolysis of(46) (R_f=0.6) to 2-hydroxyestradiol(47) (R_f=0.06). GLC (OV-25,250°) showed the conversion of(46); I=3800; to compound(47), which showed no peak, due to its high polarity and adsorption, but after trimethylsilylation had I=3040(220°), at the expected position.

2.Hydrogenolysis with lithium aluminium hydride in dry ether:-

NOTE:-All apparatus was dried.

2-Hydroxyestradiol triacetate(46) (5mg), was dissolved in dry diethyl ether (3ml). This solution was added gradually from the top of a condenser; to a 5min refluxed suspension/solution of lithium aluminium hydride (300mg) in dry ether (4ml). This condenser was fitted with a drying tube, and the reaction mixture was refluxed overnight. The excess reagent was destroyed with EtOAc (2ml), and the greyish-white ppt. of $Al(OH)_3$ was dissolved by adding 5N-HCl(2ml). The usual working-up gave 2.2mg (63%) of (47): TLC showed complete conversion to triol, as in procedure A1.

B. 3-Hydroxyestra-1,3,5(10)-triene(48)

Modified procedure of Huang-Minlon[117]: 3-hydroxyestra-1,

3,5(10)-trien-17-one(40) (1.5g) was dissolved in diethylene glycol (40ml), with heating and stirring in an oil bath at 115-120°; then hydrazine hydrate (98-100%,12ml) was added in four (3ml) portions at 15min intervals; during this time stirring and heating was continued and the flask connected to a drying tube. Nitrogen was then bubbled through the reaction mixture, to remove excess reagent and water as by-product; while the temperature was raised to 150°. When most of the reagent had been evaporated, KOH pellets (9.6g) were added, the flask was fitted with a 50cm air condenser (with drying tube), and the reaction mixture was refluxed for 2hr under nitrogen (the reaction was monitored by GLC, which showed the reaction was completed after 1hr reflux). The cooled basic reaction mixture was diluted with water and extracted three times with ether, and each extract was washed five times with water. The ether extracts were dried, and the solvent evaporated. The residue crystallised from MeOH-water, yielding whitish-yellow crystals, 1.305g(91%) m.p.128-130°C. This product was recrystallised three times from MeOH-water, yielding compound(48), m.p.134-135° (Lit.[117] 134-134.5°); IR($CHCl_3$), 3600cm^{-1} (νOH), 1600 (ν Ph). (Note : this compound(48) was unexpectedly extracted from the basic aqueous solution).

Data for compound(48) from TLC; GLC; and ^{1}H-NMR, are given in Tables 21, 28 and 22 respectively.

NOTE: The main steps found to be important for preparing compound(48), were initial formation of the hydrazone (in the absence of KOH), with large excess of the reagent(based

yellow crystals, 780mg; TLC showed compounds(15) and (16) and also unreacted(40).

The crude product (765mg) was dissolved in a mixture of $CHCl_3$-MeOH-AcOH(96:3:1, v/v) and subjected to column chromatography (200 x 2.5cm); on a column of "dry column" silica gel (300g) impregnated with a saturated solution of ascorbic acid in AcOH-$CHCl_3$-methylcyclohexane (1:2:2,v/v). Elution was carried out with the same solvent system and 20ml fractions were collected. TLC showed that fractions 26-34 contained compound(40); these were combined and re-crystallised from acetone, recovered 390mg, as unreacted material. Fractions 39-42 contained compound(16); these fractions were washed with water and extracted with ether-EtOAc (4:1,v/v), in the usual way. The organic phase yielded whitish crystals, 20mg, m.p.258-263°. These crystals failed to crystallise from EtOAc and 2% AcOH (Lit.[118] m.p.268-271°) but were recrystallised from EtOAc-hexane, yielding pale yellow crystals m.p.261-265°. Fractions 47,48 and 49 contained compound(15); these were combined, washed and extracted as above; giving fine whitish crystals, 65mg,m.p.187-191°. These also failed to crystallise from EtOAc-2%AcOH (Lit.[118] 193-196°) but were recrystallised from ether-hexane; whitish crystals were recovered, m.p.190-193°.

Fractions 35-58 contained different ratios of compounds (40) and (16), total recovered material after extraction was 15mg.

Fractions 43-46 contained different ratios of compounds (15) and (16); total recovered material after extraction was 24mg.

on advice from Prof.D.N.Kirk), by heating at 120°, then evaporation of the excess hydrazine and water with a stream of nitrogen. The KOH, in large excess, was added and the solution refluxed in extra diethylene glycol for 2hr under nitrogen.

C. 2,3-Dihydroxyestra-1,3,5(10)-trien-17-one(15) and 3,4-dihydroxyestra-1,3,5(10)-trien-17-one(16)

Procedure of Gelbke, et al.[118]; 3-hydroxyestra-1,3,5(10)-trien-17-one(40) (745mg) was dissolved in acetone (800ml), by heating and shaking; then AcOH in water, (10% v/v; 500ml) was added. After the addition of potassium nitrosodisulphonate (4g), the mixture was shaken for 15min at R.T. A second portion of potassium nitrosodisulphonate(4g) was added and shaking was continued for another 15min . The yellow quinones formed were extracted from the solution with $CHCl_3$ (3 x 300ml), then each extract was washed successively with N-HCl (2 x 200ml) and with water (3 x 200ml). AcOH (100ml) and excess KI(2.4g, powdered) were added to the combined $CHCl_3$ extracts. The mixture was then shaken for 6min during which the solution turned deep yellow; iodine formed during the reaction was reduced by the dropwise addition of sufficient 0.1N-sodium thiosulphate solution with continuous shaking, until the solution was nearly colourless. Water (200ml) was added and the $CHCl_3$ layer was separated. The aqueous phase was further extracted with CH_2Cl_2-EtOAc (1:1, (v/v), 2 x 200ml) and the combined extracts were washed successively with N-HCl (2 x 200ml) and water (4 x 300ml), dried and solvents evaporated. The crude material was recovered as

D. 2,3-Dihydroxyestra-1,3,5(10)-triene(17)and 3,4-dihydroxy-estra-1,3,5(10)-triene(18)

Compounds(17) and (18) were prepared according to the aforementioned procedure:3-hydroxyestra-1,3,5(10)-triene(48) (1.2g) was highly soluble in acetone. After oxidation; reduction; extraction and drying, the recovered deep coloured oil was 1.315g. This was purified by chromatography on "dry column" silica gel (300g), then 200ml fractions were collected. The combined fractions, which represented each compound, after TLC analysis, were washed with water, and worked up to yield the following products:

Compound(48); Fractions 26-46 (617mg), on recrystallisation from MeOH-water; yielded 515mg , m.p.134-135°.

Compound(18); Fractions 58-63 (140mg) formed yellowish crystals, m.p.128-135°. After three recrystallisations from ether-hexane, it yielded 7mg, m.p.142-143°. Anal. calcd. for $C_{18}H_{24}O_2$ (mol.wt.272):C,79.41;H,8.82. Found: C,78.47; H, 8.63%. UV λ_{max}(EtOH):282nm (ε,2868); IR ($CHCl_3$): 3810 and 3570 cm^{-1} (νO-H). The combined mother liquors yielded a further 50mg, m.p.139-141°. The mother liquor was evaporated, and the residue washed with hexane; giving 48mg of impure product, m.p.118-122°.

Preparative derivative formation for compound(18):

Compound(18) (44mg,crude m.p.118-122°) was dissolved in EtOAc (1ml), then divided into two equal parts: one part was reacted with excess Ac_2O (0.05ml), and the other with benzeneboronic acid (10mg, in EtOAc 0.2ml). These reaction mixtures were kept at R.T. for 10min , then the solvent and excess reagent were evaporated, and the following results

were obtained:

Benzeneboronate: A crude yellowish solid (36mg) was recovered; this was dissolved in ether (1ml), then eluted with ether through a short plug of "dry column" silica gel. The pale yellow product (28mg); recrystallised from acetone, gave 12mg, m.p.166-172°. Further purified by micro-analysis , by vacuum subl^{n}. at 160°/0.01 torr, and by two recrystallisations from acetone, this yielded (4.5mg) m.p.176-177°.

Anal: Calcd. for $C_{24}H_{27}BO_2$ (mol.wt.358): C,80.45; H,7.54. Found: C,80.43; H, 7.34%. GC-MS ($M^{+\cdot}$ 358).

Acetylation: The yellow crude product (33mg) was dissolved in ether (1ml), then eluted with ether through a short column of "dry column" silica gel. Yellow crystals (30mg), m.p. 172-178° were recovered, and recrystallised from ether-hexane, giving 3mg , m.p.179-181°. Micro-analysis for C and H was not satisfactory; GC-MS ($M^{+\cdot}$ 356). The mother liquor afforded pale yellow crystals (10mg) m.p.173-178°. The mother liquor was evaporated and the residue washed with hexane giving 17mg, m.p.170-178°.

Compound(17); Fractions 64-74 (262mg) yielded a highly viscous deep yellow oil, this was vacuum sublimed, then recrystallised three times from ether-hexane, yielding 40mg m.p.138-140°. Anal.:Calcd. for $C_{18}H_{24}O_2$ (mol.wt.272): C, 79.41; H, 8.82. Found: C,78.44; H, 8.76%. UV,λ(EtOH): 290nm (ε 3348); IR ($CHCl_3$): 3610 and 3570cm^{-1} (νOH). The combined mother liquors gave a further 40mg, m.p.134-137°. The mother liquor was evaporated, then the residue washed

with hexane, giving 40mg of yellowish crystals, m.p.130-134°.

Preparative derivative formation for compound(17):

Compound(17) (40mg, crude m.p.130-134°) was dissolved in EtOAc and divided into two equal parts; one part was treated with excess Ac_2O and the other with benzeneboronic acid, by the same method as for compound(18). The crude oily products were each eluted through "dry column" silica gel, then sublimed under vacuum, yielding oils which failed to crystallise from acetone, acetone-hexane; ether-hexane or EtOAc-ether. GC-MS data were obtained for compound(17) diacetate ($M^{+\cdot}$ 356) and for the benzeneboronate ($M^{+\cdot}$ 358).

Compounds (15),(16),(17),(18),(40) and (48) were further characterised by TLC and GLC(Tables 21,28,27), p.168,193,191 and ^{1}H-NMR spectrometry (Table 22 , p.169).

VI. BENZENEBORONATE DERIVATIVES

Methyl dihydrocinnamate 3,4-benzeneboronate(77)

Methyl 3,4-dihydroxydihydrocinnamate(11)(0.5g:2.5 mmole) was dissolved in EtOAc (5ml), then benzeneboronic acid (0.317g: 2.6 mmole) in EtOAc (2ml) was added, and the reaction mixture heated for 20min at 70°. The solvent was evaporated. Dissolution of the gummy product in ether, then evaporation of the solvent with nitrogen, yielded crystalline material (680mg: 95%), m.p.60-62'. GLC showed a single well-defined peak. Vacuum subl^{n}. yielded fine white crystals, m.p. 61-62°.

Anal.: Calcd.for $C_{16}H_{15}BO_4$ (mol.wt. 282): C,68.09; H, 5.32; B, 3.9. Found: C, 68.24; H, 5.38; B, 3.83%.

The benzeneboronate derivatives of the compounds in Table 2 were prepared according to the above procedure.

Table 2, Preparative benzeneboronate derivative formation from catechol type compounds

Parent compound	Compd no.	Weight used mg (mmole)	Benzene-boronic acid mg (mmole)	Corresponding benzeneboronate			
				Weight recovered mg (%crude yield)	m.p.°C (Anal.m.p.°C)[b]	Mol.formula (Mol.Wt.)	Analysis Found % / Calcd. %
Methyl 3,4-dihydroxybenzoate[c]	9	831 (4.496)	629.5 (5.15)	1050 (84)	103-105 (109.5-110.5)[d]	$C_{14}H_{11}BO_4$ (254)	C,65.90 C,66.14 H, 4.23 H, 4.33 B, 4.78 B, 4.3
Methyl 3,4-dihydroxy-phenylacetate	10	300 (1.648)	203 (1.664)	400 (90.4)	81-84 (82-83)[e]	$C_{15}H_{13}BO_4$ (268)	C,67,20 C,67.16 H, 4.95 H, 4.85 B, 3.8 H, 4.1
Methyl 3,4-dihydroxy-dihydrocinnamate	11	500 (2.55)	317 (2.6)	680 (95)	60-62 (61-62)	$C_{16}H_{15}BO_4$ (282)	C,68.24 C,68.09 H, 5.38 H, 5.32 B, 3.83 B, 3.9
Methyl 3,4-dihydroxy-cinnamate	12	263 (1.357)	170 (1.393)	349 (92)	122-125 (131-133)[f]	$C_{16}H_{13}BO_4$ (280)	C,68.7 C,68.57 H, 4.35 H, 4.64 B, 4.01 B, 4.0

Table 2 continued

N-Acetyldopamine	20	977 (5.01)	612 (5.02)	1405 (99.8)	145-148 (159-160)[g]	$C_{16}H_{16}BNO_3$ (281)	C,68.0 C,68.33 H, 5.90 H, 5.69 B, 4.11 B, 3.9 N, 5.06 N, 4.98
Estra-1,3,5(10)-triene-3,4-diol	18	22 (0.08)	10 (0.082)	28 (96.7)	166-172 (176-177)[h]	$C_{24}H_{27}BO_2$ (358)	C,80.42 C,80.45 H, 7.34 H, 7.54
4-Methyl-7,8-dihydroxy coumarin	14	260 (1.35)	165 (1.352)	370 (98.4)	143-146 (191-193)[j]	$C_{16}H_{11}BO_4$ (278)[i]	

a. GLC analysis for these crude products showed in each case a single peak corresponding to their benzeneboronate derivative. Traces of excess benzeneboronic acid (eluted as triphenylboroxine) were also observed.

b. These compounds were purified for analytical studies.

c. The isomeric methyl 2,3-dihydroxybenzoate(8), did not show a complete reaction with benzeneboronic acid, as judged by GLC. On recrystallisation of the crude reaction mixture, from acetone-hexane; the recovered material was mainly the reagent, benzeneboronic acid. Vacuum subl^n. of the crude product, was also unsuccessful as a purification method.

d. Recrystallisation from acetone-hexane; m.p.107-108.5°; then vacuum subl^n.: a middle fraction was collected.

Footnotes:-Table 2

e. Vacuum subl^{n}. at 55°/0.01 torr.

f. Recrystallisation from acetone-hexane; m.p.125-127°; then vacuum subl^{n}.; a middle fraction was collected.

g. Vacuum subl^{n}. at 130°/0.01 torr; m.p.157-160°, then second vacuum subl^{n}.

h. Recrystallisation with acetone, then vacuum subl^{n}. at 160°/0.01 torr. The sublimed material was recrystallised twice from acetone.

i. Recrystallised twice from $CHCl_3$-hexane, then vacuum subl^{n}. at 143°/0.01 torr: a middle sublimed fraction was collected. This sublimed material was recrystallised from EtOAc-hexane. GLC analysis for this purified product showed it to contain about 0.1% of benzeneboronic acid; and the micro-analysis was not satisfactory for carbon.

VII. MICRO-SCALE REACTIONS, DERIVATIVE FORMATION FOR GLC AND GC-MS ANALYSIS.

A. Boronates

Methane-, n-butane- and benzeneboronates of catechols, other diols and α-hydroxyacids were prepared in dry pyridine, EtOAc, or EtOAc-pyridine solution, by addition of 1.1molar equivalent of the appropriate boronic acid (Lit.[21,22,26,27,62]). Reactions were completed within 5-10min at R.T. and the products examined by direct GLC or GC-MS analysis.

B. Acetates and trimethylsilyl ethers

Trimethylsilylation reagents were TMCS, HMDS and BSA; and the reagent for acetylation was Ac_2O. These reagents were added in excess (5-10molar proportions) to 0.01-0.5mg of substrates possessing functional groups such as -OH,-NH_2 and -NHR). The solvents used were EtOAc, dry pyridine or EtOAc-pyridine. These reactions generally took place at R.T., for 5-15min . The complete derivatisation for some substrates with multi-functional groups required heating at 60-70° for ½-2hr . Solution concentrations were usually 2mg/ml, and 1μl was injected for direct GLC or GC-MS analysis. Sometimes the excess reagents were evaporated under a stream of nitrogen, before analysis.

C. Methoximation

3-Hydroxyestra-1,3,5(10)-trien-17-one(40) (1mg:0.0037mmole) was dissolved in dry pyridine(0.02ml), then methoxyamine hydrochloride (1.74mg: 0.037 mmole) in dry pyridine(0.174ml)

was added. The reaction mixture was heated for 2hr at 80°. The pyridine was evaporated under nitrogen, and the products were separated from pyridine-HCl by five extractions with EtOAc. TLC ($CHCl_3$) showed no significant difference between the mobilities of the product and the starting material (Rf=0.40); while GLC (OV-17:240°), showed a complete methoximation reaction for the product(41), I=3090, distinguished from the starting material(40), I=3065. The trimethylsilyl ether derivatives of the crude product(41) and of (40) both showed on GLC I=2985.

The corresponding methyloxime derivatives of 2,3- and 3,4-dihydroxybenzaldehyde(44); were prepared on a 1-3mg scale, by the above procedure, for TLC; GLC; GC-MS and derivative analysis.

VIII. EXPERIMENTS TOWARDS THE SYNTHESIS OF BORONIC ACIDS

A. Synthesis of Substrates

1. 3α-Hydroxy-3β-methyl-5α-cholestane(52) and 3β-hydroxy-3α-methyl-5α-cholestane(53):- (Procedure of Barton et al.[119] with minor modifications). 5α-Cholestan-3-one(51) (3g:8 mmole) in dry ether (40ml) was added to methylmagnesium iodide [prepared by the gradual addition of methyl iodide (1.2ml:20 mmole) in dry ether (15ml) to magnesium (0.5g:21mmole) in dry ether (10ml) during 30min with continuous stirring and cooling], and the resulting solution was refluxed for 3hr. The reaction mixture was poured on to ice, then a solution of H_2SO_4 (1.52g) and water (10ml) added with stirring. The mixture was extracted three times with ether and washed with saturated $NaHCO_3$, then with water until neutral. Extracts were dried and evaporated, yielding a white crystalline product (2.92g:94%), m.p.95-100°. TLC and GLC for this product showed it to consist almost wholly of compounds(52) and (53) (60:40). 550mg of the product, in $CHCl_3$-EtOAc (3:1,v/v) was chromatographed on "dry column" silica gel (55g); elution with the same solvent mixture gave 9 fractions. Compound(52), isolated from fraction 4 (203mg), had m.p. 120-121° (Lit.[119] 126-127°); compound(53), isolated from fractions 7,8 and 9 (115mg) had m.p.145-146° (Lit.[119] 147-149°). These compounds(52) and (53) were highly pure as judged by TLC and GLC. Fraction 3 (48mg) was about 90% compound(52) and fractions 5 and 6 (196mg) contained about equal parts of compounds(52) and (53).

2. 3-Methyl-5∝-cholest-2-ene(54):- The two stereo-isomeric alcohols(52) and (53), (2g) were dissolved in glacial acetic acid (25ml) on warming, then perchloric acid (72% w/w,10 drops) was added and the solution heated in a hot water bath (80-90°) for 30min. The solvent and excess reagent were evaporated azeotropically. The yellow solid residue was dissolved in ether, washed with $NaHCO_3$, then with water, until neutral; each washing was back-extracted three times with ether. The extracts were combined; dried and evaporated. A yellow oily residue, which gradually crystallised was recovered:1.81g (95%), m.p.69-73°. This was recrytallised from ether-MeOH, giving 1.739g (91%) of compound(54), m.p.75-77° (Lit.[119] 82-83°, from light petroleum); highly pure as judged by TLC and GLC.

3. 24-Bromo-5β-cholane(56):- Phosphorus tribromide (0.06ml: 0.647 mmole) in CH_2Cl_2 (10ml) was added dropwise during 15min with stirring to a cooled solution [-5°-0°], of 5β-cholan-24-ol (55) (224mg:0.647mmole) in CH_2Cl_2 (10ml). This mixture was stirred at 10-15° overnight. The reaction mixture was basified with saturated $NaHCO_3$, then extracted with hexane-EtOAc (4:1,v/v) and washed with water in the usual procedure. The yellow oily residue gradually crystallised and 135mg (51%) was recovered; its purity as judged by TLC and GLC was about 70% of compound(56). This product was dissolved in hexane and chromatographed on "dry column" silica gel (10g). Three fractions were collected: fraction 1

(78mg:29.5%) yielded pale yellow crystals of compound(56)m.p.62-65° (Lit.[120]83°, from ether-acetone); its purity as judged by TLC and GLC was about 95%; fraction 3 gave starting material (55), (46mg) as white solid, m.p.122-125°.

NOTE: More material (111mg) was recovered from the combined water extracts, after basification with NaOH to pH14 and extracted as above. This crude product was chromatographed as above: compound(56), (54mg) as crude oil; 70% pure,and starting material(55), (25mg) as white solid, were recovered.

4. 3-Aminoestra-1,3,5(10)-trien-17-one(70) (Procedure of Conrow and Bernstein[121]), 3-(2-Benzene-4-quinazolinyloxy)estra-1,3,5(10)-trien-17-one(68) (Derivatisation Step):- A mixture of 3-hydroxyestra-1,3,5(10)-trien-17-one(40) (1.08g:40mmole), 4-chloro-2-benzenequinazoline(72) (1.01g:4.2mmole) and anhydrous potassium carbonate (1.10g:8.0mmole) in acetone (50ml) was stirred and refluxed for 21hr. The mixture was diluted with water, and extracted with benzene (3x60ml). The extract was washed, dried, concentrated to approximately 100ml, and filtered through magnesium silicate(10g). The magnesium silicate was washed with benzene (50ml) and the total filtrate was evaporated. The resulting product was crystallised from CH_2Cl_2-hexane, to give 1.825g (96%) m.p. 198-200°(TLC, one spot). This material was judged to be sufficiently pure for the next step.

3-[4-Oxo-2-benzene-3(4H)-quinazolinyl]estra-1,3,5(10)-trien-17-one(69) (Rearrangement Step):- The estrone 3-quinazolinyl ether(68) (1.735g:3.7mmole) was heated, neat, at 320-340° (Wood's metal bath) for 6hr in a nitrogen atmosphere. The resulting amber glass was dissolved in CH_2Cl_2-EtOAc, treated with Darco and filtered through Celite. The filtrate was evaporated, giving yellow crystalline material 1.170g, m.p.130-135° (Lit.[121] m.p.245-255° for crude compound 69). TLC showed about 40% of compound(69), 40% of estrone(40) (from hydrolysis during heating) and 20% of unrearranged compound(68).

3-Aminoestra-1,3,5(10)-trien-17-one(70) (Hydrolysis Step):- The crude 3-quinazolinylestratriene(69)(1.125g) was refluxed in a solution of MeOH(60ml), NaOH (9.53g) and water (15ml) for 5hr . The mixture was acidified with 12M-HCl (25ml), allowed to stand overnight at R.T., then refluxed for 1.5hr, and worked up by extraction with CH_2Cl_2 giving a deep yellow oil (1.090g); TLC and GLC showed about 40% of compound(70) and 30% of estrone(40) [NOTE:-this recovery of compound(70) from the acidic solution, may be due to ion-pair ($Ar\text{-}\overset{+}{N}H_3\overset{-}{O}Ar$) formation]. The combined water extracts were basified with NaOH and extracted with CH_2Cl_2, giving yellow solid(81mg), m.p.95-110°, having the same composition as above. The major crude product (1.090g) was chromatographed on "dry column" silica gel (450g) and eluted with $CHCl_3$-EtOAc-MeOH-AcOH (90:6:3:1, v/v/v/v): 33 fractions were collected; fractions 21-31 (20ml each) contained mixtures of compounds (70) and (40) (~5:1,GLC) (no pure fraction was obtained). These fractions were combined and recrystallised from EtOAc-hexane, giving fine tan crystals (600mg), m.p.120-130°. Vacuum subl^n. of volatiles at 140°/0.01torr left 470mg of unsublimed crystals, but GLC and TLC analysis did not show the existence of either compounds(70) or (40). Acid hydrolysis in HCl-MeOH of unsublimed crystals, regenerated these two compounds, and after extraction, yielded 350mg, m.p.130-150°. 170mg of this crude product was purified by preparative TLC (10% acetone-benzene) giving pale yellow crystals 105mg, m.p.165-175° [GLC about 80% of compound(70) and 20% of compound(40)]. Two recrystallisations from EtOAc-hexane gave off-white crystals of compound(70),(15mg) m.p.184-187°

(Lit.[121] m.p.194-198° tan crystals) and GLC showed only one major peak in the expected region. The remaining material in the mother liquor was treated with excess Ac_2O at R.T., then solvents and excess reagent were evaporated. The residue was purified by column chromatography as above, giving pale yellow crystals of compound (N-acetyl-70) 30mg, m.p.234-236°, purity as judged by GLC and TLC was about 98%.

5. 3-Bromoestra-1,3,5(10)-trien-17-one(71):- This experiment was based on the procedure of Talik et al.[122]. Potassium bromide (250mg) and sodium metabisulphite (68mg) were dissolved in concd. HBr (0.5ml) in water (1ml), then cupric sulphate (140mg) in water (0.5ml) was added. To this solution, a crude product containing about 60% of 3-aminoestra-1,3,5(10)-trien-17-one(70) (150mg), and MeOH (0.5ml) were added. This mixture was heated in a hot water bath (80-90°) with continuous shaking and more concd. HBr (0.4ml) added; a highly viscous tan oil remained undissolved. The supernatant was transferred and cooled to 10° and a saturated solution of potassium nitrite (70mg) in water (0.2ml) added dropwise. The temperature of the reaction mixture was held at 5-15° throughout the addition. After the addition was completed, the mixture was allowed to stand for 1hr, then heated on a steam bath for 15min. This solution was basified with 50% KOH solution, then diluted with water and extracted with CH_2Cl_2, giving a partially crystallised yellow oil (25mg); (~ 40% pure). This

showed a peak with $I_{240°}^{OV-17}$ 3260, which presumably corresponded to compound(71). (Note: Further studies are required to prepare a pure sample of compound(71), and fully characterise it).

B. Hydroboration of Alkenes with catecholborane

Procedure of Brown et al.[51].

A single necked round bottom flask equipped with a rubber septum inlet, magnetic stirring bar, and outlet tube connected to a mercury bubbler was flushed with nitrogen and charged with the required alkene. After reflushing the system with nitrogen, 10-20molar excess of catecholborane was added using a dry syringe. The resulting mixture was then stirred for 3-6hr at 100° (oil bath) under nitrogen. GLC monitoring of the reaction revealed it to be essentially complete (90-99% yield) at the above period. The excess catecholborane was removed by vacuum evaporation(the products being distilled or undistilled depending on their volatility). The model compounds which have been studied are cited in Table 3, p.96 .

Data for catechol alkeneboronates derived from alkenes (54),(57),(58),(59),(60) and (61) from GLC; GC-MS; IR and ^{1}H-NMR are given in Tables 8, VIII-IX, 5,4 respectively.

Table 3, The Synthesis of 2-alkyl-1,3,2-benzodioxaboroles (catechol cyclic alkaneboronates)* from olefins via hydroboration with catecholborane

Alkene	Compd. No.	Weight mg (mmole)	Catechol-borane ml (mmole)	Time hr	% yield isol.[a] (GLC)[b]	b p.°C (torr)	GC-MS $M^{+\cdot}$ (% of base peak)
1-Octadecene	57	2190 (8.6)	1.2 (10)	4	92 (98)	solid[c]	
Cyclohexene	58	4000 (49)	6.5 (54)	5	87 (99)	100 (0.5)	202 (92)
Norbornene	59	4600 (49)	6.7 (56)	6	86 (EXO 99.5[d] ENDO 0.5)	104 (0.5)	214 (EXO 33 ENDO 68)
(1S)-(-)-β-Pinene	60	500 (3.7)	0.49 (4.1)	3	90 (ENDO 86[d] EXO 14)	162 (0.1)	256 (ENDO 25 EXO 25)

3-Methyl-5α-cholest-2-ene	54	500 (1.5)	0.6 (5)	24	96[e] (96)	viscous[c] oil	504 (38)
5β-Chol-23-ene	61	48.7 (0.15)	0.2 (1.5)	6	77[e] (99)	viscous[c] oil	448 (66)

* All compounds studied herein as boronates are understood to be cyclic esters.

a. All these isolated catechol alkaneboronates proved to be highly unstable towards air or moisture.

b. Yields as judged by GLC.

c. These products did not distil in the range 100-250°/0.01 torr.

d. The isomers are assigned on the basis of the expected mode of addition of catechol-borane to the double bond.

e. These isolated yields were calculated indirectly from displacement reactions with 2,3-butanediol, after extraction and drying.

C. Preparation of Alkeneboronic Acids

1.By direct hydrolysis of the corresponding catechol alkaneboronates:-

a.Cyclohexaneboronic acid(62):- Catechol cyclohexaneboronate (2.933g:14.5mmole) was stirred rapidly with water (30ml) and acetone (0.5ml) at R.T. for 1½hr. The white crystalline material formed was filtered and dried giving 1.750g (94%), m.p.116-120° (in a sealed capillary tube). Recrystallisation from acetone and hot water yielded 1.504g (81%), m.p.124-128° (Lit.[51] 112-114°, from hot water) (two recrystallisations as above of an authentic sample of (62) gave m.p.125-128°).

b.Octadecaneboronic acid(63):- Catechol octadecaneboronate (3g:8.1mmole) was dissolved in hexane and washed three times with water, then each washing extracted three times with hexane. The hexane extracts were dried and solvent evaporated, yielding yellowish viscous oil(2.25g). This crude product was dissolved in acetone and crystals ppted. by addition of warm water. These crystals were filtered and re-ppted. as before giving whitish crystals of (63) (2.035g : 79%), m.p.45-55°. 930mg of this product was triturated with EtOAc in dry ice-acetone, giving white crystals 685mg, m.p.65-70° (two re-pptns. as above for authentic sample of (63) gave m.p.66-69°).

Note:Compound(63) proved to be unstable in hot water; a significant amount of 1-octadecene(57) was detected by GLC in the mother liquors during purification of (63).

The catechol alkaneboronates derived from alkenes(59),

(60) and (54) underwent hydrolysis and decomposition during attempted hydrolysis in water or dilute-HCl at R.T.; and were also unstable in air or under nitrogen in a sealed tube. Reactions were monitored by GLC: see Table 8, p.130.

2.By displacement of the corresponding catechol alkaneboronate with 2,3-butanediol, then subsequent acid hydrolysis:- The catechol alkaneboronate was dissolved in EtOAc (or ether), mixed with excess (5-10molar) 2,3-butanediol, then diluted with hexane and washed four times with water, to remove catechol and excess butanediol. Each washing was extracted twice with hexane. The hexane extracts were dried and the solvent evaporated. The residue was analysed by GLC, usually showing a complete displacement reaction. These 2,3-butanediol boronates were stable in air and in solvents: hexane, EtOAc or ether. The following 2,3-butanediol boronates, prepared by the above procedure, were hydrolysed directly to the corresponding boronic acids, as described below.

Note: The formation, purity and stability and the estimated yields of the following free boronic acids were analysed indirectly by GLC, after cyclic ester formation with 2,3-butanediol (meso & dl), pinacol, catechol or cis-1,2-indanediol, in micro-scale tests. Hydrolysis experiments were monitored by GLC.

a. Octadecaneboronic acid(63):- 2,3-Butanediol octadecane-1-boronate (65mg:0.185mmole) was dissolved in ether (2ml) and acetone(5ml), then water (5ml) and 0.01N HCl (0.5ml) were added. This mixture was stirred at R.T. for 12hr ; during which much of the acetone and ether evaporated

and a gummy precipitate formed which gradually crystallised. The crystals were filtered and washed with water, dried, giving compound(63), 43mg (78%), m.p.45-50°. This compound proved to be stable in air and cold water.

b.Norbornane-2-boronic acid(64):- 2,3-Butanediol norbornane-2-boronate (40mg:0.206mmole) was dissolved in hexane (2ml) in a sealed tube with rubber septum and the air displaced with nitrogen, then a solution comprising water (0.5ml), AcOH (0.5ml) and saturated aqueous ascorbic acid (0.1ml) was added by means of a syringe. The mixture was shaken mechanically at R.T. for 12hr. About 30% of the unhydrolysed butanediol norbornane-boronate was detected by GLC. The hydrolysed boronic acid was concentrated mainly in the hexane layer, and did not give a peak on GLC; but the corresponding boronate esters of pinacol, 1,3-propanediol and catechol, gave peaks indicating the presence of compound(64). This was highly unstable in air. Pinacol norbornane-boronate ($M^{+\cdot}$255) showed high stability, and resisted hydrolysis in aqueous basic or acidic solutions at R.T. and in air.

c.(1S)-(-)-β-Pinane-10-boronic acid(65):- 2,3-Butanediol (1S)-(-)-β-pinane-3-boronate ($M^{+\cdot}$236) (120mg:0.508mmole) was dissolved in acetone (6ml), then aqueous 0.25N HCl (4ml) and ascorbic acid (~10mg) were added. This solution was stirred overnight at R.T., becoming a milky suspension after acetone evaporation. The supernatant was transferred into a separatory funnel and the remaining oil was extracted four times by shaking with aqueous 0.1N HCl,then only 15mg

of undissolved oil was remained. GLC for this showed it was mainly the unhydrolysed butanediol pinane boronate. The combined aqueous acidic extracts were extracted four times with hexane, dried and solvent evaporated, giving a slightly yellowish oil(80mg); GLC showed it contained about 60% of the free boronic acid and the rest was unhydrolysed boronate. This product was dissolved in hexane (10ml) and extracted with water (4x30ml) then each extract was washed once with hexane(10ml). The combined water extracts were extracted with ether-hexane (3x50ml;1:1,v/v) and the organic extracts dried and solvents evaporated, giving colourless oil of compound(65), 22mg (24%). Its purity as judged by GLC (after boronate ester formation) was about 98%. This compound was unstable on GLC; on TLC,it showed a single purple spot after spraying with diphenylcarbazone. The product was also unstable in air, but was stable in hexane under nitrogen.

d.3-Methyl-5α-cholestane-2β-boronic acid(66):-2,3-Butanediol 3-methyl-5α-cholestane-2-boronate ($M^{+\cdot}$ 483)(300mg: 0.621mmole) was dissolved in acetone (2ml), then aqueous 0.1N HCl (10ml), ascorbic acid (10mg) and ether (5ml) were added. This mixture was stirred overnight at R.T. becoming a milky suspension after acetone and ether evaporation. 0.05N HCl (40ml) was added, the mixture was extracted with hexane (2x50ml) and each hexane extract washed with water (2x50ml). The hexane extracts were dried and solvent was evaporated,giving a colourless oil of compound(66), 226mg (85%), its purity as judged by GLC was about 80% (after boronate ester formation), also traces of the unhydrolysed boronate and

a by-product(possibly 3-methyl-5α-cholestan-2-ol,$M^{+\cdot}$402) were detected. This boronic acid(66) decomposed gradually to the by-product, in air and in solvents such as EtOAc, ether and hexane; and on "dry column" silica gel (saturated with ascorbic acid), preparative TLC and Lipidex-5000.

cis-1,2-Indanediol 3-methyl-5α-cholestane-2-boronate (25mg), as a highly viscous colourless oil, 95% pure as judged by GLC, was obtained from the reaction of excess cis-1,2-indanediol with 2,3-butanediol 3-methyl-5α-cholestane-2β- boronate in EtOAc at 70° overnight. It was purified by hexane-water extraction, then chromatography on Lipidex-5000, then vacuum subl^{n}. at 80°/0.01torr of the volatile impurities. Indanediol 3-methyl-cholestane-boronate was stable in air, both neat and in solvents like EtOAc, hexane or ether.

e.5β-Cholane-24-boronic acid(67):- 2,3-Butanediol 5β-cholane-24-boronate ($M^{+\cdot}$428) (48.5mg:0.113mmole) was dissolved in acetone (10ml) and ether (1ml), then aqueous 0.5N HCl(5ml) and ascorbic acid (~20mg) added. This solution was stirred for 5hr at R.T. yielding a milky suspension by acetone and ether evaporation. Saturated $NaHCO_3$ (0.1ml), boric acid (~30mg) and water (5ml) were added, and the mixture was extracted with hexane (3x40ml): each extract was washed with water (4x15ml). The hexane extracts yielded a yellowish oil (largely compound(67): 40mg (95%); its purity as judged by GLC was 80%: some unhydrolysed boronate ester was observed. 1.4mg of compound(67) (98% pure) as white solid was recovered from preparative TLC of 8mg of the crude product. The remaining (32mg) crude product was

submitted to chromatography on Lipidex-5000 and "dry column" silica gel, but no purification was achieved.

This boronic acid(67) was stable to air oxidation, as solid or in solvents such as: EtOAc, hexane or ether.

3.By Grignard reaction with boron trichloride or trimethyl borate, then subsequent hydrolysis:

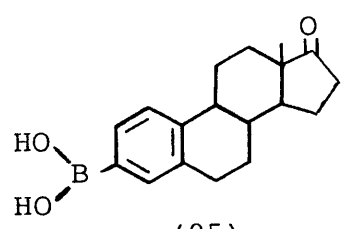
5β-Cholane-24-boronic acid(67):- 24-Bromo-5β-cholane(56) (65mg:0.159mmole) in dry ether (10ml) was added gradually to magnesium (20mg:0.833mmole) in ether (5ml), then methyl iodide (0.01ml) was added and the solution stirred and refluxed for ½hr to afford the Grignard reagent. Boron trichloride (~0.3ml) in ether (10ml) was added gradually, and the mixture was refluxed for 2hr, then poured on ice (~100g) containing NaOAc(100mg), extracted with hexane and each extract washed with water until pH=7. The hexane extracts yielded a yellowish oil (60mg). (Theoretical yield 59.4mg). As judged by GLC, this crude product contained about 50% of compound(67) together with about 30% of unreacted compound(56) and traces of 24-Ol (55) and 23-ene (61). After vacuum subln. at 100°/0.01torr of the volatile impurities, 18mg of unsublimed material was recovered, containing about 60% of the boronic acid(67) (TLC and GLC).

Chapter 4

BORONIC ACIDS AND THEIR DERIVATIVES

RESULTS AND DISCUSSION

Many organic boronic acids have been synthesised (see Chap.1, IV D). These acids and their esters are becoming of increasing significance as intermediates in organic synthesis (several examples have been reviewed by Lane and Kabalka[52]), and as selective reagents to form cyclic boronates with polar bi-functional compounds, useful for separation and identification of these compounds (see Chap.1, VI A). Boronic acids are also of potential value in cancer therapy and as hormone analogues (see Chap.1, VI E). Our main interest in this field was focused on the synthesis of steroidal boronic acids, for example, 3-deoxyestrone-3-boronic acid(95) and 5β-cholane-24-boronic acid (67), with a view to their possible application in ^{10}B-cancer therapy and in the studies of the enzymatic mechanistic behaviour of steroidal hormones. It is hoped that certain steroidal boronic acids might be developed, which will



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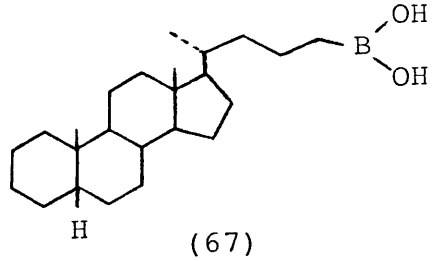

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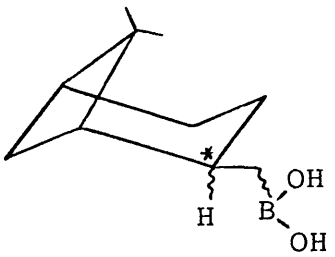
possess the properties required (cf. p.48) from a boron-containing chemical compound to be used in cancer therapy. Our other aim was to prepare chiral boronic acids, for example

1S-(-)-β-pinane-10-boronic acid (65) and 3β-methyl-5α-cholestane-2α-boronic acid (66), which were of interest



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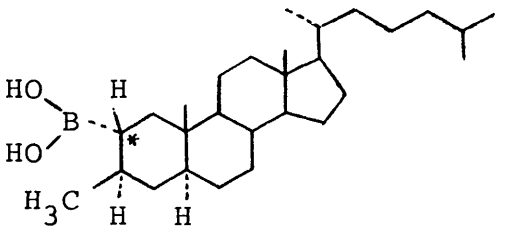
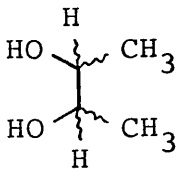


(66)

as reagents for separation of optically active polar bifunctional enantiomeric compounds, for example dl-2,3-butanediol (I).



(I)

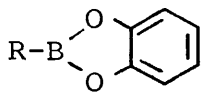
As discussed in Chapter 1, (IV D , 5&6) there are two widely applicable procedures for the synthesis of these boronic acids: Grignard and hydroboration reactions. We have used the hydroboration procedure for the attempted preparation of the boronic acids from the olefins listed in Table 3, p.96, and the Grignard procedure for preparations starting from 5β-Cholan-24-ol (55). Each of these procedures have their advantages and disadvantages. For example, in the hydroboration reaction (reviewed by Brown[51]; Lane[52]; cf. also Chap.1, IV D,6), the addition of catecholborane to the double bond was influenced by regio-and stereoselective factors (see pp.25-27), therefore, in many cases two isomers were obtained, as in the reactions of 2-norbornene(59) and β-pinene(60), (see Table 3, p.96), which yielded isomers

that were difficult to separate. On the other hand, the hydroboration reactions proceeded readily and the yields of the corresponding catechol esters were always high. In the Grignard reactions, with aliphatic or aromatic bromo compounds, which could be prepared from their corresponding hydroxy compounds, for example 5β-cholan-24-ol(55) and estrone(40), there is only one corresponding boronic acid expected, with retention of configuration, but the yields were poor.

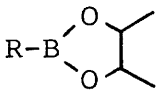
I. THE HYDROBORATION OF ALKENES WITH CATECHOLBORANE

The hydroboration of a selected number of cyclic and acyclic alkenes with excess catecholborane was effected according to the procedure of Brown and Gupta[51], which is described on p.95; the experimental conditions and the yield of each compound are listed in Table 3, p.96. The alkenes were obtained from the sources mentioned in Chap.2, p.54, except 3-methyl-5α-Cholest-2-ene(54), which was prepared as described on pp.89-90, according to the procedure of Barton *et al.*[119], in an overall 90% yield, see reactions Scheme 4.3, p.122. The reaction of catecholborane with these alkenes took place under nitrogen and the reactants were heated in an oil bath at 100°C, giving homogeneous solutions. (Note: these reactions were found not to take place in solution in methylcyclohexane). Reactions were monitored by GLC, Table 8, p.130; the yields of the catechol alkane cyclicboronates, were 96-100% as judged by GLC. The excess reagent was removed *via* vacuum distillation.

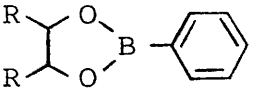
The catechol octadecane-, cyclohexane-, norbornane-2-, β-pinane-10ξ-,3-methyl-5α-cholestane-2ξ -, and 5β-cholane-24- boronates, were all stable under vacuum or under nitrogen, when kept below 10°c, in the absence of light; but they were not stable in air, in organic solvents (containing dissolved air), or in water: under these conditions they gradually changed from colourless to black liquids within a very short period. This effect may be due to aerial oxidation and subsequent hydrolysis. This phenomenon was also observed for catechol 2-bromo-propane-2-boronate by Matteson and Schaumberg[123], but was not reported by Brown and Gupta[50,51]. After displacement of the catechol group with 2,3-butanediol, pinacol or indane-cis-1,2-diol, (especially with the last two diols) esters having higher stability towards aerial oxidation were obtained, which could be stored neat or in organic solvents without decomposition. The susceptibility to oxidation of the catechol alkaneboronates (II) as compared with the other diols (III) , was possibly due to the electron resonance of the 2p electrons of the oxygen atoms with the benzene ring, leading to a reduced electron density on the boron atom, which is thus more easily attacked by nucleophiles such as atmospheric oxygen and water. It is worth mentioning here that the benzeneboronate derivatives of many diols, including catecholic compounds (IV)



II



III



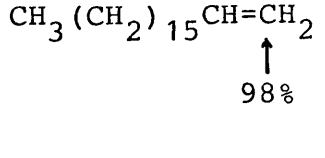
IV

(see Chap.1,V and Chap.5) have proved to be highly stable towards aerial oxidation. This is due to the direct bonding of the boron atom with the electron rich benzene ring and also with the two oxygens.

Catechol 3-methyl-5α-cholestane-2β-boronate was not stable on heating above 150°, under vacuum: this may be due to thermal isomerisation, resulting in the migration of the boron atom from one carbon atom to another with establishment of an equilibrium, as studied by Herz and Márquez[124] in the case of steroidal boranes.

A. Directive effects in the hydroboration of alkenes with catecholborane

After the standard hydroboration with catecholborane (procedure on, p.95) of the alkenes listed in Table 3, the product in each case was identified by GLC, followed by displacement reactions with other diols and by GC-MS analysis. The percentage yield of each isomeric product, was calculated from their relative peak height ratios in the total mixture for example see Fig. 4.1. The following results were obtained (Table 3, p.96):



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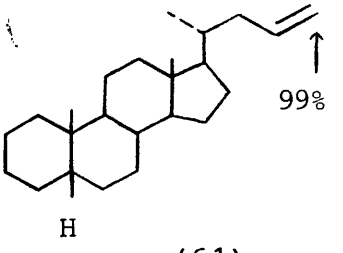

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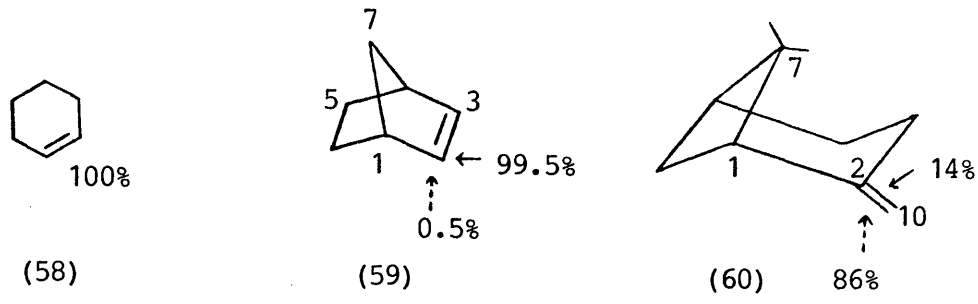
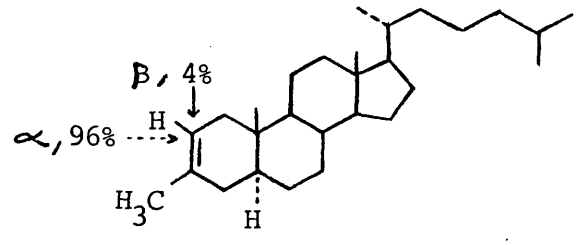


(58) (59) (60)



(54)

(Note: the addition of catecholborane to compounds(58) and (59) was also effected by Brown and Gupta[50,51], who obtained similar results).

The above results indicate that the hydroboration of the terminal alkenes, such as 1-octadecene(57) and 5β-chol-23-ene(61), proceeded in a highly regioselective manner by placing the boron atom at the terminal position[50-52]. The hydroboration of the cyclic alkenes showed that cyclohexene (58) gave the expected product in 100% yield, while norbornene (59) gave mainly the <u>exo</u> isomer 99.5%, and 0.5% of the <u>endo</u> isomer, showing the almost stereospecific nature of the hydroboration addition reaction[50-52,56]. Stereoselectivity was also observed in β-pinene(60), in which the addition of the boron atom occurred exclusively at the terminal position[5] the catecholborane approaching preferentially from the less hindered <u>endo</u> position to give the <u>exo</u> isomer in 86% yield[56,57] (Fig. 4.1).

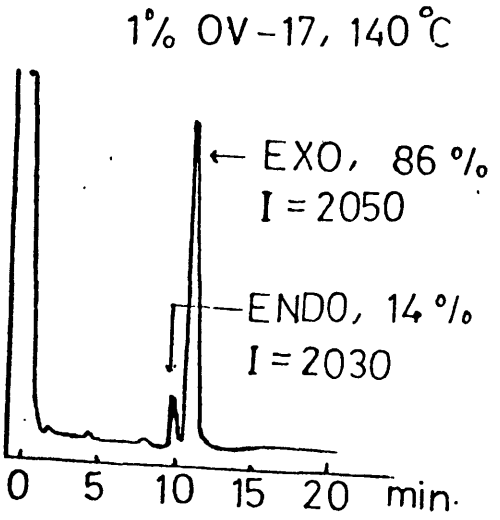


Fig. 4.1 GLC Chromatogram of mixture of exo and endo catechol β-pinane-10ξ-boronates, in EtOAc, injection volume 1µl; concentration 2µg/µl.

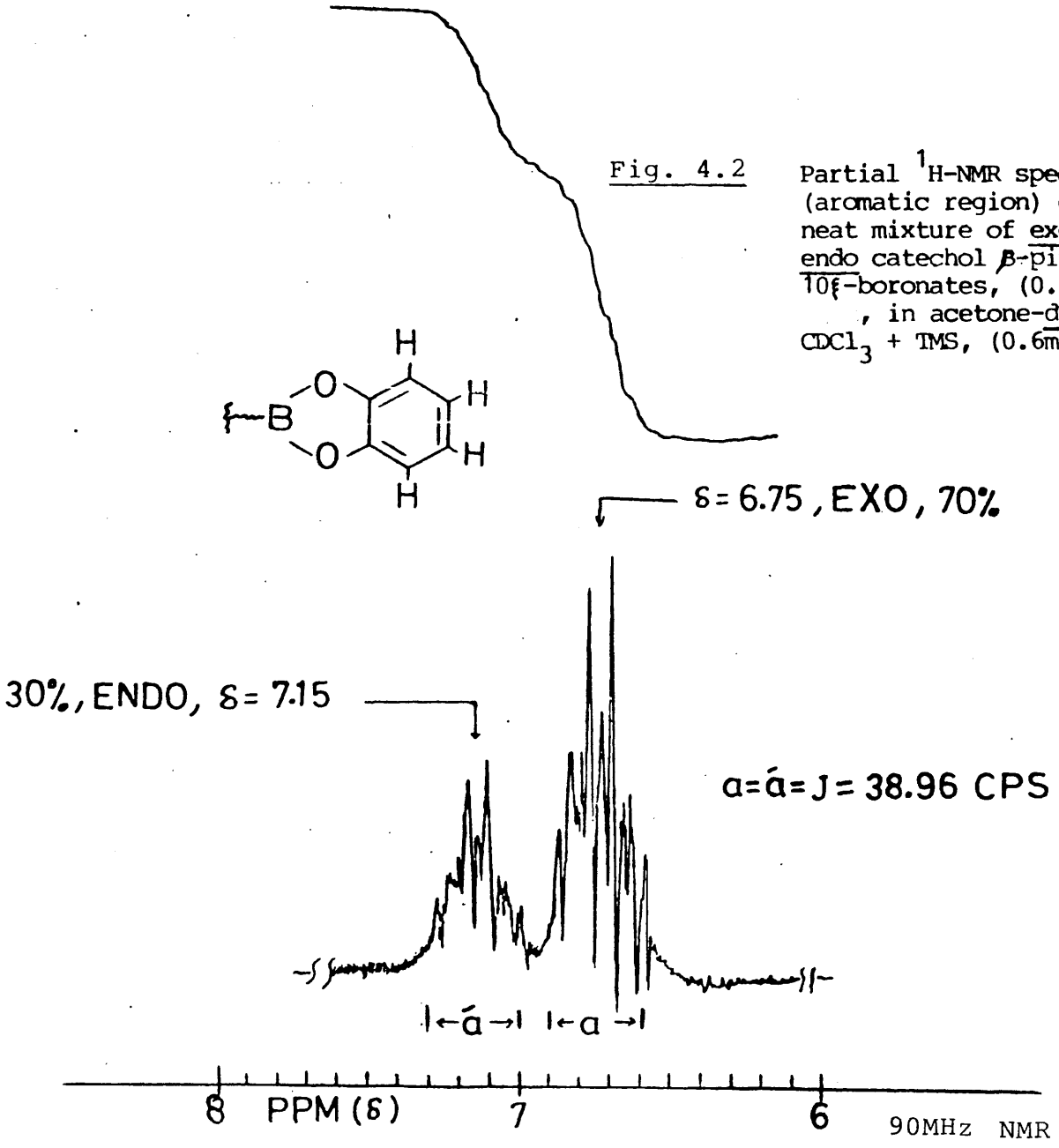
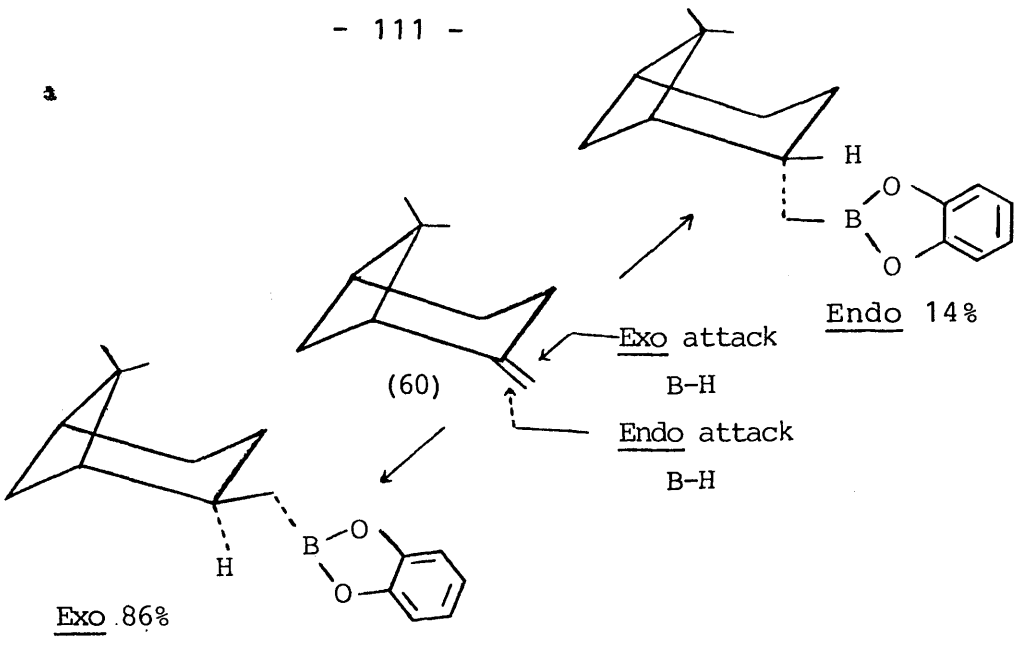
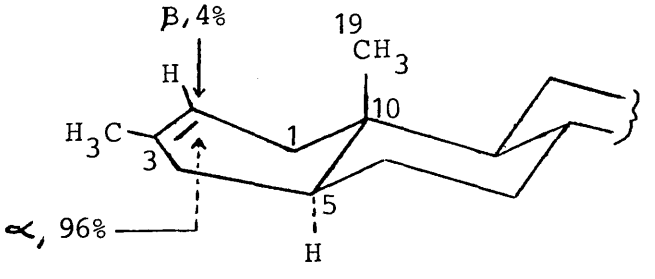


Fig. 4.2 Partial ^{1}H-NMR spectrum (aromatic region) of a neat mixture of exo and endo catechol β-pinane-10ξ-boronates, (0.1ml) , in acetone-d6 + $CDCl_3$ + TMS, (0.6ml).



However ^{1}H-NMR analysis for the purified catechol β-pinane-10ξ-boronate (Fig. 4.2), showed almost two identical and well separated meltiplet peaks at δ=6.75 and 7.15, for each of the four aromatic protons of the exo and endo isomers, respectively, in a ratio of 70:30. This ratio represents the proportion of each isomeric product in the total mixture.

In the hydroboration of 3-methyl-5α-cholest-2-ene (54), the boron atom should be placed at the less hindered C-2, position; and because of the steric affect of the axial C-19 methyl group, addition is expected to occur from the less hindered α-face. The major product, obtained in 96% yield probably represents the α-isomer.



(54)

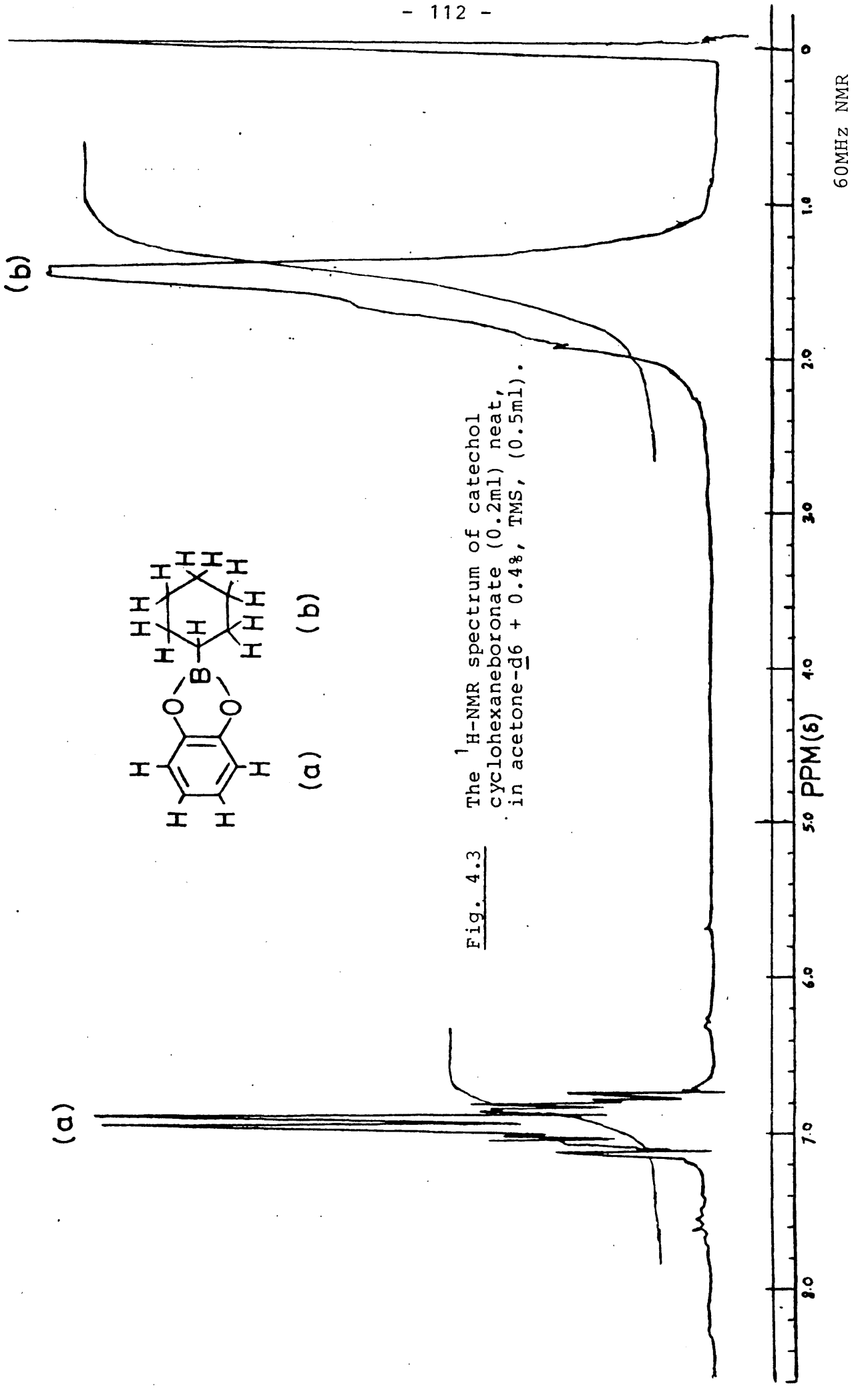
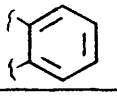
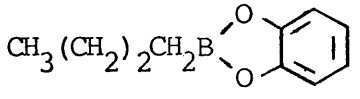
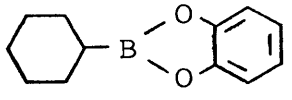
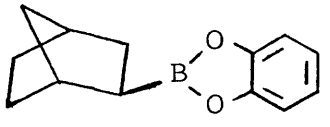
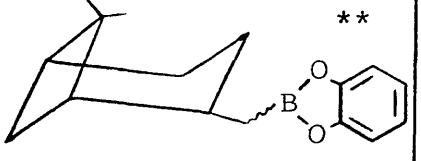
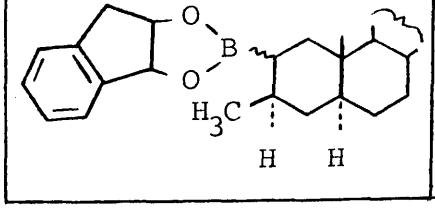


Fig. 4.3 The ^{1}H-NMR spectrum of catechol cyclohexaneboronate (0.2ml) neat, in acetone-d6 + 0.4%, TMS, (0.5ml).

The ^{1}H-NMR analyses of a few of the hydroboration products listed in Table 3 p.96 are summarised in Table 4. Data are cited only for the four aromatic protons of each compound, which gave well defined signals in contrast with the other partially defined aliphatic protons which resonated at higher field; for example see Fig 4.3. .

Table 4. ^{1}H-NMR, Characteristic Proton Chemical Shifts (δ=ppm) of diol alkane boronate type compounds. (m=multiplaet). Data measured on 60MHz instrument.

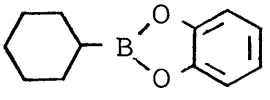
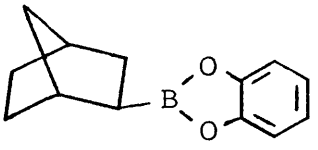
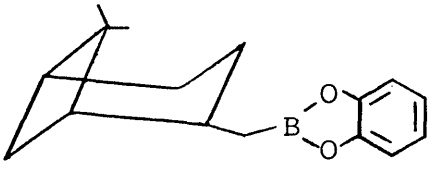
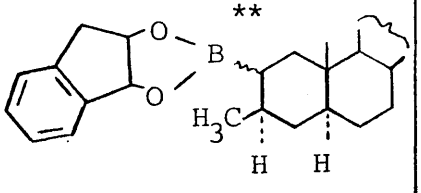
Diol alkane boronates	No.	Solvent		Reference
	89	$CDCl_3$	6.93,m	*
	83	Acetone-d6	6.96,m	50,51
	84	Acetone-d6	6.76,m 7.16,m 7.59,m	50,51
 **	85	Acetone-d6 + $CDCl_3$	70% EXO, 6.75,m & 30% ENDO, 7.15,m	This work p.96
	86	$CDCl_3$	7.26,m	This work p.96

* Prepared from butaneboronic acid and catechol,(1:1 molar) in ethyl acetate.

** Measured on R32 90MHz instrument.

The C-B and B-O infra red absorption bands,[15,19,125] of some of the hydroboration products are listed in Table 5.

Table 5*. IR Absorption bands of diol alkane boronate derivatives. ν max, cm^{-1}, measured in Nujol or in NKCl discs.

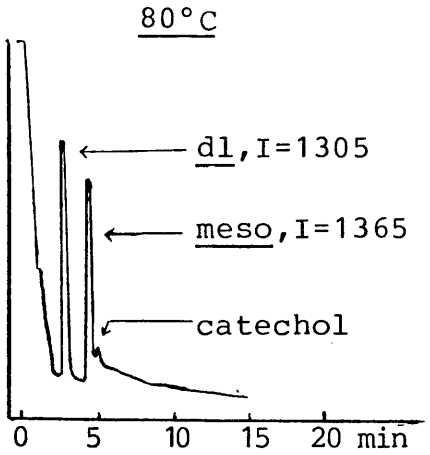
Diol alkane boronate	No.	C-B	B-O
	83	1465,S	1385,Ss 1345,w
	84	1465,S	1375,Ss 1345,ms
	85	1450,Ss	1375,ms
	86	1450,wb	1375,ms

* Strong=S; Medium=m; Weak=w; Broad=b; and Sharp=s; These abbreviations are used throughout this thesis.
** $CHCl_3$, 0.5mm cell.

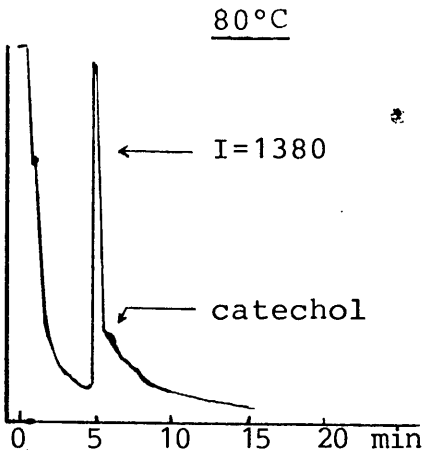
B. Hydrolysis of diol alkaneboronates to the corresponding boronic acid

1. Direct hydrolysis:- Brown and Gupta[50,51] reported that catechol cyclohexaneboronate(83) was readily hydrolysed in cold water and that cyclohexaneboronic acid(62) crystallised out of the solution. This procedure has been applied (see exptl. procedure, p.98) and 81% yield of pure compound (62) was obtained, after recrystallisation from hot water. It is important to note that after hydrolysis the crystals of the boronic acid must be immediately purified from traces of catechol, otherwise this will cause decomposition and darkening of the crystals. Octadecaneboronic acid(63) was also prepared according to the above procedure (see exptl., p. 98), in 79% yield, but this compound was not stable in hot water and a significant amount of 1-octadecene(57) was detected by GLC. Purification was achieved by trituration with EtOAc in dry ice-acetone.

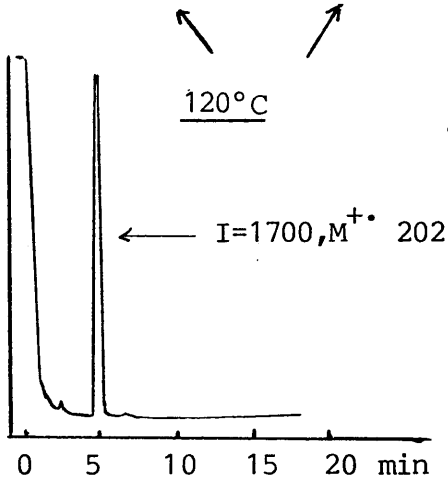
Attempts to hydrolyse catechol norbornane-2-,β-pinane-10ξ-, 3ξ-methyl-5α-cholest-2ξ- and 5β-cholane-24-boronates,(84), (85),(90) and (92) respectively, according to the above procedure failed. The esters decomposed spontaneously and their solutions turned black, in spite of the addition of ascorbic acid as an antioxidant[126] Decomposition also occurred under nitrogen in a sealed flask during hydrolysis. In the case of catechol β-pinane-10ξ-boronate, after hydrolysis the solution was extracted with hexane and washed with water, but only traces of the corresponding boronic acid were detected, by GLC, after derivative formation.

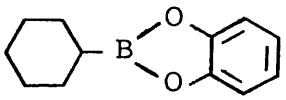


Aliquot solution from compd. (83)
+ excess meso & dl-2,3-butanediols.

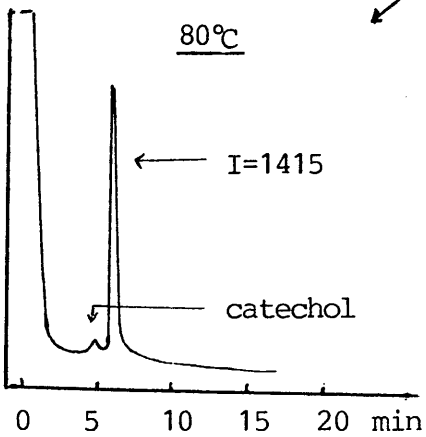


Aliquot solution from compd. (83)
+ excess pinacol.

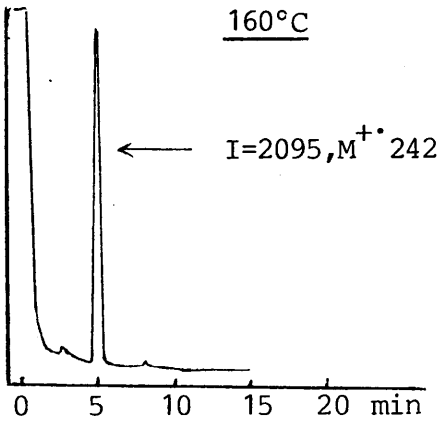




Catechol cyclohexaneboronate (83),
in EtOAc, inj$^{n.}$ vol. 1µl, concn. 2µg/µl.



Aliquot solution from compd. (83),
+ excess 1,3-propanediol.

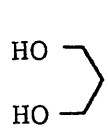


Aliquot solution from compd. (83)
+ excess 1,2-indanediol.

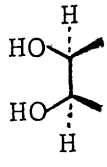
Scheme 4.1: GLC analysis diagrams of catechol cyclohexaneboronate and its displacement reactions; on 1% ov-17, 2m x 2mm; reactions at R.T. for 2-5min in EtOAc inj$^{n.}$ vol. 1µl. All conditions are the same except the oven temperature as mentioned above.

As a result of these difficulties, an indirect method was adopted for the preparation and characterisation of the boronic acids. This depended on the displacement of catechol by diols that formed more stable esters, as described below.

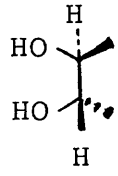
2. Displacement of the catechol group with 2,3-butanediol and subsequent hydrolysis:- The catechol group in all the catechol alkaneboronates, which are listed in Table 3, p.96, was readily displaced at room temperature, in solution, by adding, in excess, diols such as 1,3-propanediol (97), meso and dl-2,3-butanediols(98), pinacol(99), and (±)-indane-cis -1,2-diol*(100). These displacement reactions were monitored by GLC and each product was characterised by GC-MS (for example,see Scheme 4.1). The resulting alkaneboronates of



(97)

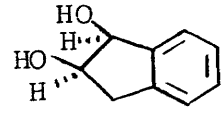


meso (98)



dl

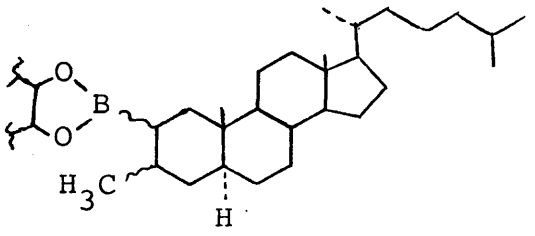
(99)



(100)

compounds (98), (99) and (100) were purified from the hydrolysed catechol by hexane/water partition, except in the case of 1,3-propanediol alkaneboronates, which proved to be unstable during extraction: these esters tended to hydrolyse readily, and the resulting boronic acid recombined with catechol in the hexane layer. Most of the displacement reactions afforded high yields of products which were recovered as colourless oils and studied by GLC, TLC and GC-MS (Tables, 8 and 10), and in some cases also by ^{1}H-NMR and IR (Tables 4 and 5 respectively).

* This compound will be referred to as indanediol throughout the following discussions.



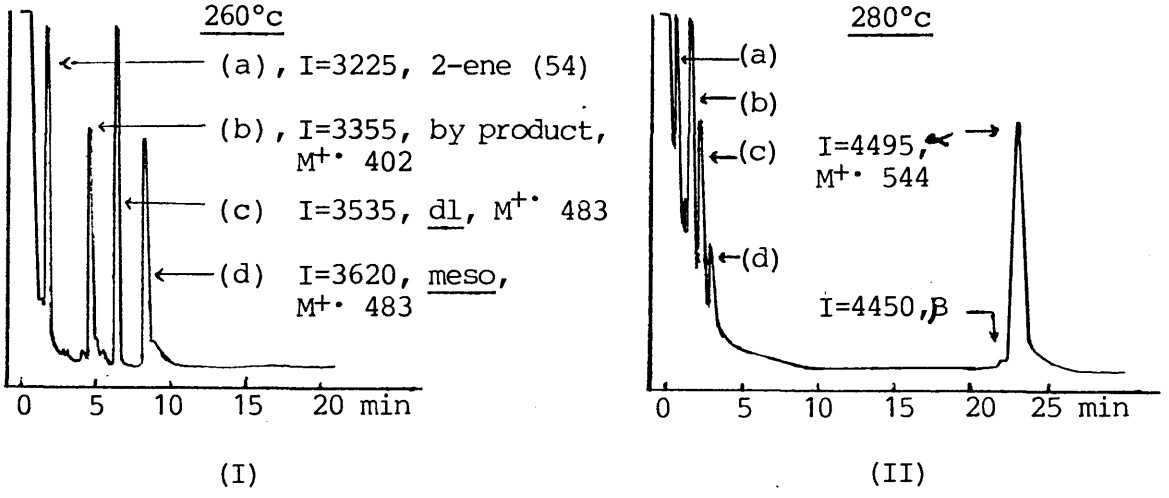
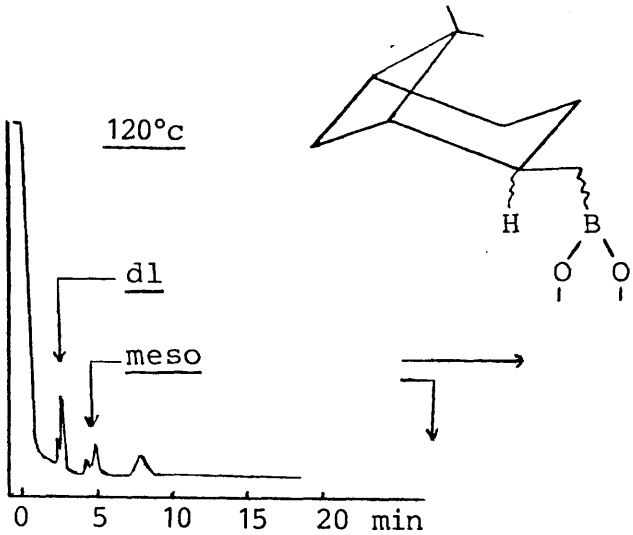
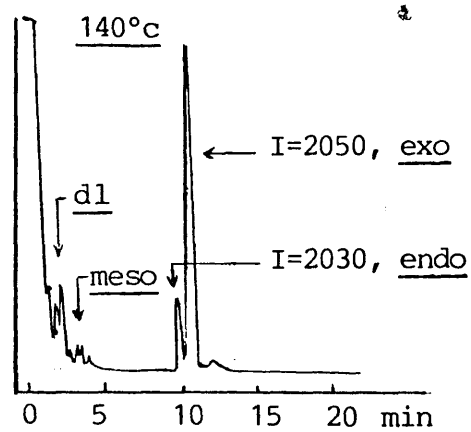


Fig 4.4: GLC analysis, on 1% OV-17, 2m x 2mm, N_2, 40ml/min., solvent EtOAc, concn.3µg/µl, $inj^{n.}$ vol. 1µl. (I) dl and meso-2,3-butanediol 3ξ-methyl-5α-cholestane-2ξ-boronates. (II) aliquot of solution from (I) + excess (±)-cis-indanediol, in EtOAc for 24hr at 70°C, at the same concentration.

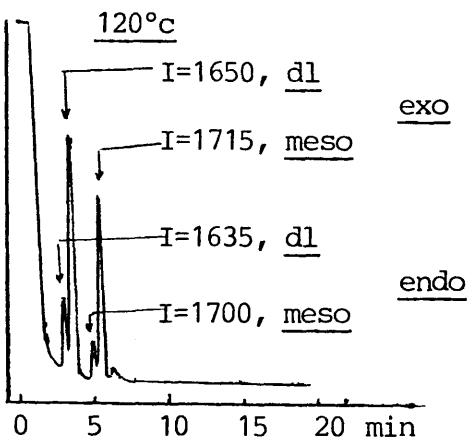
It was further observed, as expected from the relative stabilities of the cyclic esters, that pinacol(99) and indanediol (100) could easily displace 2,3-butanediol from its alkaneboronates, within a short period; except in the case of 2,3-butanediol 3β-methyl-5α-cholestane-2β-boronate, for which displacement by indanediol required 24hr at 70°C in EtOAc. These reactions were useful for GLC and GC-MS correlations, which required only a few micrograms for each test (for example, see Fig. 4.4). The meso and dl-2,3-butanediol, pinacol and (±)-indane-cis-1,2-diol alkaneboronates were found to be stable in air and inorganic solvents (e.g. EtOAc, hexane, ether). The pinacol and indanediol esters were also highly stable towards hydrolysis, even in acidic or basic aqueous solutions (pH 2-12), whereas the 2,3-butanediol alkaneboronates were hydrolysed in water or in slightly acidic aqueous solutions (pH 4-5), in the presence of ascorbic acid, acetone and ether. Therefore, 2,3-butanediol was used for the initial displacement of the catechol group, then each 2,3-butane alkaneboronate was hydrolysed according to the procedure on pp.99-103. The ease of displacement by the diol compounds (97-100) of the catechol group in the cyclic alkaneboronates and also the stability of the formed cyclic diol alkaneboronate ring, depend upon the diol substituents and decrease in the order: indanediol pinacol 2,3-butanediol 1,3-propanediol. The greater stability of the cyclic boronates of pinacol as compared with those of 2,3-butanediol is an example of the "gem.-dialkyl" or "Thorpe-Ingold" effect, which in turn reflects a generally more



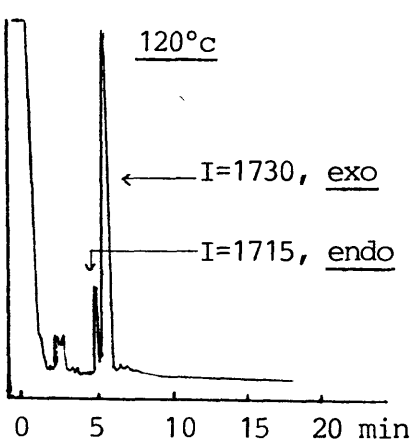
(I). β-Pinane-10f-boronic acid, after water - hexane extraction. (From butanediol boronate hydrolysis).



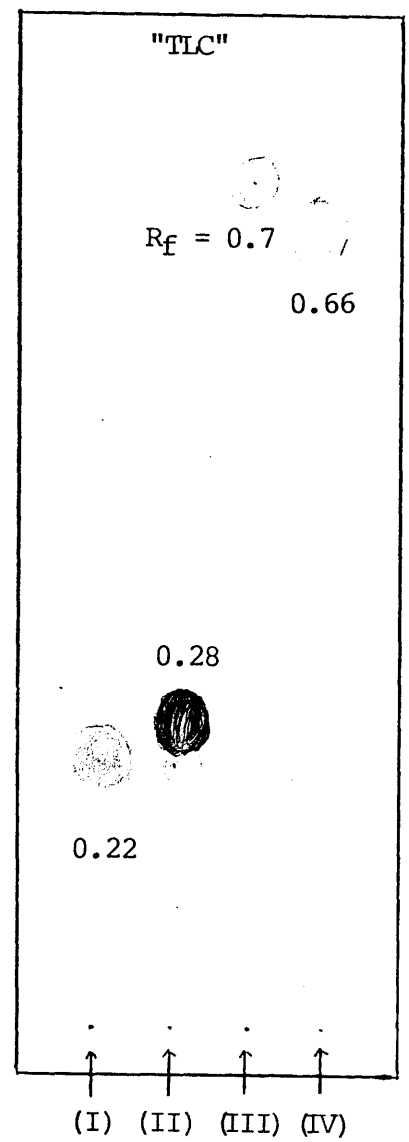
(II). Aliquot of solution from (I) + excess catechol.



(III). Aliquot of solution from (I) + excess meso & dl-2,3-butanediols.



(IV). Aliquot solution from (I) + excess pinacol.



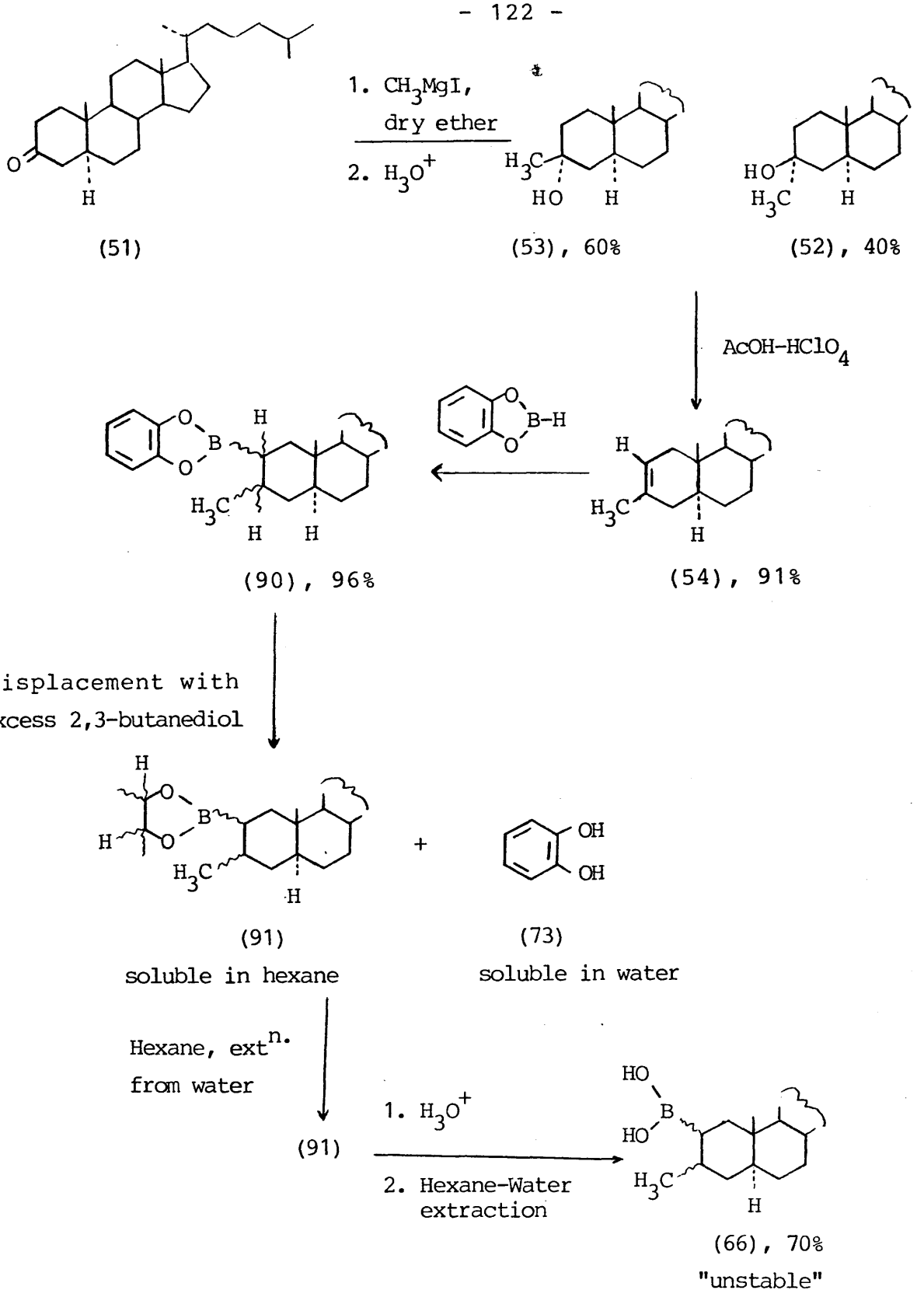
Scheme 4.2: β-Pinane-10f-boronic acid and its derivatives, GLC analysis, on 1% OV-17, 2m x 2mm, solvent EtOAc inj$^{n.}$ vol. 1µl,, conc. 2µg/1µl. TLC analysis, solvent $CHCl_3$: EtOAc 3:1, v/v; spots detected with "DPC" in EtOH.

favourable enthalpy and entropy of ring closure for branched than for unsubstituted chains[128,129].

The hydrolysis steps and the purity of the recovered boronic acids were monitored by direct TLC analysis. The boron-containing compounds were detected with diphenylcarbazone in ethanol, giving a purple coloured spot on TLC[138]; this colour was sharper in the case of the free boronic acids (see Scheme 4.2). On GLC analysis, cyclohexaneboronic acid gave a peak at $I^{OV-17}_{180°}$ = 2310, which corresponded to tricyclohexane boroxine, Table 8, p.131 but neither norbornane-2ξ-, nor β-pinane-10ξ-boronic acid showed any corresponding boroxine peak at the expected I values: 2600 and 3500 respectively. This may be due to instability of these acids on GLC. The fact that octadecane-, 3ξ-methyl-5α-cholestane-2ξ- and 5β-cholane-24-boronic acids did not show any corresponding boroxine peak on GLC, even with temperature programming up to 280° was not unexpected in the view of the high molecular weight of the corresponding boroxines. Accordingly the hydrolysed boronic acids were detected indirectly by GLC, after boronate ester formation with the diols listed on p.117 (for example, see Scheme 4.2).

C. Attempted purification of the hydrolysed boronic acids

The boronic acids studied differed markedly in their solubility in water, in that norbornane-2ξ- and β-pinane-10ξ-boronic acids, (64) and (65) respectively, were moderately

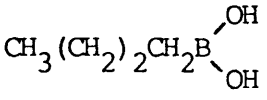
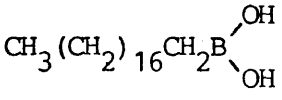
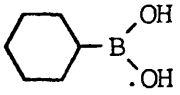
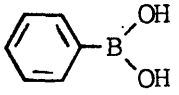


Scheme 4.3: Preparation and purification of 3ξ-methyl-5α-cholestane-2ξ-boronic acid.

soluble in water, whereas the steroidal derivatives, 3β-methyl-5α-cholestane-2β- and 5β-cholane-24-boronic acids, (66) and (67) respectively, were sparingly soluble in water. A hexane-water extraction technique was adopted for the attempted purification, according to the procedures on pp.100-103. For example; the sequence of these preparation and extraction steps, to prepare compound(66), is illustrated in Scheme 4.3. These extraction methods proved, that however efficient the extraction technique, there were still some traces of the 2,3-butanediol alkane boronates recovered with the boronic acid. These traces of boronate esters may have arisen either from the unhydrolysed ester or from the re-combination of its hydrolysis products.

The unstable boronic acids were gradually decomposed during extraction, and the decomposed material(s) was recovered along with the remaining boronic acid; for example see Scheme 4.2,I. The addition of boric acid during the extraction was examined, in the hope that it would compete with the liberated boronic acid, by removing the hydrolysed diol, but this method did not materially improve the purity of the extracted boronic acid. Among other purification techniques tried for the purification of compounds (65), (66) and (67); were preparative TLC, dry column silica gel, Lipidex-5000, using media saturated in each instance with ascorbic acid. These methods were also unsuccessful, owing to decomposition of the boronic acids[139], to give by-product(s) which had mobilities similar to those of the boronic acids (for example see Fig. 4.4, p. 118). Also vacuum sublimation failed to separate the by-product(s) and the butanediol alkaneboronate from the boronic acid.

Table 6. IR Absorption bonds of boronic acids. ν max, cm^{-1} measured in $CHCl_3$; 0.5mm cell.

Boronic Acid	No.	O-H	C-B	B-O
	81	3660 3620 S	1440 mb	1370 Sb
	63	3660 3620 wb	1450 mb	1370 mb
	62	3650 3610 wb	1440 mb	1360 1340 ms
	82	3660 3620 Sb	1435 mb	1350 Sb 1305 ms

D. Properties of the studied boronic acids

1. Stability towards aerial oxidation:- The following characteristics were observed with respect to aerial oxidation and hydrolysis then subsequent decomposition. Norbornane-2ξ-boronic acid was unstable in air and in water, but moderately stable in hexane under nitrogen. β-pinane-10ξ- and 3ξ-methyl-5α-cholestane-2ξ-boronic acids, were unstable in air and during hexane-water extraction, but were stable in hexane at R.T. (especially when stored) under nitrogen. Cyclohexane-, octadecane- and 5β-cholane-24-boronic acids all proved to be stable in air and in solutions at R.T.

2. IR absorption bands:- IR data for representative boronic acids are given in Table 6. The boronic acid OH group showed absorption bands in the same region as did most of the hydroxy aliphatic and aromatic groups viz, in the range 3620-3660cm^{-1}. The B-O asymmetric mode was easily recognised since it was the strongest band in the spectra of the free boronic acids[129,130], or their corresponding cyclic esters[19,44], in the region 1305-1370cm^{-1}. For the O- and P- hydroxy-benzeneboronic acids, B-O bond absorptionswere interpreted to be in a lower region, 1180-1220 cm^{-1} [37]. The sharp B-C stretching frequency in the cyclic boronate ester rings was observed at 1430-1440cm^{-1} [15,130], while the free boronic acids in Table 6 showed a medium-broad peak at the same region as the B-C band in the cyclic boronates.

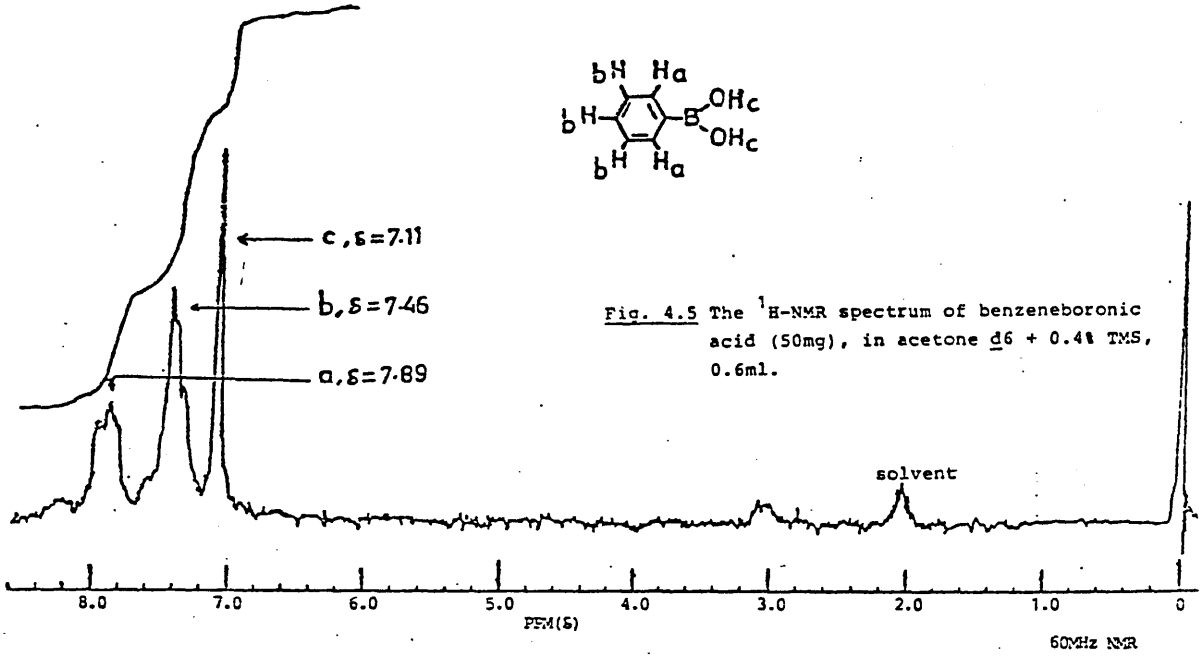


Fig. 4.5 The ^{1}H-NMR spectrum of benzeneboronic acid (50mg), in acetone d6 + 0.4% TMS, 0.6ml.

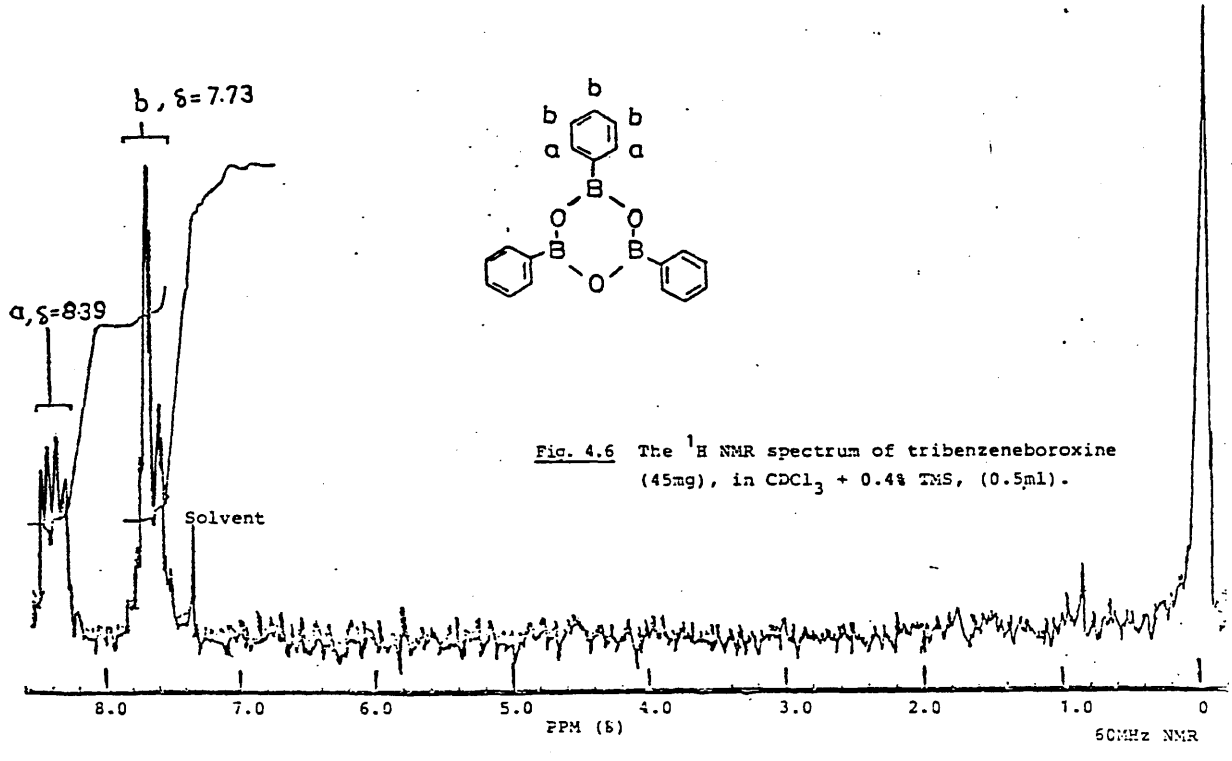
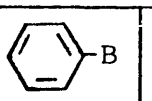
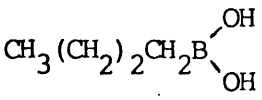
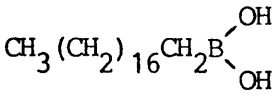
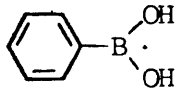


Fig. 4.6 The ^{1}H NMR spectrum of tribenzeneboroxine (45mg), in $CDCl_3$ + 0.4% TMS, (0.5ml).

3. ^{1}H-NMR analysis:- The boronic acids (64), (65) (66) and (67) were not obtained pure enough for NMR studies and esters thereof were studied instead, as in Table 4, p.113 (cf. Fig. 4.1). The ^{1}H-NMR chemical shifts of two model boronic acid compounds (81) and (82) have been studied, with octadecaneboronic acid, as in Table 7, no peaks were observed for the aliphatic boronic acid hydroxy groups, but benzene-boronic acid (8) did give a strong peak at δ 7.11, corresponding to the protons in $-B(OH)_2$ group (Table 7). These compounds proved to exist as free acids, and not as anhydride boroxines, because they showed absorption bands for the free $-B(OH)_2$ hydroxy groups in the IR region as shown in Table 6. There was also no sign of H_2O protons in the NMR spectra of these acids, which may come from dehydration and subsequent boroxine formation. The appearance of the proton peak in the $-B(OH)_2$ group of benzeneboronic acid may be due to the direct link of the $-B(OH)_2$ group to the aromatic ring, which may strengthen the OH absorption peak (see Fig. 4.5), while the corresponding anhydride benzeneboroxine did not show any $-B(OH)_2$ proton absorption, but only the five aromatic protons (see Fig. 4.6).

Table 7. ^{1}H-NMR, Characteristic proton chemical shifts(=PPM) of alkyl- and benzeneboronic acids.
Data measured in $CDCl_3$+ 0.4% tetramethylsilane and on a 60MHz instrument.

Boronic Acid	No.	$CH_3(CH_2)_n$—	-B(OH)(OH)		Ref.No.
	81	0.90, m & 1.33,m (Virtual Coupling*)	Not seen	—	170 J
	63	0.87, m & 1.27,m (Virtual Coupling*)	Not seen	—	
 **	82	—	7.11 S	3H,7.46,m 2H,7.89,m	170 K

* Virtual coupling[131]:- If a group of protons A is strongly coupled (i.e.$\Delta\nu < J$) to a second group B, which in turn is coupled to a further group of protons M, so that J_{AM}= 0, the pattern observed differs considerably from that expected. The appearance of the multiplets suggests that the group of protons M is coupled to a single group AB, rather than to only the protons of group B. The inference is that the protons of group A are influencing the multiplet pattern associated with M despite the fact that J_{AM}= 0. This effect is commonly observed in the resonance of a methyl group for a long aliphatic chain; owing to virtual coupling between the methylene groups the methyl resonance does not appear as a triplet, it may indeed be almost unrecognisable as a CH_3CH_2- group.

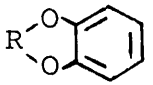
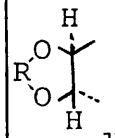
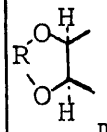
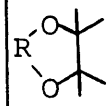
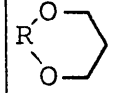
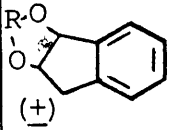
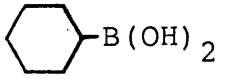
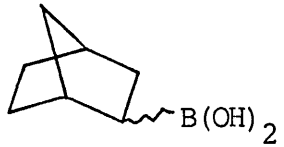
** Measured in acetone-d_6 + tetramethylsilane.

4. GLC Properties:- As discussed on p.121, the boronic acids, (63), (64), (65) and (66), (Table 8), did not give a corresponding boroxine peak, but it proved convenient to study the acids indirectly as boronate esters of various diols (see Table 8). These boronate derivatives gave well defined peaks on GLC. It is noteworthy that isomeric exo and endo products were well separated as were the diastereomeric dl and meso butane-2,3-diol esters (see Schemes 4.1 and 4.2 and Table 8). However, this GLC technique failed to separate the diastereomeric esters derived from the enantiomeric 2,3-butane diols or cis-indanediol and the chiral boronic acids (65) and (66), (Fig. 4.4 and Scheme 4.2). The apparently indistinguishable retention properties of the diastereomeric cyclic esters may perhaps reflect the flexibility of the ester ring systems. In contrast, diastereomeric carboxylate esters are frequently effective for the indirect resolution of chiral alcohols on conventional GLC columns[23]. It is possible that the unsuccessful separation of the R and S cyclic boronate diastereomers may be due to inadequate resolving power of the packed column which was used. Capillary columns, affording a larger number of theoretical plates and much higher resolution than the conventional columns, might achieve some separations

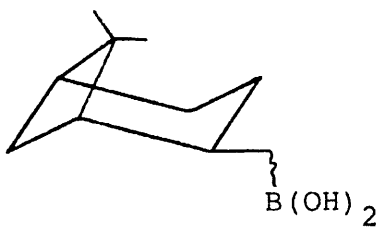
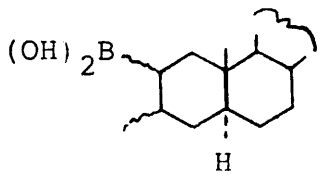
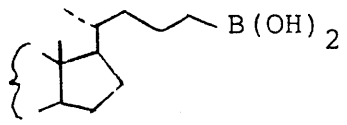
The GLC and TLC data for the starting materials which were used for the preparation of these boronic acids and esters, are listed in Table 9. Under these GLC conditions each of the two isomeric compounds (52) and (53), showed considerable dehydration to compound (54).

The TLC data for the studied boronic acids and their esters are listed in Table 10.

Table 8; GLC Retention Index values[a] for the diol derivatives of alkaneboronic acids.

Parent Boronic Acid	Acid No.	Mol. Wt.	Boroxine	I, Diol Derivatives					
					 dl	 meso			 (±)
$CH_3(CH)_2CH_2B(OH)_2$	81	102	1490 (80°)	1395 (80°)					
$CH_3(CH_2)_{16}CH_2B(OH)_2$	63	298	b	2880 (240°)					
	62	128	2310 (180°)	1700 (120°)	1305 (80°)	1365 (80°)	1380 (80°)	1415 (80°)	2095 (160°)
	64	140	b	1790 (0.5% ENDO) 1805 (99.5% EXO) (120°)	1385 1395 (80°)	1460 (80°)	1480 (80°)	1505 1515 (80°)	2185 2205 (160°)

Continued Table 8.

	65	180	b	2030 (14% ENDO) 2050 (86% EXO) (140°)	1635 1650 (120°)	1700 1715 (120°)	1715 1730 (120°)		
	66	430	b	β,4065[c] α,4120 (280°)	3535 (260°)	3620 (260°)			4450 4495 (280°)
	67	374	b	3830 (260°)	3350 (260°)	3440 (260°)	3455 (260°)		4215 (260°)

a. The retention index values(I) were measured, on 1% OV-17; 2m x 2mm; oven temperature as indicated between the small brackets ( ); injection temperature 280°; detector temperature 300°; carrier gas N_2, flow rate 28ml/min., except for compounds (66) and (67) (derivatives), was 40ml/min. The rest of the GLC conditions are described in the Chromatography section, p.58.

b. The corresponding trialkylboroxine derivative of these compounds did not show any peak, during temperature programming at 100-280°, 10°c/min. This is discussed on p.121 .

c. These assignments are based on the discussion, on p. 111 .

Table 9. GLC Retention Index Values[a] for compounds related to the preparation of the alkaneboronic acids; listed in Table 8; and TLC data[b].

Compound	Compound No.	Mol.Wt.	I^{OV-17} 240°	R_f, 3:1, v/v $CHCl_3$-EtOAc
1-Octadecene	58	252	1810 (120°)	0.76
5α-Cholestane-3-one	51	386	3370	0.59
3β-OH-3α-Me-5α-Cholestane	52	402	3260	0.34
3α-OH-3β-Me-5α-Cholestane	53	402	3230	0.51
3-Me-5α-Cholest-2-ene	54	384	3030	0.72
5β-Chol-23-ene	61	328	3075	0.72[b]
5β-Cholan-24-ol	55	346	3090	0.31[b]
24-Bromo-5β-cholane	56	409	3165	0.68[b]
Estrone[c]	40	270	3070[d]	0.37[b]
17-Oxo-3-aminoestrone[c]	70	269	3115[e]	0.28[b]
17-Oxo-3-bromoestrone	71	333	3260[f] or 3250	0.67[b]

Footnotes to Table 9:

a. GLC conditions as in Table 8-a, including compound (58); except N_2 flow rate was 40ml/min.

b. TLC conditions; the R_f values were measured in solvents as indicated, except for data with the superscript (b), obtained for hexane-EtOAc, (3:1,v/v). The total distance moved by the solvents was 16cm; spots were detected with $Ce(SO_4)_2$ in aqueous H_2SO_4, see p.58.

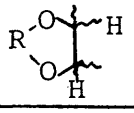
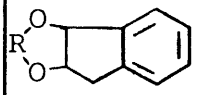
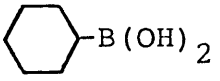
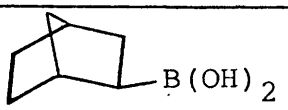
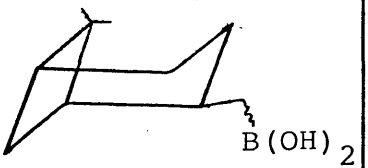
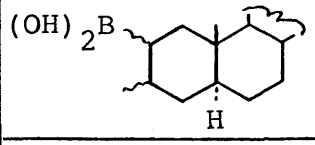
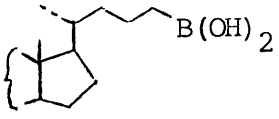
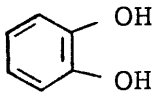
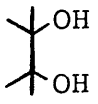
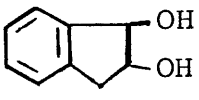
c. The R_f values for compounds: 3-(2-benzene-4-quinazolinyl-oxy)-estra-1,3,5(10)-trien-17-one,(68) and 3-[4-oxo-2-benzene-3(4H)-quinazolinyl]-estra-1,3,5(10)-trien-17-one,(69), were 0.50 and 0.17, respectively; and for the reagent 4-chloro-2-benzenequinazoline, 0.83.

d. I value for compound (40)-acetate derivative was 3205 at 260°.

e. I value for compound (70)-N-acetyl derivative was 3665 at 260°.

f. These I and R_f values were only assumed to correspond to compound (71) (see experimental procedure on p.94).

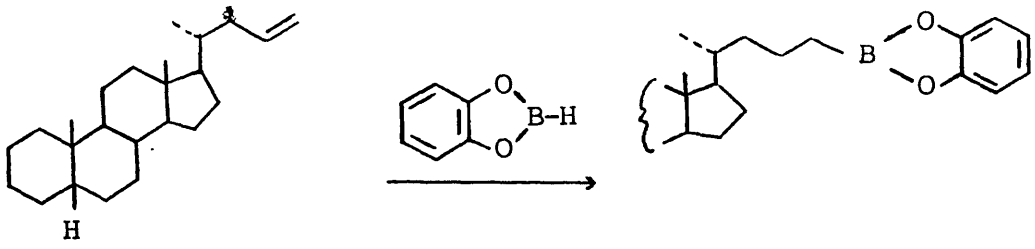
Table 10. TLC, (R_f values[a]) for alkaneboronic acids and some of their diol ester derivatives and corresponding diols.

Compound	Compound No.	R_f, 3:1, v/v, Hexane-EtOAc			
		Parent Compound	Diol Derivatives		
					
$CH_3(CH_2)_{16}CH_2B(OH)_2$	63	0.31			
	62	0.22			
	64	0.21[c]			
	65	0.22	0.7	0.66	
	66	0.08[b]	0.66		0.37
	67	0.18[b]	0.67	0.65	
	73	0.28[b]	—	—	—
	99	0.11[b]	—	—	—
	100	0.08[b]	—	—	—
$B(OH)_3$	102	0.00	—	—	—

Footnotes to Table 10:

a. & b. TLC conditions are similar to that of Table 9-b, except the spots were sprayed first with "DPC" in EtOH, for boron detection, then with $Ce(SO_4)_2$ solution.

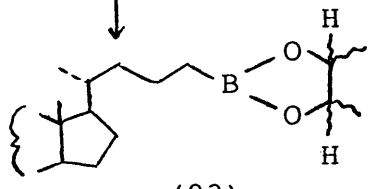
c. A decomposition spot was measured at R_f =0.53, which may represent the by-product 2-ol.



(61) (92) ,99%,GLC

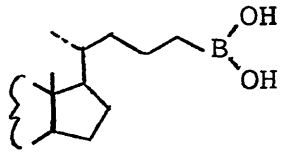
Displacement with 2,3-butanediol

Hexane/water extraction



(93)

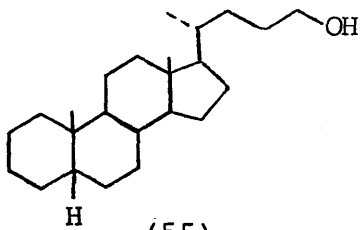
1. H_3O^+
2. Boric acid and hexane/water ext^{n}.



(67) 80%,GLC

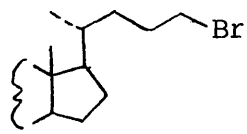
70%,GLC

1. Mg-ether-MeI
2. BCl_3
3. H_2O



(55)

PBr_3 in CH_2Cl_2



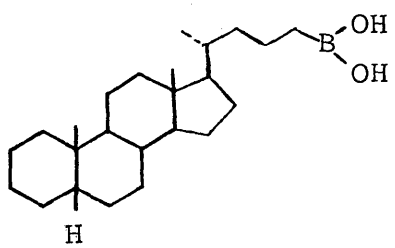
(56), 70%,GLC

Scheme 4.4

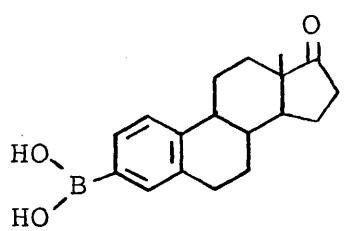
II. GRIGNARD REACTIONS WITH BORON TRICHLORIDE OR TRIMETHYLBORATE

Many alkane and areneboronic acids have been prepared from their corresponding aliphatic or aromatic hydroxy compounds via Grignard reactions, as discussed in Chapter 1, p.19.

This approach was adopted in attempts to prepare the aliphatic and the aromatic steroidal boronic acids, (67) and (95), respectively, from their corresponding hydroxy compounds as shown below:-



(67)



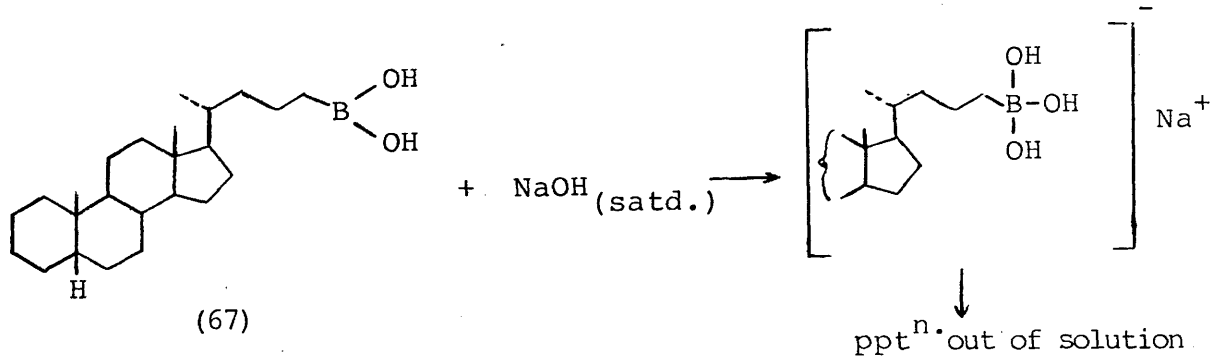
(95)

5β-Cholane-24-boronic acid (67), was prepared from 5β-cholan-24-ol (55), after displacement of the hydroxy group with bromide ion, by using phosphorus tribromide in CH_2Cl_2, affording 24-bromo-5β-cholane (56), in a yield close to that obtained by Herz and Cruz[120], who derivatised compound (55), with methanesulfonyl chloride then displaced the mesylate with lithium bromide. The purified 24-bromo compound (56), was converted into the Grignard reagent; reaction with boron trichloride, and subsequent hydrolysis gave compound (67) (see Scheme 4.4, and procedures on p.90 and 102:for GLC and TLC data see Table 9, p.132). As a reagent, boron trichloride (b.p. 12.5°) was inconveniently volatile at temperatures above 0°C, and also highly corrosive; trimethyl or tributyl borate would be preferred for use in future experiments.

Attempts at purification of compound (67) from the unreacted

24-bromo compound (56) failed, due to decomposition and subsequent hydrolysis of compound (67), during preparative TLC or column chromatography on"dry-column"silica gel, or Lipidex 5000 (both saturated with ascorbic acid).
The decomposed by-product showed identical properties on GLC and TLC, as the 24-ol, compound (55), and also had a mobility similar to the boronic acid (67). The boronic acid (67) also failed to separate from the unreacted 24-bromo compound (56) and from the decomposition by-product when subjected to vacuum sublimation. There are two possible alternative purification methods, from which the pure boronic acid (67) could be obtained. These are as follows:

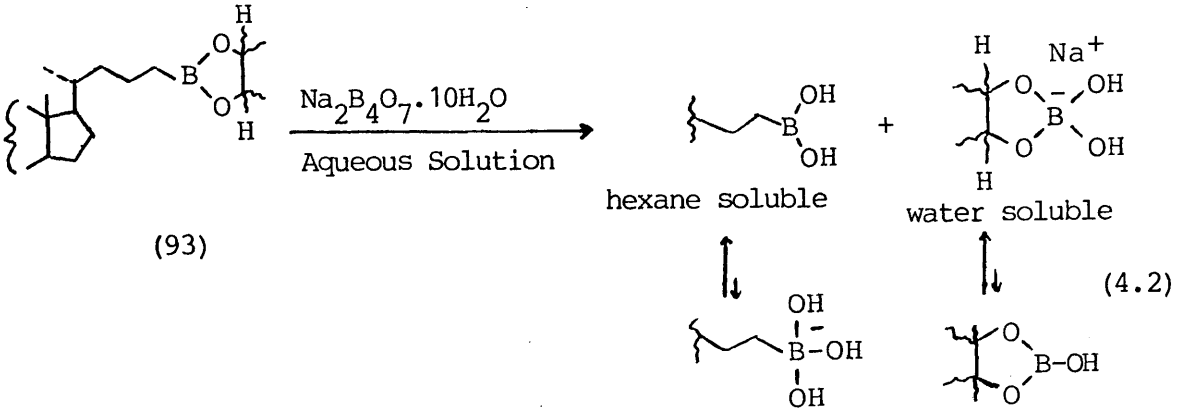
1. By converting the boronic acid into its sodium salt, after treatment with concentrated aqueous solution of sodium hydroxide[41], provided that this acid (67) is stable under strong alkaline solution. The hydrated sodium salts, could possibly be crystallised or precipitated and recovered by filtration (eq. 4.1.).



(67)

(4.1)

2. By mild hydrolysis of the 2,3-butanediol steroid-boronate with aqueous sodium tetraborate and extraction of free steroidal boronic acid with hexane, eq. 4.2.

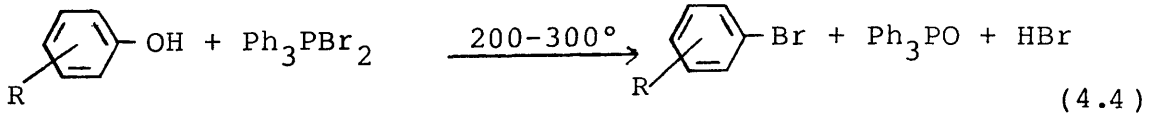


(93) (4.2)

17-Oxoestra-1,3,5(10)-trien-3-boronic acid(95): This compound could be prepared from the corresponding 3-bromo compound (71) via Grignard reaction. The available starting material was estrone (40); it appeared that the 3-hydroxy group could be replacable by bromide to give the 3-bromo compound (71), either directly with triphenylphosphine dibromide, or indirectly via the 3-amino compound (70), as described in the attempted preparations below.

1. Bimolecular displacement reactions; in which triphenyl-phosphine dibromide has been used to convert phenols to aryl halides[132-135]. The methods are outlined in eqs.4.3-4.

$$Ph_3P + Br_2 \xrightarrow[N_2,\ 0°C]{CH_3CN} Ph_3PBr_2 \qquad (4.3)$$

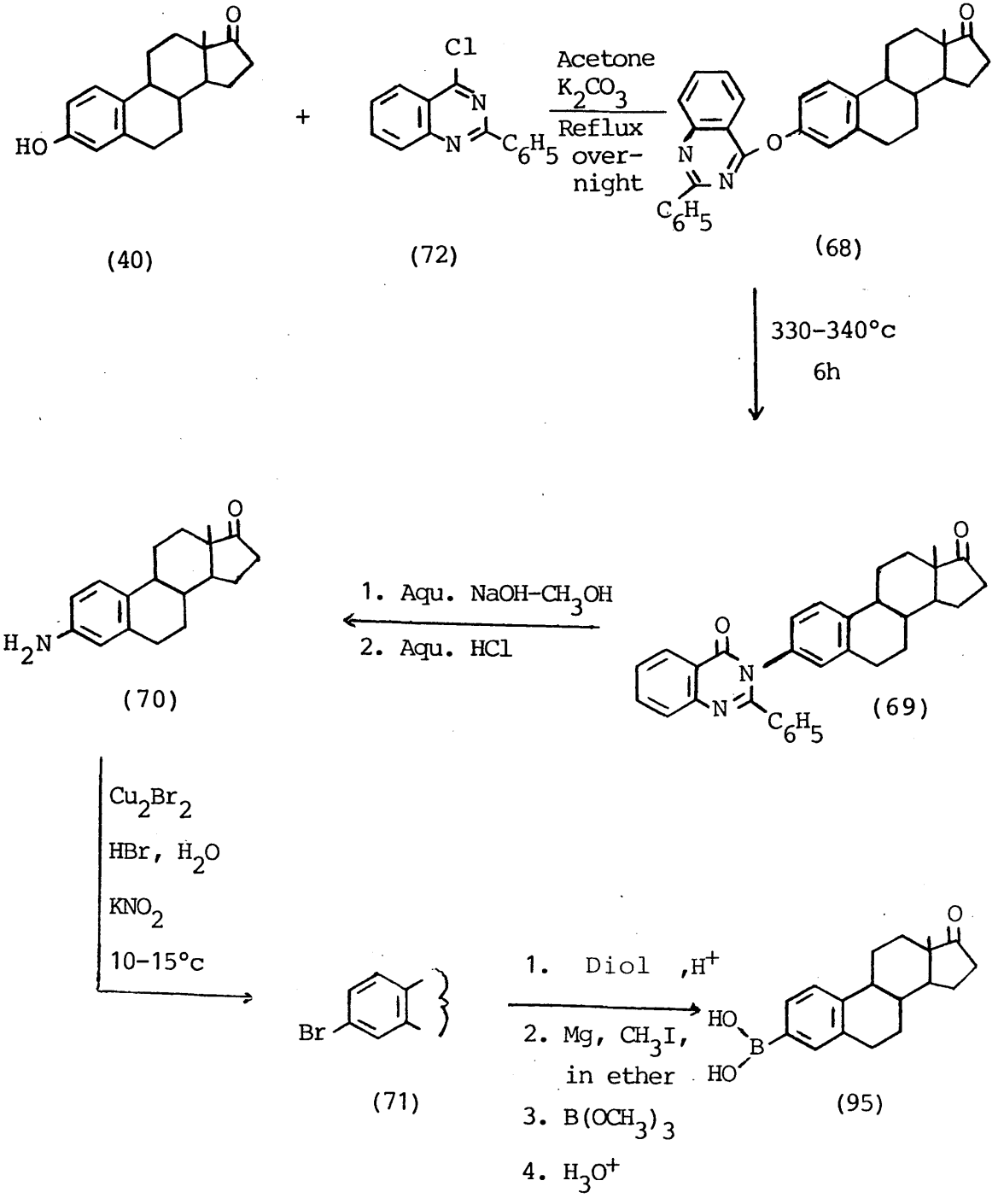


(4.4)

In the general procedure[133,134], a solution of triphenylphosphine in acetonitrile was treated with bromine at 0°C, whereupon triphenylphosphine dibromide was formed rapidly and quantitatively. Addition of an equimolar quantity of a phenol resulted in the formation of a complex which was readily decomposed on heating to the aryl halide, triphenylphosphine oxide, and hydrogen bromide. According to these procedures, attempts have been made to displace the phenolic hydroxy group with a bromo group, in the model compounds: 17-deoxyestrone (48), p-chlorophenol and 3,4-dimethylphenol, but all the reactions failed, even though the triphenylphosphine dibromide was prepared under strictly dry conditions and under nitrogen, and the heating of the solvent-free substrate and reagent took place in a vacuum-sealed flask. In most of the experiments, about half of the substrates were recovered and the remaining material was a mixture of different undefined compounds and traces of the proposed bromo products, detected by GLC and TLC. Several optimisation procedures were tried; in some CCl_4 was used instead of CH_3CN, to minimise the affect of moisture; but all failed to give a significant separable amount of the desired bromo compound.

2. Indirect procedure via Sandmeyer reaction of the 17-oxo-3-aminoestrone(70);

This amine (70) was obtained from estrone (40) in 67% overall yield by the method of Conrow and Bernstein[121], compared with a 10% yield by a dearomatization sequence, reported by Gold and Schwenk[136]. In Conrow and Bernstein's method, a mixture of estrone with 4-chloro-2-phenylquinazoline (72) and potassium carbonate in acetone was refluxed overnight



(40) (72) (68) (70) (69) (71) (95)

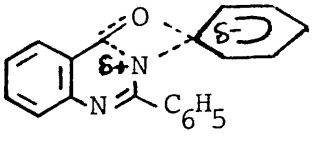
Scheme 4.5

and the ether (68) was obtained. Pyrolysis of (68), neat at 330-340°, followed by chromatography on silica gel, afforded the quinazolinyl derivative (69). Basic, followed by acidic hydrolysis of (69) provided the amine (70), see Scheme 4.5. This procedure has been applied to prepare compound (70), as described on pp.92-94. The products were analysed by GLC and TLC, (relevant data are in Table 9, p.132). The results obtained are described in the following steps:

The derivatisation step was achieved by condensation of compound (40) with compound (72), giving 96% yield of compound (68).

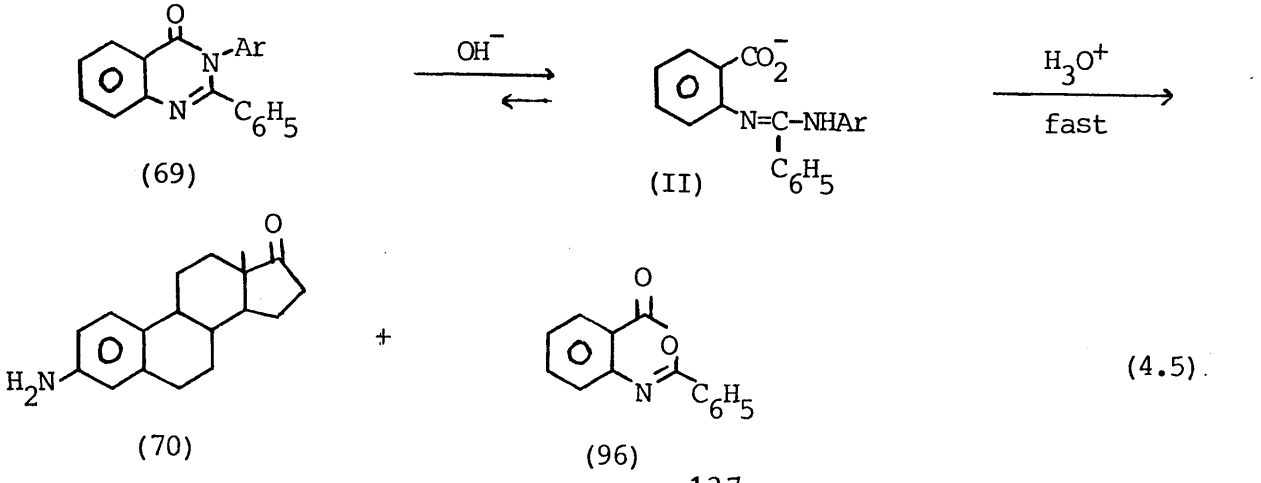
The rearrangement step, gave an estimated yield of rearranged compound (69) of about 40%, as judged by GLC, together with 40% of estrone (40) and 20% of unrearranged material (68). Conrow and Bernstein[121] reported an 80% yield of compound (69). The low yield observed by us is ascribed to the presence of traces of moisture in the derivative, which caused the hydrolysis of (68) during heating.

This sequence is a thermally induced 1,3-O to N aryl migration around the quinazoline ring. Scherrer and Beatty[137] studied the course of the rearrangement by spectroscopic methods: their data showed an intramolecular nucleophilic displacement of the incipient amide oxygen by the imine nitrogen in a four-membered transition state (I).



(I)

The hydrolysis step was carried out by the following reactions, eq. 4.5. The mildest consists of an alkaline hydrolysis



(69) (II) (70) (96) (4.5)

to the presumed amidine intermediate[137] (II) which is considerably more resistant to further base hydrolysis. Then acidification readily liberated the aniline with the formation of 2-phenyl-4H-3,1-benzoxazin-4-one (96).

The crude material from the rearrangement step was hydrolysed, with the aim of extracting the amine as its water-soluble hydrochloride and separating it from any unchanged estrone and other non-basic materials. However, the amine did not form a water-extractable HCl salt, and was recovered from the acidic solution together with estrone. Also both of these compounds (70) and (40) were recovered from a strong alkaline solution and they did not separate satisfactorily by TLC dry column chromatography , or; vacuum sublimation. These results led to the conclusion that the basic amine group in compound (70) was interacting with the acidic phenolic group in compound (40) to form a salt (III) or ion-pair which was

difficult to separate.

$$Ar — \overset{+}{N}H_3\overline{O} — Ar$$

(III)

To obtain a high yield of compound (70), the following considerations must be taken:

The rearrangement step must be done in an absolutely dry apparatus with neat compound (68); this could be achieved by heating the compound and the apparatus at 80-100°, under vacuum; then heating under vacuum in a Wood's metal bath. The main pyrolysis product (69) must be purified completely before the hydrolysis step.

The diazotisation route to prepare compound (71), (Scheme 4.5) was examined with a small amount of crude product (70), according to the method of Talik, et al.[122] (cf. procedure on p.94). This trial experiment has been done only to show the course of the Sandmeyer reaction. GLC analysis for the crude product showed a peak at $I^{ov-17}_{240°}$ 3260, which corresponds to the 3-bromo compound(71). This tentative assignment was made from GLC correlation with the other compounds listed in Table 9, p.132.

Lack of time precluded completion of these experiments during the period available for the work included in this thesis.

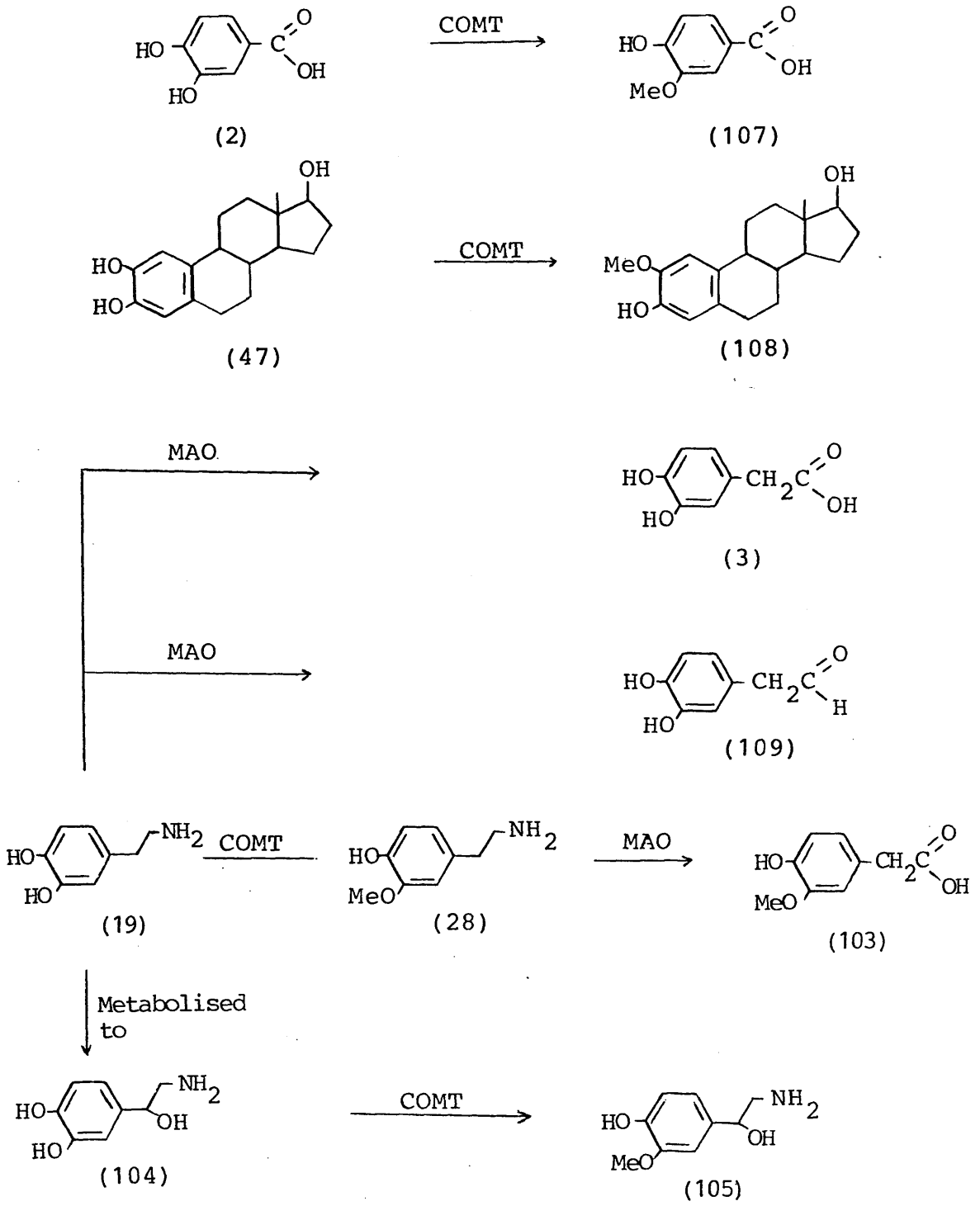
Chapter 5

CYCLIC BORONATE DERIVATIVES OF COMPOUNDS OF CATECHOL TYPE

RESULTS AND DISCUSSION

Compounds of catechol type such as catecholamines, catechol estrogens, isoquinoline alkaloids and catecholic carboxylic acids, have key physiological roles in organisms from invertebrates to man, even though they are present in very low concentrations. For example 2-hydroxy estrogens and their monomethyl esters are important intermediates of estrogen metabolism. They also act as strong inhibitors of the methylation of catecholamines by the enzyme catechol-O-methyltransferase(COMT)[118,140-144] [see Scheme 5.1]. Tetrahydroisoquinoline alkaloids have been unequivocally demonstrated[145-147] *in vivo* for the first time in Parkinsonian patients on L-Dopa treatment[147]. Several classes of tricatecholamides are showing promise as possible iron removal agents for patients with blood diseases that lead to severe iron accumulation[148]. Gossypol (which is isolated from cotton seeds[149,150]) has also been used as an orally administered male contraceptive. Attempts to elucidate the possible physiological function of these compounds have required the use of extremely sensitive and specific analytical techniques.

The development of analytical methods for any compound which has the catechol moiety is difficult because of the instability of these compounds to oxygen, particularly in the presence of base. Many analytical methods have been explored, for example; lipophilic exchange materials[151];



COMT= Catechol-O-methyltransferase

MAO = Monoamine oxidase

Scheme 5.1

thin layer chromatography[125,152]; spectrophotometry[153]. In particular, analyses of low concentrations of sample have been based on GC-MS with selective ion monitoring, often with isotope-labelled internal standards[141,154]. One approach has been to convert these catecholic cmpounds into their more stable ether or ester derivatives, which are also suitable for GLC and GC-MS studies. Derivates commonly employed are acetyl or O-trimethylsilyl ether derivatives, or both of these in sequence[145,152,155-161]; in addition, trifluoroacetyl, pentafluoropropionyl, dinitrophenyl and 4-trifluoromethyl-benzenesulphonyl[155,162] derivatives, which have been found to give higher sensitivity when detected by electron-capture methods[155]. On the other hand it is possible to apply reagents which can form cyclic derivatives selectively with the catechol group. Boronic acids are being studied in this respect [for discussion see Chap.1,V,p.27]. Other cyclic derivatives such as acetonides[163], siliconides[163], carbonates[164] and methylene[165-168] derivatives normally require strong basic or acidic conditions for their preparation, which may be unsuitable for biological extracts: moreover, the yields in many cases are poor and not reproducible. Boronic acids can react with catechols under neutral conditions, and cyclic esters may be formed within a short period.

The selectivity and ease of reaction under mild conditions of boronic acids with catechols attracted our interest in studying certain biologically important catecholamines and catechol estrogens, in parallel with some model compounds. Attention was focused on the following aspects:

1. The stability of the formed cyclic boronate derivative in organic solvents (e.g. EtOAc, $CHCl_3$, pyridine, CH_3OH, EtOH), and in aqueous media. The selective reaction of boronic acids with catechols in aqueous media is potentially important for isolating such bifunctional compounds from biological fluids.

2. The extent of these reactions at microgram levels.

3. The separation and identification of a mixture of catechols especially isomers, after cyclic boronate formation, by using GLC and GC-MS techniques; also the stability of these boronates under GLC conditions and towards electron impact mass spectrometry.

4. Isolation of crystalline catechol boronate derivatives, on a preparative scale, and characterisation by GLC and GC-MS, IR, ^{1}H-NMR and m.p.

5. The stability of catechol boronates during subsequent trimethylsilylation or acetylation of remaining polar functional groups (e.g. -OH,-NH_2). Also for GLC and GC-MS analysis, acetyl and trimethylsilyl derivatives to be used for comparison with the boronate derivative of catechols.

6. Comparison of catechol boronates with other derivatives (chiefly acetyl and trimethylsilyl) for GLC and GC-MS.

7. The stability of catechol boronates during purification on dry column silica gel or by preparative GLC.

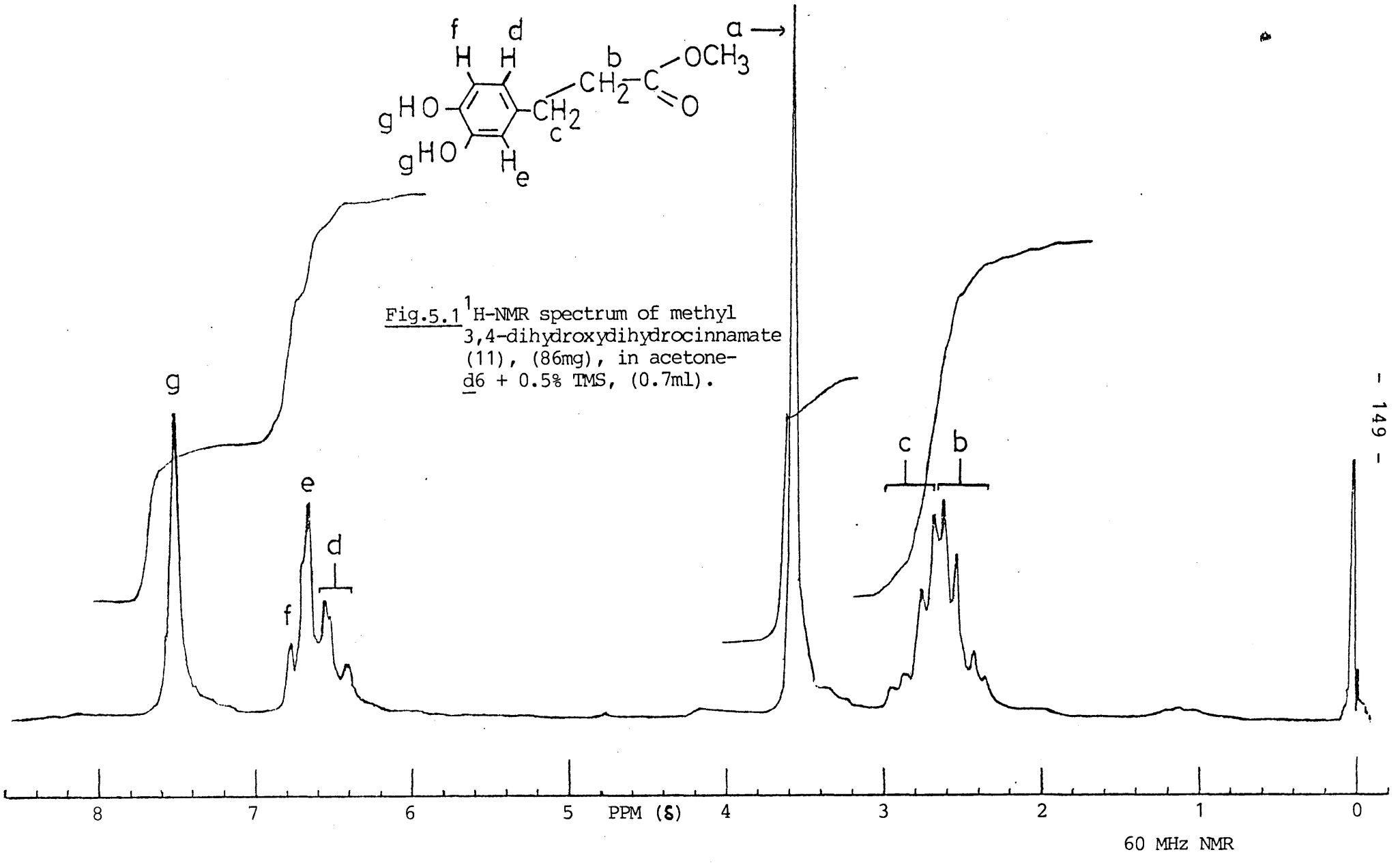
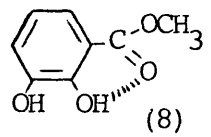


Fig.5.1 ^{1}H-NMR spectrum of methyl 3,4-dihydroxydihydrocinnamate (11), (86mg), in acetone-d6 + 0.5% TMS, (0.7ml).

I. PREPARATION OF THE SUBSTRATES USED IN THE BORONATE REACTIONS

A. Methyl esters of acids containing catechol groups

Methyl esters were prepared by standard procedures (pp. 65-66). Only the methyl ester of L-DOPA (50) showed any serious instability during bicarbonate washing. The purity of these methyl esters was checked by TLC and m.p. then GLC, after further derivative formation (Table 11 & 25). Methyl 2,3-dihydroxybenzoate showed the expected higher R_f value, by reason of the intramolecular hydrogen bond typical of salicylates.

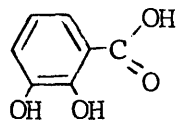
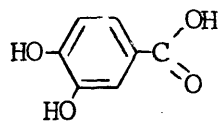
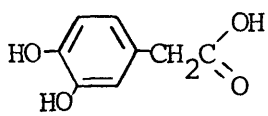
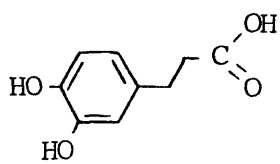
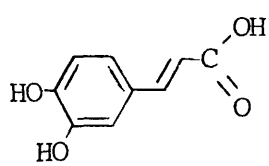


(8)

The infrared spectra[169] showed characteristic strong intense bands for the catecholic hydroxy groups at 3380-3600cm^{-1}, and weak to medium but sharp bands for the methyl ester groups at 2850cm^{-1} and 1445cm^{-1}; and strong bands for the carbonyl groups in the region 1675-1720cm^{-1}; as shown in Table 12.

The ^{1}H-NMR chemical shifts for these catecholic methyl esters have been measured, as in Table 13. The catecholic hydroxy groups[170a] showed strong peaks with chemical shifts 5.39-8.23ppm. The assignment of these hydroxylic protons was achieved by deuterium exchange with D_2O, and also by forming cylic boronate esters with benzeneboronic aicd, as shown in Table 23, p. 174 and fig(s). 5.1 & 5.9, p.175. The three aromatic protons resonated at low field in the region 6.86-7.48ppm. These protons showed different coupling constants depending on the nature of the other functional groups attached to the benzene ring[170]. The three methyl ester protons

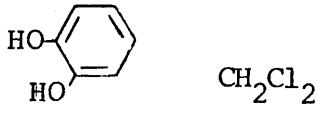
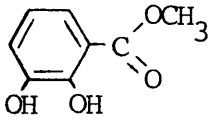
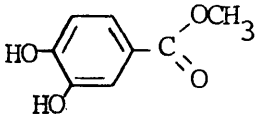
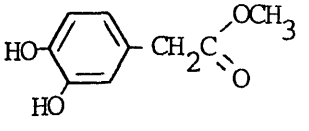
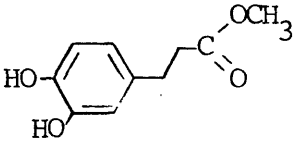
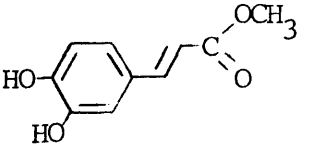
Table 11, R_f values for catecholic carboxylic acids and their methyl esters. Spots were detected with ceric sulphate in aqueous sulphuric acid.

Acids	Compound No.	R_f			
		Acid	Me ester	Me ester di-TMS[a]	Me ester di-Ac[b]
		$CHCl_3$-EtOAc (1:1v/v)		$CHCl_3$-EtOAc (3:1v/v)	
	1	0.14	0.60	0.67	0.50
	2	0.19	0.42	0.67	0.50
	3	0.09	0.41	0.67	0.47
	5	0.13	0.43	0.66	0.49
	6	0.11	0.39	0.66	0.48

a. These compounds were trimethylsilylated under strong conditions with "BSA", "HMDS" and "TMCS", in large excess in dry pyridine, at 70° overnight.

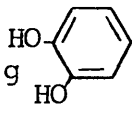
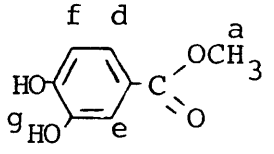
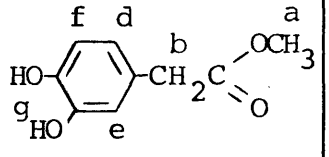
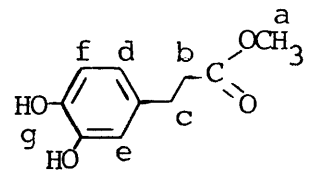
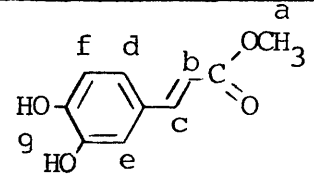
b. Acetylation was achieved with excess Ac_2O in dry pyridine and EtOAc, at 70°, overnight.

Table 12*. IR absorption bands of catechol type compounds. ν max, cm^{-1} measured in $CHCl_3$; 0.5mm cell.

Compound	No.	O-H	$O-CH_3$	C=O	Ref. No.
	73	3550 Ss	/	/	167a
	8	3550 Ss	2850 ws 1440 m	1675 S	167b free acid
	9	3590 3540 Ss	2850 ws 1445 m	1710 S	167c 3,4-dimethoxy
	10	3550 Ss 3380 Sb	2850 ws 1445 m	1710 S	167d free acid
	11	3600 Ss 3560 3400 Sb	2850 ws 1435 Ss	1720 S	167e free acid
	12	3600 3540 m	2850 ws 1440 m	1700 S	167f free acid

* The data in this table and the other related tables were measured for qualitative identifications only. The following abbreviations will be used in the other infra red tables; strong=S, medium =m, weak=w, broad=b and sharp=s.

Table 13*. ^{1}H-NMR, characteristic proton chemical shifts (δ=PPM), of catechol and catechol type methyl ester derivatives. Data measured in acetone - d_6 + 0.4% tetramethylsilane (60 MHz instrument).

Compound	No.***	a	b	c	d	e	f	g
 **	73[A]	/	/	/	6.96 S 4H-Ph			5.39 S
	9[B]	3.81 S	/	/	6.86 dd (7.80)	7.48 d (2.97)	7.34 d (2.97)	8.23 Sb
	10[C]	3.63 S	3.50 S	/	6.76 m (11.99)			7.74 S
	11[D]	3.56 S	2.54 t	2.76 t	6.50 dd (7.80)	6.70 d (2.97)	6.80 d (2.97)	7.53 S
	12[E]	3.70 S	6.09 d	6.36 d	6.78 dd (7.80)	7.09 d (2.97)	6.94 d (2.97)	7.36d 7.61d

* Key words: singlet =S, doublet =d, triplet =t, multiplet =m, doublet of doublets =dd and broad =b.

These symbols will be used in the other NMR tables. The coupling constant values (J=CpS) are indicated in parentheses.

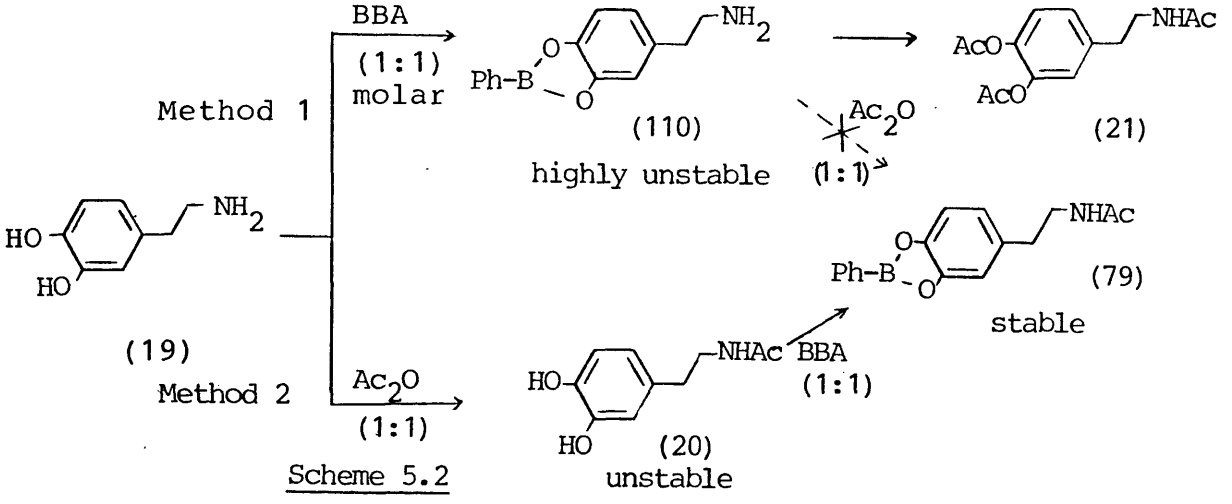
** Measured in $CDCl_3$ + 0.4% tetramethylsilane.

*** The ^{1}H-NMR data for some of these compounds or their derivatives are illustrated in reference 170. B, the free acid and methyl vanillate. C, Homoveratric acid. D, the free acid. E, the free acid and 3-hydroxy-4-methoxycinnamic acid.

showed a strong singlet at 3.56-3.81ppm[170B]. The assignment of the aliphatic proton(s) carbon atom to the aromatic ring, which caused deshielding, while the b protons resonated at higher field; as in compounds(11) and (12)[170D&E], Table 13.

B. Selective N-acetylation of dopamine

Two methods have been used to prepare N-acetyldopamine-3,4-benzeneboronate(79), as in Scheme 5.2. The first method

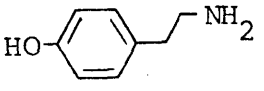
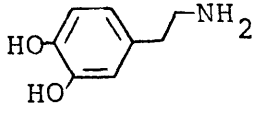
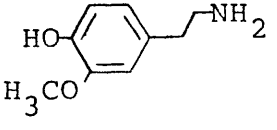
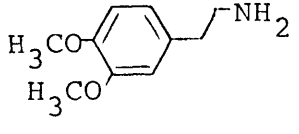


Scheme 5.2

was by forming dopamine-3,4-benzeneboronate(110), which proved to be highly unstable in air and tended to decompose to a dark oil; then subsequent acetylation in dry pyridine or EtOAc. This method yielded dopamine tri-acetate(21) as the main product. The second method was to prepare N-acetyl-dopamine(20), then to form the corresponding benzeneboronate (79), as a stable crystalline derivative (see experimental procedure p.84). The selective acetylation of the amine group, in presence of the catecholic hydroxyl groups required careful control because there was little difference in reactivities. If acetylation was carried out with 1:1 molar

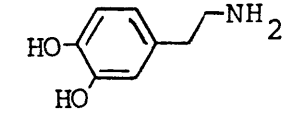
proportions of dopamine and Ac_2O, in EtOAc, pyridine, EtOAc-water, or pyridine-water, at R.T., the products were a mixture of N-acetyl, diacetyl and triacetyl-dopamine derivatives [see Tables 14,15 and Fig.5.2]. Different preparations of N-acetyldopamine(20) have been described: in the earliest report, by Karlson et al.[171], it was extracted from Calliphoral Karvae and identified as the tanning agent in purparium formation[172]; these authors prepared it in modest yield by acid hydrolysis of triacetyldopamine. This method was modified by Mills et al.[173], hydrolysis of triacetyldopamine in ethanol-10% HCl (1:4), but no yield was reported for the yellowish oily product of N-acetyldopamine(20). Bodnaryk et al.[174], prepared N-acetlydopamine directly from dopamine and Ac_2O (1:1 molar) in 10% (w/v) potassium tetraborate. Halmekoski and Saarinen[175] selectively acetylated some sympathomimetic amines (e.g. tyramine), by using acetyl chloride in 2N NaOH solution; or using Ac_2O in an aqueous solution of sodium bicarbonate; then chromatographic separation. Some of these procedures were not reproducible and the yields were poor due to contamination with the di- and tri-acetate derivatives; therefore extensive studies have been carried out, with the help of TLC, GLC NMR, IR techniques,and the model compounds: tyramine(24), 3-methoxytyramine(28) and 3,4-dimethoxy-phenethylamine(33). Selective N-acetylation was found to be achieved in good yield by means of the two-phase reaction system described in Chap.3 p.68. During these attempted acetylation experiments, and by using an excess amount of Ac_2O for a longer period; N,N-diacetyl derivatives were discovered. These peracetylated compounds (see Table 14),

Table 14, TLC R_f values* and melting point (m.p.,°C) results for the acetylated tyramine type of compoounds.

Compound	No.	R_f	-NHAc -OH		-NHAc -OAc (or OCH_3)		-$N(Ac)_2$ -OAc (or OCH_3)	
			Rf	m.p.,°C	Rf	m.p.,°C	Rf	m.p,°C
	24	0.00	0.17	128-129 (Lit.[175] 129-130)	0.26	87-88 (Lit.[173] 91)	0.58	78-81
	19	0.00	0.13	oil (Lit.[173] oil)	0.14 (Di-Ac) 0.16 (Tri-Ac)	oil 65-67	0.54	72-74
	28	0.00	0.12	oil	0.15	87-90	0.55	62-64
	33	0.03	/	/	0.13	86-89	0.52	oil

* The mobile solvent was EtOAc "Analar", and spots were detected with ceric sulphate in in aqueous sulphuric acid.

TLC*, Fig. 5.2



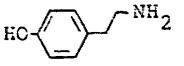
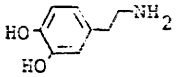
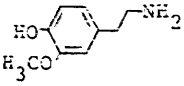
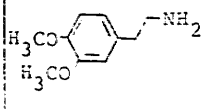
Tetra-Ac

Tri-Ac

Di-Ac

Mono -NHAc

Table 15*, GLC Retention index values for tyramine type acetate derivatives;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)					Retention Increment, ΔI			
			Parent Compound	Derivatives							
				-NHAc	Di-Ac	Tri-Ac	Tetra-Ac	NH_2 → -NHAc	NHAc→ Di-Ac	NHAc→ Tri-Ac	Di-Ac→ Tri-Ac	Tri-Ac→ Tetra-Ac
 No.24	$C_8H_{11}NO$	137		M,179	M,221	M,263						
			1455 [1]	2470 [1] 190°	2470 [1] 190°	2495 [1] 190°		1015	a 0	25	25	
			1725 [2] 150°	2245 [2] 200°	2295 [2] 200°	2350 [2] 200°		520	50	105	55	
 No.19	$C_8H_{11}NO_2$	153		M,195	M,237	M,279	M,321					
			No Peak on either column [1] or [2] Temp.Prog. 160-220°	No Peak on either column [1]or[2] Temp.Prog. 160-220°	2550 [2] & 2575 220°	2820 [1] 2610 [2] 220°	2845 [1] 2675 [2] 220°				60 35	25 65
 No.28	$C_9H_{13}NO_2$	167		M,209	M,251	M,293						
			1535 [1]	2495 [1] 190°	2675 [1] 190°	2680 [1] 190°		960	180	185	5	
			1785 [2] 150°	2305 [2] 200°	2495 [2] 200°	2535 [2] 200°		520	190	230	40	
 No.31	$C_{10}H_{15}NO_2$	181		M,223	M,265							
			1560 [1] 150°	1950 [1] 170°	1995 [1] 170°			390	45			

* [1] 1% OV-25 and [2] 1% OV-17; 2m x 2mm. The rest of the GLC conditions are described in Chromatography Section p.58.

a. On OV-25, tyramine, mono- and diacetate had the same retention time.

were not previously reported and they have been isolated and purified as described on p.74, then characterised along with the other acetylated products (Table 14), by TLC, GLC, ^{1}H-NMR, IR and GC-MS (Chap.6) as below.

1. Gas liquid chromatography:- The gas chromatographic properties of the fully acetylated catecholamines have been studied by Brooks and Horning[176]; and GLC of the partly and fully acetylated adrenergic amines was studied by Halmekoski[177]. Our gas chromatographic studies of the tyramine types of compounds (24),(19),(28) and (33), yielded retention index and increment values for the unacetylated, partly and completely acetylated derivatives, as listed in Table 15. These values were highly reproducible and indicated that the fully acetylated derivatives showed no adsorption on either OV-17 or OV-25 phase, and gave symmetrical peaks. On the other hand, the free amines and their N-acetyl derivatives, showed high adsorption and considerable tailing.[Dopamine and N-acetyl-dopamine were not detected at all]. The retention data in Table 15 showed that useful separation of acetylated derivatives were observed on OV-17, while the OV-25 column, failed to separate certain derivatives, such as N-acetyl- and diacetyltyramine.

2. Infrared spectrum:- This method has been used to distinguish between the N-acetylated, partly and fully acetylated tyramine types of compounds. The infrared spectra of the acetylated products of compounds(24),(19),(28),(33) have been compared with the reported data for N-acetyldopamine[171,173],

and N-acetyl-O,O-diacetyltyramine[173,175]. The N-acetyl groups of compounds(24),(19),(28) and (33), showed two characteristic sharp and intense bands; one for the N-H group, in the region 3340-3450cm^{-1}, and the other for the acetyl carbonyl group at 1650-1670cm^{-1}. The corresponding N,N-diacetyl derivatives showed the disappearance of the N-H band and the appearance of a strong absorption band for the N,N-diacetyl carbonyl groups, at 1695cm^{-1}. The selectively N-acetylated compounds (24),(19),(28) and (33), showed intense absorption bands for the free phenolic hydroxyl group(s) in the region, 3580-3540cm^{-1}, (Table 16). The carbonyl group bands of phenol acetates appeared in the region, 1750-1760cm^{-1} for compounds with one acetate group; and 1764-1770cm^{-1} for compounds with diacetate groups (as in Table 17).

Table 16. IR absorption bands of tyramine type of compounds. ν max, cm^{-1} measured in CH_2Cl_2; 0.5mm cell. (Ac=$COCH_3$).

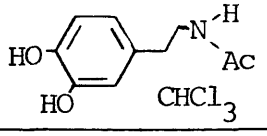
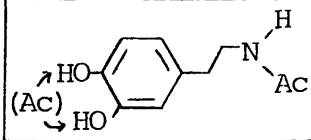
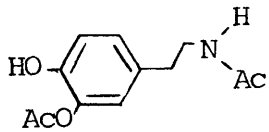
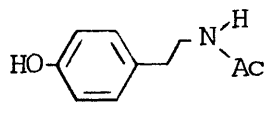
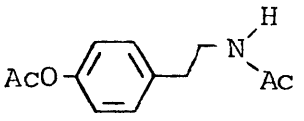
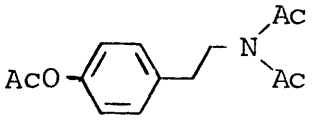
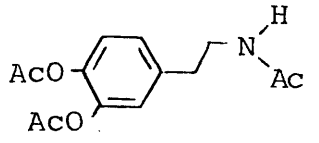
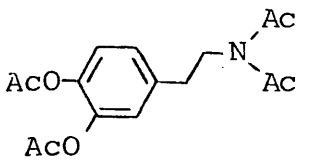
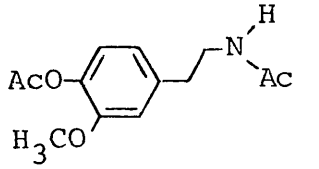
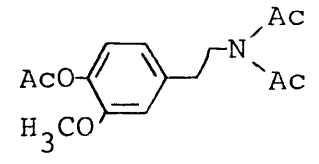
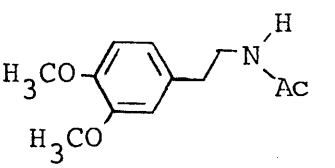
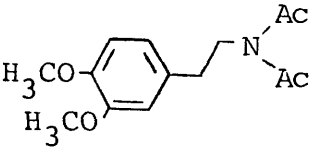
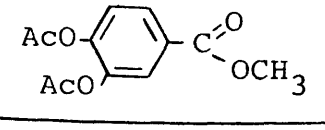
Compound	No.	$Ph(OH)_2$	PhO-H	N-H	N(Ac)H
	20	3540 m	/	3440 ms	1670w 1650ms
	23 a&b	3540 Wb	/	3440 ms	1728S 1660m
	29	/	3540 Ss	3440 Ss	1675 1670Ss
	25	/	3580 Sm	3440 Ss	1670S

Table 17*. IR absorption bands of acetate derivatives of tyramine type compounds and catechols. ν max, cm^{-1} measured in $CHCl_3$, 0.5mm cell. (Ac=$COCH_3$).
*Compound (13) used for comparative study.

Derivative	No.	N-H	O-CH_3	OC(=O)-CH_3	Di-OAc	N(H)-$COCH_3$	N(Ac)-Ac	C(=O)-OCH_3
	26	3440 Ss	/	1752 S	/	1670 S	/	/
	27		/	1750 S	/	/	1695 S	/
	21	3450 Ss	/	/	1770 S	1665 S	/	/
	22	/	/	/	1764 S	/	1695 S	/
	74	3450 Ss	2840 w	1760 Ss	/	1665 S	/	/
	30	/	2840 w	1760 S	/	/	1695 S	/
	32	3450 ms	2840 ws	/	/	1665 Ss	/	/
	33	/	2840 Ss	/	/	/	1695 S	/
	13	/	2840 w	/	1770 Ss	/	/	1720 Ss

3. Nuclear magnetic resonance spectra:- The structure of the selectively N-acetylated, partly and fully acetylated tyramine type of compounds, was further assigned from their ^{1}H-NMR spectra in acetone-$\underline{d}_6$, for compounds in Table 18; and in $CDCl_3$ for compounds in Tables 19 and 20. The assignments of chemical shifts were based on comparisons between the spectra of these related acetylated compounds and compounds in Tables 13 & 18-20 , together with reported spectra as follows: N-acetyldopamine, N-acetyltyramine, and diacetyltyramine[173], N-methyl-3,4-dimethoxy-β-phenethylamine[178]; 3,4-dimethoxyphenylacetamide[179]; 3,4-dimethoxyphenethylamine[170F]; 3-methoxytyramine HCl[170G] and dopamine HCl[170H]. The ^{1}H-NMR data of other acetylated tyramine derivatives listed in Tables 18-20 do not appear to have been previously reported. The selectively N-acetylated compounds(25), (20) and (29), showed a single broad peak in the regionsδ3.06-7.36, for the N-H proton; and their phenolic hydroxyl proton showed peak(s) at δ 7.19-8.42[170G,H], (Figs.5.3&5.6). Both types of signal were absent after exchange with D_2O. The absence of N-H proton signals from the spectra of N,N-diacetyl compounds is clear from Figs.5.3,5.5-6 & 5.8; and Tables 18 and 20. The identity of N-acetyldopamine was verified by fromation of its 3,4-benzeneboronate, in which O-H protons were absent from the spectrum and the five aromatic (Ph-B) protons appeared (see Fig.5.10). The NMR spectra of these tyramine type of compounds showed a clear distinction between the N-acetyl, N,N-diacetyl and the phenolic acetate methyl protons, which gave singlets in the regionsδ1.85-1.95[173], δ 2.30-2.36 and δ 2.21-2.30, respectively (Figs.5.3-10, Tables 18-20). The

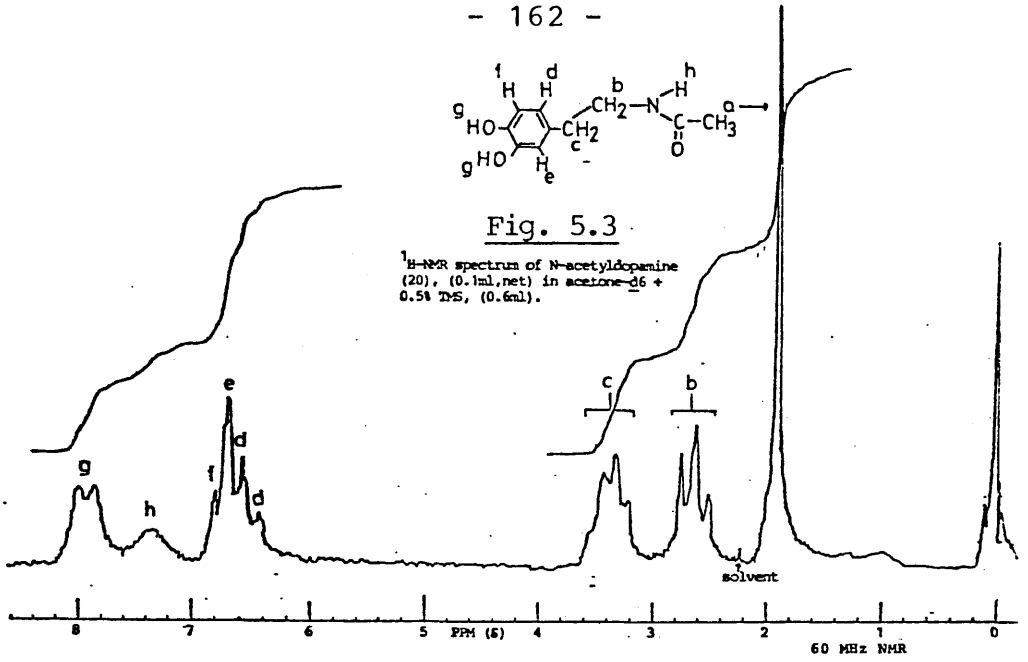


Fig. 5.3

^{1}H-NMR spectrum of N-acetyldopamine (20), (0.1ml,net) in acetone-d6 + 0.5% TMS, (0.6ml).

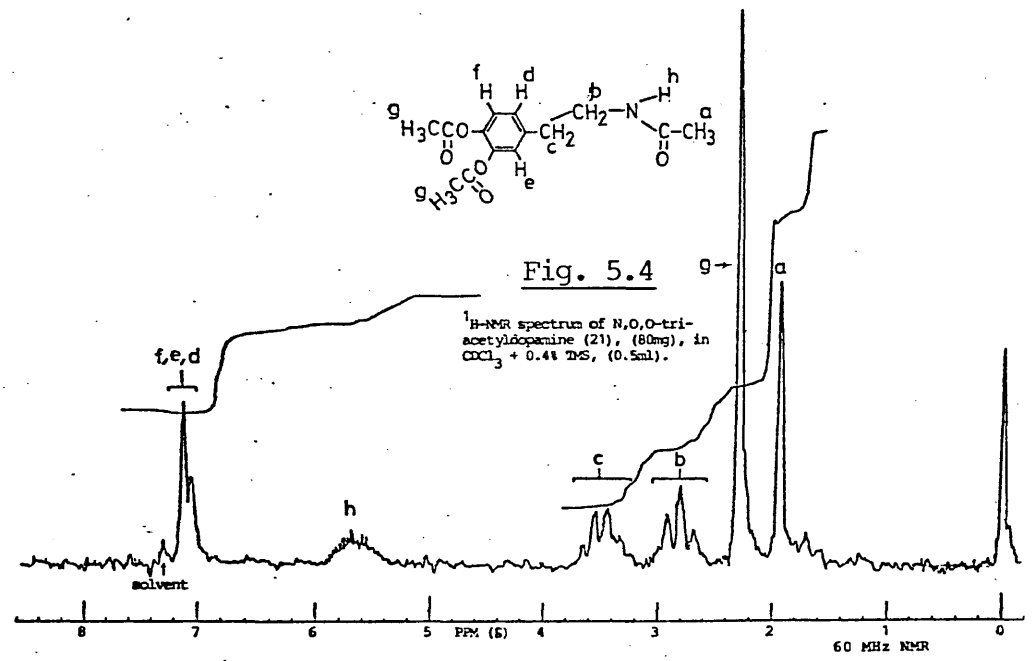


Fig. 5.4

^{1}H-NMR spectrum of N,O,O-triacetyldopamine (21), (80mg), in $CDCl_3$ + 0.4% TMS, (0.5ml).

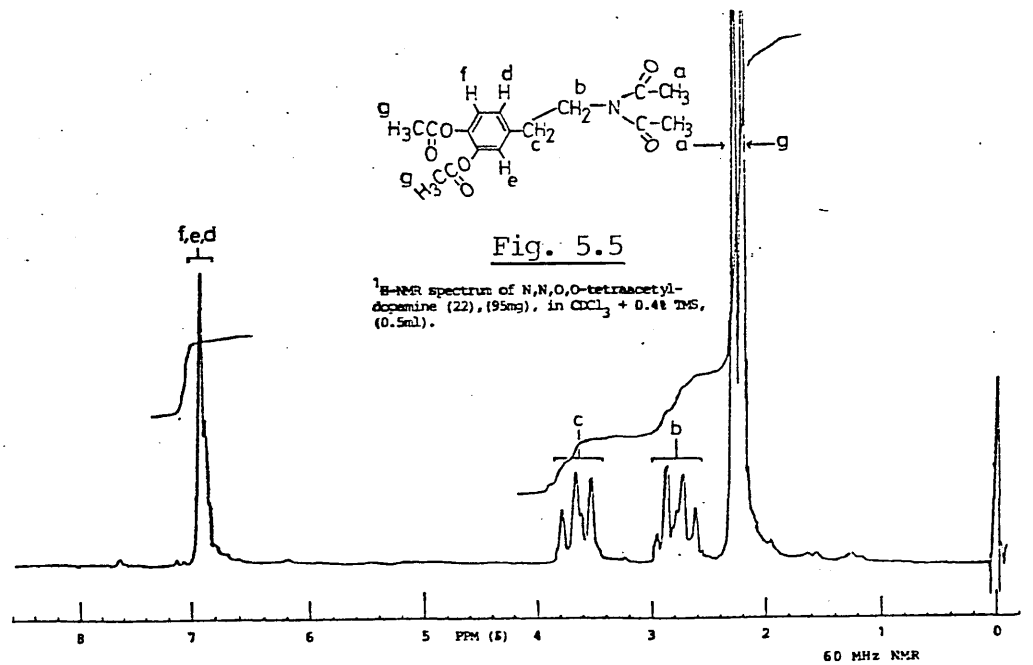


Fig. 5.5

^{1}H-NMR spectrum of N,N,O,O-tetraacetyldopamine (22), (95mg), in $CDCl_3$ + 0.4% TMS, (0.5ml).

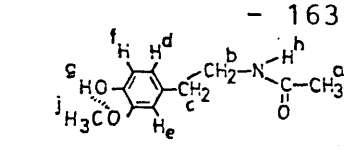


Fig. 5.6

^{1}H-NMR spectrum of N-acetyl-3-methoxytyramine (29), (29mg), in acetone-d6 + 0.4% TMS, (0.5ml).

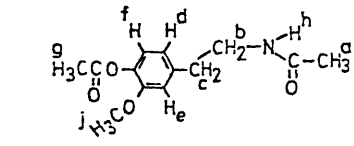


Fig. 5.7

^{1}H-NMR spectrum of N,O-diacetyl-3-methoxytyramine (74), (26mg), in $CDCl_3$ + 0.4% TMS, (0.7ml).

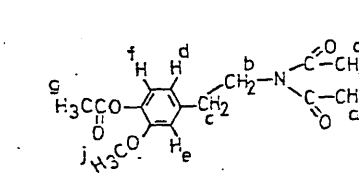


Fig. 5.8

^{1}H-NMR spectrum of N,N,O-triacetyl-3-methoxytyramine (30), (44mg) + $CDCl_3$ + 0.4% TMS, (0.7ml).

Table 18. ^{1}H-NMR, characteristic proton chemical shifts (δ=ppm) of N-acetyltyramine type derivatives. Data measured in acetone-d_6 + 0.4% tetramethylsilane, (60 MHz).

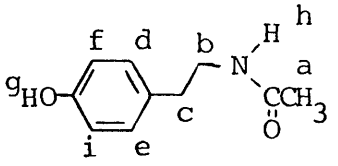
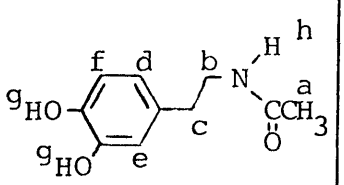
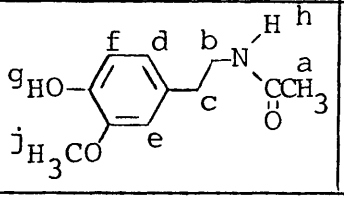
Compound	No.	a	b	c	j	d & e	f	g	h
	25	1.86 S	2.68 m	3.35 m	/	including i, 6.79 q & 7.09 q		8.42 S	3.06 S
	20	1.93 S	2.66 m	3.33 m	/	6.42 d 6.59 d	6.73 d	6.79 d	7.86S & 7.99S 7.36 b
	29	1.85 S	2.70 m	3.43 m	3.85 S	6.76		6.88	7.19 b	5.66 S

Table 19. ^{1}H-NMR, characteristic proton chemical shifts (δ=PPM) of N,O-diacetyltyramine type compound derivatives. Data measured in $CDCl_3$ + 0.4% tetramethylsilane, (60 MHz).

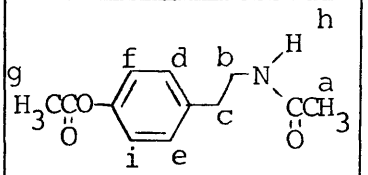
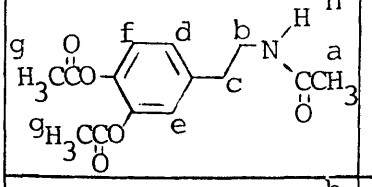
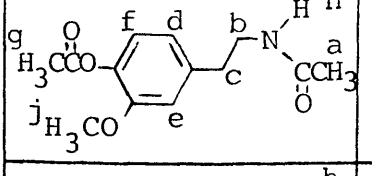
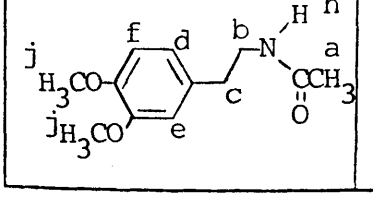
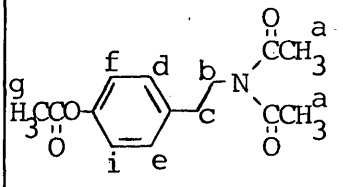
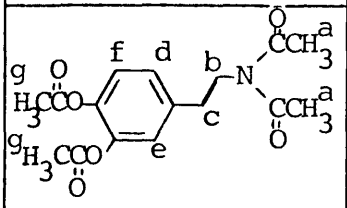
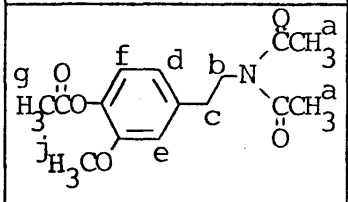
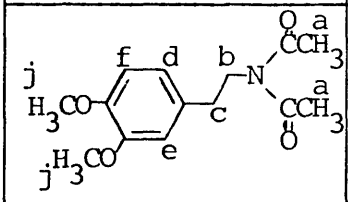
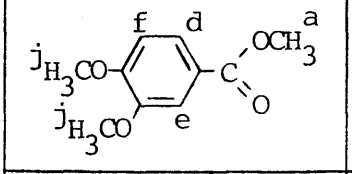
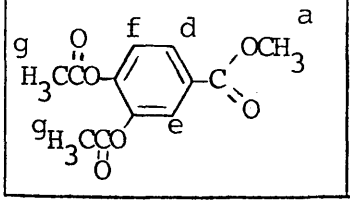
Compound	No.	a	g	b	c	j	d	e	f	h
	26	1.86 S	2.21 S	2.76 m	3.36 m	/	Including i, 6.99 dd & 7.29 dd			6.43 b
	21	1.95 S	2.30 S	2.83 m	3.46 m	/	7.16 m			5.73 b
	74	1.93 S	2.30 S	2.80 m	3.65 m	3.80 S	6.73d & 6.96d	6.83 d	7.09 d	5.69 b
	32	1.93 S	/	2.85 m	3.57 m	3.90 S	6.86 m			5.73 b

Table 20*. ^{1}H-NMR, characteristic proton chemical shifts (δ=PPM) of fully acetylated, tyramine type compounds and methyl benzoate, derivatives. Data measured in $CDCl_3$ + 0.4% tetramethylsilane, (60 MHz).

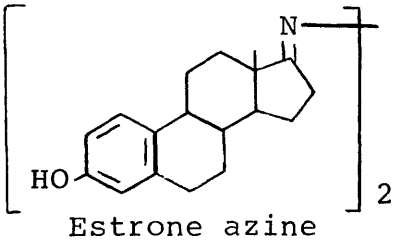
Compound	No.	a	g	b	c	j	d	e	f
	27	2.33 S	2.26 S	2.85 m	3.90 m	/	Including i, 7.06dd 7.26dd		
	22	2.30 S	2.23 S	2.76 m	3.70 m	/	6.94 m		
	30	2.36 S	2.30 S	2.85 m	3.93 m	3.90 S	6.70d 7.00d	6.89 d	7.14 d
	33	2.36 S	/	2.85 m	3.96 m	3.93 S	6.89 m		
	87	3.98 S	/	/	/	3.96 S	6.91 dd	7.63 m	7.79 m
	13	4.03 S	2.36 S	/	/	/	7.46 dd	8.09 d	8.23 d

* Compounds (13) and (87) used for comparative study.

aromatic proton meltiplets occurred in the region δ6.42-7.26[170,173,178-9]. The two methylene groups b & c (Figs. 5.3-10 and Tables 18-20), showed two multiplet centers in the region δ2.66-2.85 and δ3.35-3.96, respectively[173,178].

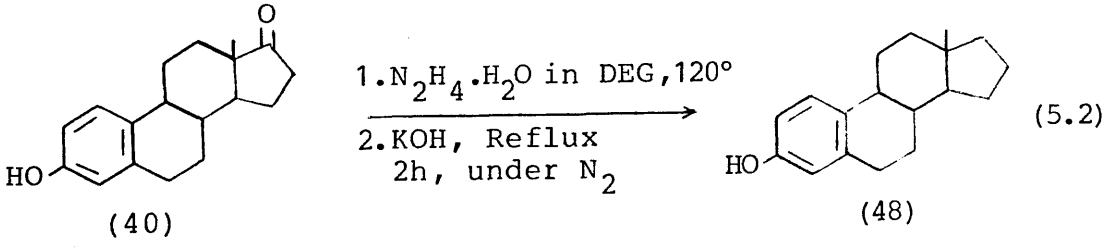
C. Catechol estrogens

In oder to obtain the boronate derivatives of 2- and 4-hydroxylated estrogens, chiefly for GLC and GC-MS studies, these substrates were prepared from estrone(40). 17-Deoxo-estrone(48) was not obtained by the procedure of Danneberg and Kohler[180]; although they reported a 99% yield of compound (48). Instead estrone azine[181] was mainly recovered.



Estrone azine

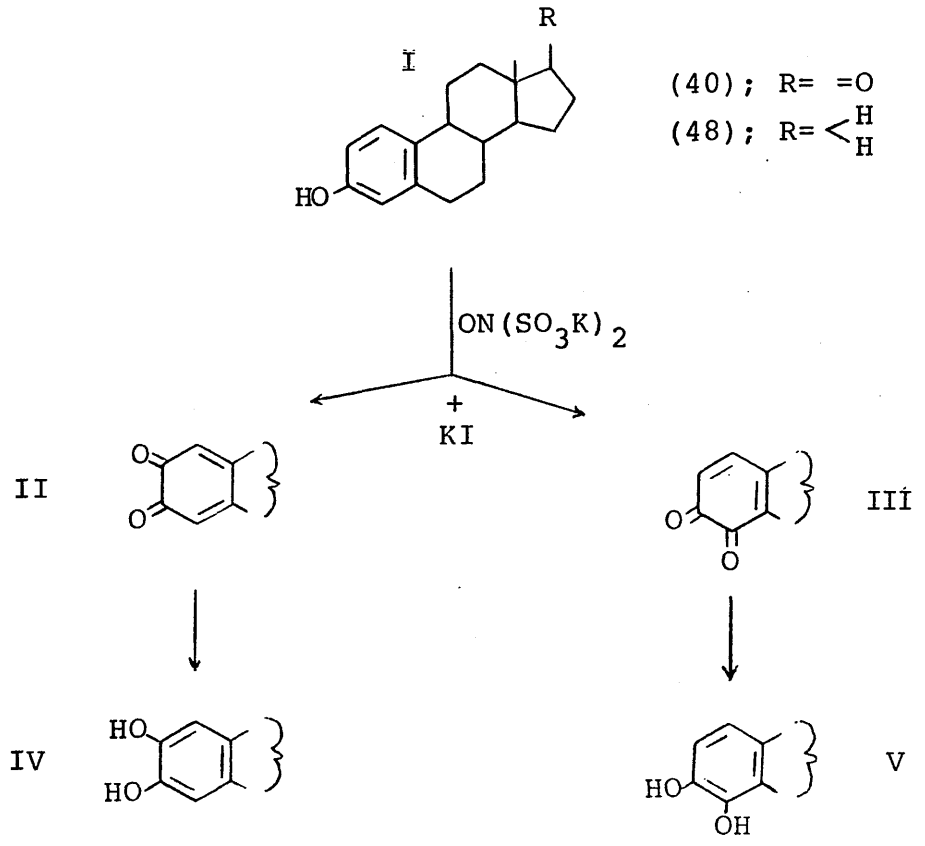
The procedure of Huang-Minlon[117] was modified (as advised by Prof. D.N.Kirk) by adopting a large excess of hydrazine hydrate. This afforded a 91% yield of compound(48), (see Exptl. pp.76-78) (eq. 5.2). The ^{1}H-NMR chemical shifts of (48)



(40) (48) (5.2)

in Table 22; are based on those of estrone[170I]; and 2- and 4- hydroxyestrone[118].

2-Hydroxyestrone(15) and 4-hydroxyestrone(16) were prepared from the corresponding aminophenols by Stubenrauch and Knuppen[140]. This procedure involved multi-step reactions, and the yields were almost the same as the one-step procedure of Gelbke, et al.[118]: oxidation of the monophenolic estrogen(I) with FREMY'S salt (potassium nitrosodisulphonate) to the 2,3- and 3,4-quinone (II and III) and subsequent reduction with KI gave the corresponding catechol estrogens (IV and V), as in Scheme 5.3 , 2- and 4-Hydroxy-17-deoxoestrone, (17) and (18), were also prepared according to this method, from 17-deoxoestrone (40).



Scheme 5.3

The ^{1}H-NMR chemical shifts of compounds(17) and (18), listed in Table 22; are based on the NMR data of compounds (15) and (16)[118]. The TLC Rf values (Table 21) showed a

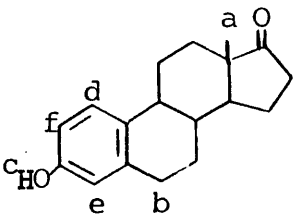
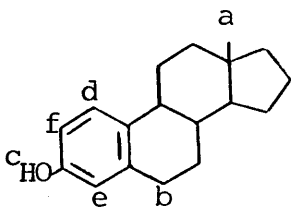
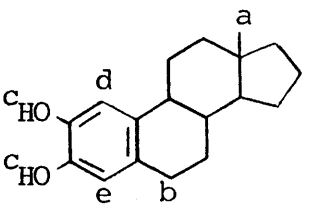
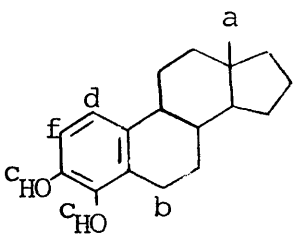
possible separation between the isomeric catechol estrogens.

2-Hydroxyestradiol(47) was prepared by two methods, from its corresponding triacetate(46): hydrolysis with HCl in methanol, (94% yield); and by hydrogenolysis with $LiAlH_4$ in ether, (63% yield) (see Exptl. p.76). The product was purified by preparative TLC, and identified by GLC, after trimethylsilylation (see Table 27). The ^{1}H-NMR spectrum of 2-hydroxy-estradiol triacetate(46), was recorded for comparative study with the compounds in Table 22. The chemical shifts of this compound (as listed in the footnote of Table 22), were assigned by comparison with that of estradiol diacetate[182].

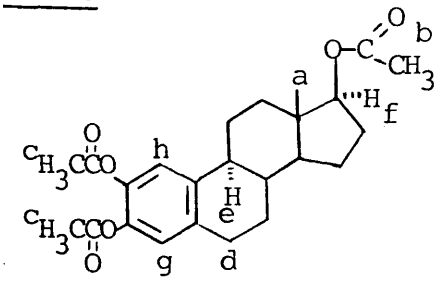
Table 21. Melting point and TLC R_f values, measured in $CHCl_3$-MeOH-AcOH, 96:3:1, (v/v/v): for estrogen compounds.

Estrogens	No.	m.p.°C	R_f
Estrone	40	254-262	0.35
17-Deoxoestrone	48	134-135 (Lit.[117] 134-134.5)	0.44
2-Hydroxyestrone	15	190-193 (Lit.[118] 193-196)	0.19
4-Hydroxyestrone	16	261-265 (Lit.[118] 268-271)	0.24
2-Hydroxy-17-deoxoestrone	17	138-140	0.22
4-Hydroxy-17-deoxoestrone	18	142-143	0.27

Table 22. ^{1}H-NMR characteristic proton chemical shifts (δ=PPM), of some catechol estrogens. Data measured in $CDCl_3$ + tetramethylsilane, (90 MHz).

Compound	No.	a	b	c	d	e	f
	40	0.89 S	2.78 b	7.72 Sb	6.65	6.58	7.03
	48	0.72 S	2.80 mb	5.011 Sb	6.62	6.52	7.08
	17	0.73 S	2.69 mb	5.17 Sb	6.79 S	6.55 S	/
	18	0.73 S	2.78 mb	4.95S 5.02S b	6.70 d	/	6.83 d

No. 46



a	b	c	d & e
0.80,S	2.06,S	2.26,S	2.86,b

f	g	h
4.73,b	6.91,s	7.09,s

D. Gossypol

Commercial "gossypol acetate" (complexed with acetic acid), was liberated by recrystallisation from ether. Yellow fine crystals of gossypol(34) were recovered; m.p.184-187° (Lit.[115] 182-4° and Lit.[116] 184°). Its structure was further verified by TLC, IR[183,115] and UV data[183]. For comparative TLC and GLC studies of gossypol hexatrimethylsilylether and the attempted bis-methaneboronate; gossypol hexa-acetate(35) was formed, from acetylation of gossypol with excess Ac_2O in pyridine (see Exptl. p.73). This product was purified by dry column chromatography, and two forms of compound(35) were recovered: one as white crystals m.p.275-280° (Lit.[116] 276-279°); and the other as bright yellow fine crystals, m.p.195-200° (Lit.[116] 184-186°). The IR, UV, ^{1}H-NMR data of gossypol hexa-acetate, corresponded with published data[115]. The TLC and GLC data of gossypol and its derivatives are discussed on p. 199.

E. Methylenation of catechols

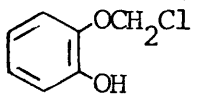
The methylenation of catechol type of compounds was one of the procedures attempted to afford cyclic derivatives for comparison with cyclic boronate derivatives in the current research. However, attempts were made to carry out the methylenation of the model compounds: catechol and methyl 3,4-dihydroxy-benzoate, with dichloromethane, by the following methods, in which high yields were reported:

(i) [Bonthrone and Cornforth[165]], using sodium hydroxide pellets in a dipolar aprotic solvent such as dimethyl sulphoxide;

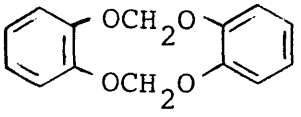
(ii) [Femino, et al.[166]], using potassium carbonate in dimethylformamide (DMF);

(iii) [Clark et al.[167]],using an excess of potassium or caesium fluoride in DMF, in which the reaction was accelerated by the formation of an H-bond between the fluoride anion and the aromatic molecule which directs electrons from the electron rich fluoride anion to the organic part of the complex.

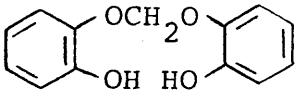
These procedures, in our hands, gave very low yields (~1-5%) of the methylenated product. This was due to the formation of many by products (I-VI), together with o-quinone and unreacted material.



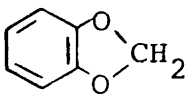
I



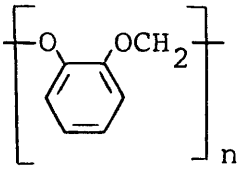
II



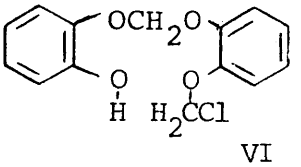
III



IV

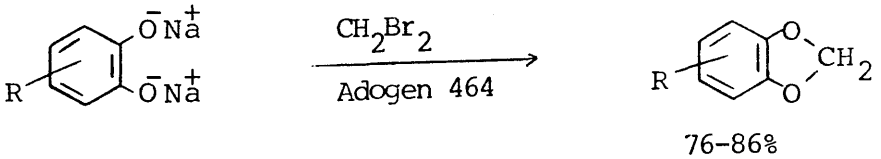


V



VI

There is a procedure by Bashall and Collins[184], in which phase-transfer catalysis is used for methylenation of catechols with dibromomethane via the reaction of the disodium salts of catechols, in the presence of Adogen 464 [$CH_3(C_8\text{-}C_{10})_3N^+Cl^-$] (eq.5.3). This method has not been examined in the present work.



76-86%

(5.3)

II. PREPARATION OF CRYSTALLINE CYCLIC BENZENEBORONATES

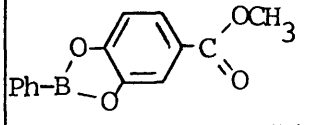
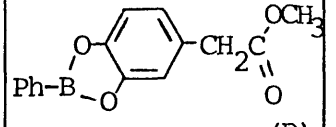
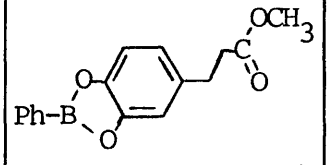
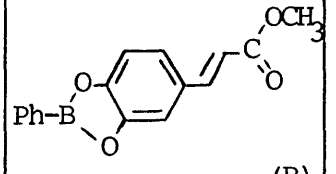
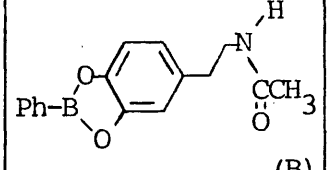
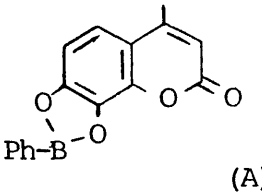
Crystalline cyclic boronate esters of catechol type of compounds: 3,4-dihydroxy methyl esters (9-12); N-acetyl-dopamine(20); estra-1, 3,5(10)-triene-3,4-diol(18) and 4-methyl-7,8-dihydroxy coumarin (14), were obtained (Table 2, p.84). These new compounds were prepared under neutral conditions in EtOAc, at 70° for a short period, from the spontaneous reaction of 0•1 molar excess of benzene-boronic acid with these catecholic type of compounds (9-14, 18 & 20), (see Chap.1, V, p.27). The recovered yield of these cyclic boronates were high (84-99.8%). These proved to be stable in organic solvents (e.g. EtOAc, pyridine, hexane, toluene), and towards aerial oxidation and atmospheric moisture, and remained stable on storage. They proved to be unstable on TLC, or dry column silica gel chromatography; they tended to hydrolyse, and to undergo subsequent partial oxidation. Methyl 2,3-dihydroxybenzoate(8), failed to form a stable cyclic benzene boronate on a preparative scale although a boronate peak was detectable on GLC; indicating that partial formation of a cyclic ester had occurred. Tetrahydroxy terephthalic acid diethyl ester failed to form a cyclic boronate derivative; either on an analytical scale or in preparative amounts, presumably because of the unreactivity of the strongly intramolecularly hydrogen-bonded phenol groups.

The IR absorption bands of the cyclic boronate derivatives of compounds (9-12, 18 & 20), are listed in Table 23. These compounds showed two characteristic bands, for Ph-B and B-O

absorption, in the region 1430-1455cm^{-1}, respectively (also see Chap.4, p.114 & 125). The other bands were characteristic for the remaining functional groups of the compounds listed in Table 23; (cf Tables 12&16).

The cyclic boronate derivatives of the compounds listed in Table 24, showed characteristic NMR chemical shifts for the five aromatic protons of the benzene ring, which bonded directly to the boron atom (Ph-B)[78,170K]. The two ortho protons to the boron atom, were deshielded, and resonated at δ8.03-8.17; while the other three protons resonated at higher field in the region δ 7.29-7.69, (see Figs.5.9-10. The three catecholic aromatic protons resonated at higher field in the region δ 6.91-7.96[139,170,173,178-9]; as shown in Table 24; see (Figs. 5.9-10); and for comparison see Figs 5.1 & 5.3. For boronates NMR, see Chap.1, VI, p.44. The GLC data are discussed in the next section (III); and the GC-MS in Chap.6.

Table 23. IR absorption bands of catechol type compounds, as benzeneboronate derivatives. νmax, cm-1 measured in CH_2Cl_2 (A) or $CHCl_3$ (B); 0.5mm cell.

Boronate Derivative	No.	N-H	O-OCH_3	C(=O)-OCH_3	N-C(=O)-CH_3	Ph-B*	B-O*
 (B)	75	/	2850 w	1715 S	/	1440 1400 S	1370 1335 S
 (B)	76	/	2840 w	1715 S	/	1430 mb 1390 ms	1365 1325 ms
 (A)	77	/	2840 w	1738 1730 S	/	1430 mb 1390 ms	1365 1330 ms
 (B)	78	/	2850 w	1700 S	/	1435 mb 1395 ms	1365 1330 ms
 (B)	79	3440 ms	/	/	1675 1670 S	1435 wb 1395 ms	1365 ms 1345 ms
 (A)	80	/	/	/	/	1455 ms 1435 w	1380 ms 1335 ws

* Ref. No. 15,19,125.

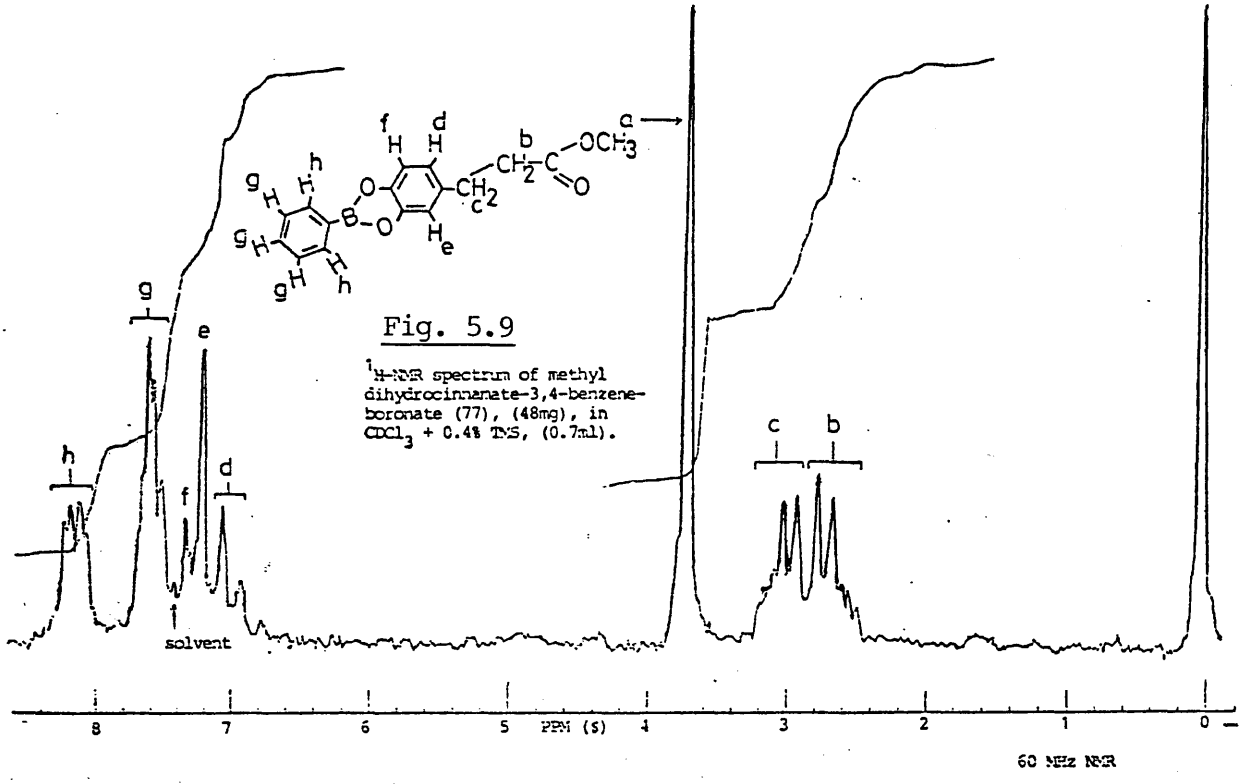


Fig. 5.9

^{1}H-NMR spectrum of methyl dihydrocinnamate-3,4-benzeneboronate (77), (48mg), in $CDCl_3$ + 0.4% TMS, (0.7ml).

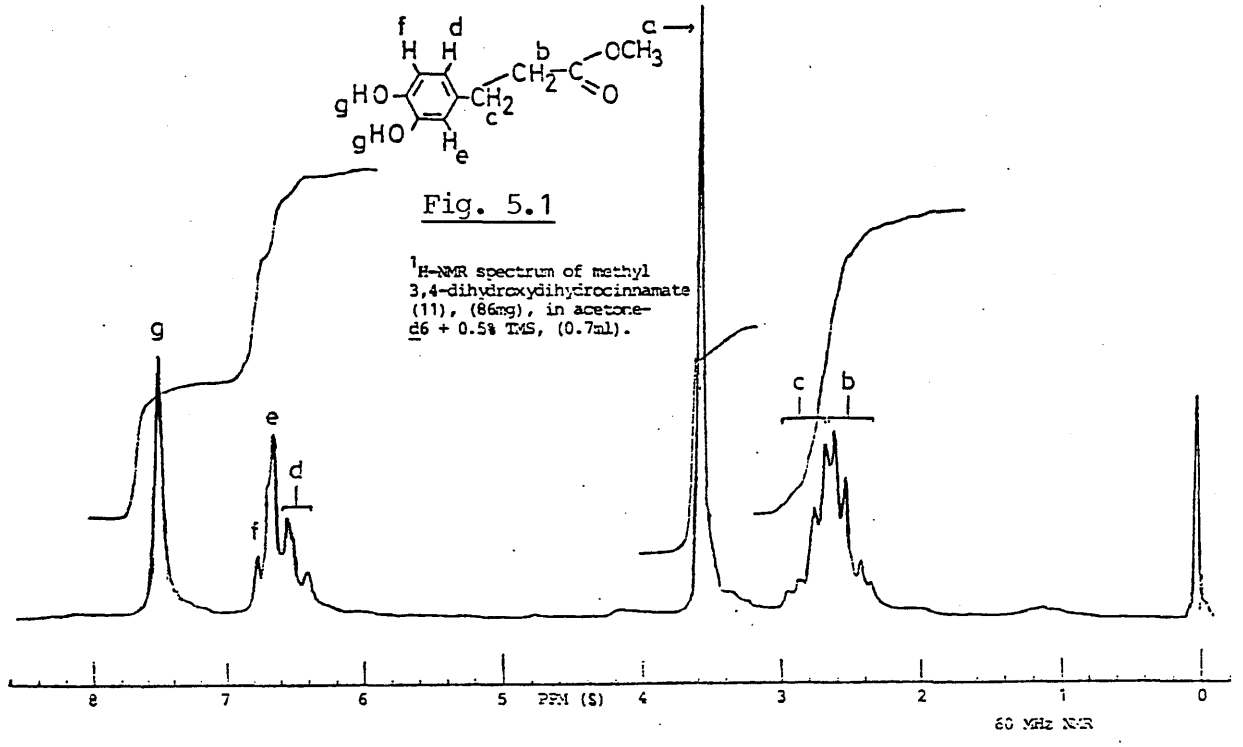


Fig. 5.1

^{1}H-NMR spectrum of methyl 3,4-dihydroxydihydrocinnamate (11), (86mg), in acetone-$\underline{d}$6 + 0.5% TMS, (0.7ml).

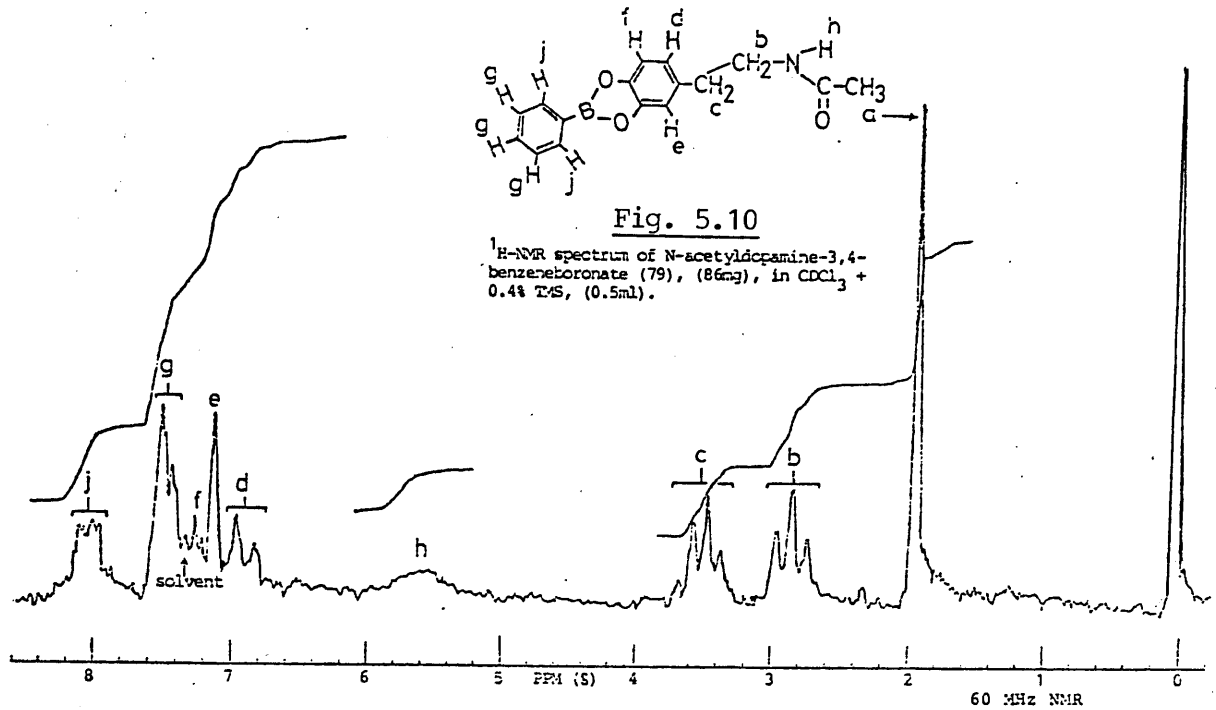


Fig. 5.10

^{1}H-NMR spectrum of N-acetyldopamine-3,4-benzeneboronate (79), (86mg), in $CDCl_3$ + 0.4% TMS, (0.5ml).

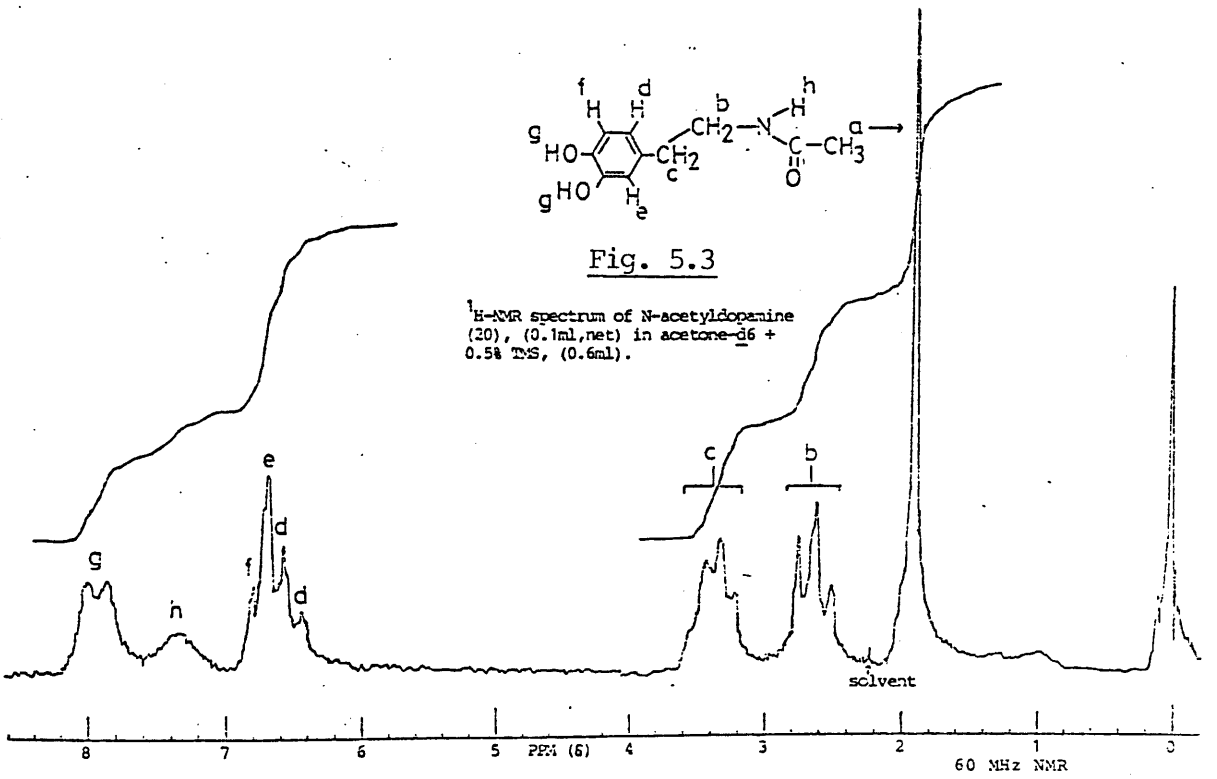
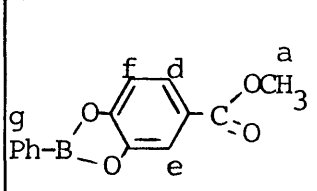
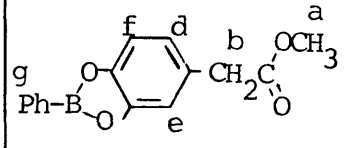
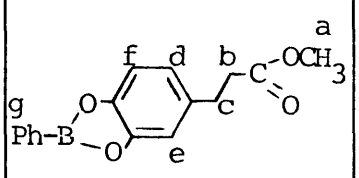
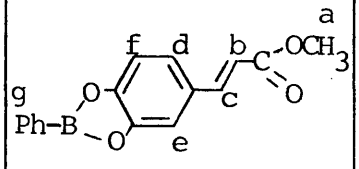
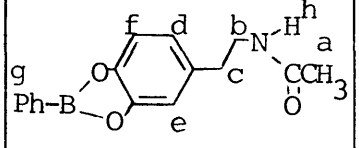
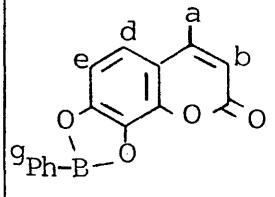


Fig. 5.3

^{1}H-NMR spectrum of N-acetyldopamine (20), (0.1ml,net) in acetone-d6 + 0.5% TMS, (0.6ml).

Table 24. ^{1}H-NMR, characteristic proton chemical shifts (δ=PPM), of benzeneboronate derivatives of methyl benzoate, N-acetyl-tyramine type compounds and 4-methyl coumarin. Data measured in $CDCl_3$ + 0.4% tetramethylsilane, (60 MHz).

Compound	No.	a	b	c	d	e	f	g	h
 *	75	3.94 S	/	/	7.26 dd	7.96 d	7.85 d	7.54m 3H 8.10dd 2H	/
	76	3.70 S	3.66 d	/	7.16 dd	7.58 d	7.49 d	7.29m 3H 8.09m 2H	/
	77	3.71 S	2.73 q	2.99 q	7.02 dd (7.8)	7.23 d (2.97)	7.36 d (1.98)	7.63m 3H 8.13m 2H	/
	78	3.85 S	6.33 d	6.59 d	7.39	7.96	7.49	7.63m 3H 8.17m 2H	/
	79	1.95 S	2.83 m	3.46 m	6.91 dd	7.13 d	7.28 d	7.56m 3H 8.09dd 2H	5.93 Sb
	80	2.33	6.09	/	6.99	7.36	/	7.69m 3H 8.03m 2H	/

* Measured on an R32 90Mc instrument.

III. GAS LIQUID CHROMATOGRAPHY OF CATECHOL TYPE OF COMPOUNDS

In the current work, cyclic boronates of a range of model catechols, including methyl dihydroxybenzoates, catecholamines and catechol estrogens, have been examined (see Tables 25-7 & 29). In general they were suitable for characterisation by GLC and GC-MS (see Chap. 6), but not for quantitative analysis. This limitation resulted from the strong adsorption of many of the boronates on the chromatographic columns (OV-1, OV-17 and OV-25), which was especially severe when additional "free" hydroxy or amino groups were present (e.g. 2,3,4-trihydroxyacetophenone; 2-hydroxyestradiol). It was found that the catechol boronates were largely hydrolysed in aqueous media. In some cases it is considered that this was assisted by hydrogen bonding of a catechol OH with an adjacent polar group, such as amino or carbonyl, as in methyl 2,3-dihydroxybenzoate and 2,3-dihydroxybenzaldehyde. In addition to hydrolysis, products were susceptible to decomposition probably via autoxidation of free catechol groups; thus boronates of brazilin (111), and apomorphines (Tables 26&30) appeared to be lost within a few hours of formation, even in dry non-aqueous solvents. Most of the catechol boronates which have been prepared, undergo either total decomposition or displacement reactions after the addition of reagents for trimethylsilylation or acetylation (see Figs. 5.15-6).

Catechol boronates undergo ring opening on the active surface of silica gel during TLC or column chromatography, and (unlike acetates and carboxylic esters in general) cannot be purified by these techniques. The ring opening is probably hydrolytic

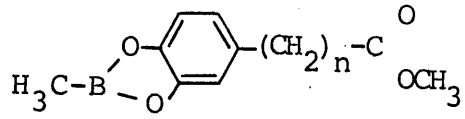
but is followed by rapid oxidation especially in basic media. Some protection from oxidation can be achieved by treating the silica gel with a saturated solution of ascorbic acid in, acetic acid-chloroform-cyclohexane, (1:2:2, v/v/v)[126]. For further GLC discussion see Chap.1, p.41.

The boronate derivatives studied, could be divided into three main groups, according to the number of the other reactive groups; (e.g. -OH, $-NH_2$, -NHR). The corresponding trimethylsiyl and acetyl derivatives were also studied for these groups of compounds, for comparative GLC studies. These compounds were:

A. Catechols with no other reactive groups

1. Catechol(73); 1,2-dihydroxynaphthalene(112); 2- and 4-hydroxy-17-deoxoestrone(17 & 18):- The methane- and-benzene-boronate derivatives of these compounds, showed almost symmetrical peaks, which were highly stable on GLC, with low adsorption on the columns (OV-17 and OV-25), see Figs. 5.13 & 5.14 . The boronate derivatives of compounds(73), (112), (17) and (18) were easily displaced with acetyl or trimethylsilyl groups, after the addition of Ac_2O or "BSA" (see Figs. 5.15-6). On the other hand the boronate derivatives of these compounds were fairly stable on storage in EtOAc. The retention index and increments for these compounds and their derivatives are in Tables 28 & 30.

2. Methyl esters of dihydroxy aromatic acids:- The free catecholic compound, methyl 3,4-dihydroxy benzoate(9), and the analogous phenylacetate(10), dihydrocinnamate(11) and cinnamate(12) showed high adsorption on GLC columns (OV-1, OV-17 & OV-25), with considerable tailing; and sometimes their



a; n=0

b; n=CH_2

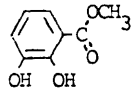
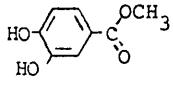
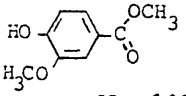
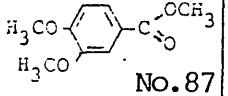
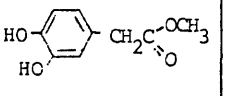
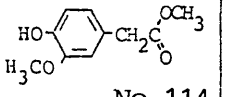
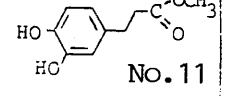
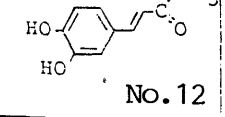
c; n=$(CH_2)_2$

d; n=$(CH)_2$

Fig. 5.11 GLC Chromatogram, on 1% OV-25, 2m x 2mm Temp.120°C. Methyl 3,4-dihydroxy- of:(a)benzoate (b);phenylacetate (c);dihydrocinnamate (d);cinnamate;1.5,1.5,2.0 & 6.0 µg/µl + methaneboronic acid (excess).

I values were not reproducible. Their corresponding cyclic methane- and benzeneboronate derivatives were formed easily and showed good analytical data for GLC; they were well separated on OV-17 & OV-25, with partial adsorption, which caused slight tailing of their peaks [see Fig. 5.11]. The I values of these boronate derivatives were highly reproducible, as in Table 25. The data for methaneboronate derivatives, were recorded at lower temperature, than the corresponding benzeneboronates. The high volatility and low molecular weights of methaneboronates are especially useful for GC-MS analysis. The cyclic boronate group of compounds (9)-(12) was easily displaced, by acetyl or trimethylsilyl groups, after mixing with excess reagents (Ac_2O or "BSA", respectively), forming the diacetate or di-trimethylsilyl ether derivatives; in pyridine-EtOAc, within a short period. These derivatives showed no adsorption, and gave symmetrical peaks and highly reproducible I values (Table 25). Results from this Table, show an average constant retention increment, I=825, between each of the methane- and benzeneboronates for all these compounds, which is useful to predict approximate retention times; and shows at the same time the stability of the GLC data. The other compounds (87,113-4), listed in Table 25, were used for comparative studies.

Table 25*, GLC Retention index values for catechol methyl ester derivatives;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)					Retention Increment, ΔI				Comment
			Parent Compound	Derivatives								
				MB	TMS	Ac	BB	MB→ TMS	TMS→ Ac	Ac→ BB	MB→ BB	
 No.8	$C_8H_8O_4$	168	1520 [2] 140° 1310 [3]	M,192 1620 [1] 1570 [2]	M,312 1850 [1] 1790 [2]	M,252 2140 [1] 2020 [2]	M,254 2450 [1] 2335 [2]	230 220	290 230	310 315	830 765	
 No.9	$C_8H_8O_4$	168	2075 [2] 210° 1710 [3]	M,192 1620 [1] 1565 [2]	M,312 1900 [1] 1850 [2]	M,252 2175 [1] 2075 [2]	M,254 2435 [1] 2335 [2]	280 285	275 225	260 260	815 770	
 No.113	$C_9H_{10}O_4$	182	1910 [1] 1745 [2] 180°	/	M,254 1905 [1] 1830 [2] 150°	M,224 2065 [1] 1940 [2] 180°	/	/	160 110	/	/	
 No.87	$C_{10}H_{12}O_4$	196	1990 [1] 1865 [2] 180°	/	/	/	/					
 No.10	$C_9H_{10}O_4$	182	1590 [2] 140° 1405 [3]	M,206 1750 [1] 1675 [2]	M,326 1960 [1] 1895 [2]	M,266 2325 [1] 2180 [2]	M,268 2570 [1] 2450 [2]	210 220	365 285	245 270	820 775	
 No.114	$C_{10}H_{12}O_4$	196	1985 [1] 1845 [2] 180°	/	M,268 1980 [1] 1885 [2] 150°	M,238 2190 [1] 2040 [2] 180°	/	/	210 155	/	/	
 No.11	$C_{10}H_{12}O_4$	196		M,220 1850 [1] 1770 [2]	M,340 2050 [1] 1975 [2]	M,280 2415 [1] 2275 [2]	M,282 2675 [1] 2550 [2]	200 205	365 300	266 275	825 780	
 No.12	$C_{10}H_{10}O_4$	194	2405 [2] 1755 [3]	M,218 1985 [1] 1900 [2]	M,338 2220 [1] 2150 [2]	M,278 2535 [1] 2405 [2]	M,280 2820 [1] 2690 [2]	235 250	315 255	285 285	835 790	

* The GLC conditions were as follows:-

Columns coded as;

[1] 1% OV - 25; 2m x 2mm; oven temperature for the methaneboronate (MB) 120°; trimethylsilyl ether (TMS) 140°; and the acetate (Ac) and benzeneboronate (BB) 160°.

[2] 1% OV - 17; 2m x 2mm; oven temperature for the methaneboronate (MB) 120°; trimethylsilyl ether (TMS) 140°; and acetate (Ac) and benzeneboronate (BB) 200°.

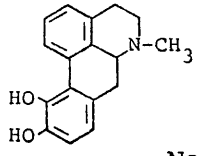
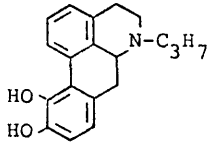
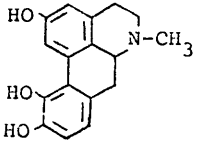
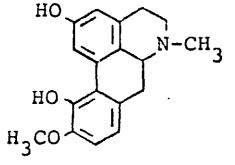
[3] 1% OV - 1; 3m x 2mm; oven temperature 100°.

The rest of the GLC conditions are described in Chromatography section, Page 58.

3. Apomorphine derivatives:- The GLC retention data of the methane- and benzeneboronate, trimethylsilylether and acetate derivatives of apomorphines are given in Table 26. The methane- and benzeneboronate derivatives of apomorphine (115) and N-n-propyl-norapomorphine(116), showed satisfactory GLC data, adequate for qualitative analysis, but not quantitative; because of their partial adsorption on the OV-25 phase. These derivatives were not stable in moist media, or on storage overnight in dry solvents (e.g. EtOAc, Pyridine). Successive trimethylsilylation or acetylation for the boronate derivatives of compounds (115-6), showed chemical instability of the boronate ring, and replacement by trimethylsiyl ether or acetate group. The trimethylsilyl ethers studied by Evans and Vouros[145] and Green, et al.[146]; and acetate derivatives of compounds (115-6), exhibited good GLC characteristics, showing perfect peaks useful for qualitative and quantitative analysis, with almost no adsorption. The trimethylsilyl ether derivatives showed instability on storage and underwent hydrolysis; while the corresponding acetate derivatives remained highly stable. Regularity and reproducibility was observed between the retention increments of the highly volatile derivatives, in that the decrement between methaneboronate and bis-trimethylsilyl ether, was 85; while the decrement between the less volatile benzeneboronate and acetate was 430, for each of compounds(115) and (116), as shown in Table 26.

4. Catechol estrogens:- The stable di-O-ethyl derivatives of catechol estrogens were studied by GLC, by Rosenfeld and

Table 26*, GLC Retention index values for aporphine type compound derivatives

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M) Derivatives ** MB	TMS	Ac	BB	Retention Increment, ΔI MB→TMS	TMS→Ac	Ac→BB	MB→BB
 No.115	$C_{17}H_{17}NO_2$	267	M,291 2920	M,411 2835	M,351 3450	M,353 3880	-85	615	430	960
 No.116	$C_{19}H_{21}NO_2$	295	M,319 3025	M,439 2940	M,379 3530	M,381 3965	-85	590	435	940
 No.117	$C_{17}H_{17}NO_3$	283	M,307 No Peak Temp.Prog 200-250°	M,499 2995	M,409 3845	M,369 No Peak Temp.Prog 240-280°		850		
 No.118	$C_{18}H_{19}NO_3$	297	/	M,441 3125	M,381 3745	/	/	620	/	/

* The GLC conditions were as follows:-

** These derivatives were measured on 1% OV-25; 2m x 2mm; oven temperature for the methaneboronate(MB) and trimethylsilyl ether (TMS) was 200°C and for the acetate (Ac) and benzeneboronate (BB) was 250°C.

The rest of the GLC conditions are described in Chromatography section, page 58.

Taguchi[156], for qualitative and quantitative measurements. Gelbke, et al.[153] studied the trifluoroacetate derivatives of 2-hydroxyestrone and 2-hydroxyestradiol, by GLC analysis. The methane- and benzeneboronates of 2- and 4-hydroxyestrone (15 & 16) were obtained analytically in good yields by Fotsis et al.[161], by using capillary GC, but no GC data were reported, except for trimethylsilyl ether derivatives of a number of estrogens (e.g. 2- and 4-hydroxyestriols). In our GLC studies the free catecholic estrogens 2- and 4-hydroxyestrone (15 & 16), and 2- and 4-hydroxy-17-deoxoestrone (17 & 18), showed peaks with considerable tailing, and poor volatility, which is largely associated with the free catecholic hydroxyl groups, their GLC I values are listed in Table 27. The GLC separation of the free isomers (15 & 16) or (17 & 18), was poor or lacking for example see Fig. 5.12(a&b). The methane-boronate derivative of compounds (15-18), failed to separate the isomeric 2- and 4-hydroxy compounds, although it increased their volatility and reduced their adsorption, as in Fig. 5.13. On the other hand, the corresponding benzeneboronate, did succeed in separating the two isomers, but at higher temperature (Table 27), and for example see Fig. 5.14. The benzene-boronate derivatives of the four isomeric compounds (15-18), were well separated on GLC (OV-17), showing symmetrical peaks with slight tailing, (Fig. 5.15). These boronate derivatives proved to be unstable on addition of excess Ac_2O or "BSA", yielding the corresponding diacetyl or di-trimethylsilyl derivatives; for example see Fig. 5.16. The GLC data for the boronate; trimethylsilyl and acetate derivatives for the catechol estrogens listed in Table 27; were highly reproducible.

1% OV-17, 2m x 2mm

250°C

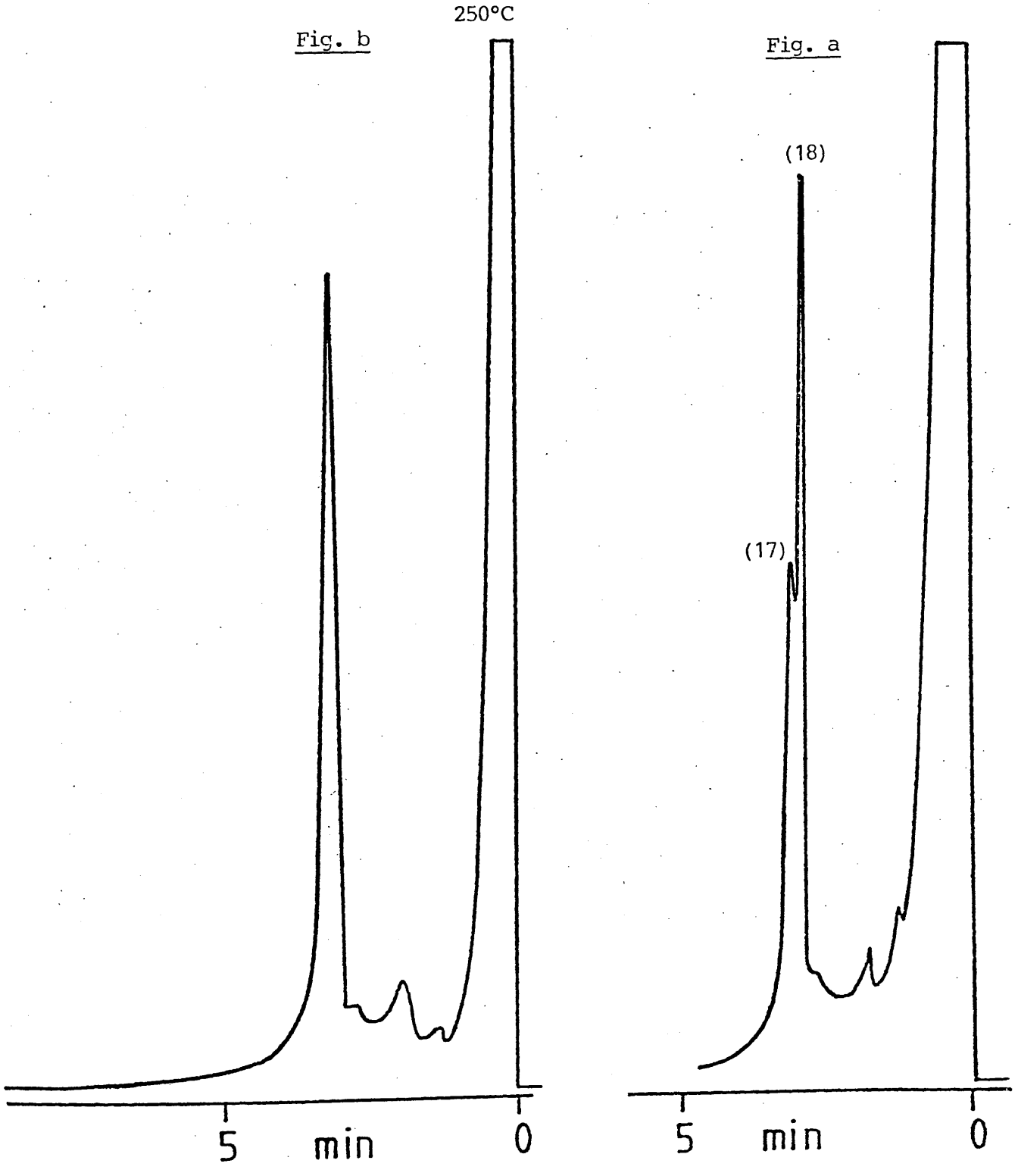


Fig. 5.12 (a&b) GLC Chromotogram for a mixture of 2-and 4-hydroxy-17-deoxoestrone (17&18), in EtOAc; total conc$^{n.}$ 1µg/µl.

Fig. 5.13 GLC Chromatogram of mixture of 2-& 4-hydroxy-17-deoxoestrone, (17 & 18) + methaneboronic acid (1:1.2)molar, in EtOAc, at R.T. for 2min; conc$^{n.}$ 1µg of each in µl. 1% OV-17, 2m x 2mm, 200°C.

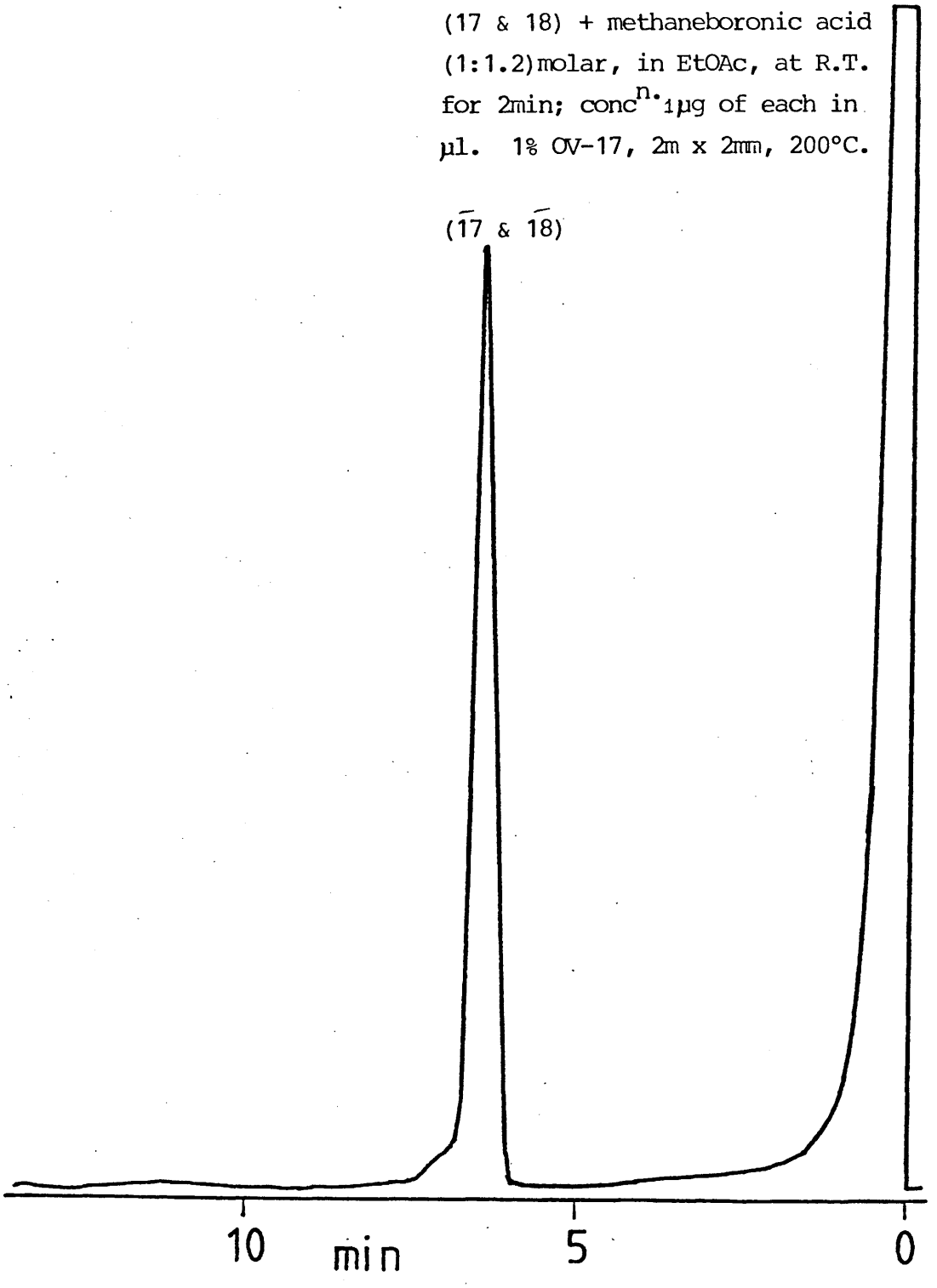
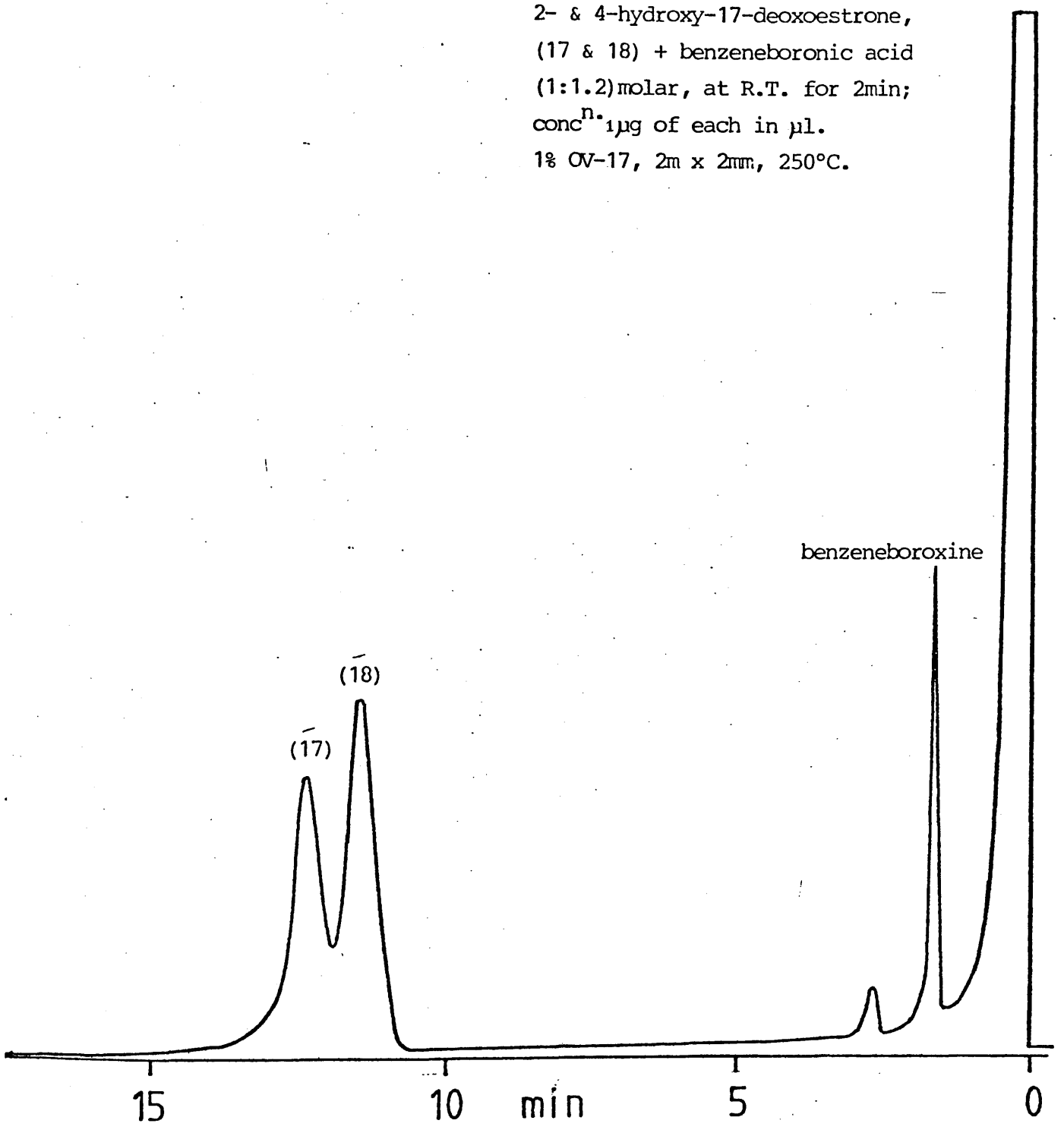


Fig. 5.14 GLC Chromatogram for mixture of 2- & 4-hydroxy-17-deoxoestrone, (17 & 18) + benzeneboronic acid (1:1.2) molar, at R.T. for 2min; conc$^{n.}$ 1µg of each in µl. 1% OV-17, 2m x 2mm, 250°C.



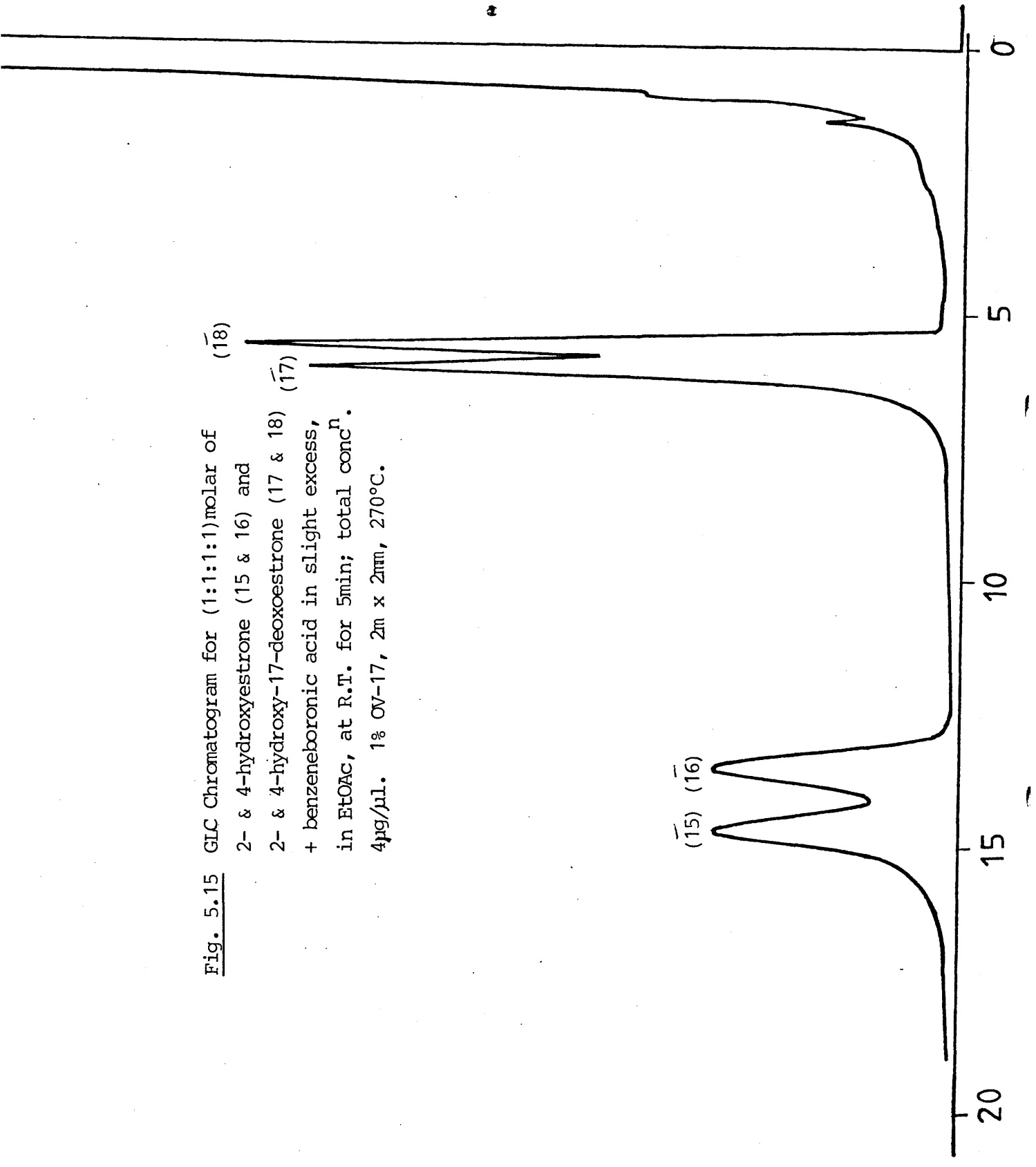


Fig. 5.15 GLC Chromatogram for (1:1:1:1)molar of 2- & 4-hydroxyestrone (15 & 16) and 2- & 4-hydroxy-17-deoxoestrone (17 & 18) + benzeneboronic acid in slight excess, in EtOAc, at R.T. for 5min; total conc^{n}. 4µg/µl. 1% OV-17, 2m x 2mm, 270°C.

Fig 5.16 GLC Chromatogram of the benzeneboronate solution of compounds: 2- & 4-hydroxyestrone (15 & 16) and 2- & 4-hydroxy-17-deoxoestrone (17 & 18) + excess Ac_2O in EtOAc-Pyridine, at 70° for 20min, total $conc^{n.}$ 4µg/µl. 1% OV-17, 2m x 2mm, 250°C.

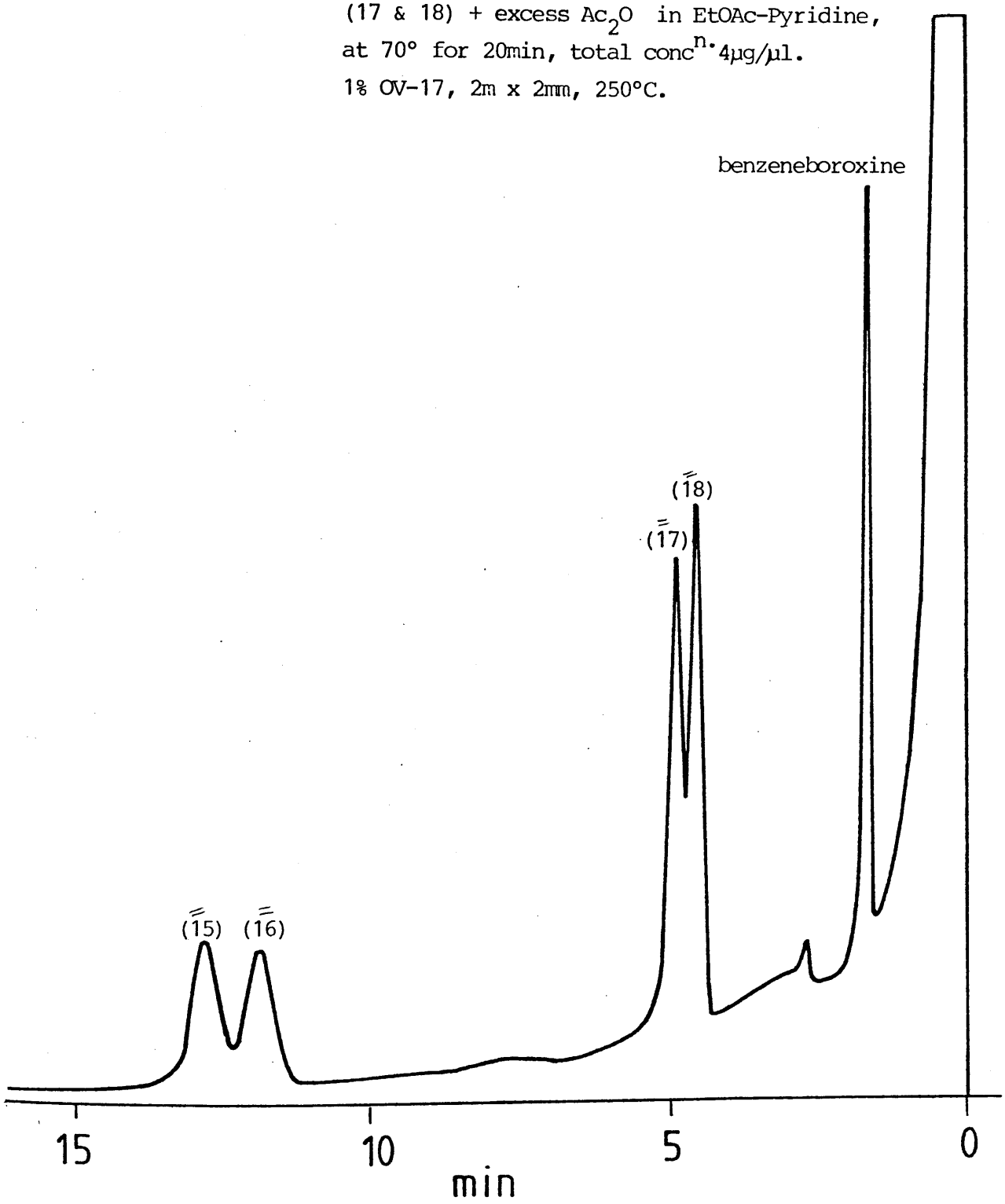
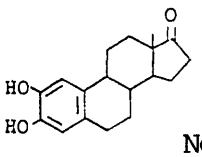
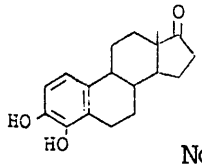
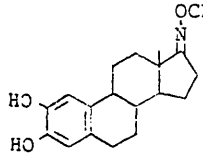
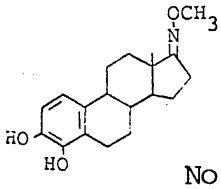
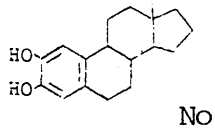
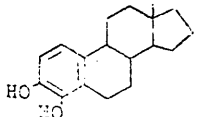
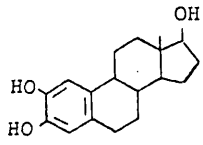
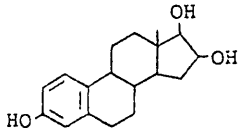


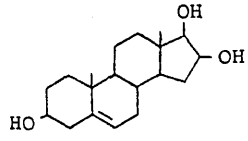
Table 17*, GLC Retention index values for 2-and 4-hydroxyestra-1,3,5(10)-trien-17-one, together with 17-deoxo-, 17-methyl oxime and 17-hydroxy type compounds;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M) Derivatives				Retention Increment, ΔI				Comment
			MB	TMS	Ac	BB	MB→ TMS	TMS→ Ac	Ac→ BB	MB→ BB	
 No.15	$C_{18}H_{22}O_3$	286	M,310 3160 [1] 2995 [2]	M,430 3210 [1] 3060 [2]	M,370 3795 [1] 3540 [2]	M,372 4155 [1] 3905 [2]	50 65	585 480	360 365	995 910	(a)
 No.16	$C_{18}H_{22}O_3$	286	M,310 3150 [1] 2985 [2]	M,430 3260 [1] 3105 [2]	M,370 3770 [1] 3515 [2]	M,372 4120 [1] 3875 [2]	110 120	510 410	350 360	970 890	(b)
 No.42	$C_{19}H_{25}NO_3$	315	M,339 3180 [1] 3015 [2]	M,459 3235 [1] 3095 [2]	M,399 3800 [1] 3550 [2]	M,401 4160 [1] 3920 [2]	55 80	565 455	360 370	980 905	
 No.43	$C_{19}H_{25}NO_3$	315	M,339 3180 [1] 3015 [2]	M,459 3290 [1] 3135 [2]	M,399 3770 [1] 3525 [2]	M,401 4120 [1] 3885 [2]	110 120	480 390	350 360	940 870	
 No.17	$C_{18}H_{24}O_2$	272	M,296 2775 [1] 2655 [2]	M,416 2835 [2] 2735 [2]	M,356 3400 [1] 3200 [2]	M,358 3760 [1] 3560 [2]	60 80	565 465	360 360	985 905	(c)
 No.18	$C_{18}H_{24}O_2$	272	M,296 2765 [1] 2640 [2]	M,416 2875 [1] 2765 [2]	M,356 3370 [1] 3170 [2]	M,358 3730 [1] 3530 [2]	110 125	495 405	360 360	965 890	(d)

Continued Table 27*,

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)				Retention Increment, ΔI				Comment
			MB	TMS	Ac	BB	MB→TMS	TMS→Ac	Ac→BB	MB→BB	
 No.47	$C_{18}H_{24}O_3$	288	M,312 * 3115 [1] 220°	M,504 3025 [1] 220°	M,414 3825 [1] 250°	M,374 * 4190 [1] 250°	-90	800	365	1075	*(e)
 No.120	$C_{18}H_{24}O_3$	288	M,312 No Peak Temperature Programme 180-250°	M,504 3110 [1] 220°	M,414 3780 [1] 250°	M,374 No Peak Temperature Programme 220-280°		670			

Continued Table 8,

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt.(M) Retention Increment, ΔI							Comment
			Parent Compound	Derivatives			MB→TMS	OH→OMB	OH→OTMS	TMS→Ac	
				MB	TMS	Ac					
 No.121	$C_{19}H_{30}O_3$	306	3410 [1] 3175 [2]	M,330 3100 [1] 2940 [2]	M,522 3030 [1] 2965 [2]	M,432	-70 25	310 235	380 210		

Continued Table 27

* Oven temperature for the parent compounds (if measured) and the trimethylsilyl ether (TMS) and methaneboronate(MB) derivatives was 240° and for the acetate (Ac) and benzeneboronate (BB) was 270°.

The remaining conditions were as in Table 25.

COMMENTS

(a) I value for the free catechol was measured at 250°; 3660 [1] & 3370 [2].

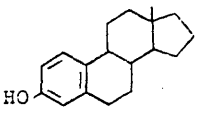
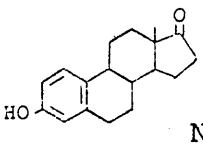
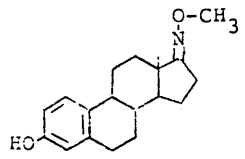
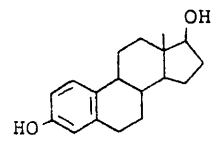
(b) I value for the free catechol was measured at 250°; 3625 [1] & 3345 [2].

(c) I value for the free catechol was measured at 250°; 3225 [1] & 3000 [2].

(d) I value for the free catechol was measured at 250°; 3200 [1] & 2975 [2].

(e) The methaneboronate(MB) & benzeneboronate (BB) derivatives showed very weak, broad peaks but these were not reproducible.

Table 28*, GLC Retention index values for estrone and estrone type compounds and derivatives;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)			Retention Increment, ΔI			
				Derivatives					
			Parent Compound	TMS	Ac	OH→ OTMS	OH→ Ac	TMS→ Ac	Comment
 No.48	$C_{18}H_{24}O$	256	2715 [2] 240°	M,328 2620 [2] 240°	M,298 2840 [2] 260°	-95	125	220	
 No.40	$C_{18}H_{22}O_2$	270	3295 [1] 3065 [2] 240°	M,342 3115 [1] 2955 [2] 240°	M,312 3420 [1] 3205 [2] 270°	-180 -110	125 140	305 250	
 No.41	$C_{19}H_{25}NO_2$	299	3310 [1] 3090 [2] 240°	M,371 3145 [1] 2985 [2] 240°	M,341 3420 [1] 3210 [2] 270°	-165 -105	110 120	275 225	
 No.119	$C_{18}H_{24}O_2$	272		** M,416 3100 [1] 2950 [2] 240°	M,356 3480 [1] 3280 [2] 270°			380 330	(a)**

*GLC conditions as in table 25.

Comments

(a) GLC showed the mono-TMS peak, I values were; 2940 [1] and 2855 [2] at 240°.

For each isomeric compound the average retention increment between trimethylsilylether and acetate groups was 435 (OV-17) and 530 (OV-25); between the acetate and the benzeneboronate groups was 360, on both columns; and between the methane-and benzeneboronate groups was 890 (OV-17) and 960 (OV-25). A number of estrogens: estrone(40), 17-deoxoestrone (48) and estradiol(119); free and as their trimethylsilyl ether and acetate derivatives were also studied, and their GLC I values are listed in Table 28. These compounds have been studied for comparison with the catechol estrogens in Table 27.

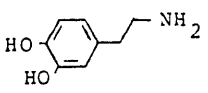
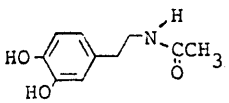
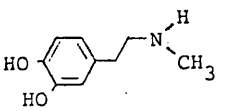
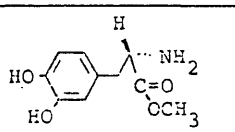
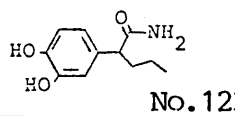
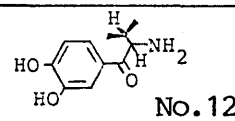
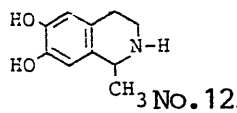
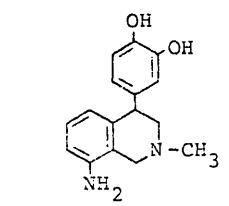
B. Catechols with other (mono) reactive group

1. 2-Hydroxyestradiol(47); 16-epiestriol(120) and 2,10,11-trihydroxyapomorphine(117):- The di-O-ethyl[156], trimethylsilylether[161], acetate[186] and trifluoroacetate[153] derivatives of compound(47) and some poly hydroxy catecholic estrogens; also the trimethylsilyl derivatives of compound (117) studied by Green, et al.[146] and Evans and Vouros[145]; have been studied by GLC. They failed to show any corresponding boronate derivative peak on GLC columns (OV-17 & OV-25). This disappearance of the boronate peak was possibly due to the free single hydroxyl group in the three catecholic compounds(47), (120) and (117); which possibly causes high adsorption and polymerisation on the column. At the same time the non catecholic trihydroxyl steroid compounds: 5-androstene-3β,16β,17β-triol(121) and 5β-pregnane-3∝-17∝, 20∝-triol(122) both showed good GLC peaks for their cyclic boronate derivatives. On the other hand 2- and 4-hydroxy-

estrone showed good GLC boronate peaks (Table 27:- also see this chapter, p. 183. To overcome this problem, selective trimethylsilylation[156] (or acetylation) of the free hydroxyl group was attempted under mild conditions, but the products shown by GLC were mainly the partly and fully trimethyl-silylated (or acetylated) products, apparently due to the instability of the boronate ring during farther derivatisation of these catecholic compounds,[see this chapter, p.184]. In other compounds; e.g. 3∝,17∝, 21-Trihydroxy-5β-pregnan-20-one (Anthony,et al.[21]): and prostaglandin F boronate derivatives were stable towards trimethylsilylation of the free hydroxyl group. The trimethylsilyl ether and acetate derivatives of compounds (47), (120) and (117), showed complete derivatisation and excellent peaks, with no adsorption on GLC columns (OV-17 & OV-25); see Tables 26 and 27.

2. Dopamine derivatives:- Catecholamines were mainly studied by GLC, as their fully acetylated derivatives [141,147, 162,176-7]. To obtain higher volatility, the catecholamines were firstly selectively N-acetylated, then O,O-di-trimethyl-silylated[145-7,155,157-8,187]. In our technique for selective cyclic boronate derivative formation with the catechol group, of the compounds listed in Table 29; the boronate derivatives of these compounds with the free amino group were unstable during storage in solution (e.g. EtOAc, pyridine, hexane); the solutions became dark. These compounds also showed considerable tailing and adsorption on columns (OV-17, OV-25), due to the high polarity of the free amino group. If selective acetylation was attempted for these catecholamine boronate compounds; the result was a complete displacement

Table 29*, GLC Retention index values for dopamine and dopamine type derivatives;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M) Derivatives MB	TMS	Ac	BB	Retention Increment, ΔI MB → TMS	TMS → Ac	Ac → BB	MB → BB	Comment
 No.19	$C_8H_{11}NO_2$	153	M,177 1765 [1] 1635 [2] 160°	M,297* 1910 [1] 1825 [2] 160°	M,279 2820 [1] 2610 [2] 220°	M,239 2600 [1] 2425 [2] 220°	145 190	910 785	-220 -185	835 790	*(a)
 No.20	$C_{10}H_{13}NO_3$	195	M,219 2275 [1] 2135 [2] 160°	M,339* 2405 [1] 2300 [2] 160°	M,279 2820 [1] 2610 [2] 220°	M,281 3150 [1] 2930 [2] 220°	130 165	415 310	330 320	875 795	*(b)
 No.106	$C_9H_{13}NO_2$	167	M,191 1925 [1] 120° 1720 [2] 140°	M,311* 1910 [1] 120° 1845 [2] 140°	M,293 2785 [1] 180° 2640 [2] 200°	M,253 2525 [1] 180° 2420 [2] 200°	-15 125	875 795	-260 -220	600 700	*(c)
 No.50	$C_{10}H_{13}NO_4$	211	M,235 2090 [1] 160°	M,355* 2240 [1] 160°	M,337 2950 [1] 220°	M,297 2940 [1] 220°	150	710	-10	850	*(d)
 No.123	$C_{11}H_{15}NO_3$	209	M,233 2325 [1] 170°	M,353 2235 [1] 170°	M,293 2850 [1] 220°	M,295 3190 [1] 220°	-90	615	340	865	
 No.124	$C_{11}H_{15}NO_3$	209	M,233 2155 [1] 170°	M,353 2175 [1] 170°	M,335 3110 [1] 240°	M,295 (3375) [1] 240°	20	935	(265)	(1220)	BB was a weak broad peak.
 No.125	$C_{10}H_{13}NO_2$	179	M,203 1925 [1] 150°	M,323 2085 [1] 150°	M,305 3065 [1] 220°	M,265 2825 [1] 220°	160	980	-240	900	(e)
 No.126	$C_{16}H_{18}N_2O_2$	270	M,294 3155 [1] 2930 [2] 250°	M,414* 3165 [1] 2975 [2] 250°	M,396	M,356	10 45				*(f)

* The GLC conditions as in Table 25.

COMMENTS

(a) GC-MS showed the O,O - Di-TMS derivative only. The NH-TMS seemed unstable. The I values for the tri-propanyl derivative, at 220° were; 3045 [1] and 2930 [2].

(b) The free dopamine and N-acetyl dopamine, did not show any peak on temperature programming 160 - 220° on either columns [1] or [2]. The I values for N-acetyl dopamine 3,4-dipropanoyl derivative, at 220° were; 2990 [1] and 2775 [2].

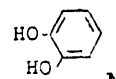
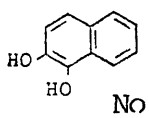
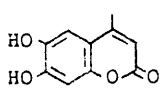
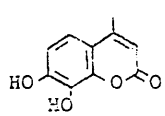
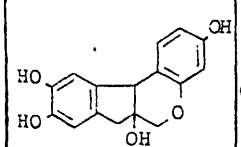
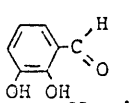
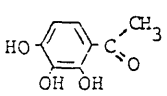
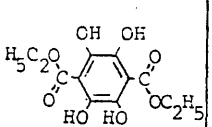
(c), (d) & (f) GC-MS, showed the O,O-Di-TMS derivative only.

(e) The I value for the n-butaneboronate derivative, M,277, was 2275 [1] at 170°.

of the boronate group by acetyl groups. The methane- and benzeneboronate derivatives of the selectively N-acetylated, or methylated catecholamines (e.g. N-acetyldopamine(20) and ∝-methyldopamine(106); showed high stability on storage and good GLC peaks with only partial tailing on columns (OV-17 & OV-25) [Table 29]. The acetate derivative of the catecholamines listed in Table 29, proved to be highly stable in solution and in GLC, showing good GLC peaks, without adsorption. Their corresponding trimethylsilyl ether derivatives showed good GLC peaks for the N-acetylated or methylated compounds [e.g. (20) & (106)]; but the compounds with the free amino group showed the O,O-di-trimethylsilyl ether derivatives only and the N-TMS ethers proved to be unstable under the GLC conditions[145,187], as stated in Table 29.

3. Brazilin (111):- This compound has two catecholic; one phenolic and one aliphatic hydroxyl groups (see structure and GLC data on Table 30). It formed a cyclic boronate derivative with methaneboronic acid, and showed a moderately good GLC peak with tailing on column OV-25, while the benzeneboronate did not show any corresponding peak due to higher molecular weight and polarity. The boronate derivative of brazilin proved to be unstable in solution, suffering hydrolysis and subsequent decomposition, after a few hours. The trimethylsilyl derivative showed a good GLC peak without adsorption, while the acetate derivative showed no peak on GLC (OV-25).

Table30*, GLC Retention index values for some catechol type compounds;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)					Retention Increment, ΔI				Comment
			Parent Compound	Derivatives								
				MB	TMS	Ac	BB	MB → TMS	TMS → Ac	Ac → BB	MB → BB	
 No.73	$C_6H_6O_2$	110	1370 [1] 90°	M,134 1085 [1] 90°	M,254 (a)	M,194 1725 [1] 150°	M,196 1885 [1] 150°			160	800	(a)
 No.112	$C_{10}H_8O_2$	160	No Peak Temp. Programme 140-200° [1] & [2]	M,184 1755 [1] 1685 [2] 140°	M,304 1965 [1] 1905 [2] 140°	M,244 2370 [1] 190° 2215 [2] 200°	M,246 2585 [1] 190° 2470 [2] 200°	210 220	405 310	215 255	830 785	
 No.128	$C_{10}H_8O_4$	192		M,216 2385 [1] 2265 [2] 200°	M,336 2540 [1] 2460 [2] 200°	M,276 2905 [1] 2720 [2] 240°	M,278 3315 [1] 3105 [2] 240°	155 195	365 260	410 385	930 840	
 No.14	$C_{10}H_8O_4$	192		M, 216 2425 [1] 2305 [2] 200°	M,336 2450 [1] 2365 [2] 200°	M,276 2895 [1] 2700 [2] 240°	M,278 3345 [1] 3135 [2] 240°	25 60	445 335	450 435	920 830	
 No.111	$C_{16}H_{14}O_5$	286	No Peak Temp. Programme 200-230° [1]	M,310 3390 [1] 230°	M,502 2985 [1] 230°	M,412 No Peak Temp. Programme 230-290°	M,372 No Peak Temp. Programme 230-290°	-405				Tri-TMS $M^{+\cdot}$=502
 No.44	$C_7H_6O_3$	138	1450 [1] 1360 [2] 120°	M,162 1580 [1] 1445 [2] 120°	M,282 1775 [1] 1725 [2] 150°	M,222 2030 [1] 1905 [2] 180°	M,224 2290 [1] 2175 [2] 180°	195 260	255 180	260 270	710 730	
 No.124	$C_8H_8O_4$	168	1820 [1] 1670 [2] 120°	M,192 1805 [1] 1720 [2] 120°	M,384 1970 [1] 1910 [2] 150°	M,294 2475 [1] 2330 [2] 200°	M,254 2665 [1] 2530 [2] 200°	165 190	505 420	190 200	860 810	
 No.130	$C_{12}H_{14}O_8$	282		M,334 No Peak on either column [1]&[2] Temp. Prog. 120-200°	M,574 2390 &[1] 2475 2305 &[2] 2400 200°	M,454 2960 &[2] 2995 240°	M,458 No Peak on either column [1]&[2] Temp. Prog. 120-280°		655 595			

* The GLC conditions as in Table 25.

Comments

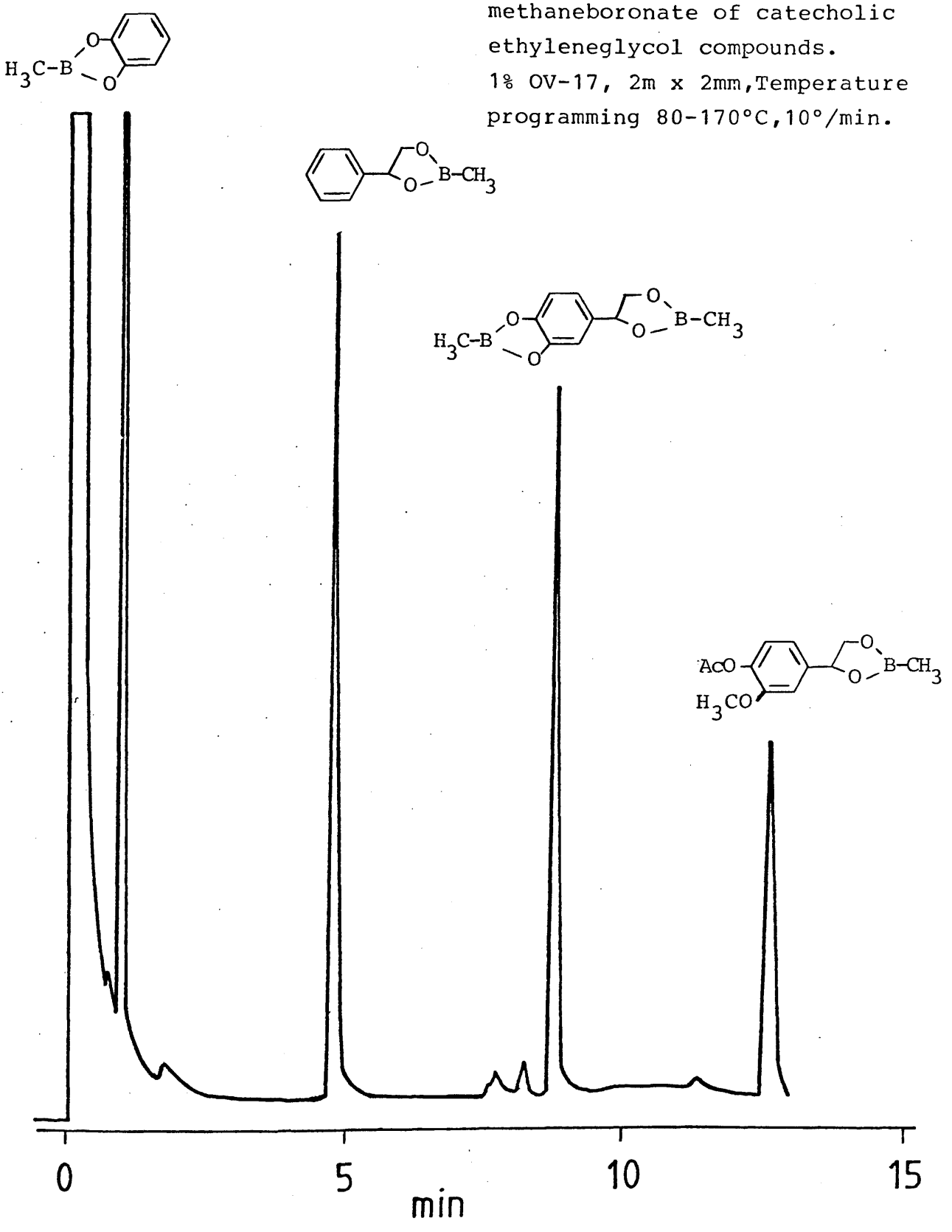
(a) The trimethylsilyl ether (TMS) peak did not appear at 80 - 120°, mainly the reagent with the solvent peak.

C. Compounds with other proximal dihydroxy boronate forming groups

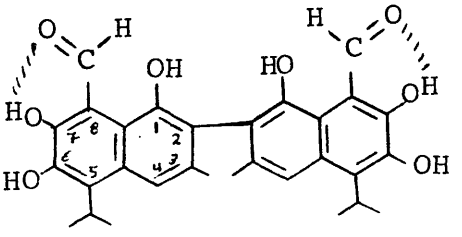
1. Styrene glycol(131); 3,4-dihydroxystyrene glycol(132), and dl-4-hydroxy-3-methoxystyrene glycol(38):- Biondi, et al.[26] studied the condensation of compounds(132) and (38) with methane- or n-butaneboronic acids with or without previous acetylation of the phenolic groups; giving suitable derivatives for GLC and GC-MS analysis. In our studies for suitable derivatives to separate mixtures of compounds(131,132 & 38), by GLC technique, trimethylsilyl and acetyl derivatives were examined. Derivative formation was found to be incomplete for (132) and (38), and always there were some partly trimethylsilyled or acetylated by products. Methaneboronic acid selectively formed stable cyclic boronate derivatives with compounds(131,132&38); and formed a bis-methaneboronate with compound(132). The boronates showed good GLC peaks with slight tailing, except for compound(38), which was highly adsorbed on the GLC column with considerable tailing, due to the polarity of its 4-hydroxy group. Selective acetylation of the 4-hydroxyl group of compound(38), remedied this. GLC showed good separation for a mixture of compounds(131),(132),(39) and catechol(73), after methaneboronate formation, as shown on Fig. 5.17. The bis-benzeneboronate of compound(132) did not show any corresponding peak on GLC analysis, due to high molecular weight and polarity. For GLC data of these compounds see Table 31.

2. Gossypol(34):- This compound or its corresponding: methyloxime(136) (see p.87); or hemiacetal-methyl ether(37); (crude, from gossypol + MeOH + p.TSA, at R.T. for 20min); or apogossypol(36)[188] (crude), derivatives failed to form

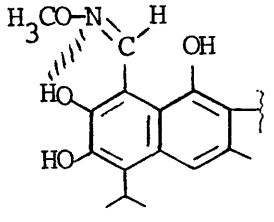
Fig. 5.17 GLC Chromatogram of the methaneboronate of catecholic ethyleneglycol compounds. 1% OV-17, 2m x 2mm, Temperature programming 80-170°C, 10°/min.



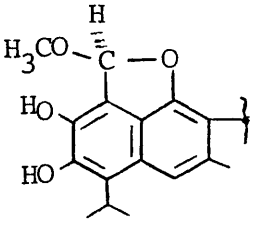
a bis-cyclic boronate derivative with methaneboronic acid; as judged by GLC on column OV-25, temperature programming 200-300°; but these gossypol derivatives showed on GLC good symmetrical peaks corresponding to hexa-trimethylsilyl ether derivatives with $I^{1\%OV-25}_{280°}$ =4040; 4005; (4100&4125) and 3875, respectively. Gossypol is a highly polar compound, and there is also strong intramolecular hydrogen bonding between its polar functional groups[183], especially between the aldehydic carbonyl groups and the adjacent catecholic hydroxyl groups. The conversion of the aldehyde groups into O-methyloximes led to a remarkable reduction in polarity as judged by TLC in the mobile phase $CHCl_3$-EtOAc (2:1,v/v): gossypol R_f 0.25, bis-O-methyloximes 0.66, gossypol hexa-acetate 0.50.



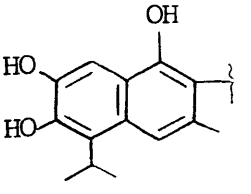
(34)



(136)

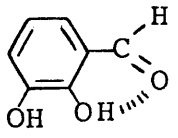


(37)

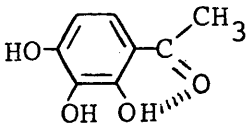


(36)

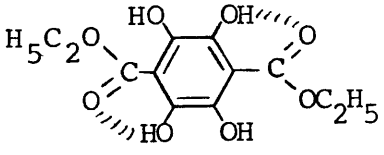
Tetrahydroxyterephthalic acid diethyl ester(130), also failed to show on GLC, a peak corresponding to its cyclic bis-methaneboronate derivative; this is ascribed to the hydrogen bonding between the di-esters and the catecholic hydroxyl groups. This kind of hydrogen bonding was not so strong, to prevent 2,3-dihydroxybenzaldehyde(44), or 2,3,4-trihydroxyacetophenone(129), from forming detectable cyclic boronate derivatives, (see Table 30); but these boronates were not stable in solution on storage. Compounds(44,129& 130), all showed good GLC peaks, as trimethylsilyl ether or acetate derivatives, on both columns (OV-17 & OV-25); (Table 30).



(44)



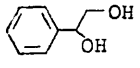
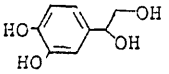
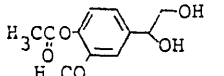
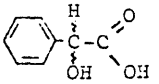
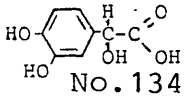
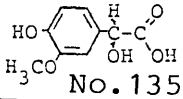
(129)



(130)

These above results suggests, that the best derivative for the analysis of gossypol by GLC is the trimethylsilyl ether; which has a high volatility and formed easily; but the only disadvantage is its high molecular weight(950).

Table31* GLC Retention index values for styrene glycol and catechol α-hydroxyacid type derivatives;

Parent Compound	Formula	Mol.Wt.	Retention Index, I and Mol.Wt. (M)					Retention Increment, ΔI				Comment
			Parent Compound	Derivatives				MB → TMS	TMS → Ac	Ac → BB	MB → BB	
				MB	TMS	Ac	BB					
 No.131	$C_8H_{10}O_2$	138	1700 [1] 1565 [2] 140°	M,162 1400 [1] 80°	M,282	M,222 Di 1840 [1] 1745 [2] 140°	M,224 2235 [1] 170°			395	835	(a)
 No.132	$C_8H_{10}O_4$	170	No Peak Temp. Programme 120-240°	M,218 1745 [1] 1680 [2] 140°		M,254 Tetra 1915 [2] 140°						(b)
 No.39	$C_{11}H_{14}O_5$	226	2095 [2] 180°	M,250 2045 [2] 180°	M,370 2065 [2] 180°		M,312 2600 [2] 230°	20			555	
 No.133	$C_8H_8O_3$	152	No Peak Temp. Programme 80-130°	M,176 1570 [1] 80°	M,296 No Peak 80°		M,238 2335 [1] 170°				765	(c)
 No.134	$C_8H_8O_5$	184		M,232 1855 [1] 120°	M,572 No Peak 80°							(d)
 No.135	$C_9H_{10}O_5$	198		M,222 2055 [1] 120°	M,414 No Peak 80-120°							(e)

* GLC conditions as in Table25.

Comments

(a) The Mono Ac M,180 I=1780 [1] 1665 [2], 140°

(b) The Di Ac M,212 I=1645 [1] 1565 [2], 140°

(c), (d) & (e) The trimethylsilyl ether (TMS) reaction product showed reagent peak only.

Chapter 6

GAS CHROMATOGRAPHY- MASS SPECTROMETRY OF CYCLIC BORONATE DERIVATIVES OF DIOLS AND OF COMPOUNDS OF CATECHOL TYPE

RESULTS AND DISCUSSION

In selecting a derivative for GC-MS it is not only necessary to consider the increased volatility obtained, but also the structural information which can be acquired from specific ions associated with the fragmentation of the new functionality. Our choice of cyclic boronate derivatives along with acetate and trimethylsilyl ether derivatives for GC-MS analysis of compounds of catechol type allowed comparative evaluation of their utility. The mass spectra of catechol amines and catechol estrogens have been studied by GC-MS, as their TMS ethers[145,146,156,161], as acetate derivatives[83,141,158,162], or as a combination of both derivatives, in the compounds with multifunctional polar groups (e.g. OH, NH_2)[160]; they were also studied as labelled compounds[141,145,158].

* The following abbreviations will be used throughout this chapter:-

Acetate = Ac; trimethylsilylether = TMS ether; methaneboronate = MB and benzeneboronate = BB.

The potential selectivity and ease of reaction of boronic acids (e.g. methane-,n-butane-, cyclohexane- and benzene-boronic acid), with diols by forming cyclic boronate derivatives, for GC-MS studies was first realised when satisfactory results were obtained by Brooks and Watson[30,97]. The cylic nature of these boronate derivatives; in some cases

directs the mode of mass-spectrometric fragmentations[40,66,189]. Although the relative intensity of certain fragments is influenced by the substituent on the boron atom, the general mode of break down was found to be the same for many methane-, n-butane-, cyclohexane- and benzeneboronates studied[40,66,190]: examination of these boronate analogues has facilitated the interpretation of mass spectra by a "substituent shift" technique. In addition, the mass spectra frequently contained enhanced molecular ions: the identification of these, and of other ions containing boron, was facilitated by the characteristic isotope ratio of boron($^{11}B:^{10}B$,4:1)[97, 189, 190].

The mass spectra of the cyclic boronate derivatives of catechol amines and catechol estrogens have not been studied before. We were interested in characterising, by GC-MS, a number of catecholic type of compounds and diols, as their boronate derivatives (see Tables I-IX)*.

The selectivity of formation of cyclic boronates and also their ease of displacement by acetylating or trimethylsilylating reagents (see Chap. 5), which allows the application of sequential derivatisation procedures, are useful features for analysis of biologically important catechols, which usually exist in low concentrations. In our GC-MS studies of the boronate derivatives of catecholic compounds, the corresponding diacetates and di-TMS ethers were included for comparison (see Tables I-VII). Formation of a benzeneboronate increases the molecular weight by 86, as compared

* See Appendix for Tables I-IX and Figs. 6.2-5.

with 84 for a diacetate and 144 for a bis-TMS ether; the increment for each methaneboronate is only 24 units. Methaneboronates are potentially more suitable as derivatives than the corresponding diacetate and bis-TMS ether derivatives, especially for compounds with high molecular weight; such as brazilin (Table VI), catechol estrogens (Table VII, and Figs. 6.2-3) and apomorphines (Table IV, and Fig. 6.1). All the compounds studied in Tables I-IX, showed good molecular ions. Under electron impact GC-MS the boronate derivatives of the compounds listed in Tables I-VII showed higher stability than their corresponding diacetates and di-TMS ethers. Thus the calculated average abundances of molecular ions as percentages of the base peaks in these compounds are as follows: MBs54%, BBs50%, diacetates 8% and di-TMS ethers 33%.

A range of boronic acids with different molecular weights and structure (e.g. methaneboronic acid, molecular weight 60; 3-methyl-5α-cholestane-2-boronic acid, molecular weight 430) have been used to study the catecholic type of compounds and vicinal diols. Under electron impact GC-MS the C-B bond was highly stable in the MB and BB derivatives (see Tables 1-VII); but it was less stable in the cyclohexane-; norbornane-2-; β-pinane-10-; 3-methyl-5α-cholestane-2-, and 5β-cholane-24-boronates; these showed fragment ions representing their corresponding alkenes; as in the following examples (also see Tables VIII-IX):-

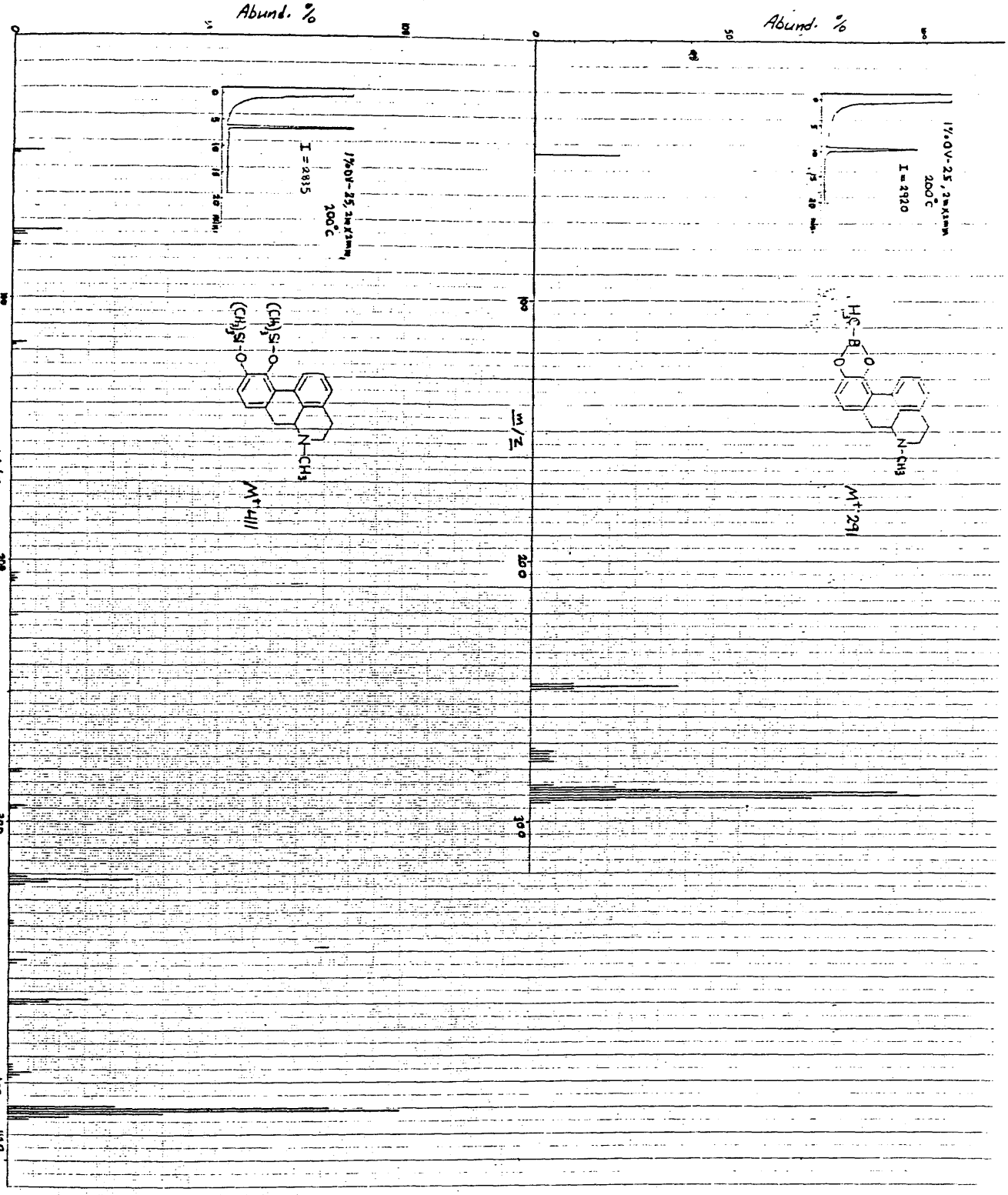
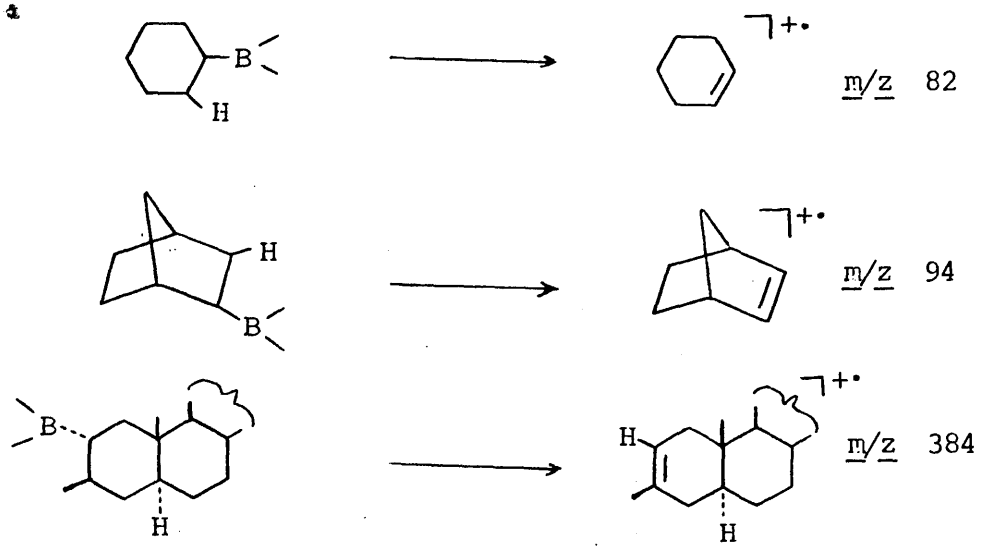


Fig. 6.1 Gas chromatograms and mass spectra of apomorphine derivatives.

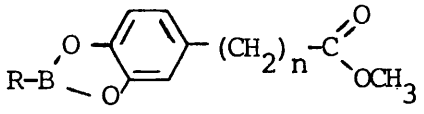


The mass spectral fragmentation pattern was identical for the stereoisomeric boronate derivatives of the exo and endo isomers of catechol norbornane-2- and β-pinane-10-boronate (see Table VIII) ; and was also the same for the stereoisomeric dl and meso 2,3-butanediol boronates of these compounds. GLC afforded distinction between some isomers (see Chap. 4, pp.117-121, also Scheme 4.2,p.120). Similarly the MB, BB derivatives of the isomeric pairs 4-methyl-6,7- (and -7,8-)-dihydroxycoumarin (Table VI), 2- and 4-hydroxyestrone and 2- and 4-hydroxy-17-deoxo-estrone (Table VII, see also Figs. 6.4-5), showed no significant differences in fragmentation modes; but GLC succeeded in separating the isomers (see Chap.5, Fig.5.15,p.189).

The mass spectral fragmentation modes of compounds which are listed in Tables I-IX, showed several ions which showed appropriate mass shifts with different substituents on the boron atom, suggesting that they contained the boronate moiety; as studied in the following compounds:-

A. Boronates of methyl esters of acids containing catechol groups:- These compounds showed high abundance of molecular ions (Table I) and high stability of the boronate ring:

Table 6.1. Relative abundance of boron-containing ions from boronates of methyl esters of acids containing catechol groups.



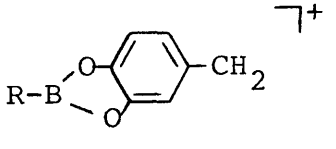
R; a = CH_3 or b = C_6H_5

I, n = 0 II, n = 1 III, n = 2 IV, n = $(CH)_2$

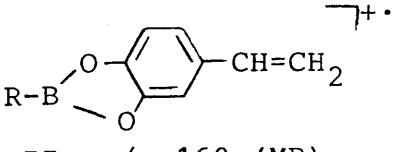
Compound	Derivative R	m/z, (% abundances) M-15	M-31	M-59	M-73
I	a	174 (4)	161 (88)	/	/
	b	/	223 (38)		
II	a	/	/	147 (100)	/
	b			209 (100)	
III	a	/	189 (5)	161 (14)	147 (100)
	b		251 (3)	223 (14)	209 (86)
IV	a	203 (0.5)	187 (45)	159 (3)	/
	b		249 (24)	221 (3)	

the main fragmentation occurred at the ester group, by loss of the CH_3, OCH_3 and CO_2CH_3 groups (and in the dehydro cinnamates, also loss of $CH_2CO_2CH_3$). The complimentary ions contained the boronate group; as shown in Table 6.1 . These boron-containing ions could be verified from their "substituent shifts" from the MB to the BB (Δ m + 62); for example compound I, MB and BB, Table 6.1, showed m/z 161 and 223 [M-31] respectively. The isotopic ratio of $^{11}B:^{10}B$, for m/z, 161/160 (4:1, see Table I), also indicated the presence of the boron atom.

B. Compounds related to dopamine:- These compounds are cited in Table III. The MB and BB of dopamine (previously studied as its tri-pentafluoro derivative[141]); N-acetyl-dopamine (previously studied as its di-O,O-TMS ether[160]); α-methyldopamine (previously studied as its tri-pentafluoro derivative[142]), and methyl L-Dopa all showed fragments resulting from the alkyl amide side chain and gave fragments as in the ion(I), m/z 147(MB) and m/z 209(BB), which represents a benzylic ion; and cinnamic radical ions(II) at m/z 160(MB) and m/z 222(BB). These ions retained the boronate moiety.



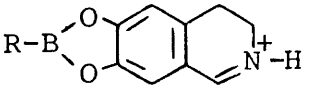
I, m/z 147 (MB)
209 (BB)



II, m/z 160 (MB)
222 (BB)

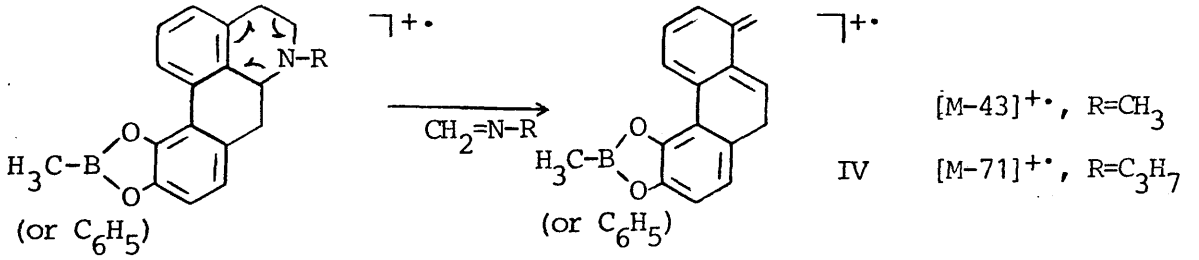
C. Salsolinol:- The MB and BB derivatives of this compound showed mainly the loss of the methyl group α to the secondary amino group, yielding an ion(III), which retained the boronate group and was detected as the base peak, (see Table IV).

Sjöquist and Magnuson[162] have studied the tri-pentafluoropropionyl derivative of salsolinol, for which the base peak also resulted from elimination of the CH_3 group.



III, m/z 188 (MB)
250 (BB)

D. N-methyl-and n-propylapomorphine:- The methaneboronates showed a diagnostically significant ion which is obtained from a retro Diels-Alder fragmentation (ion IV, Scheme 6.1). This fragmentation originates from both $[M]^{+\cdot}$ and $[M-1]^{+}$, and can be used to distinguish between n-methyl and n-propyl substituents (see Table IV). The mass spectra of di-TMS ethers of these compounds were also studied by Green et al.[146].



IV $[M-43]^{+\cdot}$, R=CH_3
$[M-71]^{+\cdot}$, R=C_3H_7

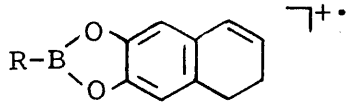
Scheme 6.1

3,4-dihydroxynomifensine also showed fragmentation, by similar elmination of $[M-43]^{+\cdot}$, as the apomorphine derivatives (see Table IV).

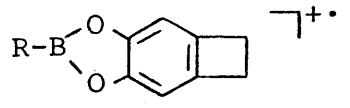
E. Styrene glycols:- The methaneboronate derivatives for these compounds, listed in Table V, all showed base peaks corresponding to the molecular ion, while minor fragmentation occurred at the glycol boronate group. The mass spectra of the MB and n-butaneboronate derivatives of these compounds along with their acetate derivatives were studied by Biondi et al.[26].

F. 4-Methyl dihydroxycoumarins:- The MB and BB derivatives of these compounds as in Table VI, showed high stability under electron impact, and the only major fragmentation was the loss of CO affording $[M-28]^{+\cdot}$ in both examples.

G. 2- and 4-Hydroxyestrone and 2- and 4-hydroxy-17-deoxoestrone:- The MB and BB derivatives of these pairs of isomers all showed molecular ions as their base peaks (see Table VII), but also fragmented between rings B and C, affording ions at m/z 186 (MB) and m/z 248 (BB), as in the radical ion (V). Other ions at m/z 160 (MB) and m/z 222 (BB), are postulated to be of type (VI). The mass spectrum of deuterium labelled



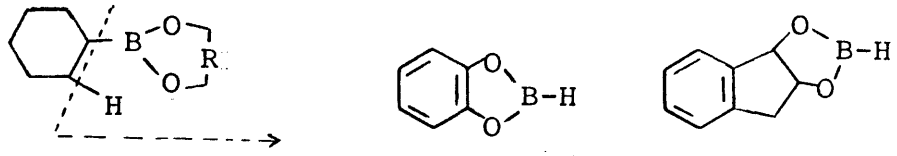
V, R, m/z 186 (MB)
248 (BB)



IV, R, m/z 160 (MB)
222 (BB)

2-hydroxyestrone was determined by GC-MS, by Knuppen et al.[154] and its 2,3-diethyl-17β-TMS ether, GC-MS, was also studied[156]; and 4-hydroxyestradiol as Tri-TMS ether by Fotsis et al.[161].

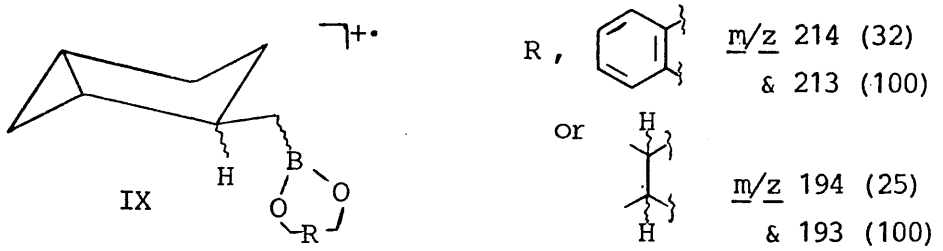
H. Diol alkaneboronates:- The catechol and cis-indane diol cyclohexane-; norbornane-2-; β-pinane-10-; and 3-methyl-5α-cholestane-2-boronate, all showed fragmentation mainly occurring at the C-B bond, with abstraction of a hydrogen atom from the C-H group which is α to the C-B bond; this was indicated by the formation of radical ions of catecholborane (VII) m/z 120; and the indanodioxoborolane (VIII), m/z 160; as depicted in Scheme 6.2 (see Tables, VIII-IX).



R=C_6H_4 or C_9H_8 VII m/z 120 VIII m/z 160

Scheme 6.2

In addition the catechol and 2,3-butanediol β-pinane-10-boronates showed a characteristic base peak at m/z 213, attributable to IV.

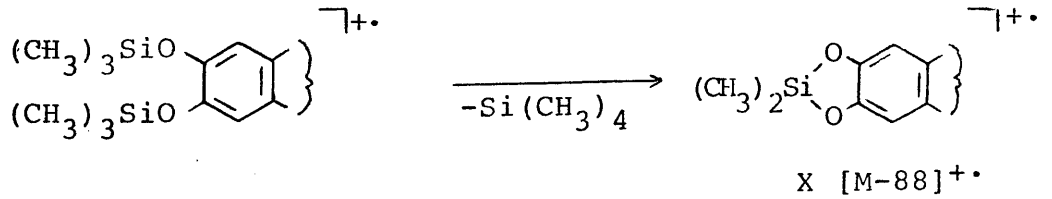


IX

R, m/z 214 (32) & 213 (100)

or m/z 194 (25) & 193 (100)

The mass spectra of the TMS ether derivatives for the catecholic type of compounds which are cited in Tables I, III, VI & VII, all showed an ionically induced intramolecular cyclization process, involving a TMSO group and an adjacent TMSO function, resulting in elimination of the elements of $si(CH_3)_4$, $[M-88]^{+\cdot}$, and formation of a cyclic silyldioxy ion [M-88] (X)[145,160]; as shown in Scheme 6.3. This ion was diagnostic for the catechol moiety in these compounds.



X $[M-88]^{+\cdot}$

Scheme 6.3

The acetates of the compounds in Tables I,III-VII showed the loss of one or two molecules of Ketene[26]; and in many cases after the loss of the Ketene groups, the ions corresponding to parent compounds were detected.

CONCLUSION

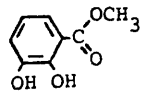
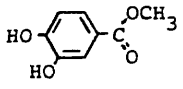
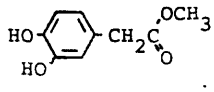
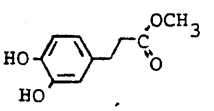
The MB and BB; diacetate and di-TMS ether derivatives for each compound gave closely similar fragmentation patterns and it was the same for most of the catecholic type of compounds cited in Tables I & III-VII (with the expected mass shifts). Stereoisomeric compounds showed closely similar fragmentation patterns, but in most cases were clearly distinguished by GLC. The value of cyclic boronate derivatives in characterisation of small quantities of catecholic compounds by GC-MS is enhanced by their easy displacement by acetylating or trimethylsilylating reagents, allowing the subsequent examinations of other derivatives for the same mixture. Major features of the mass spectra of the derivatives are reported. Examination of MB and BB analogues has facilitated the interpretation of mass spectra by a "substituent shift" Technique. Interpretation of fragmentations is further aided by the characteristic isotope pattern of boron-containing fragments arising from the relative abundances of the ^{10}B to ^{11}B isotopes. The general mode of break down is the same for both the MB and BB studied by GC-MS.

APPENDIX

GC-MS Tables

Mass Spectra Lined Diagrams

Table I, Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB); benzeneboronate (BB); acetate (Ac) and trimethylsilylether (TMS) derivatives for the following catechol methylesters;

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 168	MB[d]	4	1570	192 (100)	192	194 (2) 193 (12) 191 (27) 178 (1) 177 (2) 176 (1) 137 (1) 135 (2) 134 (1) 133 (1) 88 (1) 73 (1) 70 (2) 61 (3) 45 (2) 44 (2) 43 (5)
	BB[e]	3	2335	254 (100)	254	256 (2) 255 (18) 253 (26) 224 (6) 223 (34) 222 (10) 177 (2) 168 (3) 136 (10) 78 (3) 44 (2) 43 (2)
	Di-TMS[f]	2	1790	312 (8)	297	314 (1) 313 (2) 301 (1) 300 (2) 299 (11) 298 (26) 284 (1) 283 (2) 282 (2) 281 (3) 267 (1) 240 (1) 225 (1) 224 (1) 195 (2) 194 (4) 193 (23) 99 (1) 75 (2) 73 (3)
 Mol.wt. 168	MB[g]	6	1565	192 (100)	192	194 (2) 193 (10) 191 (30) 177 (4) 162 (10) 161 (88) 160 (22) 147 (1) 70 (2) 61 (3) 45 (2) 44 (3) 43 (5)
	BB[h]	7	2335	254 (100)	254	265 (3) 255 (18) 253 (28) 225 (1) 224 (6) 223 (38) 222 (11) 209 (1) 208 (1) 177 (2) 88 (1) 78 (2) 70 (1) 61 (2) 45 (1) 44 (1) 43 (3)
	Di-Ac[i]	12	2075	252 (3)	168	253 (1) 221 (2) 212 (1) 211 (5) 210 (31) 179 (4) 170 (2) 169 (11) 167 (1) 138 (2) 137 (12) 136 (1) 44 (1) 43 (19) 42 (1)
	Di-TMS	33	1850	312 (41)	193	314 (0.5) 313 (12) 297 (2) 281 (3) 196 (0.5) 194 (17) 73 (12)
 Mol.wt. 182	MB[j]	42	1675	206 (50)	147	207 (7) 205 (14) 148 (11) 146 (27)
	BB[k]	43	2450	268 (97)	209	269 (18) 267 (28) 210 (16) 208 (19)
	Di-Ac[l]	44	2180	266 (3)	182	267 (0.5) 238 (0.4) 225 (5) 224 (28) 207 (2) 183 (12) 165 (3.5) 123 (20)
	Di-TMS[m]	45	1895	326 (70)	179	329 (2) 328 (8) 327 (20) 311 (2) 269 (2) 268 (6) 267 (20) 207 (2) 181 (5) 180 (16) 105 (3) 73 (13)
 Mol.wt. 196	MB[n]	46	1770	220 (56)	147	222 (2) 221 (8) 219 (16) 190 (1) 189 (5) 188 (1) 163 (2) 162 (2) 161 (14) 160 (70) 159 (20) 148 (10) 146 (27) 135 (1) 134 (3) 133 (1) 77 (1)
	BB[o]	47	2550	282 (100)	282	284 (3) 283 (20) 281 (28) 252 (0.5) 251 (3) 250 (1) 225 (1) 224 (2) 223 (14) 222 (61) 221 (17) 210 (14) 209 (86) 208 (23) 196 (2) 78 (1) 77 (2)

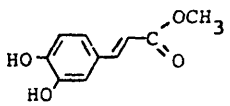
Parent Compound	Derivative type	Spectrum number	I^c	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
	Di-Ac	48	2275	280 (3)	196	281 (0.5) 233 (4) 232 (24) 208 (1) 207 (6) 198 (2) 197 (13) 165 (3) 164 (2) 137 (2) 136 (17) 135 (1) 123 (1) 122 (13) 121 (1) 43 (20)
	Di-TMS [p]	49	1975	340 (97)	179	343 (2) 342 (13) 341 (30) 310 (1) 309 (3) 282 (0.5) 281 (1) 28[illegible] (2) 269 (4) 268 (10) 267 (30) 223 (0.5) 222 (1) 221 (5) 193 (2) 181 (5) 180 (17) 73 (15)
 Mol.wt. 194	MB [q]	10	1900	218 (100)	218	220 (2) 219 (15) 217 (27) 203 (0.5) 200 (1) 188 (7) 187 (45) 186 (13) 173 (2) 172 (1) 160 (5) 159 (3) 158 (4) 157 (1) 44 (1) 43 (1)
	BB [r]	11	2690	280 (100)	280	282 (3) 281 (20) 279 (27) 262 (0.5) 250 (5) 249 (24) 248 (7) 222 (4) 221 (3) 220 (2) 219 (1) 84 (1) 44 (1) 43 (1)
	Di-Ac [s]	14	2405	278 (5)	194	279 (1) 250 (1) 247 (1) 237 (6) 236 (20) 221 (1) 220 (3) 219 (1) 205 (3) 196 (2) 195 (13) 193 (1) 189 (1) 178 (4) 137 (1) 136 (8) 135 (1) 134 (4) 60 (3) 43 (10)
	Di-TMS	39	2150	338 (32)	337	340 (3) 339 (14) 323 (2) 309 (1) 308 (1.4) 307 (4) 221 (7) 220 (19) 219 (95) 73 (15)

Table I;

COMMENTS

a. The derivatives were analysed by GLC on a Packard 419 gas chromatograph, equipped with a glass column (2m x 2mm, i.d.) packed with 1% OV-17, on Gas Chrom Q (100-120 mesh). Injection temperature 280°c, dectector temperature 300°c, Carrier gas N_2, flow rate 28ml/min and Kovats retention indices "I" were recorded as in (C).

b. Mass spectra were recorded at electron energy 20 eV, using an LKB 9000 gas chromatograph - mass spectrometer "GC-MS", fitted with a glass column (2m x 4mm,i.d.) of 1% OV-1 on Gas Chrom Q (100-120 mesh) The flash heater at 250°c , the molecule separator at 270°c, and ion source at 265°c. The helium carrier gas flow rate was 30ml/min. The trap current was 60 mA, filament current 4A and accelerating voltage 3.5 KV.

The abundance for the ions were written between small brackets ( ).

c. The retention index "I" values for these derivatives were measured at different oven temperatures, as follows; The methaneboronates at 120°c, the TMS-ethers at 140°c, the benzeneboronate and the acetates at 200°c.

Metastable ions were observed as follows:-

(d) 135.0(calcd. for 192→161, 135.01); (e) 163.0(calcd. for 268→209, 162.99); (f) 126.0(calcd. for 297→193, 125.

(g) 135.0(calcd. for 192→161, 135.01); (h) 196.0(calcd. for 254→223, 195.78); (i) 175.0(calcd. for 252→210, 175.

135.0(calcd. for 210→168, 134.4); & 112.0(calcd. for 169→137, 111.72); (j) 105.0(calcd. for 206→147, 104.

(k) 163.0(calcd. for 268→209, 162.99); (l) 148.0(calcd. for 224→182, 147.9) ; (m) 219.0(calcd. for 326→267, 218.

(n) 115.0(calcd. for 189→147, 114.3); (o) 175.0(calcd. for 282→222, 174.7) ; & 154.0(calcd. for 251→196, 153.

(p) 210.0(calcd. for 340→267, 209.7); (q) 160.5(calcd. for 218→187, 160.7) ; (r) 221.5(calcd. for 280→249, 221.

(s) 200.0(calcd. for 278→236, 200.35) & 160.0(calcd. for 236→194, 159.47).

Table II, Retention index values [a] and mass spectrometric data [b] for methyl-2,3-& 3,4-dihydroxybenzoate, and N-acetyl-dopamine

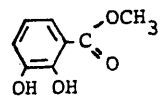
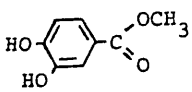
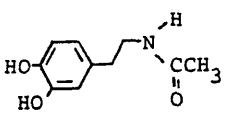
COMPOUNDS	Spectrum number	I [c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 168	1 [d]	1520 [140°]	168 (72)	136	170 (1) 169 (7) 138 (2) 137 (12) 110 (1) 109 (1) 108 (5) 80 (1) 75 (2) 73 (1) 70 (1) 61 (2) 45 (1) 44 (1) 43 (4)
 Mol.wt. 168	5 [e]	2075 [210°]	168 (100)	168	169 (1) 138 (1) 137 (66) 136 (1)
 Mol.wt. 195	34	No Peak high Adsorption	195	136	196 (3) 178 (2) 167 (1) 161 (1) 150 (1) 149 (2) 137 (14) 135 (4) 124 (7) 123 (35) 107 (3) 106 (1) 105 (3) 93 (3) 92 (1) 91 (4) 79 (3) 78 (2) 77 (9) 60 (7)

Table II

COMMENTS

a. The GLC Conditions were the same as in Table I.(a).

b. The GC-MS data were recorded at the same conditions as in Table I (b), except for N-acetyl dopamine was measured by the mass spectrometry unit-high resolution, as an oil.

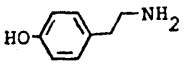
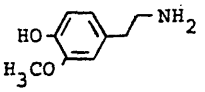
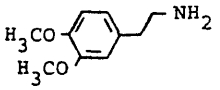
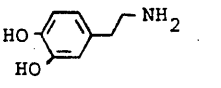
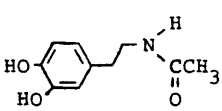
c. The retention index "I" values for these compounds were measured at different oven temperatures "C", these were indicated between the large brackets [ ]. N-acetyl dopamine didn't show any peak on 1% ov-17 nor 1% ov-25, at temperature programming 100-260°, due to very high adsorption.

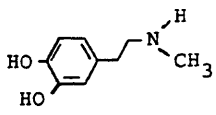
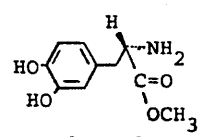
Metastable ions were observed as follows:-

(d) 86.0(calcd. for 136→108, 85.8);

(e) 111.5(calcd. for 168→137, 111.72).

Table III, Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB); benzeneboronate (BB); acetate (Ac) and trimethylsilylether (TMS) derivatives for the following tyramine type compounds.

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 137	Di-Ac	15	2295 [200°]	221 (2)	120	223 (0.2) 222 (1) 193 (0.1) 179 (1.5) 164 (1) 163 (6) 162 (50) 161 (1) 150 (1) 135 (1) 121 (10) 118 (1) 108 (3) 107 (9) 73 (1) 72 (6) 43 (3) 42 (1)
	Tri-Ac	16	2350 [200°]	263 (0.2)	120	265 (0.1) 264 (0.4) 222 (0.2) 221 (0.3) 179 (0.2) 178 (0.2) 163 (6) 162 (45) 150 (2) 137 (0.2) 136 (0.2) 122 (1) 121 (10) 118 (0.5) 114 (0.3) 108 (2) 107 (8) 102 (1) 89 (0.2) 84 (5) 60 (1) 43 (5) 42 (1)
	Mono-O-TMS[d]	94	[140°]	209 (4)	180	210 (1) 208 (1) 207 (2) 205 (1) 195 (0.5) 194 (4) 192 (1) 190 (1) 182 (5) 181 (20) 179 (30) 167 (1) 166 (5) 165 (24) 164 (1) 79 (4) 73 (20) 30 (75)
 Mol.wt. 167	Di-Ac[e]	17	2495 [200°]	251 (5)	150	252 (1) 210 (3) 209 (20) 193 (1) 192 (7) 151 (11) 138 (2) 137 (8) 135 (2) 72 (2) 60 (1) 43 (1)
	Tri-Ac[f]	18	2535 [200°]	293 (3)	150	252 (2) 251 (2) 209 (1) 193 (2) 192 (10) 152 (1) 151 (11) 138 (1) 137 (5) 135 (1) 84 (1) 72 (1) 44 (2)
 Mol.wt. 181	Mono-Ac	19	1950[g] [170°]	223 (10)	164	224 (2) 166 (2) 165 (12) 152 (2) 151 (15) 150 (1) 149 (7) 137 (1) 121 (1) 120 (1) 107 (1) 103 (1) 93 (1) 91 (1) 78 (1) 72 (1) 43 (1)
	Di-Ac	20	1995[g] [170°]	265 (9)	164	266 (2) 166 (1) 165 (13) 152 (2) 151 (15) 150 (1) 149 (7) 121 (1) 84 (1) 72 (3) 43 (3)
 Mol.wt. 153	MB[h]	86	1635 [160°]	177 (7)	30	178 (1) 176 (2) 175 (2) 174 (1) 173 (1) 161 (0.2) 160 (0.5) 159 (0.2) 149 (4) 148 (40) 147 (20) 146 (3) 79 (3)
	BB	25	2425 [220°]	239 (2)	30	238 (1) 237 (2) 236 (1) 235 (2) 222 (1) 221 (1) 211 (9) 210 (49) 209 (20)
	Di-o,o-TMS[i]	85	1825 [160°]	297 (20)	268	299 (3) 298 (6) 296 (2) 295 (4) 283 (1) 282 (4) 281 (0.5) 280 (1) 270 (10) 269 (30) 267 (32) 253 (3) 195 (1) 194 (4) 193 (19) 181 (1) 180 (4) 179 (13) 147 (0.5) 102 (0.5) 30 (78)
 Mol.wt. 195	MB	84	2135 [160°]	219 (9)	160	220 (2) 218 (3) 205 (0.1) 204 (1) 203 (0.5) 176 (0.3) 175 (0.5) 161 (10) 159 (28) 148 (2) 147 (8) 146 (2) 134 (0.5) 117 (0.2) 73 (0.5) 72 (6) 30 (22)

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
	BB	24	2930 [220°]	281 (6)	222	282 (2) 280 (2) 238 (1) 237 (1) 224 (3) 223 (18) 221 (28) 210 (3)
						209 (7) 208 (3) 204 (2) 203 (13) 202 (3) 162 (2) 78 (2) 77 (1)
						72 (4) 43 (2)
	Di-o,o-Ac [j]	21	2610 [220°]	279 (2)	136	280 (2) 238 (3) 237 (10) 221 (3) 220 (14) 219 (3) 208 (1) 197 (1)
						196 (2) 195 (9) 194 (2) 193 (1) 192 (2) 180 (1) 179 (5) 178 (39)
						177 (1) 166 (3) 165 (1) 163 (2) 162 (6) 138 (1) 137 (10) 135 (1)
						134 (1) 133 (1) 124 (3) 123 (6) 118 (1) 102 (2) 100 (1) 85 (1)
						84 (1) 73 (1) 72 (5) 60 (8) 45 (1) 44 (1) 43 (8) 42 (2)
	Tri-Ac [k]	22	2675 [220°]	321 (1)	136	237 (2) 222 (1) 221 (5) 220 (28) 208 (1) 195 (1) 194 (1) 138 (1)
						137 (10) 135 (1) 124 (2) 123 (5) 102 (6) 84 (3) 73 (1) 72 (4)
						60 (3) 43 (8) 42 (1)
	Di-o,o-TMS [l]	83	2300 [160°]	339 (15)	280	341 (2) 340 (4) 326 (1) 325 (2) 324 (7) 309 (1) 308 (1) 283 (2)
						282 (12) 281 (30) 269 (1) 268 (3) 267 (13) 266 (0.5) 265 (1) 208 (1)
						194 (2) 193 (10) 192 (1) 170 (0.5) 169 (3) 117 (0.5) 75 (5) 74 (5) 73 (45) 30 (1)
 Mol.wt. 167	MB	87	m 1925 [120°]	191 (2)	44	192 (0.2) 190 (0.6) 189 (0.3) 188 (0.3) 161 (0.3) 160 (0.4) 149 (0.3) 148 (4)
						147 (2) 146 (0.3) 79 (1)
	BB	88	m 2525 [180°]	253 (1)	44	254 (0.3) 252 (0.5) 251 (0.7) 250 (0.4) 249 (0.4) 248 (0.2) 224 (0.1) 223 (0.2)
						222 (0.5) 221 (0.2) 211 (1) 210 (4) 209 (2) 208 (0.5) 79 (1) 78 (1)
	Tri-Ac	90	m 2785 [180°]	293 (4)	44	295 (1) 294 (1) 269 (1) 252 (4) 251 (25) 233 (4) 221 (6) 220 (18)
						209 (7) 197 (2) 192 (3) 179 (6) 178 (46) 166 (3) 160 (8) 137 (5)
						136 (50) 123 (2) 116 (3) 87 (4) 86 (55) 74 (20) 60 (35)
	Di-o,o-TMS	89	m 1910 [120°]	311 (1)	44	312 (0.5) 310 (1) 309 (2) 298 (0.5) 297 (1) 296 (3) 288 (6) 287 (15)
						286 (55) 253 (2) 222 (1) 194 (1) 193 (5) 180 (1) 179 (2) 117 (0.5)
						116 (4) 79 (2) 75 (4) 74 (3) 73 (35)
 Mol.wt. 211	MB	91	1980 [170°]	235 (6)	88	236 (1) 234 (2) 233 (3) 219 (0.2) 218 (1) 217 (0.5) 187 (0.5) 177 (4)
						176 (25) 175 (7) 161 (0.2) 160 (1) 159 (0.4) 149 (6) 148 (55) 147 (64)

Continued Table III

Parent Compound	Derivative type	Spectrum number	I^c	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
						146 (17) 108 (1) 102 (0.5) 31 (1) 30 (2)
	BB	92	2825 [250°]	297 (5)	209	298 (1) 296 (2) 295 (3) 294 (1) 239 (3) 238 (20) 237 (5) 236 (1)
						235 (3) 222 (3) 211 (10) 210 (64) 208 (30) 102 (3) 88 (85) 79 (2) 7E (6)
	Tri-Ac n	100	2780 [250°]	337 (0.7)	194	338 (0.2) 324 (0.2) 323 (0.7) 296 (1) 295 (4) 279 (3) 278 (15) 277 (2)
						264 (0.3) 263 (1) 254 (1) 253 (5) 251 (1) 250 (4) 237 (8) 236 (50)
						222 (0.6) 221 (2) 220 (0.7) 209 (0.5) 208 (1) 195 (12) 180 (0.6) 179 (3)
						166 (3) 165 (10) 131 (3) 124 (3) 123 (33) 102 (5) 99 (2) 89 (2)
						88 (6) 79 (2) 71 (2) 43 (16)
	Di-o,oTMS	93	2150 [170°]	355 (6)	267	357 (1) 356 (2) 354 (0.5) 353 (2) 342 (0.3) 341 (0.6) 340 (2) 309 (2)
						308 (1) 307 (1) 298 (0.5) 297 (1) 296 (3) 295 (1) 294 (0.5) 293 (1)
						281 (0.5) 270 (3) 269 (12) 268 (30) 251 (0.5) 250 (0.5) 219 (0.5) 218 (1)
						209 (0.5) 208 (2) 180 (2) 179 (10) 161 (2) 147 (0.5) 133 (0.5) 117 (0.5)
						88 (1) 75 (4) 74 (0.5) 73 (7)

Table III

COMMENTS

a. The GLC conditions were the same as in Table I(a), except some derivatives were analysed on other columns such as, (g) on 1% OV-1, on Gas-Chrom Q (100-120,mesh), (3m x 2mm, i.d.) column, and (m) on 1% OV-25, on Gas Chrom Q (100-120,mesh),(2m x 2mm, i.d.) column.

b. The GC-MS data were recorded at the same conditions as in Table I(b), except some compounds like Epinine and L-Dopa-methylester derivatives were analysed on 1% OV-17, on Gas Chrom Q (100-120,mesh), (2m x 4mm,i.d.) column.

c. The retention index "I" values for these derivatives were measured at different oven temperatures "°C" as shown on Table II, between the large brackets [ ].

Metastable ions were observed as follows:-

(d) 151.0(calcd. for 180→165, 151.3); (e) 174.0(calcd. for 251→209, 174.02) & 118(calcd. for 192→150, 117.18)

(f) 215.0(calcd. for 293→251, 215.02) & 117.0(calcd. for 192→150, 117.18); (h) 124.0(calcd. for 177→148, 123.8

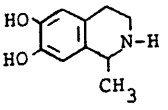
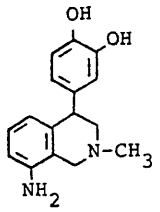
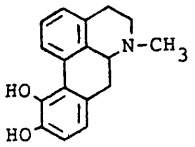
134.0(calcd. for 195→162, 134.5

(i) 242.0(calcd. for 297→268, 241.8); (j) 144.0(calcd. for 220→178, 144.02);

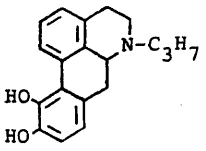
104.0(calcd. for 178→136, 103.91); (k) 144.0(calcd. for 220→178, 144.02) & 104.0(calcd. for 178→136, 103.9

(l) 231.0(calcd. for 309→267, 230.7); (n) 200.5(calcd. for 278→236, 200.3) & 159.5(calcd. for 236→194, 159.5

Table IV , Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB); benzeneboronate (BB); acetate (Ac) and trimethylsilylether (TMS), derivatives for the following catecholamine compounds;

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 179	MB	63	1925 [150°]	203 (4)	188	201 (61) 200 (53) 199 (33) 198 (8) 189 (13) 187 (29) 174 (4) 173 (3); 172 (2) 161 (2) 160 (2) 159 (2) 79 (9)
	BB	64	2825 [220°]	265 (4)	250	264 (17) 263 (65) 262 (42) 261 (38) 260 (10) 251 (18) 249 (31) 237 (1); 236 (4) 235 (1) 188 (3) 79 (14) 78 (8)
	Tri-Ac[d]	65	3065 [220°]	305 (48)	248	307 (2) 306 (10) 292 (2) 291 (10) 290 (53) 264 (2) 263 (12) 262 (1); 249 (17) 222 (2) 221 (16) 220 (1) 192 (2) 179 (1) 178 (1.2) 177 (1.5); 165 (0.5) 164 (7) 163 (4) 150 (2) 79 (2)
	Di-TMS[e]	66	2085 [150°]	323 (12)	308	325 (1) 324 (4) 322 (15) 321 (28) 320 (8) 319 (8) 311 (3) 310 (13); 309 (30) 249 (1) 236 (1) 235 (0.5) 234 (2.5) 233 (1) 232 (5) 231 (1); 102 (3) 73 (8)
 Mol.wt. 270	MB	40	3155 [250°]	294 (50)	251	295 (18) 293 (25) 279 (6) 252 (25) 250 (81) 249 (20) 236 (6) 235 (7); 234 (7) 160 (16) 134 (8) 89 (19)
	Di-o,o-TMS	41	3165 [250°]	414 (12)	160	416 (2) 415 (5) 413 (2) 399 (5) 373 (5) 372 (15) 371 (38) 370 (7); 342 (4) 309 (6) 307 (4) 299 (5) 284 (2) 283 (7) 282 (2.2) 281 (5); 280 (2) 251 (2) 250 (1) 210 (2) 209 (8) 161 (15) 159 (5) 158 (5); 116 (2) 75 (16) 73 (5)
 Mol.wt. 267	MB[f]	29	2920 [200°]	291 (72)	290	293 (5) 292 (22) 289 (94) 288 (33) 287 (22) 286 (6) 277 (6) 276 (5); 275 (5) 274 (5) 273 (6) 272 (1) 249 (11) 248 (38) 247 (11) 44 (22)
	BB[g]	30	3880 [250°]	353 (72)	352	356 (4) 355 (13) 354 (45) 351 (50) 350 (41) 349 (20) 348 (4) 337 (3); 336 (3) 335 (6) 334 (3) 312 (9) 311 (18) 310 (11) 250 (3) 136 (3); 134 (2) 80 (3) 79 (9) 44 (18)
	Di-Ac	32	3450 [250°]	351 (100)	351	353 (5) 352 (25) 350 (48) 349 (13) 348 (6) 336 (3) 309 (12) 308 (37); 307 (5) 295 (2) 294 (3) 293 (4) 292 (10) 291 (3) 290 (2) 289 (1); 267 (6) 266 (31) 265 (9) 225 (1) 224 (9) 223 (4) 60 (22) 44 (25); 43 (8) 42 (6)
	Di-TMS	31	2835 [200°]	411 (100)	411	414 (5) 413 (16) 412 (40) 410 (82) 409 (28) 398 (1) 397 (3) 396 (6); 395 (1) 394 (1) 393 (1) 370 (5) 369 (10) 368 (21) 354 (2) 353 (4); 339 (1) 338 (1) 324 (4) 323 (10) 322 (32) 321 (5) 320 (2) 295 (2)

Continued Table IV ;

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
						294 (4) 281 (3) 280 (3) 221 (2) 208 (1) 207 (2) 206 (1) 205 (2) 118 (1) 117 (4) 80 (2) 79 (2) 76 (4) 75 (3) 74 (12) 45 (2) 44 (8)
 Mol.wt. 295	MB [h]	35	3025 [200°]	317 (100)	317	322 (5) 321 (12) 320 (9) 319 (35) 318 (40) 316 (28) 291 (9) 290 (38) 289 (25) 288 (65) 287 (20) 275 (3) 274 (3) 273 (6) 272 (4) 262 (4) 261 (20) 260 (4) 249 (1.8) 248 (8.3) 247 (2.3) 72 (7)
	BB [i]	36	3965 [250°]	381 (25)	379	382 (5) 380 (40) 378 (30) 377 (25) 352 (17) 351 (15) 350 (40) 349 (10) 335 (4) 334 (5) 323 (10) 310 (5) 72 (5)
	Di-Ac [j]	37	3530 [250°]	379 (100)	379	381 (5) 380 (30) 378 (30) 377 (35) 352 (5) 351 (25) 350 (80) 337 (5) 336 (22) 335 (27) 322 (2) 321 (5) 320 (2) 309 (2) 308 (15) 295 (5) 294 (15) 293 (22) 292 (10) 291 (5) 279 (5) 266 (5) 265 (3) 264 (4) 249 (3) 248 (4) 224 (3) 219 (6) 72 (12)
	Di-TMS	38	2940 [200°]	439 (100)	439	441 (15) 440 (40) 438 (65) 437 (50) 424 (5) 412 (13) 411 (30) 410 (75) 383 (2.5) 371 (10) 369 (5) 368 (15) 351 (7) 350 (22) 293 (2.5) 291 (2.5) 73 (28) 72 (10)

COMMENTS

a. The GLC Conditions were the same as in table I(a), except the column packing was 1% OV-25, on Gas-Chrom Q, (100-120, mesh).

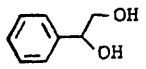
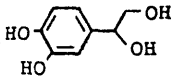
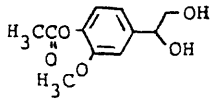
b. The GC-MS data were recorded at the same conditions as in table I(b).

c. The retention index "I" values for these derivatives were measured at different oven temperatures "°C", these indicated between the large brackets [ ].

Metastable ions were observed as follows:-

(d) 276.0(calcd. for 305→290, 275.7); 234.0(calcd. for 263→248, 233.9); 212.0(calcd. for 290→248, 212.1); 171.0(calcd. for 248→206, 171.1) & 131.0(calcd. for 206→164, 130.6); (e) 295.0(calcd. for 321→308, 295.5); (f) 260.0(calcd. for 291→275, 259.9) & 211.0(calcd. for 291→248, 211.4); (g) 318.0(calcd. for 353→335, 317.9) & 373.5(calcd. for 353→311, 373.99); (h) 235.0(calcd. for 290→261, 234.9); (i) 396 (calcd. for 352→323, 396.4); (j) 323.0(calcd. for 379→350, 323.2) & 397.0(calcd. for 379→335, 396.1).

Table V , Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB) and benzeneboronate (BB), derivatives for styrene glycol and catechol glycol type compounds;

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 138	MB	26	1400 [80°]	162 (100)	162	164 (1) 163 (11) 161 (97) 160 (24) 159 (1) 147 (2) 146 (1) 133 (1); 132 (5) 131 (2) 121 (1) 120 (7) 119 (9) 105 (1) 104 (1) 103 (1); 92 (3) 91 (8) 90 (34) 85 (1) 84 (3) 83 (1) 78 (3) 57 (9); 56 (3) 43 (1)
	BB	27	2235 [170°]	224 (100)	224	226 (2) 225 (18) 223 (70) 222 (15) 209 (2) 195 (1) 194 (5) 193 (8); 192 (3) 180 (1) 179 (1) 176 (1) 167 (2) 166 (5) 165 (2) 147 (1); 146 (3) 145 (1) 133 (2) 120 (6) 119 (8) 118 (2) 106 (1) 105 (2); 104 (1) 92 (2) 91 (9) 90 (11) 89 (1)
 Mol.wt. 170	Di-MB	28	1745 [140°]	218 (100)	218	220 (2) 219 (15) 217 (99) 216 (40) 215 (10) 204 (2) 203 (16) 202 (8); 201 (1) 189 (2) 188 (11) 187 (5) 186 (1) 177 (2) 176 (11) 175 (15); 174 (6) 173 (14) 172 (8) 171 (2) 162 (1) 161 (2) 160 (3) 159 (1); 148 (2) 147 (8) 146 (22) 145 (6) 136 (1) 135 (7) 134 (2) 133 (1); 94 (1) 85 (1) 84 (1) 79 (1) 78 (1) 77 (1) 76 (1) 57 (12); 56 (4) 44 (1) 43 (1)
 Mol.wt. 226	MB	99	2045[d] [180°]	250 (5)	208	251 (1) 249 (2) 210 (2) 209 (12) 207 (36) 206 (5) 193 (4) 192 (2); 191 (6) 190 (3) 178 (2) 177 (15) 176 (10) 175 (2) 167 (0.5) 166 (4); 165 (4) 164 (1) 163 (2) 151 (1) 150 (1) 149 (2) 148 (1) 147 (2); 146 (0.5) 138 (0.5) 137 (3) 136 (2) 135 (4) 125 (0.5) 124 (2) 123 (1) 43 (6)

COMMENTS

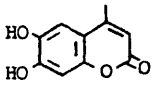
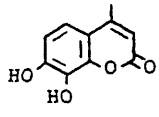
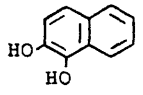
a. The GLC Conditions were the same as in Table I(a), except the column packing was 1% OV-25, on Gas-Chrom Q (100-120,mesh).

b. The GC-MS data were recorded at the same conditions as in Table I(b), except 4-acetyl-3-methoxy phenylglycol -methyl boronate which were analysed on 1% OV-17, on Gas Chrom Q (100-120,mesh),(2m x 4mm,i.d.) Column.

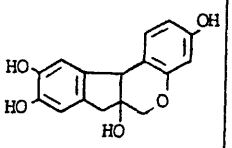
c. The retention index "I" values for these derivatives were measured at different oven temperatures "°C", these indicated between the large brackets [ ].

d. Measured on 1% OV-17, 2m x 2mm.

Table VI ; Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB); benzeneboronate (BB); acetate (Ac) and trimethylsilylether (TMS) derivatives for 4-methylcoumarins;1,2-dihydroxynaphthalene and brazilin.

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 192	MB[d]	67	2385 [200°]	216 (100)	216	218 (2) 217 (15) 215 (25) 189 (7) 188 (55) 187 (14) 186 (4) 79 (7)
	BB[e]	71	3315 [240°]	278 (100)	278	280 (4) 279 (22) 277 (30) 252 (1) 251 (9) 250 (48) 249 (18) 248 (4) 79 (4) 78 (7)
	Di-Ac[f]	72	2905 [240°]	276 (5.5)	192	277 (1) 249 (0.5) 248 (1.5) 235 (4) 234 (20) 220 (1) 193 (13) 165 (1) 164 (9) 163 (1) 79 (1) 43 (10) 42 (5)
	Di-TMS[g]	68	2540 [200°]	336 (100)	336	339 (3) 338 (15) 337 (30) 324 (2) 323 (5) 322 (14) 321 (45) 309 (3) 308 (7) 264 (0.7) 248 (2) 221 (1) 220 (4) 74 (10) 73 (96)
 Mol.wt. 192	MB[h]	69	2425 [200°]	216 (100)	216	217 (15) 215 (28) 189 (8) 188 (60) 187 (22) 186 (4) 161 (0.5) 160 (3) 159 (1) 79 (7)
	BB[i]	73	3345 [240°]	278 (100)	278	280 (3) 279 (20) 277 (30) 251 (10) 250 (45) 249 (15) 248 (2) 79 (1) 78 (7)
	Di-Ac[j]	74	2895 [240°]	276 (4)	192	278 (0.3) 277 (1.5) 248 (0.3) 235 (4) 234 (19) 220 (0.2) 219 (0.2) 194 (1) 193 (14) 165 (1) 164 (7) 163 (1) 136 (0.2) 118 (0.2) 79 (1) 43 (5) 42 (2)
	Di-TMS[k]	70	2450 [200°]	336 (48)	321	339 (1) 338 (6) 337 (15) 324 (3) 323 (13) 322 (31) 294 (1) 293 (3) 279 (1) 250 (0.4) 249 (0.5) 220 (0.4) 147 (0.8) 73 (24)
 Mol.wt. 160	MB	95	1755 [140°]	184 (30)	185	186 (14) 115 (0.5) 114 (5) 113 (0.2)
	BB	96	2585 [190°]	246 (100)	246	248 (4) 247 (20) 245 (30) 123 (0.5) 114 (1) 113 (0.5)
	Di-Ac[l]	97	2370 [190°]	244 (9)	160	246 (1) 245 (2) 216 (0.5) 203 (4) 202 (26) 184 (1) 162 (3) 161 (14) 159 (3) 132 (1) 131 (2) 120 (5) 60 (5) 43 (10)

Continued Table VI ;

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
	Di-TMS	98	1965 [140°]	304 (80)	73	306 (10) 305 (26) 291 (0.5) 290 (1) 289 (4) 232 (0.5) 218 (1) 217 (2) 216 (13) 201 (0.5) 149 (1) 141 (1) 75 (5) 74 (10)
 Mol.wt. 286	MB [m]	52	3390 [230°]	310 (100)	310	312 (6) 311 (25) 309 (39) 293 (13) 292 (49) 291 (31) 290 (8) 282 (4) 281 (5) 268 (4) 267 (5) 253 (9) 201 (4) 200 (8) 199 (3) 172 (6) 79 (11)
	Tri-Ac [n]	53	-	412 (29)	328	413 (7) 396 (7) 395 (18) 394 (31) 384 (58) 371 (10) 370 (36) 367 (10) 366 (36) 353 (14) 352 (63) 343 (9) 342 (33) 333 (17) 329 (28) 325 (8) 324 (39) 323 (6) 311 (14) 310 (64) 309 (12) 293 (12) 292 (38) 291 (22) 287 (19) 286 (83) 268 (28)
	Tri-TMS [o]	54	2985 [230°]	502 (100)	502	504 (28) 503 (52) 485 (8) 484 (16) 483 (8) 465 (4) 430 (3) 414 (4) 413 (8) 398 (4) 397 (15) 320 (4) 309 (5) 307 (4) 292 (2) 267 (1) 235 (1) 233 (1) 179 (1) 75 (12) 73 (19)

COMMENTS

a. The GLC Conditions were the same as in Table I(a), except the column packing was 1% OV-25, on Gas-Chrom Q (100-120,mesh).

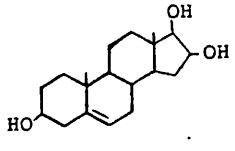
b. The GC-MS data were recorded at the same conditions as in Table I(b), except 1,2-dihydroxynaphthalene derivatives, which were analysed on 1% OV-17, on Gas-Chrom Q (100-120,mesh), (2m x 4mm,i.d.) column.

c. The retention index "I" values for these derivatives were measured at different oven temperatures "°C", these were indicated between the large brackets [ ].

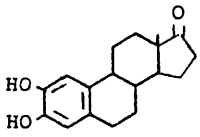
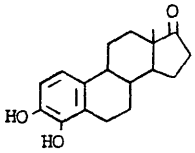
Metastable ions were observed as follows:-

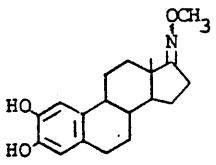
(d) 164.0(calcd. for 216→188, 163.6); 140.0(calcd. for 192→164, 140.1); (e) 225.0(calcd. for 278→250, 224.8); (f) 158.0(calcd. for 234→192, 157.5); (g) 307.0(calcd. for 336→321, 306.7); (h) 164.0(calcd. for 216→188, 163.6) 158.0(calcd. for 234→192, 157.5); (i) 225.0(calcd. for 278→250, 224.8); 140.0(calcd. for 192→164, 140.1); (j) 198.0(calcd. for 276→234, 198.4); (k) 307.0(calcd. for 336→321, 306.7); (l) 167.0(calcd. for 244→222, 167.2); 127.0(calcd. for 202→160, 126.7); (m) 276.0(calcd. for 310→293, 276.9); (n) 251.0(calcd. for 286→258, 251.1); (o) 466.0(calcd. for 502→484, 466.6) & 339.0(calcd. for 465→397, 338.9).

Table VII ; Retention index values[a] and mass spectrometric data[b] for methaneboronate (MB); benzeneboronate (BB); acetate (Ac) and trimethylsilylether (TMS) derivatives for the following Estrone derivatives and for 5-Androsten-3β,16β,17β-triol.

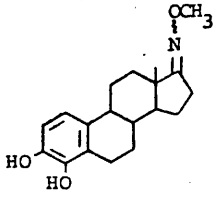
Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 306	MB	50	3100 [240°]	330 (51)	312	332 (3) 331 (13) 329 (15) 328 (6) 327 (1) 315 (11) 314 (6) 313 (24) 311 (27) 299 (4) 298 (17) 297 (74) 296 (22) 286 (8) 285 (4) 284 (6) 283 (10) 273 (12) 272 (8) 271 (12) 270 (16) 269 (8) 259 (3) 258 (8) 257 (9) 256 (6) 255 (12) 254 (3) 246 (14) 245 (70) 244 (28) 243 (8) 238 (6) 237 (24) 228 (5) 227 (9) 226 (6) 220 (8) 219 (50) 218 (30) 217 (7) 213 (17) 191 (11) 185 (11) 173 (10) 172 (8) 171 (9) 161 (14) 160 (13) 159 (37) 158 (7) 157 (12) 147 (11) 146 (11) 145 (37) 144 (5) 143 (12) 135 (11) 134 (8) 133 (17) 132 (6) 131 (12) 125 (24) 124 (10) 123 (7) 122 (9) 121 (56) 120 (22) 119 (18) 111 (9) 110 (7) 109 (10) 108 (18) 107 (60) 106 (18) 105 (14) 95 (10) 94 (9) 93 (28) 92 (6) 91 (6) 83 (13) 81 (11) 79 (10)
	Tri-TMS	51	2915 [240°]	522 (15)	239	525 (1) 524 (3) 523 (7) 521 (1) 520 (1) 509 (1) 508 (2) 507 (4) 506 (1) 435 (4) 434 (5) 433 (30) 432 (65) 420 (2) 419 (4) 418 (4) 417 (12) 394 (1) 393 (3) 392 (1) 391 (1) 344 (10) 343 (20) 342 (45) 330 (20) 329 (65) 328 (15) 327 (30) 316 (1) 315 (1) 314 (1) 313 (1) 312 (1) 304 (10) 303 (20) 302 (10) 301 (12) 290 (1) 289 (2) 288 (3) 287 (1) 238 (15) 237 (45) 226 (5) 225 (5) 224 (2) 223 (2) 222 (5) 221 (2) 220 (2) 214 (10) 213 (50) 212 (10) 206 (12) 205 (12) 198 (10) 197 (20) 196 (12) 192 (10) 191 (40) 186 (4) 185 (12) 184 (4) 183 (10) 182 (5) 181 (6) 171 (10) 170 (6) 169 (15) 168 (10) 160 (4) 159 (10) 158 (15) 157 (13) 156 (8) 155 (5) 147 (10) 146 (3) 145 (14) 144 (11) 143 (15) 142 (3) 133 (7) 132 (4) 131 (18) 130 (6) 129 (20) 121 (2) 120 (2) 119 (4) 118 (1) 117 (8) 109 (2) 108 (2) 107 (5) 106 (1) 105 (2) 104 (1) 103 (5) 95 (5) 94 (1) 93 (4) 92 (1) 91 (2) 81 (6) 75 (12) 73 (15)

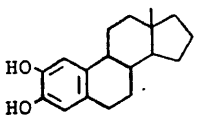
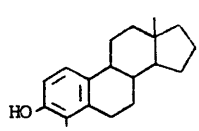
Continued Table VII ;

Parent Compound	Derivative type	Spectrum number	I[c]	M+·	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 286	MB	55	3160 [240°]	310 (100)	310	312 (4) 311 (12) 309 (20) 308 (8) 307 (3) 295 (1) 294 (0.5) 293 (2) 292 (2) 291 (1) 283 (0.6) 282 (3) 281 (1.7) 277 (1.3) 268 (1) 267 (1.5) 266 (4) 265 (1.5) 264 (0.6) 255 (1) 254 (6) 253 (8.5) 252 (3.5) 251 (4) 250 (2.5) 240 (1) 239 (1.5) 238 (0.5) 237 (0.5) 227 (1) 226 (5) 225 (15) 224 (4) 214 (1.5) 213 (2.5) 212 (7.5) 211 (3) 210 (4) 209 (1.5) 201 (1.5) 200 (5.5) 199 (4.5) 198 (6.5) 197 (4.5) 187 (4) 186 (15) 185 (7) 184 (2.5) 173 (2.5) 161 (2) 160 (10) 159 (2.5) 150 (2) 149 (1) 148 (2) 147 (4.5) 146 (1) 123 (2) 97 (4.5) 85 (2)
	BB	56	4155 [270°]	372 (100)	372	374 (5) 373 (30) 371 (30) 370 (5) 344 (1.5) 343 (1) 342 (1) 328 (1.5) 318 (1) 316 (3) 315 (3.5) 314 (2) 313 (2) 312 (2) 288 (3) 287 (7) 286 (2) 275 (1) 274 (3.5) 273 (2) 272 (2) 262 (2.5) 261 (2) 260 (2.5) 259 (2) 249 (2) 248 (7.5) 247 (3) 246 (1) 235 (1) 223 (1) 222 (5) 221 (1)
	Di-Ac [d]	57	3795 [270°]	370 (7)	286	372 (0.3) 371 (2) 369 (0.3) 368 (1) 344 (0.5) 343 (1.5) 342 (5) 341 (0.5) 340 (0.8) 330 (1) 329 (7) 328 (26) 327 (1) 326 (3) 311 (0.4) 310 (1.2) 309 (0.4) 288 (4) 287 (22) 285 (4) 284 (4) 268 (0.5) 258 (0.5) 257 (0.5)
	Di-TMS	58	3210 [240°]	430 (100)	430	432 (15) 431 (40) 429 (15) 428 (10) 417 (0.5) 416 (1.5) 415 (3.5) 402 (0.5) 375 (0.1) 374 (0.3) 358 (0.5) 346 (0.5) 345 (1.5) 342 (1.5) 341 (4) 332 (0.6) 325 (1.3) 307 (1) 306 (2) 305 (0.5) 281 (0.6) 280 (0.8) 267 (0.8) 245 (1) 231 (0.5) 229 (1.4) 217 (0.4) 205 (0.7) 73 (12)
 Mol.wt. 286	MB	59	3150 [240°]	310 (100)	310	312 (5) 311 (26) 309 (28) 308 (4) 295 (1.5) 292 (2.5) 282 (2.5) 281 (2.5) 268 (2) 267 (2) 266 (6.5) 265 (1) 255 (1) 254 (6.5) 253 (9.5) 252 (4) 251 (4) 239 (2) 226 (5) 225 (16) 224 (5) 214 (2) 213 (3) 212 (13) 211 (3) 210 (3) 201 (1.5) 200 (6.5) 199 (4.5) 198 (4.5) 197 (2.5) 187 (4) 186 (16) 185 (6) 150 (2) 149 (6) 148 (1) 112 (1) 111 (3) 110 (2) 109 (2) 99 (1.5) 98 (1.5) 97 (5) 96 (1.5) 95 (1.5) 85 (4) 84 (1.5) 83 (2)
	BB	60	4120 [270°]	372 (100)	372	374 (7) 373 (30) 371 (30) 370 (5) 330 (1) 329 (1) 328 (3) 327 (1)

Parent Compound	Derivative type	Spectrum number	I[c]	$M^{+\cdot}$	m/z Base Peak	Other m/z (including isotope peaks)
						317 316 315 314 313 288 287 286 (1) (3.5) (5) (1.5) (2) (4) (10) (4) 275 274 273 262 261 260 249 248 (2.5) (6) (2.5) (3.5) (3) (2) (3) (9.5) 247 223 222 221 97 (3) (1) (2) (1) (2)
	Di-Ac [e]	61	3770 [270°]	370 (6)	286	371 343 342 329 328 310 269 268 (2) (1) (3) (7) (24) (1) (0.5) (0.6)
	Di-TMS	62	3260 [240°]	430 (100)	430	433 432 431 417 416 415 414 403 (3) (15) (40) (1) (1.5) (3) (1) (0.4) 402 374 346 345 342 341 333 332 (1.2) (0.4) (1) (2.5) (2.2) (6.3) (0.4) (1) 326 325 306 246 245 231 230 229 (1) (2.6) (2) (0.3) (1.3) (0.8) (0.5) (1.8) 193 97 73 (0.5) (1.3) (15)
 Mol.wt. 315	MB	75	3180 [240°]	339 (77)	308	341 340 338 337 336 324 310 309 (4) (19) (25) (9) (3) (4) (6) (27) 307 306 292 291 290 280 274 273 (42) (9) (4) (11) (4) (5) (3) (7) 272 271 270 269 268 260 259 258 (16) (19) (11) (7) (2) (2) (8) (3) 257 229 220 219 218 217 216 207 (2) (6) (6) (16) (11) (15) (3) (3) 206 205 204 194 193 192 147 140 (16) (6) (3) (1) (10) (2) (4) (7) 127 114 97 96 87 84 (5) (4) (2) (9) (9) (21)
	BB [f]	77	4160 [270°]	401 (100)	401	403 402 400 399 398 386 372 371 (5) (28) (35) (10) (5) (4) (15) (48) 370 369 368 354 353 352 316 315 (83) (63) (24) (12) (22) (10) (5) (12) 314 313 312 311 310 301 300 249 (16) (20) (10) (9) (7) (8) (8) (8) 248 247 235 141 140 128 127 126 (32) (16) (10) (6) (6) (2) (13) (2) 110 109 108 97 96 (8) (6) (5) (6) (48)
	Di-Ac [g]	78	3800 [270°]	399 (9)	315	401 400 398 397 384 373 372 371 (0.5) (2) (0.5) (1) (0.5) (0.5) (1) (4) 370 369 368 367 359 358 357 356 (0.5) (1) (2) (3) (2) (8) (30) (1) 355 341 340 339 327 326 325 324 (2) (1) (1) (2) (2) (4) (10) (1) 317 316 285 284 283 268 267 230 (4) (25) (14) (30) (40) (1) (2) (1) 229 228 227 175 174 162 149 141 (3) (2) (2) (2) (2) (7) (1) (1) 140 127 123 112 110 96 84 43 42 (2) (2) (1) (1) (1) (2) (2) (10) (12)
	Di-TMS [h]	76	3235 [240°]	459 (100)	459	462 461 460 445 444 431 430 429 (1) (5) (40) (1) (3) (1) (3) (7) 428 427 374 373 372 371 370 329 (9) (16) (2) (1) (1) (1) (2) (1) 319 307 306 293 268 126 96 75 73 (2) (1) (4) (2) (2) (2) (2) (5) (11)

Continued Table VII ;

Parent Compound	Derivative type	Spectrum number	GC I	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 315	MB	79	3180 [240°]	339 (48)	308	341 (2), 340 (10), 338 (10), 337 (2), 325 (1), 324 (4), 323 (1), 310 (5); 309 (25), 307 (40), 306 (5), 292 (3), 291 (6), 290 (2), 280 (5), 278 (1); 277 (2), 276 (1), 253 (6), 252 (14), 251 (16), 250 (10), 249 (5), 240 (2); 239 (7), 238 (3), 225 (1), 212 (1), 207 (1), 200 (7), 199 (15), 198 (8); 197 (8), 190 (3), 189 (16), 188 (7), 173 (6), 172 (1), 141 (4), 140 (7); 127 (8), 110 (3), 109 (2), 108 (4), 100 (3), 96 (9), 84 (20)
	BB [i]	80	4120 [270°]	401 (71)	370	402 (21), 400 (21), 386 (3), 373 (9), 372 (41), 371 (41), 369 (6), 355 (1); 354 (3), 353 (7), 352 (2), 328 (1), 315 (2), 314 (13), 313 (15), 312 (8); 302 (1), 301 (7), 300 (1), 262 (6), 261 (8), 260 (7), 259 (7), 249 (2); 248 (10), 247 (5), 236 (1), 235 (6), 234 (1), 140 (5), 127 (5), 110 (4); 109 (5), 108 (3), 97 (3), 96 (29), 85 (21)
	Di-Ac [j]	81	3770 [270°]	399 (8)	315	400 (2), 384 (0.5), 371 (1), 369 (1), 368 (3), 367 (1), 358 (7), 357 (30); 327 (3), 326 (5), 325 (6), 317 (4), 316 (23), 285 (2), 284 (4), 283 (3); 268 (1), 229 (3), 228 (3), 227 (2), 176 (2), 175 (1), 162 (7), 145 (11); 140 (1), 110 (1), 96 (4), 84 (3)
	Di-TMS	82	3290 [240°]	459 (100)	459	461 (17), 460 (44), 445 (2), 444 (4), 429 (4), 428 (10), 427 (13), 426 (22); 413 (1), 412 (2), 411 (1), 387 (1), 371 (2), 370 (6), 320 (3), 319 (3); 306 (8), 231 (2), 207 (4), 127 (5), 126 (5), 112 (2), 96 (4), 75 (7), 73 (19)

Parent Compound	Derivative type	Spectrum number	I^c	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
 Mol.wt. 270	MB	101	2775 [240°]	296	296	298 (4); 297 (22); 295 (28); 294 (7); 293 (2); 282 (0.5); 281 (2.5); 280 (0.7); 269 (0.6); 268 (2.9); 267 (1.6); 254 (0.4); 253 (2); 252 (0.8); 251 (0.8); 240 (0.7); 239 (2.3); 238 (1); 226 (4); 225 (8); 224 (2); 213 (3); 212 (6); 211 (2.5); 201 (2); 200 (10); 199 (12); 198 (7); 197 (5); 187 (3); 186 (11); 185 (8); 184 (3); 174 (2.5); 173 (3); 172 (1); 161 (1.5); 160 (4); 159 (1.3); 150 (1.2); 149 (8); 148 (2.5); 147 (3); 136 (0.7); 135 (2.5); 134 (0.8); 121 (1.5); 109 (1.6); 108 (0.6); 107 (1.4); 97 (0.5); 96 (1.5); 95 (6); 94 (1); 93 (1); 81 (1.5); 67 (1)
	BB	102	3760 [270°]	358	358	360 (5); 359 (30); 357 (30); 356 (7); 344 (0.5); 343 (1); 342 (0.5); 331 (0.5); 330 (2.5); 329 (0.5); 315 (0.7); 301 (1); 288 (1.2); 287 (3.3); 286 (1); 275 (1); 274 (2); 273 (1); 263 (1); 262 (3); 261 (5.5); 260 (4.5); 259 (3.5); 248 (1.5); 248 (4.5); 247 (4); 246 (1.5); 237 (0.5); 236 (2.5); 235 (1.5); 223 (1.5); 222 (3.5); 221 (1); 210 (1.5); 209 (3.5); 208 (1.5); 150 (0.5); 149 (3.5); 135 (0.5); 134 (0.7); 109 (0.7); 108 (0.3); 107 (0.7); 96 (0.3); 95 (3.2); 94 (1); 93 (0.5); 79 (0.5); 78 (1)
	Di-Ac [k]	103	3400 [270°]	356	272	358 (0.4); 357 (2); 355 (0.3); 354 (0.5); 341 (0.4); 329 (0.7); 328 (2.5); 316 (1); 315 (6); 314 (24); 313 (1); 312 (2); 300 (0.3); 299 (1.2); 298 (0.5); 274 (2); 273 (21); 271 (2); 270 (2); 255 (0.3); 244 (0.7); 235 (1.2); 188 (0.2); 175 (0.4); 175 (0.5); 174 (0.6); 173 (0.3); 162 (0.4); 161 (0.4); 160 (0.4); 159 (0.2); 150 (0.3); 149 (0.5); 136 (0.4); 135 (0.3); 123 (0.6); 95 (0.5); 60 (1)
	Di-TMS	104	2835 [240°]	416	416	418 (15); 417 (37); 403 (0.5); 402 (1.4); 401 (3.5); 389 (0.2); 388 (0.6); 346 (0.4); 345 (0.7); 344 (0.4); 329 (0.4); 328 (1.5); 327 (4.0); 326 (0.7); 320 (0.5); 319 (1.2); 318 (0.3); 317 (0.4); 307 (0.7); 306 (0.7); 305 (0.7); 294 (0.4); 293 (0.2); 281 (0.5); 280 (0.5); 268 (0.5); 267 (1.2); 232 (0.5); 231 (2); 219 (0.3); 218 (0.2); 217 (0.3); 205 (0.7); 179 (0.4); 147 (0.6); 109 (0.3); 95 (1.2); 75 (2); 74 (1); 73 (7)
 Mol.wt. 270	MB	105	2765 [240°]	296	296	298 (4); 297 (22); 295 (30); 294 (10); 293 (3); 282 (0.5); 281 (3); 280 (0.6); 268 (1.5); 267 (1.5); 259 (0.5); 258 (1.5); 257 (0.7); 256 (1); 240 (0.5); 239 (2); 238 (1); 226 (4); 225 (8); 224 (2.5); 213 (3); 212 (6.5); 211 (2.5); 201 (2); 200 (12); 199 (13); 198 (7); 197 (5); 188 (1); 187 (4); 186 (15); 185 (8); 184 (3); 174 (2); 173 (3); 172 (1.5); 161 (2); 160 (4.5); 159 (1); 150 (1); 149 (9.6); 148 (3); 147 (2.5); 136 (1); 135 (3.5); 134 (0.5); 133 (0.5); 121 (2)

Continued Table VII ;

Parent Compound	Derivative type	Spectrum number	I^c	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
						109 (2.5) 108 (1) 107 (2) 97 (1) 96 (2.5) 95 (11) 94 (2) 93 (1.5)
						81 (2) 69 (1) 67 (1)
	BB	106	3730 [270°]	358	358	360 (4) 359 (30) 357 (25) 344 (0.3) 343 (1.7) 342 (0.3) 331 (0.3) 330 (1.2)
						329 (0.6) 316 (0.2) 315 (1) 314 (0.2) 289 (0.4) 288 (2.5) 287 (4.3) 286 (1)
						275 (2) 274 (3) 273 (1) 263 (1) 262 (4.5) 261 (6.5) 260 (2.8) 259 (2)
						249 (1.8) 248 (5.4) 247 (3.3) 246 (1) 236 (1) 235 (1.5) 234 (0.5) 223 (1)
						222 (2) 221 (0.5) 211 (0.3) 210 (1) 209 (1.5) 208 (0.5) 150 (0.6) 149 (0.9)
						148 (0.3) 136 (0.3) 135 (1.3) 134 (0.3) 133 (0.2) 121 (0.6) 109 (0.8) 108 (0.3)
						107 (0.7) 97 (0.3) 96 (0.5) 95 (2.8) 94 (0.6) 93 (0.4) 81 (0.5) 73 (0.5)
						67 (0.5)
	Di-Ac [1]	107	3370 [270°]	356	272	358 (0.2) 357 (1.4) 341 (0.3) 329 (0.4) 328 (1.3) 316 (0.7) 315 (5) 314 (20)
						300 (0.3) 299 (1) 298 (0.5) 297 (0.3) 296 (0.8) 274 (3) 273 (22) 255 (0.6)
						254 (0.8) 236 (1) 201 (0.3) 176 (0.7) 175 (0.7) 162 (0.7) 161 (0.3) 160 (0.7)
						149 (0.7) 95 (0.8) 60 (1.2)
	Di-TMS	108	2875 [240°]	416	416	418 (15) 417 (40) 402 (2) 401 (4) 389 (0.4) 388 (1.3) 346 (0.5) 345 (1.3)
						344 (1.2) 329 (1) 328 (3.5) 327 (8) 320 (0.8) 319 (1.7) 307 (0.6) 306 (0.8)
						305 (0.5) 294 (0.4) 293 (0.4) 281 (0.4) 268 (0.4) 267 (0.8) 257 (0.5) 251 (0.6)
						245 (0.5) 233 (0.5) 232 (0.7) 231 (3.7) 219 (0.3) 218 (0.3) 217 (0.5) 206 (0.3)
						205 (1.6) 193 (0.2) 179 (0.5) 147 (0.7) 135 (0.3) 109 (0.4) 95 (1.5) 73 (14)

COMMENTS

a. The GLC conditions were the same as in Table I(a), except the column packing was 1% OV-25, on Gas Chrom Q (100-120,mesh).

b. The GC-MS data were recorded at the same conditions as in Table I(b).

c. The retention index "I" values for these derivatives were measured at different oven temperatures "°C", these were indicated between the large brackets [ ].

Metastable ions were observed as follows:-

(d) 250.0(calcd. for 328→286, 249.4) ; (e) 250.0(calcd. for 328→286, 249.4);

(f) 341.0(calcd. for 401→370, 341.4) ; (g) 378.0(calcd. for 357→315, 377.9);

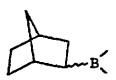
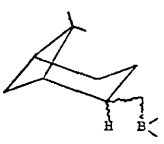
(h) 398.0(calcd. for 459→427, 397.2) ; (i) 341.0(calcd. for 401→370, 341.4);

(j) 278.0(calcd. for 357→315, 277.9) & 255.0(calcd. for 315→283, 254.2) ;

(k) 277.0(calcd. for 356→314, 276.96)& 236.0(calcd. for 314→272, 235.6) ;

(l) 277.0(calcd. for 356→314, 276.96)& 236.0(calcd. for 314→272, 235.6) .

Table VIII, Retention index values[a] and mass spectrometric data[b] for catechol(I); 2,3-butanediol(II); and cis- and indanediol(III) esters of cyclohexane-, norbornane-2-β, and β-pinane-10β-boronic acids.

Compound	Diol Derivative	Spect. No.	I^{a}_{ov-17}	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
	(I) $C_{12}H_{15}BO_2$	112	1700 (120°)	202 (90)	120	67 (9) 82 (10) 118 (20) 119 (7) 133 (0.5) 134 (1) 146 (1) 159 (0.5) 160 (1.5) 161 (0.5) 173 (0.5) 174 (0.7) 201 (18) 203 (12)
	(II) $C_{15}H_{19}BO_2$	113	2095 (160°)	242 (11)	116	117 (12) 132 (3) 133 (1) 159 (0.5) 160 (2) 161 (0.4) 241 (3) 243 (2)
Cyclohexaneboroxine $C_{18}H_{33}B_3O_3$		111	2310 (180°)	330 (22)	82	67 (7) 80 (3) 81 (4) 83 (11) 120 (0.9) 201 (0.3) 202 (0.7) 203 (0.3) 245 (0.5) 246 (1.3) 247 (1.8) 248 (1.4) 249 (1.6) 259 (0.6) 260 (0.7) 261 (0.8) 262 (1.3) 272 (0.7) 273 (1.2) 274 (0.6) 286 (1.5) 287 (3.8) 288 (4) 289 (1) 300 (2) 301 (7) 302 (8) 303 (2) 314 (1) 315 (1.2) 328 (4) 329 (15) 331 (5)
	(I) $C_{13}H_{15}BO_2$	109,I ENDO	1790 (120°)	214 (68)	81	67 (85) 80 (6) 82 (8) 94 (28) 101 (5) 119 (14) 120 (45) 121 (5) 133 (15) 144 (12) 145 (26) 146 (50) 147 (10) 160 (22) 172 (16) 173 (30) 184 (10) 185 (26) 186 (17) 199 (81) 213 (20) 215 (10)
		109,II EXO	1805 (120°)	214 (35)	67	80 (5) 81 (70) 82 (5) 94 (23) 101 (3) 119 (11) 120 (40) 121 (2) 133 (8) 144 (8) 145 (17) 146 (41) 147 (8) 148 (8) 159 (5) 160 (14) 172 (8) 173 (14) 184 (5) 185 (20) 186 (8) 199 (4) 213 (11) 215 (5)
	(III) $C_{16}H_{19}BO_2$	110,I ENDO	2185 (160°)	255 (4)	116	79 (8) 115 (30) 117 (13) 132 (8) 160 (4) 170 (4)
		110,II EXO	2205 (160°)	255 (3)	116	67 (8) 68 (2) 77 (8) 79 (5) 80 (1) 81 (1) 93 (2) 94 (7) 95 (2) 103 (7) 104 (5) 115 (23) 117 (15) 129 (2) 131 (3) 132 (6) 133 (4) 141 (1.5) 142 (1) 158 (1.5) 159 (2) 169 (2.5) 181 (4.5) 185 (1.5) 186 (2.5) 187 (1) 195 (0.4) 213 (0.8) 225 (1) 226 (1) 254 (0.5) 256 (0.5)
	(I) $C_{16}H_{21}BO_2$	114,[c] ENDO,I EXO,II	I, 2030 II,2050 (140°)	256 (24)	213	67 (50) 69 (42) 81 (35) 82 (55) 83 (16) 95 (15) 96 (7) 108 (1) 109 (20) 110 (3) 120 (3) 121 (1) 122 (50) 123 (12) 133 (4) 134 (4) 135 (1) 136 (1) 137 (1) 145 (2) 146 (4) 147 (3) 158 (4) 159 (12) 160 (15) 161 (4) 172 (5) 173 (8) 174 (12) 175 (5) 185 (12) 186 (36) 187 (10) 188 (8) 199 (25) 200 (45) 201 (30) 212 (26) 214 (32) 225 (1) 227 (5) 228 (2) 240 (4) 241 (14) 242 (3) 255 (5) 257 (4)
	(II) $C_{14}H_{25}BO_2$	115,[c] DL ENDO,I EXO, II Meso ENDO,III EXO, IV	I, 1635 II,1650 III,1700 IV,1715 (120°)	236 (8)	193	67 (30) 69 (22) 81 (35) 82 (70) 83 (20) 93 (16) 95 (23) 107 (10) 109 (8) 109 (30) 110 (5) 111 (6) 120 (10) 121 (5) 122 (70) 123 (10) 124 (5) 136 (4) 137 (10) 138 (4) 139 (5) 149 (4) 151 (4) 165 (6) 166 (18) 167 (5) 168 (4) 179 (22) 180 (22) 181 (22) 192 (30) 194 (25) 220 (5) 221 (20) 222 (4) 235 (2) 237 (1)

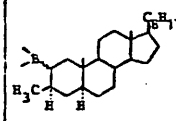
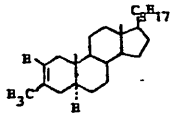
Footnotes to Table

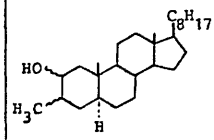
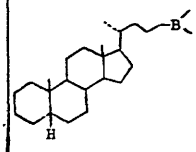
a. The retention index values(I) were measured on column 1% ov-17, 2m x 2mm, oven temperature as indicated between the small brackets(°), and the rest of GLC conditions are described in Chapter 2, p.58.

b. The mass spectra conditions, are described on p.59.

c. The DL and meso; Exo and Endo; isomers all gave identical mass spectra.

Table IX. Retention index values[a] and mass spectrometric data[b] for catechol(I), 2,3-butanediol(II), and cis-indanediol(III) esters of 3-methyl-5α-cholestane-2β-boronic acid and 5β-cholane-24-boronic acid

Compound	Diol Derivative	Spect. No.	I^{a}_{ov-17}	$M^{+\cdot}$	m/z Base Peak	Other ions m/z (including isotope peaks)
	(I) $C_{34}H_{53}BO_2$	117	4120 (280°)	504[c] (37)	166	81 (9) 82 (8) 83 (6) 95 (12) 106 (6) 107 (9) 108 (15) 109 (12) 110 (8) 120 (15) 121 (14) 122 (21) 123 (12) 135 (29) 136 (88) 137 (15) 151 (65) 152 (14) 161 (14) 162 (12) 163 (12.5) 167 (15) 176 (9) 177 (25) 178 (6) 179 (25) 180 (9) 188 (3) 189 (8) 190 (4) 203 (9) 215 (9) 229 (65) 230 (53) 231 (41) 242 (1) 243 (1) 244 (17) 245 (10) 246 (5) 247 (47) 248 (41) 249 (9) 262 (32) 263 (7) 271 (8) 281 (47) 282 (15) 314 (4) 315 (18) 316 (3) 327 (5) 328 (10) 329 (3) 347 (10) 348 (41) 349 (35) 350 (12) 354 (10) 362 (3) 363 (12) 364 (7) 368 (18) 383 (41) 384 (15) 385 (17) 401 (53) 402 (18) 428 (5) 429 (2) 487 (6) 488 (15) 489 (6) 503 (12) 505 (16)
	(II) $C_{32}H_{57}BO_2$	116	DL ,3535 & Meso,3620 (260°)	483[d] (60)	328	81 (4) 82 (3) 83 (3) 95 (7) 100 (0.5) 101 (2) 106 (3) 107 (4.5) 108 (5) 109 (6) 111 (4) 119 (2) 120 (4) 121 (6) 122 (9) 123 (6.5) 126 (26) 135 (12) 136 (26) 137 (6) 147 (4) 148 (4) 149 (8) 150 (3) 151 (21) 152 (6) 166 (40) 167 (5) 177 (10) 179 (5) 181 (3) 189 (4) 190 (4) 191 (2) 192 (2) 193 (5) 194 (4) 195 (6) 219 (5) 220 (20) 221 (24) 222 (5) 229 (7) 230 (5) 231 (7) 232 (4) 233 (6) 234 (8) 235 (5) 247 (8) 248 (5) 260 (15) 261 (40) 262 (42) 263 (15) 274 (4) 275 (3) 276 (3) 287 (2.5) 288 (2.5) 289 (4) 290 (2) 300 (2) 301 (5) 302 (2) 313 (4) 314 (10) 315 (7) 316 (1) 327 (20) 329 (75) 330 (30) 342 (7.5) 343 (25) 344 (6.5) 367 (1) 368 (4) 369 (2) 370 (4) 371 (1) 383 (5.5) 384 (2.5) 385 (2.5) 399 (1.5) 401 (5) 428 (5) 429 (2) 430 (2) 467 (10) 468 (35) 469 (10) 482 (15) 484 (20) 485 (6)
	(III) $C_{37}H_{57}BO_2$	120	4495 (280°)	544[e] (94)	388	95 (6) 115 (7) 116 (60) 117 (27) 121 (8) 122 (10) 148 (4) 149 (6) 150 (3) 161 (7) 162 (3) 163 (6) 229 (4) 230 (4) 231 (10) 232 (4) 262 (40) 263 (10) 282 (5) 283 (2) 287 (2) 288 (6) 289 (4) 290 (4) 293 (4) 301 (8) 319 (16) 320 (47) 321 (17) 322 (15) 327 (40) 328 (12) 355 (2) 373 (6) 374 (13) 375 (7) 387 (10) 389 (93) 390 (40) 391 (7) 402 (10) 403 (33) 404 (12) 528 (30) 529 (46) 530 (33) 543 (30) 545 (40)
 $C_{28}H_{46}$		118	3030 (240°)	384	384	81 (15) 82 (10) 83 (10) 94 (7) 95 (24) 96 (5) 97 (11) 105 (17) 106 (24) 107 (17) 106 (17) 109 (15) 110 (4) 111 (15) 119 (12) 120 (47) 121 (18) 122 (62) 123 (26) 134 (11) 135 (20) 136 (30) 137 (10) 147 (12) 148 (10) 149 (15) 150 (4) 151 (16) 161 (35) 162 (25) 163 (37) 164 (16) 165 (6) 166 (20) 175 (11) 176 (26) 177 (16) 189 (12) 190 (7) 203 (40) 204 (12) 215 (7) 216 (3) 217 (12) 218 (3) 229 (53) 230 (42) 231 (90) 232 (57) 233 (16) 244 (22) 245 (6) 246 (14) 260 (5) 261 (2) 262 (14) 263 (3) 271 (17) 272 (4) 273 (2) 274 (7) 275 (5) 287 (4) 288 (2) 289 (1) 300 (10) 301 (3) 302 (28) 313 (10) 314 (20) 315 (95) 316 (26) 328 (1) 327 (6) 328 (4) 329 (1) 340 (15) 356 (22) 369 (8) 370 (11) 371 (2)

						385 (35) 386 (26)
 $C_{28}H_{50}O$		119	3355 (260°)	402[f] (5)	245	81 (5) 82 (5) 83 (4) 95 (6) 96 (2) 97 (5) 108 (12) 109 (10) 110 (3) 111 (5) 121 (5) 122 (10) 123 (12) 124 (5) 134 (10) 135 (10) 136 (27) 137 (16) 138 (7) 150 (4) 151 (20) 152 (4) 165 (2) 166 (33) 167 (4) 177 (7) 178 (5) 179 (15) 180 (10) 181 (5) 188 (5) 189 (4) 190 (4) 191 (2) 203 (4) 204 (4) 217 (4) 218 (6) 219 (3) 229 (10) 230 (5) 231 (16) 232 (6) 244 (6) 246 (80) 247 (25) 248 (5) 260 (30) 261 (7) 262 (6) 263 (4) 313 (2) 314 (2.5) 315 (5) 316 (1) 327 (6) 328 (3) 329 (1.5) 383 (5) 384 (20) 385 (7) 400 (60) 401 (20)
	(I) $C_{30}H_{45}BO_2$	121	3830 (260°)	448[g] (65)	217	81 (3) 82 (4) 83 (3) 94 (1.5) 95 (6) 96 (1.5) 97 (2) 107 (2.5) 108 (18) 109 (8) 121 (5) 122 (10) 123 (9) 133 (2) 134 (5) 135 (5) 136 (7) 137 (5) 145 (4) 146 (15) 147 (2) 148 (5) 149 (9) 150 (10) 151 (22) 152 (26) 162 (4) 163 (4) 165 (15) 175 (4) 176 (2) 177 (2) 187 (1.5) 188 (1.5) 189 (3) 190 (1.5) 191 (1) 202 (2) 203 (8) 204 (2) 215 (5) 216 (20) 218 (70) 219 (20) 220 (3) 227 (3.5) 228 (11) 229 (2.5) 230 (2) 231 (1) 232 (6.5) 233 (1.5) 257 (2) 258 (1) 259 (2) 336 (1) 337 (4.5) 338 (1.5) 351 (0.5) 352 (2.5) 353 (0.5) 391 (1) 392 (1) 432 (7.5) 433 (25) 434 (9) 447 (20) 449 (22) 450 (5)
	(II) $C_{28}H_{49}BO_2$	122	DL,3350 Meso,3440 (260°)	428[h] (40)	217	81 (3.5) 82 (3) 83 (2.5) 94 (2) 95 (6) 96 (1.5) 97 (3) 107 (2) 108 (21) 109 (9.5) 110 (2.5) 121 (7) 122 (17) 123 (10) 135 (5) 136 (5.5) 137 (5) 148 (7) 149 (12) 150 (15) 151 (27) 152 (5) 161 (2) 162 (5.5) 163 (4.5) 164 (2) 165 (1) 167 (12) 168 (2) 169 (1) 170 (1) 175 (4) 176 (3) 177 (2) 178 (1) 189 (3) 190 (2) 191 (1) 203 (8) 204 (3) 205 (1.5) 218 (65) 219 (16) 231 (1) 232 (9) 233 (3.5) 257 (1.5) 258 (1.5) 259 (2) 263 (0.5) 264 (2) 316 (1) 317 (4) 318 (1) 330 (1.5) 331 (2.5) 332 (0.5) 412 (7) 413 (20) 414 (7) 427 (12) 429 (12)

Footnotes to Table IX

a. The retention index values(I) were measured on column 1% ov-17, 2m x 2mm, oven temperature as indicated between the small brackets (°), nitrogen flow rate 40ml/min, and the rest of the GLC conditions are described in Chapter 2, p.58.

b. The mass spectra conditions are described on p.59.

Metastable ions

c. 473 (calcd. for 504 —— 488, 472.5)

d. 454 (calcd. for 483 —— 468, 453.5)

e. 513 (calcd. for 544 —— 529, 514.4)

f. 369 (calcd. for 400 —— 384, 368.6)

g. 418 (calcd. for 448 —— 433, 418.5)

h. 398 (calcd. for 428 —— 413, 398.5)

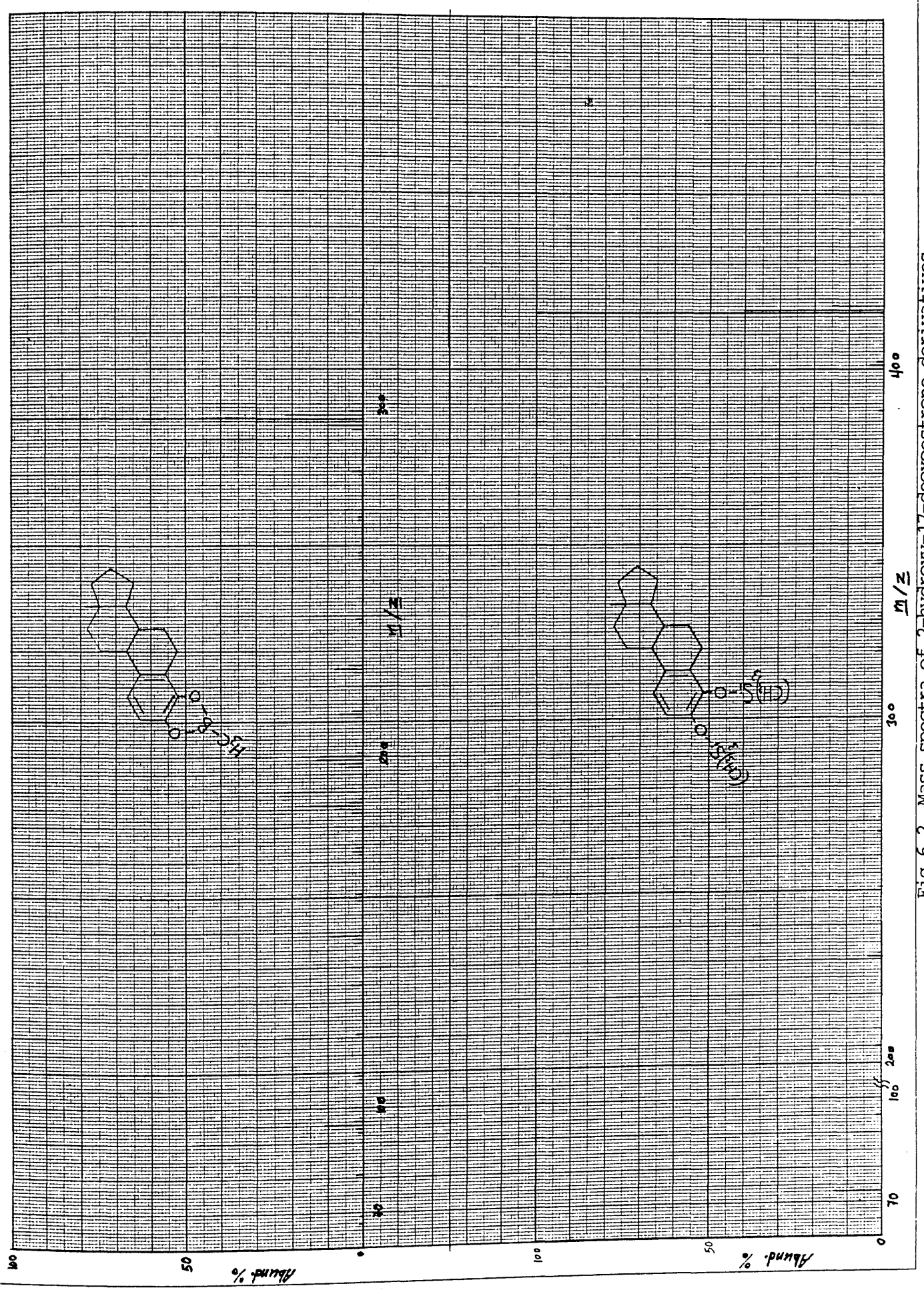


Fig.6.2 Mass spectra of 2-hydroxy-17-deoxoestrone derivatives.

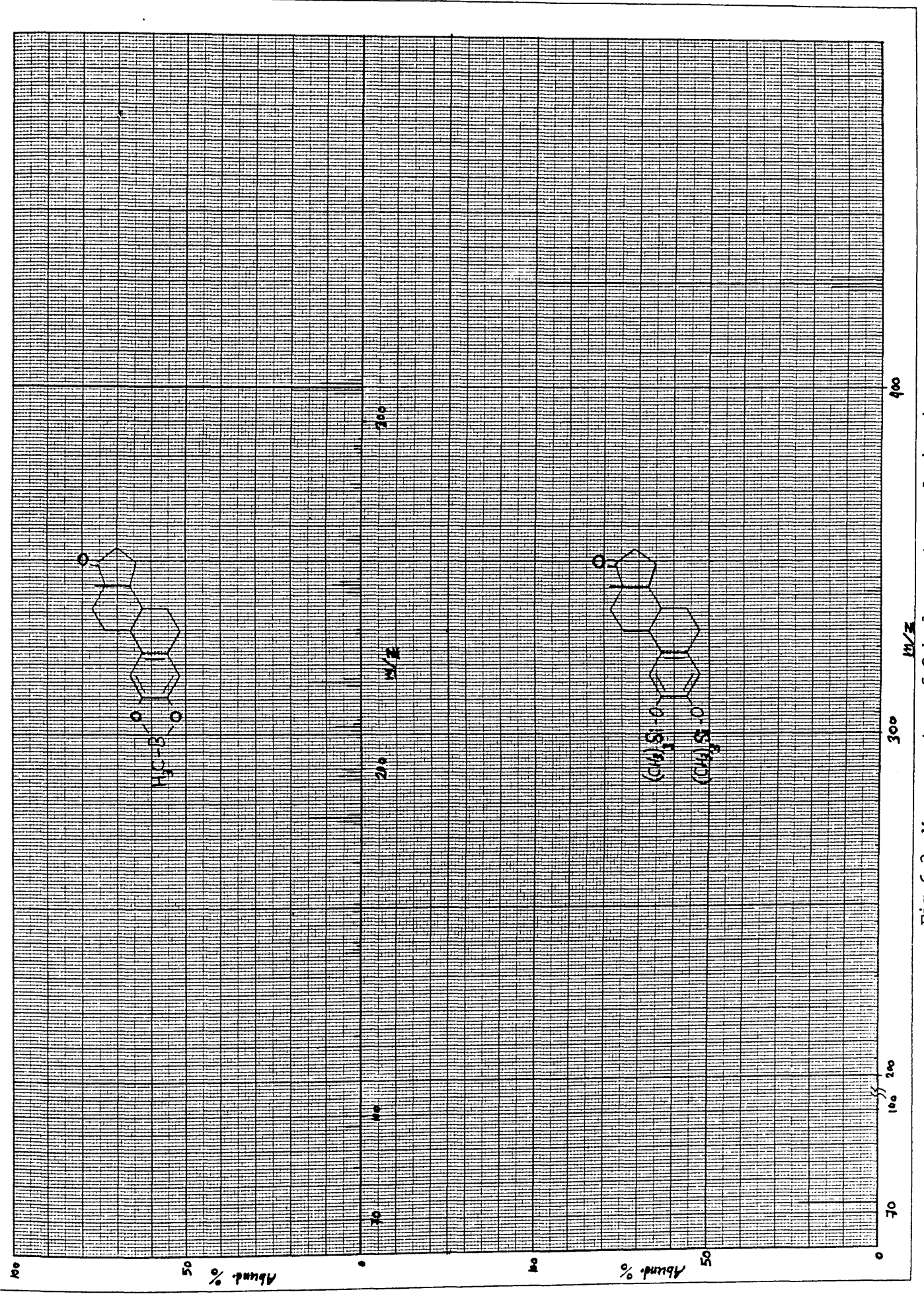


Fig.6.3 Mass spectra of 2-hydroxyestrone derivatives.

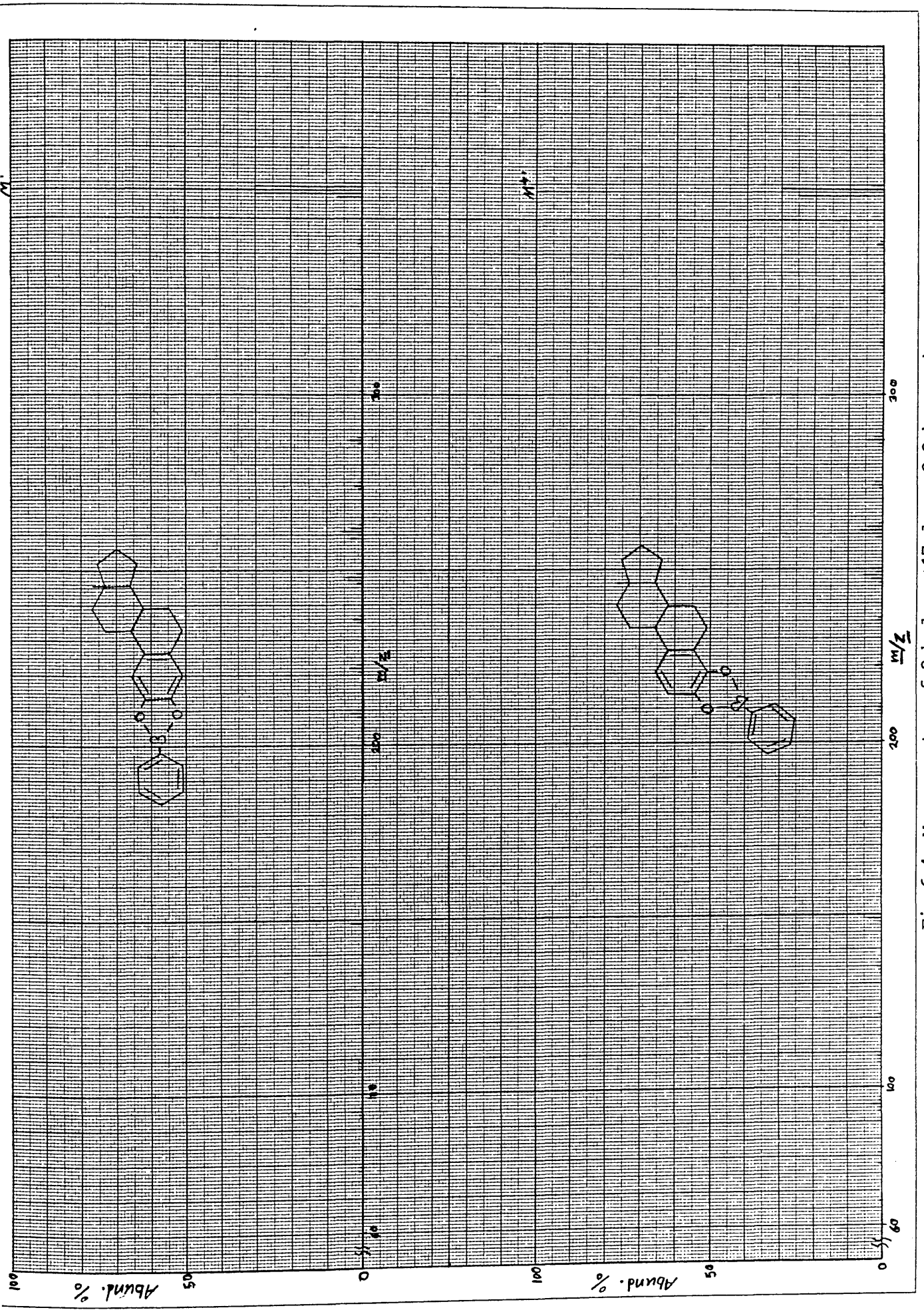


Fig.6.4 Mass spectra of 2-hydroxy-17-deoxo-2,3-benzeneboronate.

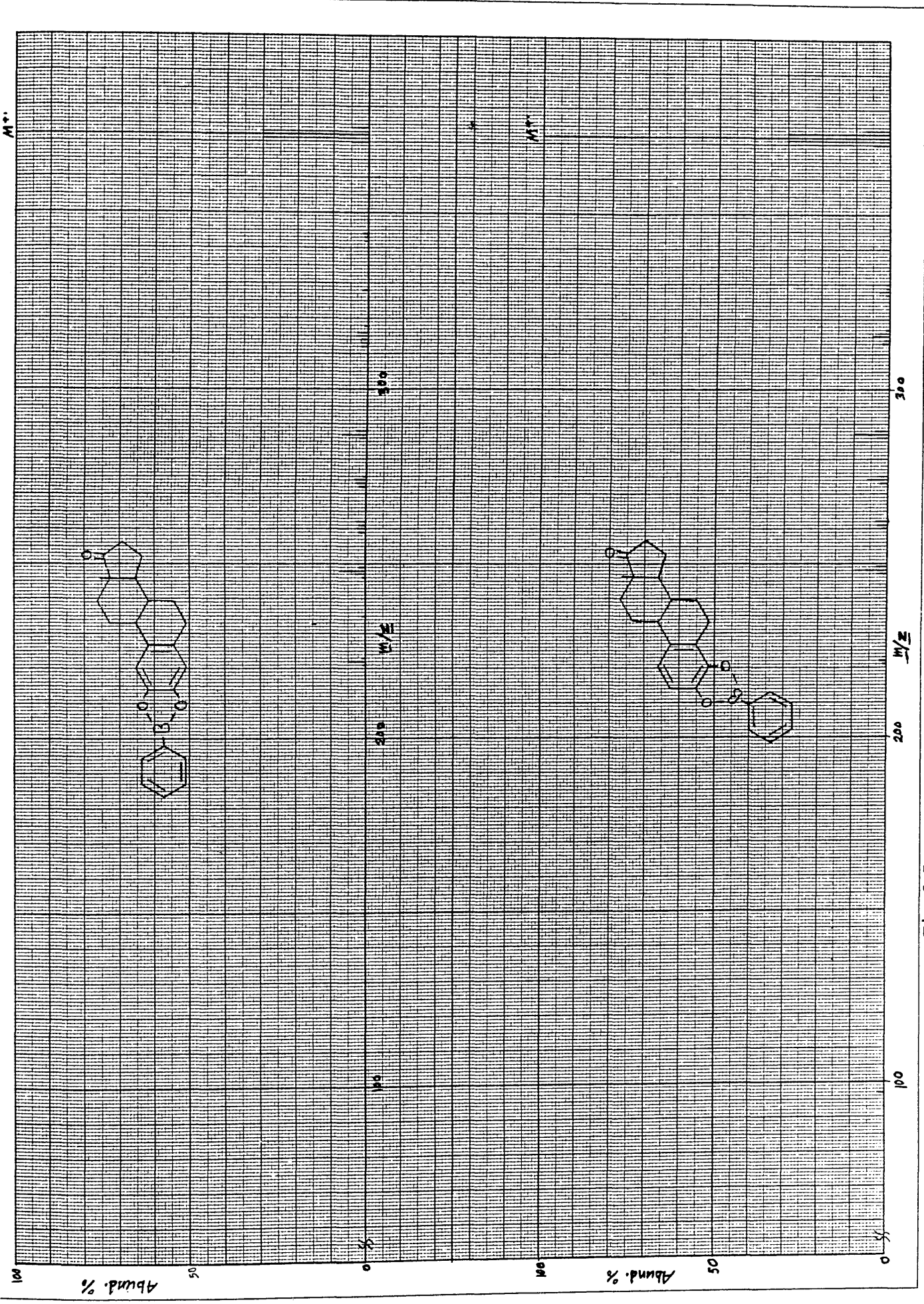
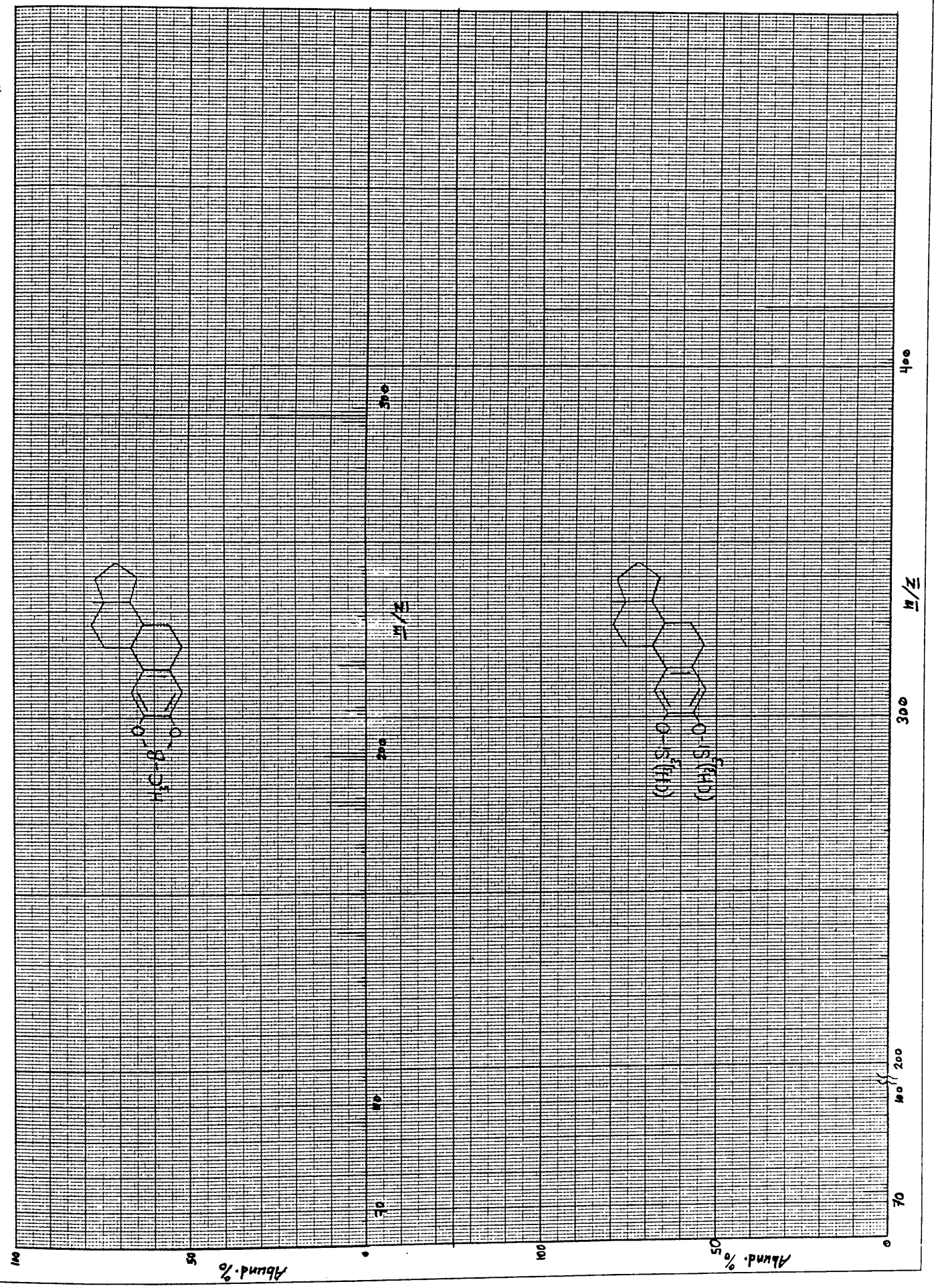
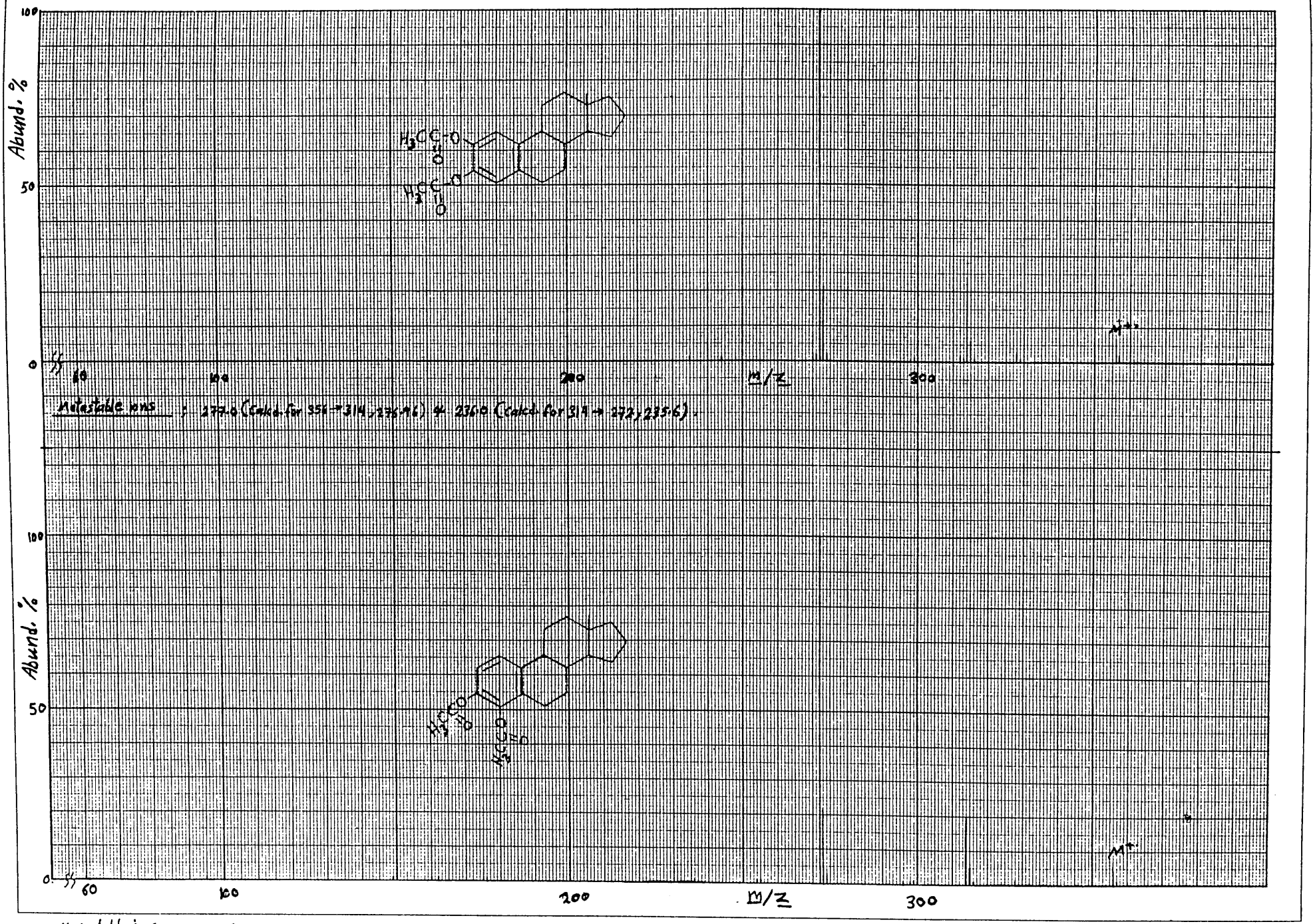
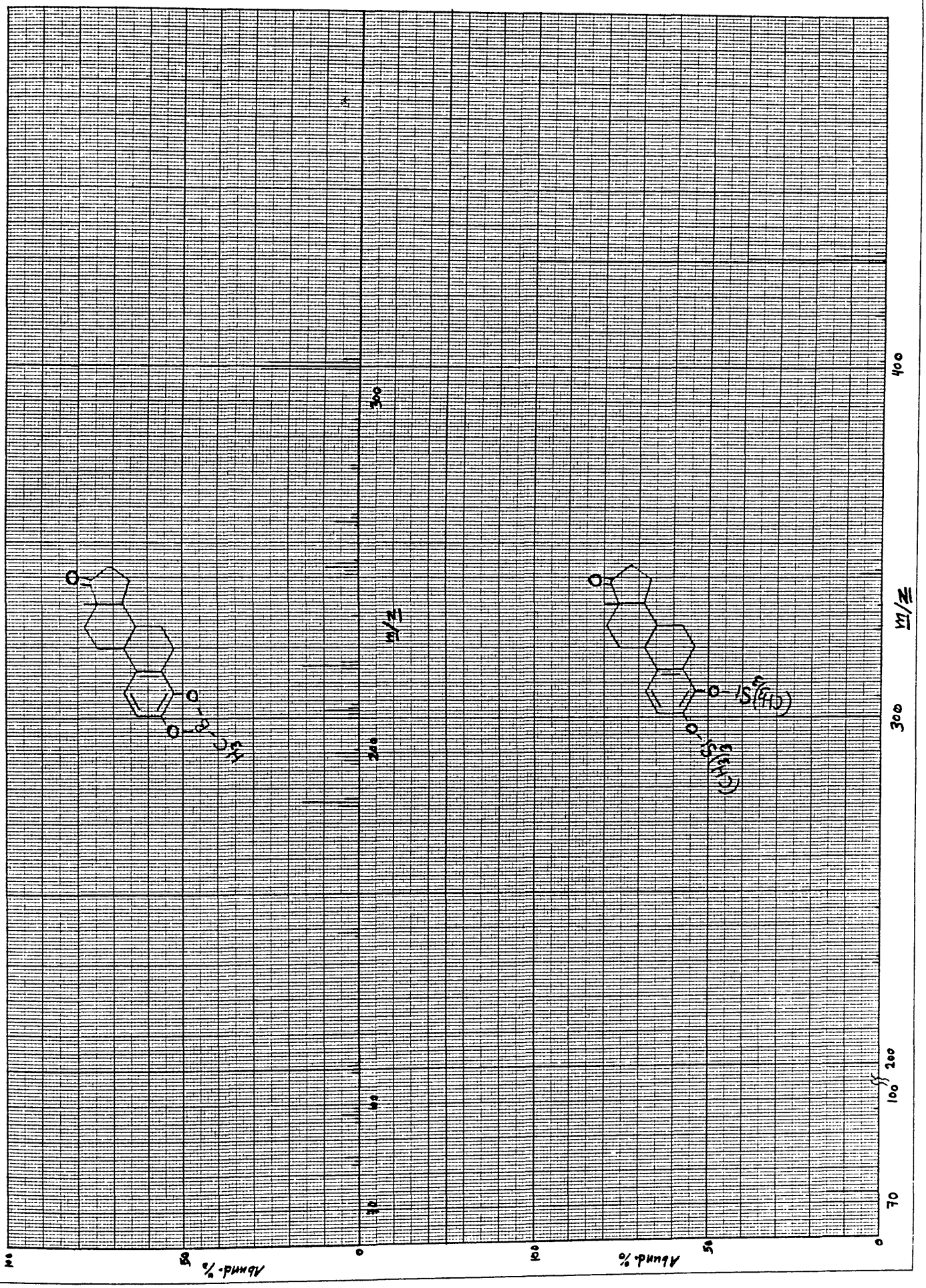


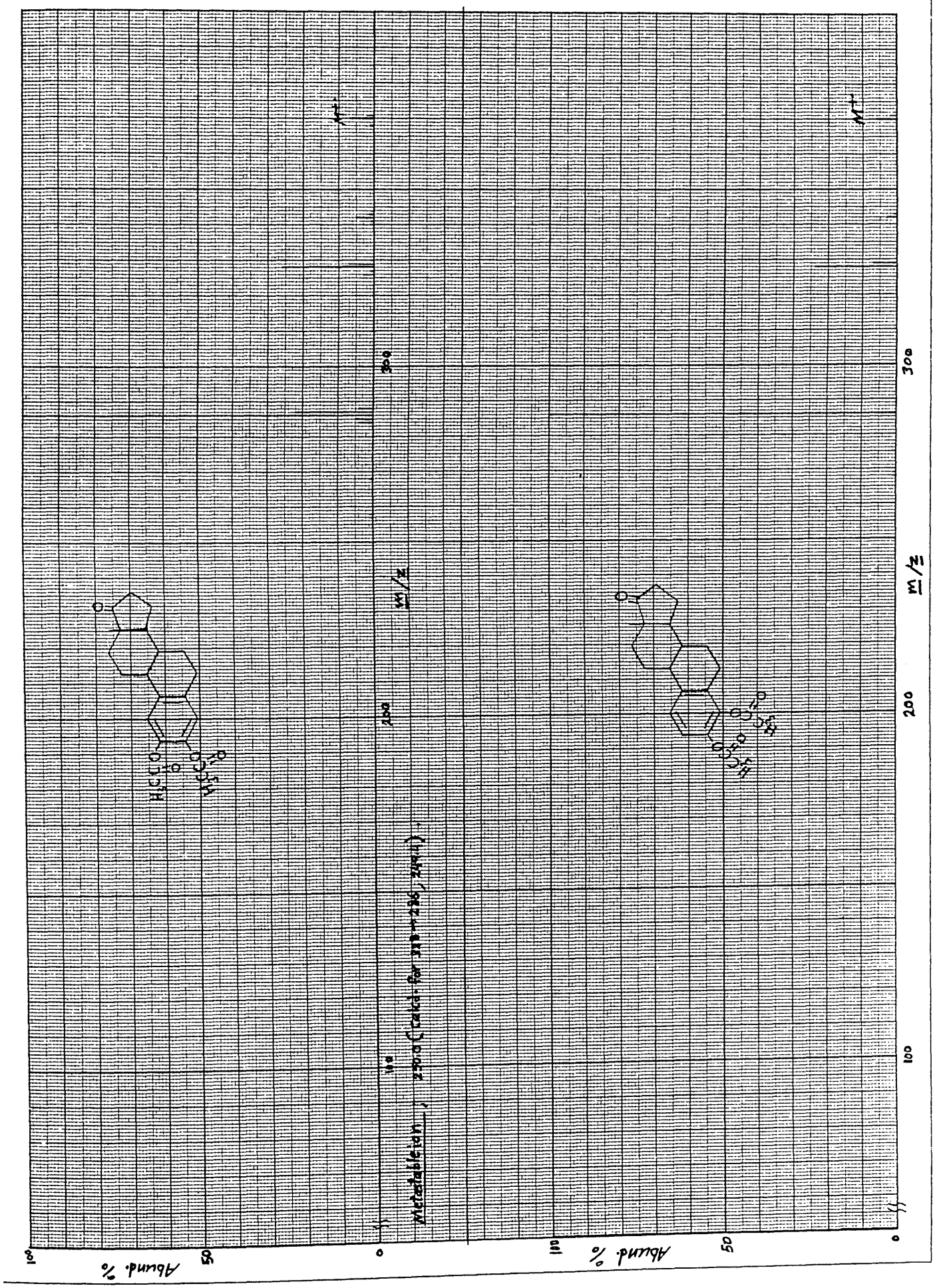
Fig.6.5 Mass spectra of 2-hydroxyestrone-2,3-benzeneboronate.

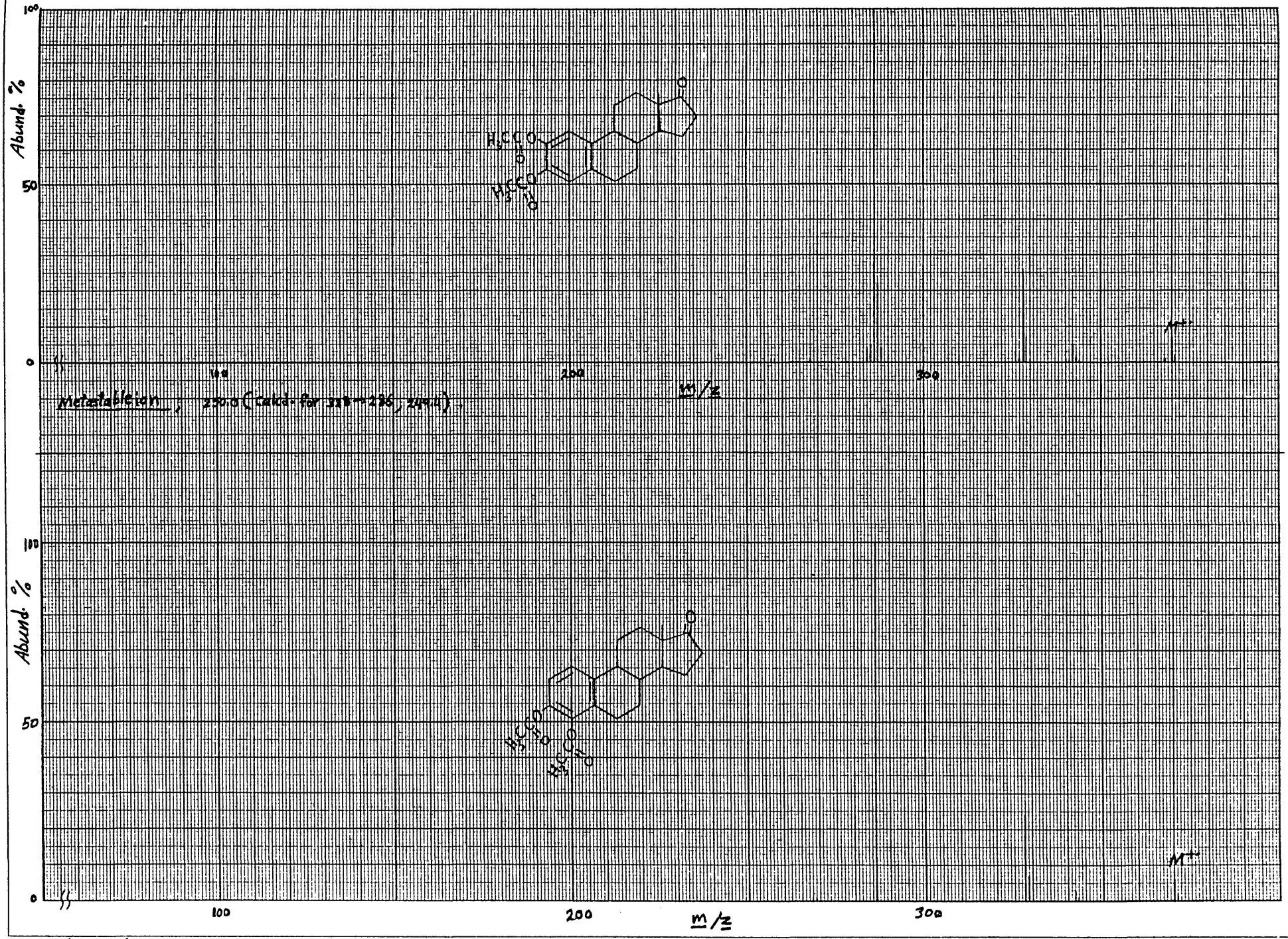

Abund. %
m/z
Abund. %
m/z



Metastable ions ; 277.0 (Calcd. for 356 → 314, 276.96) & 236.0 (Calcd. for 314 → 272 , 235.6).


Abund. %
m/z
70
100
200
300
400
Si(CH3)3
Si(CH3)3


Abund. %
m/z
Metastable ion, 2200 (calcd. for 318→286, 2249)
Abund. %
m/z
Metastable ion, 2200 (calcd. for 318→286, 2249)



Metastable ion ; 250.0 (calcd. for 328 → 286, 249.4) .

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