RATIONAL APPROACHES to the CORRELATION of

CHEMICAL STRUCTURE with BIOLOGICAL ACTIVITY

A thesis submitted to the University of Glasgow

for the degree of

DOCTOR of PHILOSOPHY

in the

Faculty of Science

by

IVOR BROWN B.Sc.(Hons.).

November, 1962.

Experimental Pharmacology,
Institute of Physiology,
The University,
Glasgow, W.2.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

The convention for citing references employed in this thesis is that of the Journal of the Chemical Society.

Acknowledgements.

I wish to thank Professor Stanley Alstead, C.B.E., and Professor R. C. Garry, F.R.S.E., for providing the opportunity to carry out this research. I am also grateful to Mr. J. J. Lewis for his interest and encouragement and to Professor R. A. Raphael, F.R.S., for permitting free use to be made of facilities in the Department of Chemistry.

I am deeply indebted to Dr. M. Martin-Smith for suggesting the problems contained in this thesis and for his constant advice and encouragement. I also wish to thank Dr. G. Eglinton for his collaboration in the infrared spectroscopy studies, Drs. G. L. Buchanan, R. Hodges, K. H. Overton, W. Parker and R. I. Reed for helpful discussions, and Mr. J. M. L. Cameron, B.Sc., and his assistants for performing the microanalyses. Mrs. F. Lawrie kindly ran the infrared spectra and Dr. R. J. Hamilton examined the plant waxes by gas - liquid chromatography. My thanks are due to all my colleagues in the University, particularly to Dr. T. C. Muir, for their helpful criticisms, and to Miss Doreen Barclay, Miss June Galbraith and Miss Irene Wilson for technical assistance.

Finally, I wish to thank Glaxo Laboratories Ltd. for the generous award of a Research Fellowship during the period of this work and for providing supplies of Bacopa monnieri, and Messrs T. & H. Smith for gifts of codeine and thebaine.

Summary.

This thesis is divided into four distinct and self-contained parts whose unifying theme is contained in the general title "Rational Approaches to the Correlation of Chemical Structure with Biological Activity."

Part I contains a physico-chemical approach to the problem, describing infrared spectroscopy studies made on several series of ortho-bromophenols with the object of determining the influence of steric compression, electronic and solvent effects, temperature, competitive intermolecular hydrogen-bonding and change of state on the intramolecular hydrogen bond normally found in these compounds. The postulate is made, based on suggestions in the literature, that the bactericidal activity of phenols could be dependent upon the ability of the phenolic hydroxyl group to bind with constituents of the living organism, and from the results obtained in the infrared studies, a prediction is made of the expected order of bactericidal potency of the compounds investigated.

Part II describes a scheme for transforming the morphine ring system into the steroid ring system with a view to obtaining biologically-interesting 19-nor 9-substituted steroids bearing substituents capable of being utilised as centres for further reactions. The intermediates in the scheme would also be of considerable interest in view of the many attempts to produce analgesics devoid of addicting

properties. The occurrence of an unexpected dimerisation in the first step of the projected conversions proved the original scheme to be unfeasible. Alternative procedures were not investigated owing to the lack of simplicity promised in the original. The dimerisation, which actually occurred in attempts to epimerise the hydrogen atom at $C_{(14)}$ in codeinone, is fully rationalised in terms of other work in the literature.

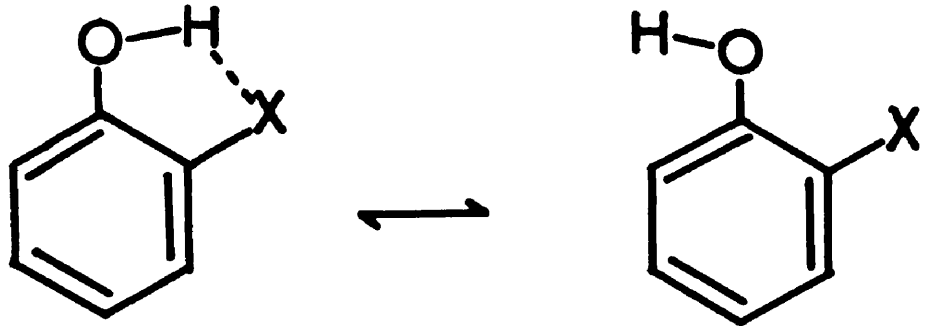
Part III describes a traditional approach, that of chemically investigating a plant which has been reported to contain pharmacologically-active alkaloids and which has been used in native medical practice. The chemical examination of Bacopa monnieri (L) Pennel is reported in this instance, the alkaloidal content, however, being too small to permit of detailed investigation. The presence and characterisation of other organic components of the plant are reported.

Part IV justifies the rationale of isosteric replacement in biologically-active molecules and deals with attempts to prepare 3-(2'-aminoethyl)-5-hydroxythionaphthen, the thionaphthen isostere of 5-hydroxytryptamine. Synthetic approaches to the thionaphthen isostere of 4-hydroxy-tryptamine are also described. The first reported instance of a 16-membered hydrogen-bonded dimer which was encountered during this work is recorded, and the formation of ortho diazo-oxides by the action of nitric acid on acetamido compounds is discussed.

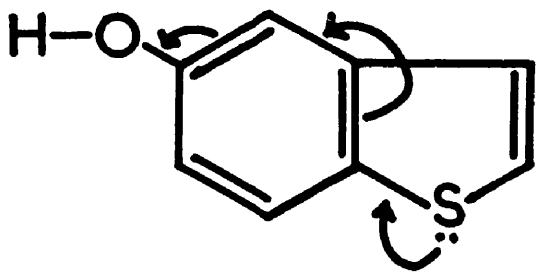
Contents.

PART I.

INVESTIGATION of HYDROGEN BOND STRENGTHS by INFRARED SPECTROSCOPY as a POSSIBLE CORRELATION with BACTERICIDAL ACTIVITY in SERIES of ortho-BROMOPHENOLS.



I II



III

Introduction.

It is well known[1-7] that intramolecular hydrogen-bonding between the hydroxyl function and the halogen atom occurs in ortho-chloro-, -bromo- and -iodophenols and that those compounds in which the second ortho position remains unsubstituted show two hydroxyl stretching frequencies in the infrared spectrum when examined in non-polar solvents. The presence of these two bands can be attributed[8] to the co-existence of the conformations I and II, the two absorption bands arising from differences in the character of the hydroxyl group in each conformation. The higher frequency band has its origin in the "free" or trans form II, and the lower frequency peak arises from the intramolecularly hydrogen-bonded cis form I in which there is a quasi-five-membered ring. Intramolecular hydrogen bond formation is of course sterically impossible in the isomeric para-halophenols where intermolecular association through hydrogen bond formation occurs instead -- the degree of association falling off with increasing dilution of the solution. This intramolecular hydrogen-bonding in ortho-halophenols, as against intermolecular hydrogen-bonding in the corresponding para-halophenols, is reflected for example in the lower melting points and boiling points and higher critical solution temperatures in water of the former.[9]

Not only are there differences in the physical properties of isomeric ortho- and para-halophenols, but pronounced differences in their biological activity are also displayed. Thus para-halophenols are more potent anthelmintics,[10] germicides,[11-15] and fungicides[16] than their ortho isomers. Again, the toxicity of the monohalophenols to goldfish[17] and to frogs and rabbits[12] has been found to increase in the order ortho < meta < para. The inference which can be immediately be drawn from these facts is that the reduction in potency and toxicity of ortho-halophenols over the meta and para isomers may also be connected in some way with the interaction between the hydroxyl group and the ortho-halogen atom.

Unfortunately, there is considerable ambiguity in the terms employed in the literature to describe various aspects of anti-microbial activity. The terms "germicide," "bactericide," "antiseptic," etc. as employed in this thesis reflect solely the usage of the authors responsible for the original publications, and in no way implies that the terms have necessarily been correctly applied.

Attempts have been made to correlate the biological properties of halogenated phenols with a variety of structural features which include the position of the halogen atom or atoms on the benzene ring and the size, position and number of substituent alkyl groups present in the molecule. That

the introduction of halogen atoms results in an increase in bactericidal potency coupled with a decrease in toxicity as compared to the parent phenol was clearly demonstrated by the early systematic investigations of Bechold and Ehrlich.[18] Moreover, it was later shown[19] that substitution of a normal alkyl chain into the para position of ortho-chlorophenol increased germicidal efficiency in direct proportion to the number of atoms in the alkyl chain. This phenomenon led Klarmann and his co-workers[13] to draw the following conclusions concerning the bactericidal potency of halophenols:-

1. Bromophenols compare favourably with the corresponding chlorophenols in bactericidal efficiency.
2. Introduction of alkyl groups into halophenols further increases their potency.
3. Ortho-alkyl derivatives of para-halophenols are more effective as bactericides than the corresponding para-alkyl derivatives of ortho-halophenols.
4. A straight chain substituent on the benzene ring in halophenols is more effective in increasing potency than a branched chain or than several alkyl groups in any given isomeric series.
5. In the case of the higher homologues, the bactericidal action manifests a "quasi-specific" character in that, beginning with a certain number of carbon atoms in the alkyl

chain, (the number being characteristic for each organism), further increase in the number reduces the activity to zero for some organisms, and raises it to comparatively high values for others.

This phenomenon of "quasi-specificity" was further investigated by Pyman[20] using non-halogenated phenols containing a straight chain alkyl group. Among Gram-negative organisms, bactericidal activity increased with increasing chain length only until a definite point was reached, but increased continuously for Gram-positive organisms. Ferguson[21] has explained these findings on the basis of a germicidal "cut-off point" which occurs earlier in a homologous series the more resistant is the test organism.

Not only the position of the halogen, but also that of the alkyl group, is important for germicidal potency. For example, para-chloroxylenol, in which the methyl groups occupy both ortho positions, shows a considerably reduced activity compared to the isomeric compound in which only one ortho position is substituted.[22] An ortho-chlorine atom would seem to be even more effective in this respect since its replacement by a tertiary butyl group more than doubles the antibacterial potency.[23] In this case, however, the change in lipid solubility consequent upon the introduction of the alkyl group must be taken into account.

Investigations concerning the comparative fungicidal potencies of the monohalogenated phenols[24] have revealed an increase in biological activity with increase in atomic weight ($Cl < Br < I$). On account of their relative insolubility in water[25] and their unpleasant and persistant odour, iodophenols are however rarely used as germicides. Fluorophenols have been tested as germicides[26] and anthelmintics,[27] but although more potent than the unsubstituted parent phenols, they are less effective than their chloro- and bromo-isosteres. No comprehensive study comparing all the halophenols appears to have been undertaken, but since a close relationship has been observed to exist between fungicidal and bactericidal activity,[24] the general effectiveness of halogen substitution would seem to increase in the order of atomic weights. ($F < Cl < Br < I$).

From the wealth of information available,[28] certain important criteria for the bactericidal and fungicidal activities of phenolic compounds, including phenol itself, have emerged. It has long been observed[29] that the development of germicidal potency initally involves the absorption of the chemical substance by the bacteria, and that the effect is proportional to the quantity absorbed.[30] The degree of absorption of the bactericide has in turn been related to the position of the functional groups in the molecule.[30] Further investigations have centered on correlating bactericidal

activity with enzyme deactivation[31] and lysis of the cell wall,[32] the latter effect causing leakage of the intracellular amino acids into the external environment. Phenols having a high partition coefficient also exert a correspondingly large depressant effect on several different stages of bacterial metabolism,[33] and it has been postulated[34] that such substances will possess the same bactericidal efficiency when present in similar molecular concentrations in the lipid phase. This hypothesis had in fact been already been substantiated when it was shown[35] that, in accordance with Ferguson's Principle,[21] equitoxic solutions of phenols were those in which the thermodynamic activities, rather than the actual concentrations, were the same. This relationship is, however, liable to be affected by other physico-chemical factors -- such as intramolecular hydrogen-bonding -- since it has been found that, despite their differences in potency, the partition coefficients of the isomeric monochlorophenols between gelatin and water are approximately equal.[11]

The synergistic effects of wetting agents[36] on the bactericidal action of halophenols has been related[37] to a reduction in interfacial tension which passes through a minimum (in the case of soaps) at the point at which micellar aggregation occurs. The wetting agent enhances the action of the free phenol rather than the phenolate,[36] and in general phenols are more active at pH values below 7.[15,38] It has

been observed,[39] however, that salt formation with aromatic amines, pyridines, pyrroles and hydrazine hydrate potentiates the bactericidal action of phenol.

There seems to be no connection between the antiseptic action of phenol itself and the solvent employed.[40,41] This is not the case with para-chlorophenol, however, whose antiseptic properties are apparent only in solvents of high dielectric constant.[40] Temperature too is important,[42] an increase from 20° to 37°C approximately doubling the effect of phenol against E. coli.

The relationships of structural changes in the phenol molecule and changes in the external environment of the test organism to the biological activity of the bactericide have so far been discussed in terms of the gross effect which has been observed. Considerable attention has also been paid to the intimate mode of action of the phenol molecule, especially with reference to the phenolic hydroxyl group. Klarmann et al.[43] proposed that phenols form molecular additive compounds with reactive groups in the organic matter or protoplasm of the biological species involved. This idea was extended[44] to include the mechanism of adsorption on a surface followed by chemical reaction with an active protein, the hydroxyl group being assumed to play an important part in a manner analogous to charcoal adsorption. Experiments indicated[44] that, for concentrations below 1%, the amount of

phenol adsorbed per gram of charcoal corresponded roughly to the order of bactericidal potency (Rideal-Walker measurements). A hydrogen bond between the hydroxyl group and a protein molecule within the cell was invoked[45] as a means of binding the phenol to the microorganism.

The effect of an ortho-chlorine atom in diminishing the bactericidal potency of xylenols has already been mentioned. That an interaction between the hydroxyl group and the chlorine atom is responsible for the decreased biological activity of these compounds compared to their meta and para isomers was first suggested by Suter.[46] The existence of steric hindrance of the hydroxyl group in certain 4-chloro-2,6-dialkylphenols has been used[47] to explain the decreased antifungal activity of these compounds, and it was further suggested[47] that it would be of additional interest to extend this kind of study to include a series of phenols in which the hydroxyl group is subject to various degrees of steric hindrance. Two series of 2,4-dihalophenols possessing a regularly increasing size of alkyl group in the 6-position have in fact been prepared for testing as bactericidal agents,[48] but the results do not appear to have been published.[48a]

Since the intramolecular hydrogen bond in ortho-halophenols, in contrast to the intramolecular bond in chelated systems, is very sensitive to changes in solvent, temperature, steric and electronic effects, it seemed desirable to seek a

possible correlation between the strength of this bond in a series of ortho-halophenols and the accompanying changes in bactericidal potency. It is conceivable that the reduction in bactericidal and fungicidal activity consequent upon the introduction of halogen atoms or alkyl groups into the ortho positions of a phenol molecule is a reflection of the lessened affinity of the phenolic hydroxyl group for certain cell constituents of the organism. If so, it might be expected that antimicrobial potency within such a series of ortho-halophenols would be inversely proportional to the strength of the intramolecular hydrogen bond, or directly proportional to the ease with which intermolecular hydrogen-bonding could be induced at the expense of the "intra-bonding," thus affording a correlation of biological activity with a readily measurable physical property.

Similar correlations of physical properties with biological activity have been sought in other fields. For example, the physico-chemical treatment of the mechanisms of narcosis has attracted considerable attention.[21,49-51] The narcotic potencies of chemically different molecules displaying a non-specific action[52,53] have been related to their lipid solubilities,[50] to their chemical potentials in solution,[21] to their ability to form hydrated microcrystals,[51] and in the case of gases to their van der Waals' constants.[52] The subject has also been treated mathematically,[54] as has the

distribution coefficients of 5,5-disubstituted barbituric acids and their relationship to the Taft constants of the substituents.[55] The importance of planarity[56] of the molecule on the one hand, and of non-planarity[57] on the other, has been emphasised in consideration of the antibacterial activity of the acridines and of the plant growth regulation abilities of certain aromatic acids respectively. Stereoisomerism[53] has been found to be an important aspect in such diverse fields as insecticides,[58] plant growth regulators[59] and analgesics.[53] Reviews have also been published on the relationship between organo-metallic chelation complexes and biological response,[60] and the part played by oxidation-reduction potentials in the antibacterial activity of substituted quinones has been studied.[61]

The majority of drugs in use at the present time are either weak acids or weak bases, for example the barbituric acids and the alkaloids, and it has been found that the biological action of these compounds is due mainly, but not wholly, to either the ionized or the unionized form[62] which have widely different solubilities and therefore different partition coefficients. The unionized form is particularly important for transport across cell membranes since it is uncharged and therefore not influenced by the charge on the polarised cell wall. The ratio of the ionized to the unionized form of the drug is a function of its pK_a and of the

pH of the medium in which it is studied, and for these reasons dissociation constants are important properties of many drugs. Studies of pK_a and pH have been undertaken in many fields including lipid/water distribution[63] and blood/cerebrospinal fluid distribution[64] of drugs in dogs, phenol[65] and surface active agents[66] as bactericides, weak acids as fungistatic agents,[67] sulphonamides,[68] sympathomimetic amines,[69] local anaesthetics,[70] muscle relaxant activity of d-tubocurarine,[71] alkaloidal toxicity for Paramoecium,[72] excretion of nitrogenous bases by dogs[73] and benzacridines as carcinogens.[74] Carcinogenic activity has also been related to the electronic characteristics of aromatic polycyclic hydrocarbons,[75] and to the electron density of the azo group in diazo compounds.[76]

Although the recognition of the intra- and intermolecular hydrogen bond is of fairly recent origin, its presence was soon invoked to explain certain observations in biological systems.[77] The concept has proved particularly useful in considering the numerous possible conformations of proteins and nucleic acids,[78] nitrogen atoms forming hydrogen bonds with oxygen atoms of other residues. It has been suggested[79] that drugs containing hydroxyl or amino groups bind themselves by means of hydrogen bonds to proteins, thereby altering the natural conformation of the polymer, and this idea has been employed to explain the trypanocidal activity of

phenanthridines[80] and the insecticidal action of chlorinated hydrocarbons.[81] The possible influence of hydrogen bonding on the absorption[82] and mechanism of action[83] of certain sulphonamides has also been discussed, while such a bond has been invoked to explain predenaturative changes in protein,[84] the pH dependence of lipase activity[85] and the "lock and key" structure of antibodies.[86] A possible mechanism for the clotting process of blood in terms of hydrogen-bonding has been proposed,[87] and the aggregation of "sickle cell" haemoglobin molecules[88] may also be attributed to this phenomenon. Certain processes of muscular contraction and relaxation,[89] and even permanent waving of the hair,[90] have been visualised in terms of the making and breaking of intermolecular hydrogen bridges.

Intramolecular hydrogen bonds usually confer favourable solubility characteristics on a compound since the external polar field is decreased, permitting a favourable distribution between water and lipids and thus allowing rapid diffusion and penetration of the drug. Examples are chloral hydrate, salicylic acid, the antirheumatic drug 2,5-dihydroxybenzoic acid and the tetracycline antibiotics which are rapidly absorbed by the body and quickly reach their site of action.

The application of molecular spectroscopy,[91] especially in the infrared region,[92] to biological problems has been of increasing interest and value in recent years, being employed

in such investigations as the relationship between odour and molecular vibration[93] and the conformation of acetylcholine in solution.[94] Of particular relevance to this thesis is the spectroscopic study of alkyl-substituted phenols and the relationship of bactericidal activity to molecular structure.[95] It was concluded that alkyl groups possessing electron-repelling properties disturb the electronic nature of the molecule, rendering it susceptible to the effects of external influences and thereby enhancing its biological activity. It would seem more feasible to suppose, however, that the reduction of activity produced by alkylation of the hydroxyl group is simply a reflection of the lack of ability of the phenol to bind itself to some site in the micro-organism, presumably by hydrogen-bonding.

It has also been observed that shifts to lower frequencies of the carbonyl and hydroxyl absorptions in 3-alkylsalicylic acids as the bulk of the 3-substituent is increased[96] are paralleled by an increase in potency both with respect to oxygen consumption in the rat and with respect to glycogenolytic activity in the mouse.[97] An exactly analogous investigation of the hydroxyl stretching frequency of several series of ortho-bromophenols is reported in this thesis and by means of the results obtained, an attempt has been made to place the compounds in the order of their expected bactericidal potency.

Several studies have been concerned with the effect of temperature on the ratio of cis to trans conformations in ortho-halophenols as evidenced by the changes in relative intensity of the bonded and non-bonded hydroxyl absorptions,[98] and the results have been used to calculate the energy[99] and the relative strengths[100] of the hydrogen bonds involved. The influence of other factors has, however, seen little attention, and the studies reported in this thesis are concerned with the influence of steric compression, electronic and solvent effects, temperature, competitive intermolecular hydrogen bonding and change of state on the position and intensity of the hydroxyl stretching absorptions, and the results are interpreted in terms of the strength of the intramolecular hydrogen bond. Bromophenols were chosen in preference to other halophenols since they are easily prepared and give a strong intramolecular hydrogen bond, although the claim[100] that they exhibit the strongest bond of any of the ortho-halophenols is not in agreement with earlier work.[4,5]

Mention has already been made of the existence of conformations I and II in unsubstituted ortho-halophenols. Recent careful quantitative measurements of the peak areas of rigorously purified ortho-halophenols[3] have shown that although the ratio of the high frequency absorption to the low frequency absorption is lower than earlier measurements

had indicated, the existence of two bands -- and hence two conformations -- is real. Thus the contention[2] that the higher frequency absorption is spurious and results from the presence of the corresponding *para*-substituted phenol as an impurity is completely refuted. Further, it has now been established [101-103] that the hydroxyl group lies in the plane of the ring in phenols possessing bulky *ortho*-substituents, and that *cis* and *trans* conformations exist in such compounds even when the *ortho*-substituent is unlikely to form an intramolecular hydrogen bond in the *cis* conformation.

The antibacterial potency of the compounds studied is being determined with the kind cooperation of Mr. D. H. O. Gemmell, Royal College of Science and Technology, Glasgow.

It will be of interest to compare the results with earlier studies[39,40,45,65] seeking to relate the biological activity of phenols to their pK_a values in view of the established relationship between $\Delta\nu$ and the Hammett σ function on the one hand,[104] and the Hammett σ function and pK_a on the other.[105]

Measurements and Results.

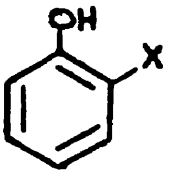
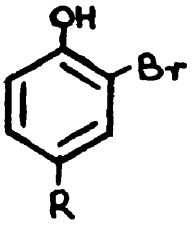
The results of the infrared studies in solution are shown in Tables I-IV and VI-IX which list the frequencies (ν, $cm.^{-1}$) of the "free", the intramolecularly hydrogen-bonded and the intermolecularly hydrogen-bonded hydroxyl absorptions where appropriate, the half-band widths ($\Delta\nu_{\frac{1}{2}a}$, $cm.^{-1}$) and the apparent molecular extinction coefficients (εa, l. $mole^{-1} cm.^{-1}$) rounded to the nearest five units and measured from a solvent-solvent base-line superimposed on the record of the absorption of the solution and determined with solvent in the reference beam.

A. Measurements in Carbon Tetrachloride.

Table I includes data obtained with the four ortho-halophenols (compounds 39, 40, 1, 38) and the results on the effect of variation of the halogen atom are in good agreement with those obtained by Puttnam[101] and by Jones and Watkinson.[100]

It is readily seen from Table I that introduction of a para-alkyl substituent into the ortho-bromophenol molecule (compounds 1-7) has little effect on either the "free" or the "intra-bonded" hydroxyl absorptions, as the frequencies, half-band widths and intensities remain practically constant. The small upward frequency shift (5-8 $cm.^{-1}$) for the "intra-

TABLE I. UNSUBSTITUTED o-HALOPHENOLS and p-SUBSTITUTED o-BROMOPHENOLS. ν_{OH} in CCl_4

Parent	Index No.		Free			Bonded			$\varepsilon_f/\varepsilon_b$?
		X	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a^+	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a^+	
	39	F		-		3592	23	200	-
	40	Cl	(3609)	*	(15)	3547	19	170	0.096
0.005M	1	Br	(3604)	*	(10)	3529	19	160	0.063
5mm cell	38	I	(3600)	*	(10)	3507	22	140	0.072
		R							
	2	Me	(3605)	*	(10)	3536	22	155	0.065
	3	Et	(3607)	*	(10)	3537	22	155	0.065
	4	iPr	(3607)	*	(10)	3534	23	150	0.067
	5	sBu	(3607)	*	(10)	3536	22	150	0.067
	6	tBu	(3607)	*	(10)	3534	22	150	0.067
0.005M	7	cycloHex	(3606)	*	(10)	3534	22	160	0.063
5mm cell	8	Ph	(3599)	*	(10)	3528	21	200	0.050
	9	Br	(3597)	*	(10)	3528	23	175	0.057
	10	NO_2	(3583)	*	(10)	3511	21	220	0.045

Footnotes.

+ Extinction coefficients are apparent and are approximate to the nearest 5 units.

ƥ Ratio of extinction coefficients of free OH to bonded OH.

* Not measured.

Values in parenthesis are approximate.

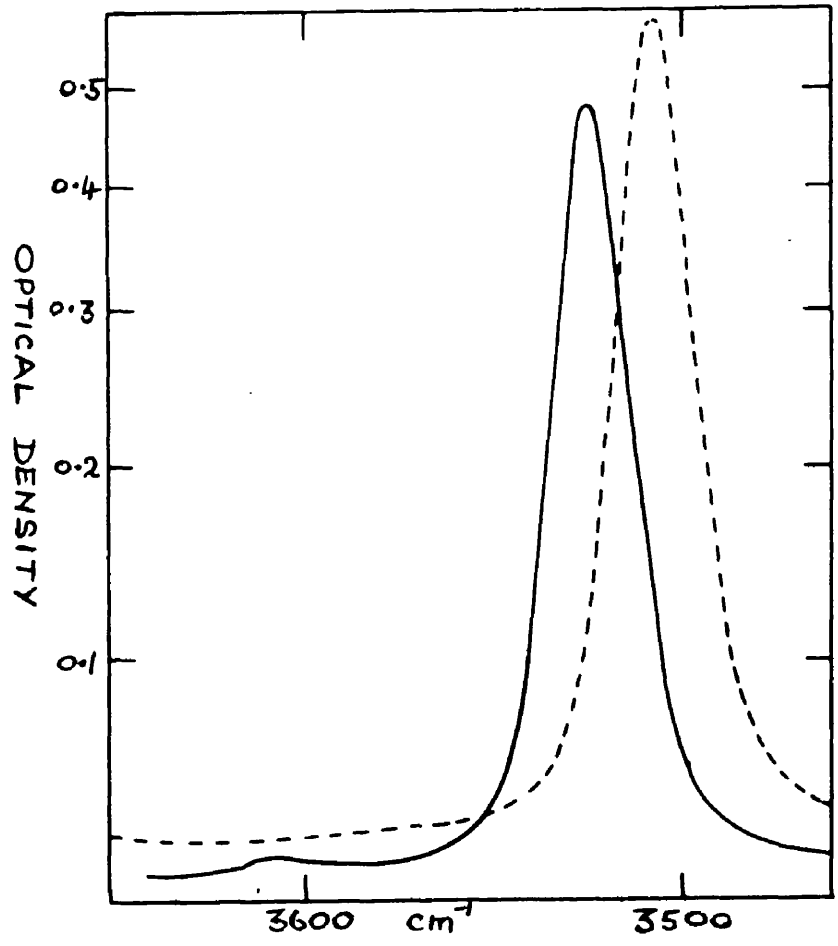


Figure 1. Hydroxyl absorptions of 2,4-dibromo-6-methylphenol (——) and 2,4-dibromo-6-t-butylphenol (- - -) in carbon tetrachloride (Table II, compounds 11 & 15)(0.005M. in 5mm. cell).

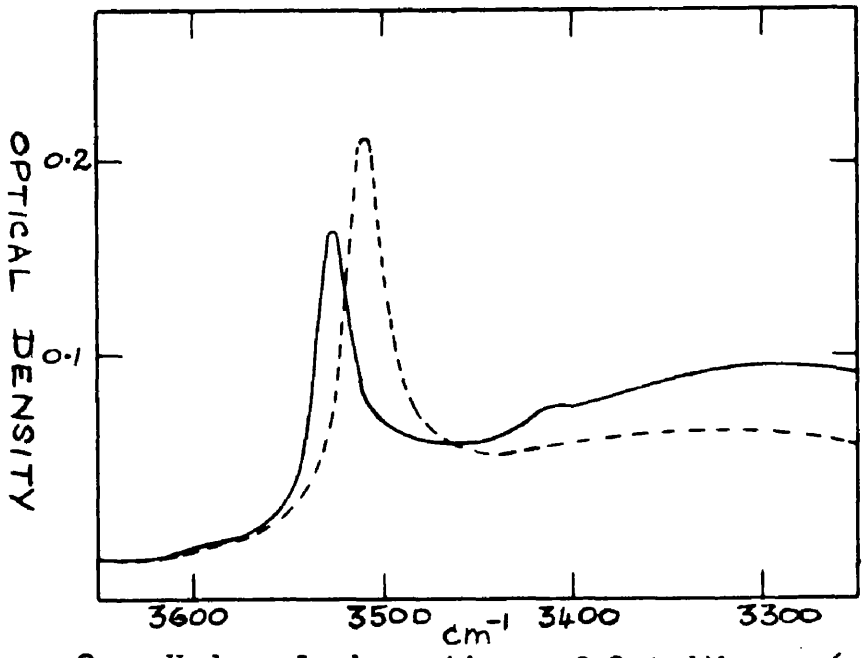
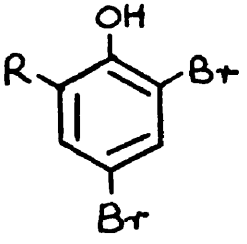
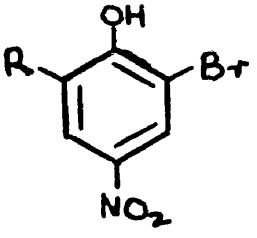
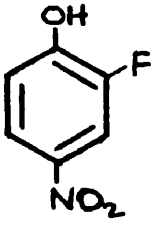


Figure 2. Hydroxyl absorptions of 2,4-dibromo-6-methylphenol (——) and 2,4-dibromo-6-t-butylphenol (- - -) in ether-carbon tetrachloride (Table IX, compounds 11 & 15)(0.003M. in 2.3M. ether-CCl_4 in 5mm. cell).

bonded" band of compounds 2-7 with respect to ortho-bromophenol (compound 1) is in accord with the established weak electron-releasing properties of the alkyl group which lead to a slight decrease in the acidity of the hydroxyl group.[106] With electron-withdrawing substituents such as phenyl-, bromo- and especially the nitro-group in the 4-position (compounds 8-10), both the free and the bonded absorptions move to lower frequencies and there is the expected intensification as the acidity of the hydroxyl group is increased.[101] The same behaviour with respect to the 6-alkyl-2,4-dibromophenols (compounds 11-17, Table II) is seen with the 6-alkyl-2-bromo-4-nitrophenols (compounds 21-26, Table II). This is the expected converse of the situation described by Oki and Hirota[107] who showed that the "intra-bonded" OH frequency in a series of aryloxyacetic acids increased whilst the intensity of absorption decreased as the basicity of the ether oxygen atom was progressively decreased by substitution of progressively stronger electron-withdrawing groups into the para-position.

Introduction of alkyl substituents into the 6-position (compounds 9, 11-18 and 10, 21-26, Tables I and II) rather than in the 4-position, results in a distinct progressive lowering of the frequency of the "intra-bonded" absorption with increasing bulk of the substituent (Figure 1). In terms of $\Delta\nu$ where $\Delta\nu = \nu_B$ for compound 9 minus ν_B for the

TABLE II. 6-SUBSTITUTED 4-BROMO and 4-NITROPHENOLS. ν_{OH} in CCl_4

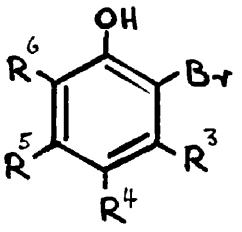
Parent	Index No.	R	ν	$\Delta\nu_{1/2}$	ε_a
	11	Me	3528	20	185
	12	Et	3527	19	200
	13	iPr	3524	21	195
	14	sBu	3524	21	195
	15	tBu	3509	19	220
	16	cycloHex	3524	22	195
	17	Ph[a]	3516	22	165
	18	Br	3515	20	225
	21	Me	3506	21	230
	22	Et	3505	21	235
	23	iPr	3502	22	235
	24	sBu	3503	22	215
	25	tBu	3488	22	235
	26	cycloHex	3502	24	230
	41		3574	34	185
			3300	-	(5)

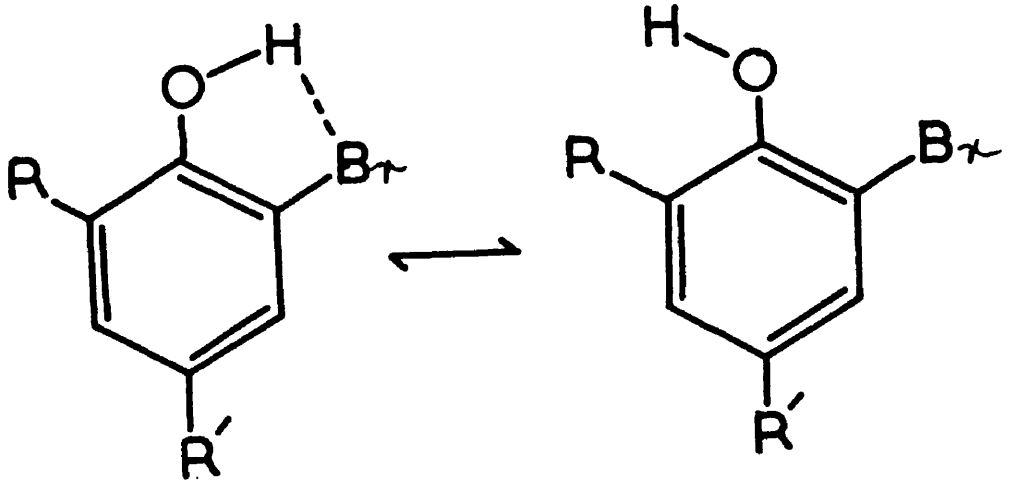
a - Additional hydroxyl band at 3547 cm^{-1} $\Delta\nu_{1/2}$ = 18 (measured by band reflection), ε_a = 65, which is ascribed to an intramolecular hydrogen bond to the phenyl group.

6-substituted compound, the effect in the para-bromo series is negligible (0-1 cm.$^{-1}$) for methyl and ethyl, small (4 cm.$^{-1}$) for isopropyl, cyclohexyl and s-butyl, and comparatively large (20 cm.$^{-1}$) for t-butyl, which corresponds to the sequence;- 3 or 2 hydrogens, a single hydrogen and no hydrogen on the benzylic carbon atom. As is to be expected from the already established linear relationship between Hammett σ contributions for para substituents and ν_{OH} values,[104,108] the para-nitro compounds form an almost exactly parallel series with $\Delta\nu$ values some 4cm.$^{-1}$ greater.

These results provide a further demonstration of the phenomenon of steric compression of the intramolecular hydrogen bond such as has already been observed in the chelated 3- and 6-alkyl-substituted salicylic acids and their methyl esters.[96] Thus the sudden increase in $\Delta\nu$ in passing from a 6-isopropyl group (compounds 13 and 23) to a 6-t-butyl group (compounds 15 and 25) for example, has its close parallel in the salicylate series. Again, as in the salicylate series, the highest frequency shifts are observed in compounds bearing substituents in both the 3- and the 6-positions where crowding is at its highest. These compounds are shown in Table III. It is seen that there is a shift to lower frequency of the order of 30cm.$^{-1}$ on proceeding from 2,4-dibromophenol (compound 9) to 2,4-dibromo-3-methyl-6-t-butylphenol (compound 20) or from 2-bromo-4-t-butylphenol

TABLE III. VARIOUSLY SUBSTITUTED o-BROMOPHENOLS. ν_{OH} in CCl_4

Parent	Index No.	R^3	R^4	R^5	R^6	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a
	19	Me	Br	H	Me	3518	20	190
	20	Me	Br	H	tBu	3500	19	220
	27	H	Br	Me	Me	3527	20	195
	28	Me	Br	Me	Me	3517	20	190
	29	Me	Me	H	Me	3524	21	170
	30	Me	tBu	H	tBu	3499	19	190



IV V

(compound 6) to 2-bromo-4,6-di-t-butyl-3-methylphenol (compound 30). An additional methyl group in the 5-position has little effect (e.g. compounds 11 and 27 as compared with compound 9).

No "free" hydroxyl absorption could be detected in any of the compounds in Table II and it is apparent that even a methyl group in the 6-position of an ortho-bromophenol is sufficient to ensure that the molecule exists solely in conformation IV. This is presumably a reflection of the energy saved through the intramolecular hydrogen bond rather than an inherent steric effect since it has been established[101] that both cis and trans conformations exist in ortho-t-butylphenol as evidenced by the existence of hydroxyl stretching absorptions at 3607 $cm.^{-1}$ (main band) and 3647 $cm.^{-1}$ The high frequency of the latter band which arises from the conformation in which the OH group is directed towards the t-butyl group has been ascribed to a slight opening out of the C-O-H angle.[109] It is interesting that not only do the two conformations in ortho-t-butylphenol possess considerably different energies as reflected in the difference in OH absorption frequencies ($\Delta\nu = 40$ $cm.^{-1}$) but there is also an appreciable energy barrier to free rotation of the OH group from one conformation to the other as seen by examination of models. (see Figure 3). In ortho-alkyl-phenols in which the alkyl substituent is smaller than

t-butyl, only one hydroxyl absorption at 3614 cm.$^{-1}$ is present[109] and this may represent a superposition of two virtually identical frequencies as the two conformations probably possess very similar energies.[110]

The absence of hydroxyl absorption other than that due to the intramolecularly hydrogen-bonded conformation in the compounds shown in Table II is also of interest in connection with the known effects of 2-alkyl substitution and 2,6-dialkyl substitution on the ability of phenols to undergo intermolecular hydrogen-bonding.[109] Thus intermolecular association of phenols with ether is only slightly reduced by a single ortho substituent (methyl to t-butyl), is markedly reduced by two ortho substituents where these are methyl, ethyl or isopropyl, and is almost completely prevented in 2,6-di-t-butylphenol.

Two compounds in the present study merit specific comment. In 2,4-dibromo-6-phenylphenol (compound 17), intramolecular hydrogen-bonding occurs not only with the bromine atom in the 2-position, but also with the π-electrons of the phenyl sustituent in a fashion analogous to that reported for ortho-phenylphenol.[111,112] In 2,4,6-tribromophenol (compound 18), there are two bromine atoms in the ortho positions and, since these are equivalent, only a single hydroxyl absorption band is observed. It is of interest,however, that this occurs at a lower frequency

(3515 cm.$^{-1}$) than that of the hydroxyl absorption in 2,4-dibromophenol (compound 9) which appears at 3528 cm.$^{-1}$ giving a further example of compression of the intramolecular hydrogen bond.

It is worth noting that the absorption bands due to the intramolecular species are almost invariably symmetrical and of consistently small (21 ± 2 cm.$^{-1}$) half-band width. This value compares with that of 26 cm.$^{-1}$ for the "free" hydroxyl in compound 2, of ca. 17 cm.$^{-1}$ for the hydroxyl frequencies of non"intra-bonded" phenols and of ca. 50 cm.$^{-1}$ for the hydroxyl band at 3560 cm.$^{-1}$ assigned to the intermolecular OH...π bond of the associated species present in dilute solutions of phenol in benzene.[103] Presumably the greater width of the band of the intermolecularly-bonded hydroxyl is to be ascribed to the numerous conformations available to the bimolecular species. Certainly the severely restricted "intra-bonded" hydroxyl groups in the 6-t-butyl-substituted ortho-bromophenols (e.g. compounds 15 and 25, Table II; compound 20, Table III) have absorption bands as narrow as that of ortho-bromophenol itself despite the shift to lower frequency. (Figure 1).

Table IV gives data for the hydroxyl absorptions of several bicyclic bromophenols. It does not seem feasible to disentangle fully the various effects which may be present such as bond fixation, inductive and mesomeric effects,

TABLE IV. VARIOUS BICYCLIC o-BROMOPHENOLS. ν_{OH} in CCl_4

Compound	Index No.	Free ν	Free $\Delta\nu_{\frac{1}{2}}$	Free ε_a	Bonded ν	Bonded $\Delta\nu_{\frac{1}{2}}$	Bonded ε_a	$\varepsilon_F/\varepsilon_B$ [p]
1-Bromo-2-hydroxy-5,6,7,8-tetrahydronaphthalene	31	3608	*	(5)	3523	21	155	0.032
1-Bromo-2-naphthol	32		-		3518	19	160	-
3-Bromo-2-naphthol	33	3598	*	15	3532[t]	20	220	0.068
4-Bromo-5-hydroxythionaphthen	34	3614	20	35	3533	22	135	0.26
4-Bromo-5-hydroxy-3-nitrothionaphthen	35		-		3507	24	145	-
3,4-Dibromo-5-hydroxy-thionaphthen	36		-		3503	21	155	-
1-Bromo-2-hydroxydibenzofuran	37	3609	*(10)		3537	21	145	0.069

Footnotes.

- \- No band present.
- * Not measured.
- [p] Ratio of extinction coefficients of free OH to bonded OH.
- t Unsymmetrical band, tail on low frequency side.

Values in parenthesis are approximate.

bond angle changes and steric influences, but several points may be made. The absence of "free" hydroxyl absorption and the lower frequency of the bonded hydroxyl absorption in 1-bromo-2-naphthol (compound 32) whereas 3-bromo-2-naphthol (compound 33) shows both "free" and bonded absorptions, can be interpreted in terms of a peri interaction of the bromine atom with the 8-hydrogen in the former compound, and the greater double bond character of the 1,2 than the 2,3 bond.

The thionaphthen system is an interesting case, for here the sulphur atom may act as an electron source with a consequent decrease in the acidity of the phenolic hydroxyl group. (III). Indeed the frequencies of the hydroxyl absorptions of β-naphthol (3608 $cm.^{-1}$) and 5-hydroxy-thionaphthen (3612 $cm.^{-1}$) in carbon tetrachloride solution could be taken as an indication that the latter is less acidic than the former. There are of course bond angle changes accompanied by a decreased peri interaction as compared with β-naphthol. 4-Bromo-5-hydroxythionaphthen (compound 34) has the highest ratio -- in the present series -- of non-bonded to bonded hydroxyl absorption, but with the introduction of a 3-bromo- or 3-nitro-group (compounds 35 and 36), the bonded form is once again the only one observed. Both electronic and steric factors (peri interaction) may be operative. A peri interaction between a bromine atom and a nitro-group has been invoked in order to rationalise the formation of 4,4-disubstituted-5-ketodihydrothionaphthens.[113]

B. Measurements in Other Solvents.

Unfortunately, a solvent such as carbon tetrachloride, which is routinely employed in infrared solution studies, differs considerably in its physical properties from biological fluids and so the results obtained in the previous section would not be expected *per se* to throw much light on the situation pertaining *in vivo*. In order to gain an insight at least into the direction of the changes in the positions, half-band widths and apparent extinction coefficients of the hydroxyl stretching frequencies of *ortho*-bromophenols which might be expected to occur in passing from carbon tetrachloride to *in vivo* conditions, certain selected compounds were studied in the solvents hexane, carbon tetrachloride, chloroform, carbon disulphide and acetonitrile which form a series of increasing polarity. Some of the physical constants of these solvents are listed in Table V.

The results for three representative *ortho*-bromophenols are summarised in Table VI. Also shown in Table VI are the corresponding data for two pairs of 2-alkyl- and 2,6-dialkylphenols which were included for comparison purposes. These results are in good agreement with earlier studies on the effect of solvent on the hydroxyl absorptions of various simple phenols and hindered 2,6-dialkylphenols.[114]

TABLE V. CHARACTERISTICS of SOLVENTS for INFRARED STUDIES.

Effect on ν_{OH} of Phenols ArOH.

	Hexane	CCl_4	$CHCl_3$	CS_2	CH_3CN
Polarity (Dipole moment)	0.08	0	1.15	0	3.37
Dielectric Constant	1.89_{20}	2.238_{20}	4.806_{20}	2.641_{20}	37.5_{20}
Hydrogen-bonding	None	Weak (?) ArOH... Cl-CCl_3	Weak ArOH... Cl-$CHCl_2$ ArOH... H-CCl_3	Weak ArOH... S=C=S	Medium ArOH... N≡C-CH_3
Observed overall effects ν	Highest (except for vapour)	Shifts to lower frequency	Shifts to lower frequency	Frequency slightly lower than in $CHCl_3$	Fall of 200 $cm.^{-1}$
$\Delta\nu_{\frac{1}{2}}$	Narrow	Slightly broader	Considerable broadening	Breadth that in CS_2	Very broad bands

TABLE VI. HYDROXYL STRETCHING ABSORPTIONS of SUBSTITUTED PHENOLS in VARIOUS SOLVENTS.

Compound No.	Phenol with substituents	Hexane 0.05M. 0.5mm.			CCl_4 0.005M. 5mm.			$CHCl_3$ 0.05M. 0.5mm.			CS_2 0.0125M. 2mm.			CH_3CN 0.05M. 0.5mm.		
		ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a
42	None	3621	13.5	*	3612	17	205	3599	32	150	3594	28	154	3143 ‡h,P	140	175
43	2-Me	3623	15.5	*	3612	17	*	3601	33	*	3596	31.5	*		*	
44	2-tBu	3655	17	25	3648	18†	20	(3638)	(20)	(15)	3641.5	22†	20	3415 P	133	170
		3616	12	195	3608	15	160	3598	28	135	3588	28.5	125			
45	2,6-diMe	3629	13	*	3618.5	16.5	*	3609	40	*	3609	23.5	*		*	
46	2,6-di-tBu	3653	12	305	3647	15	240	3641	22	195	3642	15	242	3624	40†	100
														3470 P	165†	25
1	2-Br	(3616)	*	(5)	(3604)	*	(10)	(3587)	*	15	3578	*	10	3355 ‡h,P	200	130
		3532	13	235	3529	19	160	3523	35	120	3518	20	205			
4	2-Br-4-iPr	3617	*	(5)	3607	*	(10)	(3587) ‡l	*	15	3582 ‡l	*	10	3372 P	165†	130
		3540 ‡l	16	200	3534	23	150	3527	35	115	3525	21	170			
20	2,4-diBr-3-Me-6-tBu	3503	10.5	358	3500	19	220	3499	30	165	3492	18.5	243	3496	17†	85
														(3422) P	120	55

Footnotes.

* Not measured.

‡ Unsymmetrical band. h = high frequency side; l = low frequency side.

† Measured by band reflection.

P These values involve a strong intermolecular association with the solvent and are placed here for convenience. The value for phenol in hexane is of a saturated solution.

The values for compound 20 in acetonitrile are for a 0.025M. solution in 50% CH_3CN/CCl_4.

Values in parenthesis are approximate.

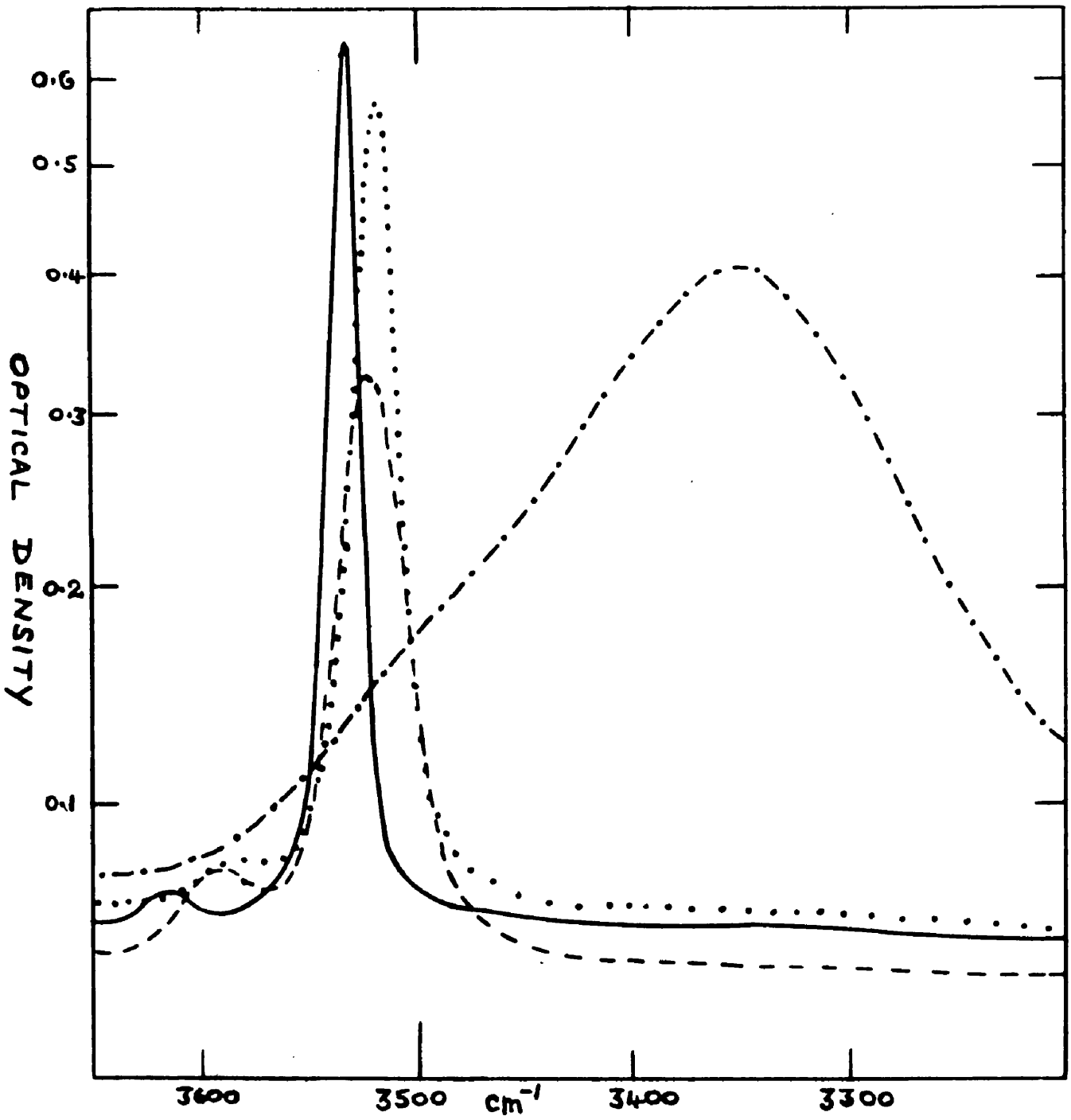


Figure 4. Hydroxyl absorptions of 2-bromophenol (Table VI, compound 1) in n-hexane (——), chloroform (- - -), acetonitrile (-.-.-)(all 0.05M. in 0.5mm. cells) and carbon disulphide (.....)(0.0125M. in 2mm.cell).

Of the solvents selected, acetonitrile is by far the most polar and the most basic (Table V), being able to associate with phenol molecules through intermolecular hydrogen-bonding, and two distinct effects on passing from the other solvents to acetonitrile are immediately apparent. In the case of 2-t-butylphenol (compound 44), 2-bromophenol (compound 1) and 2-bromo-4-isopropylphenol (compound 4), the double hydroxyl absorption arising from the coexistence of the conformations corresponding to I and II, which is present in all other solvents, disappears in acetonitrile giving rise to a single broad intense band shifted to lower frequency. This is illustrated in Figure 4 which shows the variation in the shape and intensity of the hydroxyl absorption with solvent in 2-bromophenol. On the other hand, with 2,6-di-t-butylphenol (compound 46) and 2,4-dibromo-6-t-butyl-3-methylphenol (compound 20), two hydroxyl absorption bands can be detected in acetonitrile whilst there is but a single peak in the other solvents. This is illustrated in Figure 5 which shows the variation in the shape and intensity of the hydroxyl absorption with solvent in 2,4-dibromo-6-t-butyl-3-methylphenol.

These effects on passing to acetonitrile from the other solvents and which would also be expected to occur in aqueous biological media are due to intermolecular hydrogen-bonding between the acetonitrile molecules and the molecules

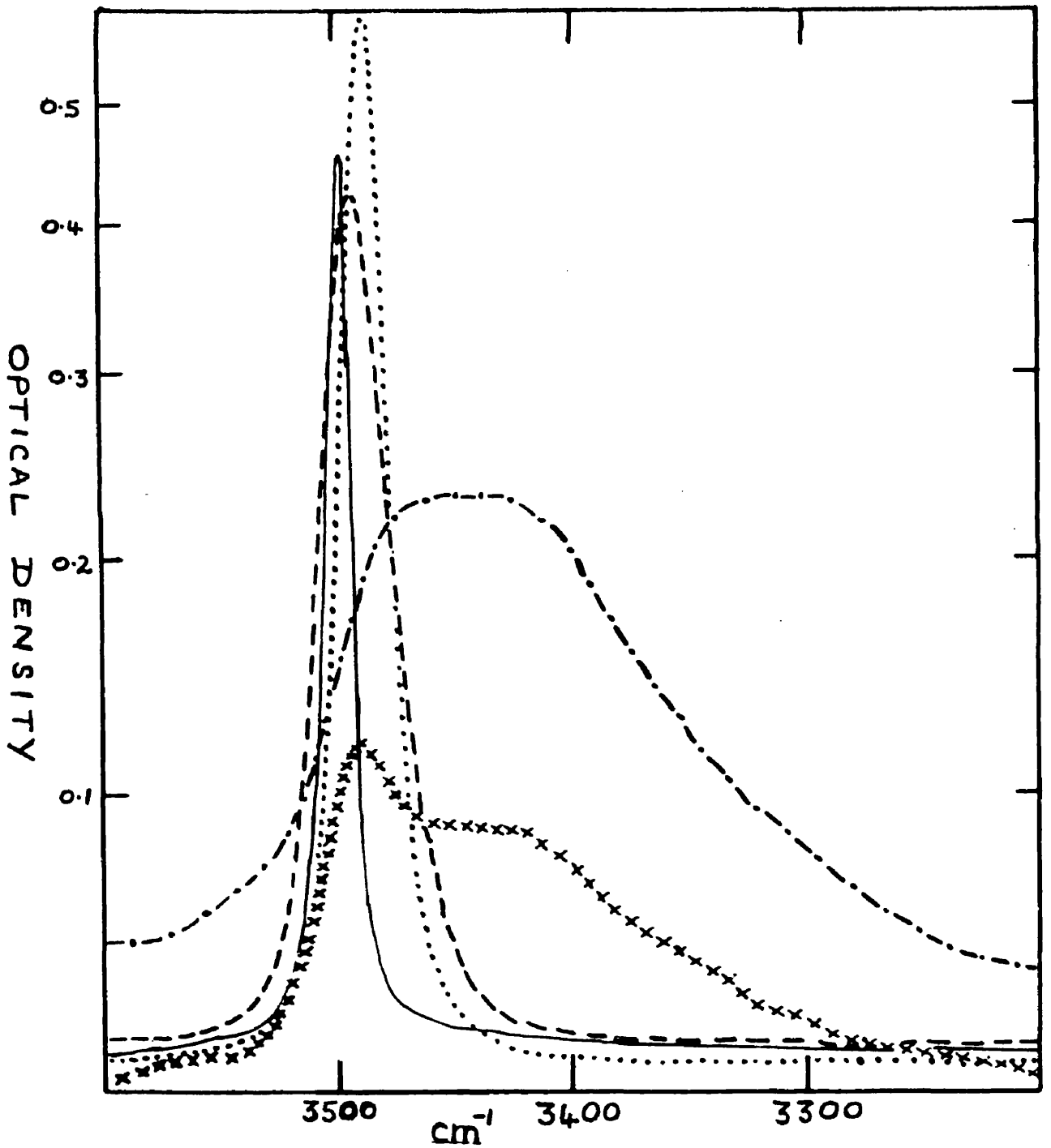


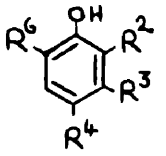
Figure 5. Hydroxyl absorptions of 2,4-dibromo-3-methyl-6-t-butylphenol (Table VI, compound 20) in n-hexane (——), chloform (- - -), acetonitrile (-.-.-)(all 0.05M. in 0.5mm. cells) carbon disulphide (....)(0.0125M. in 2. mm. cells), and 1:1 carbon tetrachloride-acetonitrile (XXX)(0.025M. in 0.5mm cells).

of the phenol. In the case of compounds 1, 4 and 44 in which there is relatively little steric hindrance to the approach of solvent molecules to the OH function, intermolecular hydrogen-bonding occurs readily in acetonitrile. The shape and position of the broad absorption peak make it apparent that intermolecular-bonding is occurring to the virtual exclusion of the intramolecular hydrogen-bonding seen in the other solvents, although the existence of a small proportion of the intramolecularly-bonded species cannot be ruled out. With compounds 46 and 20 there is considerable steric hindrance to intermolecular hydrogen-bonding, with the result that two species are present in acetonitrile. The low frequency peak arises from the intermolecularly hydrogen-bonded species whilst the high frequency peak arises from unassociated phenol molecules. Compound 20 was also examined in 50% acetonitrile-carbon tetrachloride solution in order to obtain sufficient resolution of the two bands for accurate measurement, and these are the values recorded in Table VI.

It is readily apparent from Table VI that the hydroxyl stretching frequencies generally decrease steadily in the order hexane $>$ carbon tetrachloride $>$ chloroform = carbon disulphide, whereas the apparent half-band widths rise in a different order, namely hexane $<$ carbon tetrachloride = carbon disulphide $<<$ chloroform. This variation in half-

band widths makes impossible any comparison of apparent extinction coefficients, but the changes in frequencies and half-band widths with solvent are listed in Table VII in which they have been so tabulated that, where different conformations exist, the bands are assigned to a given conformation. It is significant that the "intra-bonded" hydroxyl frequencies, (compounds 1, 4 and 20), consistently undergo much smaller solvent shifts than those of the free hydroxyl groups. This is presumably a reflection of the existence of a lower degree of solvation in the "intra-bonded" conformation I where the hydroxyl group is both more electrically neutral and more restricted conformationally and hence less solvent sensitive than is the hydroxyl group in the alternative trans conformation II. It is of interest that in 2-t-butylphenol (compound 44), the hydroxyl absorption arising from the conformation in which the hydroxyl group is directed towards the alkyl group is also less solvent sensitive than is the hydroxyl absorption ascribable to the conformation in which the hydroxyl group is directed away from the t-butyl group. It is only when the alkyl group is t-butyl or buttressed isopropyl in ortho-alkylphenols that such dual hydroxyl absorption is encountered -- lower alkyl groups give but a single peak. The values reported here for compound 44 are in good agreement with those previously given for 2-t-butyl-4-methylphenol.[114] The buttressed

TABLE VII. SOLVENT - INDUCED CHANGES in ν_{OH} and $\Delta\nu_{\frac{1}{2}a}$.



Compound No.	R^2	R^3	R^4	R^6	Conformation of OH	$\Delta(CCl_4-C_6H_{14})$ ν	$\Delta(CCl_4-C_6H_{14})$ $\Delta\nu_{\frac{1}{2}a}$	$\Delta(CHCl_3-C_6H_{14})$ ν	$\Delta(CHCl_3-C_6H_{14})$ $\Delta\nu_{\frac{1}{2}a}$	$\Delta(CS_2-C_6H_{14})$ ν	$\Delta(CS_2-C_6H_{14})$ $\Delta\nu_{\frac{1}{2}a}$
42	H	H	H	H		-9	3.5	-22	18.5	-28	14.5
43	Me	H	H	H	++	-11	1.5	-22	17.5	-27	16
1	Br	H	H	H	Directed	-12	*	-29	*	-38	*
4	Br	H	iPr	H	away	-10	*	-30	*	-35	*
44	tBu	H	H	H	from R^2	-8	3	-18	16	-28	16.5
45	Me	H	H	Me	Directed	-8.5	3.5	-20	27	-20	10.5
44	tBu	H	H	H	towards R^2	-7	1	-17	3	-13.5	5
46	tBu	H	H	tBu		-6	3	-12	10	-11	3
1	Br	H	H	H	H-bonded	-3	6	-9	22	-14	7
4	Br	H	iPr	H	to R^2	-6	7	-13	19	-15	5
20	Br	Me	Br	tBu		-3	8.5	-4	19.5	-9	8

Footnotes.

* Not measured.

++ Two conformations are feasible and indeed almost certainly present but only one absorption is observed.

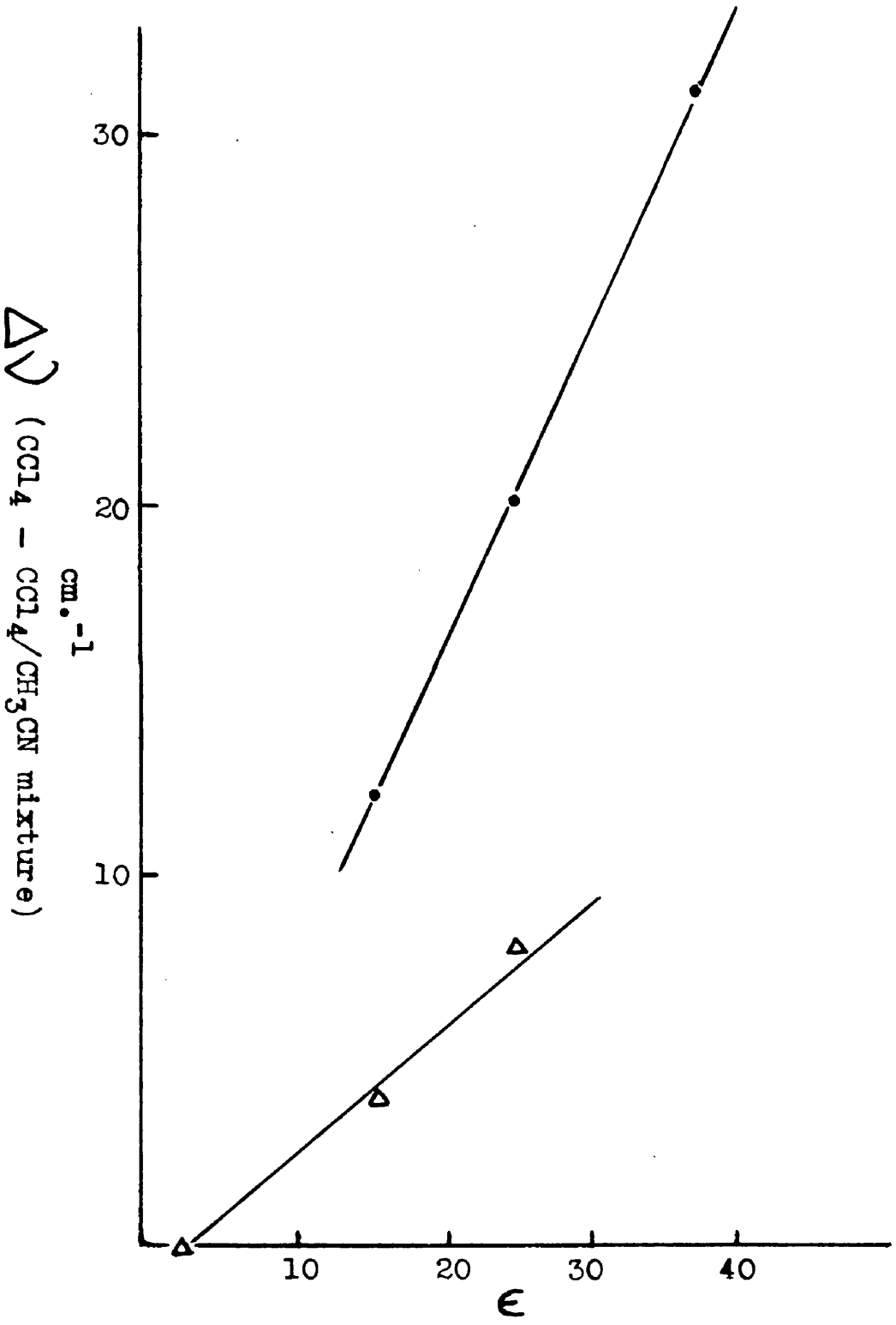


Figure 6. Graph illustrating effect of dielectric constant ϵ on ν_{OH} of 2-bromophenol (compound 1) in CCl_4/CH_3CN binary mixtures. Δ – "intra-bonded" band; • – "inter-bonded" band.

"intra-bonded" hydroxyl group of compound 20 not surprisingly shows the smallest solvent shifts and in this connection it is to be noted that carbonyl frequencies in strongly chelated systems where the basicity of the oxygen atom is reduced also undergo negligible solvent shifts,[96] as does the hydroxyl frequency in 2-nitrophenol.[115]

The solvent shifts observed for the non "intra-bonded" conformations of 2-bromophenol (compound 1) and 2-bromo-4-isopropylphenol (compound 4) closely parallel those for phenol (compound 42), but are significantly larger in magnitude, in agreement with the greater acidity of the halo compounds.

Examination of 2-bromophenol in both carbon tetrachloride and acetonitrile singly, and in several different binary mixtures of these two solvents, indicates that dielectric constant effects are of minor importance. Thus after plotting the shifts in frequency of both the "intra-bonded" and "inter-bonded" bands relative to the carbon tetrachloride value against the calculated dielectric constant of the mixed solvent, there is found to be a change of about one wave number per four dielectric constant units for the "intra" band and about one wave number per two units for the "inter" band. The extrapolated value for the "inter" band to acetonitrile in 100% carbon tetrachloride at $\epsilon = 2.24$ was found to be 3386 $cm.^{-1}$ (Figure 6).

The width of the hydroxyl absorption band does not seem to be related to the position of ν_{OH}. Thus it is apparent from Table VI that in n-hexane, although the absorption of the "intra-bonded" hydroxyl group of compound 20 occurs some 150 $cm.^{-1}$ lower than the "free" hydroxyl of compound 46, the former band is slightly narrower than the latter. Since hydrogen-bonded hydroxyl bands are almost invariably quoted as being broader than the "free", with $\Delta\nu_{\frac{1}{2}}$ proportional to $\Delta\nu$, this observation may be a significant pointer to the lack of freedom of the heavily buttressed hydroxyl group. This behaviour, (also observed between compounds 42 and 20), continues in carbon tetrachloride and carbon disulphide, but in chloroform the hydroxyl absorption of compound 20 broadens like that of phenol without breaking the "intra" bond, i.e. chloroform causes broadening without much shift in wavelength. This broadening is seen throughout the series studied except for compound 46 which is a heavily substituted symmetrical molecule. This may mean that the effect is due to an asymmetrical arrangement of polar chloroform molecules around the hydroxyl group despite the fact that the proton is still bound to the bromine atom. The formation of specific associations between chloroform and esters and ketones has recently been discussed in some detail by Whetsel and Kagarise.[116]

Chloroform solutions were found to absorb atmospheric

moisture with great rapidity giving rise to strong absorptions at 3608 cm.$^{-1}$ ($\Delta\nu_{\frac{1}{2}a}$ = 20 cm.$^{-1}$) and 1603 cm.$^{-1}$ ($\Delta\nu_{\frac{1}{2}a}$ = 16 cm.$^{-1}$) due to solvated water molecules, and although the utmost care was taken to work with anhydrous solutions, inaccuracies in the measurements made in chloroform solutions are possible.

C. Temperature Studies.

Two compounds, 2-bromophenol and 2,4-dibromo-6-t-butylphenol, were examined in tetrachlorethylene at various temperatures in order to discern any changes in the relative proportions of the "free" and "intra-bonded" hydroxyl absorptions. The results are shown in Table VIII.

With both compounds only very small effects, qualitatively similar to those found for the four ortho-halophenols by Jones and Watkinson[100] , were observed, but the relative changes in the proportion of the "intra-bonded" and "free" hydroxyl absorptions in 2-bromophenol are too small for accurate measurements of bond energies. There is a small but definite increase in the frequency of the single hydroxyl absorption arising from the intramolecularly hydrogen-bonded conformation IV (R = tBu) of 2,4-dibromo-6-t-butylphenol. There was no evidence of the appearance of absorption due to conformation V (R = tBu) despite the slight fall in apparent molecular extinction coefficient of the bonded absorption with increase in temperature.

TABLE VIII. TEMPERATURE STUDIES on 2-BROMOPHENOL and 2,4-DIBROMO-6-t-BUTYLPHENOL in TETRACHLORETHYLENE.

	2-Bromophenol					2,4-Dibromo-6-t-butylphenol				
	"Free"			"Intra-bonded"			"Free"		"Intra-bonded"	
Temp. °C	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a	ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a		ν	$\Delta\nu_{\frac{1}{2}a}$	ε_a
24	(3603)	*	6	3526.5	18.5	171	-	3507.5	19.5	207
40	(3603)	*	7	3527	19.5	161	-	3508	19.5	202
70	(3603)	*	10	3529.5	19	157	-	3509	19.5	195
90							-	3511	19.5	191

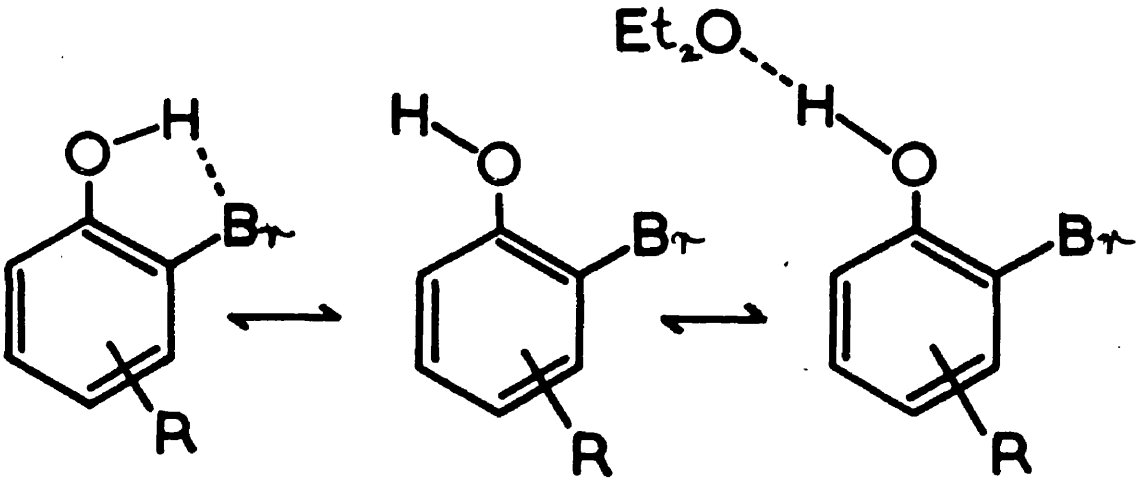
Footnotes. * Not measured.

Figures in parenthesis are approximate.

The apparent extinction coefficients (ε_a) have been rounded to the nearest whole number.

D. Competitive Intermolecular Hydrogen-Bonding Studies in Ether-Carbon Tetrachloride Mixtures.

If intermolecular hydrogen-bonding between the bromophenol and certain tissue constituents of the microorganism is playing an important role in determining biological activity, it would be expected that the degree of potency would be related to the ability of the phenol to form such a bond under competitive conditions. Accordingly, and as an extension of the studies with acetonitrile solutions, certain compounds were examined in diethyl ether - carbon tetrachloride mixtures in order to ascertain the effect of competitive intermolecular hydrogen-bonding on the positions and intensities of the hydroxyl stretching absorptions. Similar studies of phenol - ether association have been previously reported for a number of simple 2-alkyl- and 2,6-dialkylphenols[109] in which the factors limiting the extent of intermolecular hydrogen-bonding were the bulk of the groups in the ortho positions of the phenol molecule and the size of the alkyl groups of the ether. In addition to selected ortho-bromophenols, the study reported here was extended to include 2-fluorophenol and 2-fluoro-4-nitrophenol in an endeavour to provide further verification that the single hydroxyl absorption band (3592 $cm.^{-1}$ in CCl_4; Table I) of 2-fluorophenol arises from conformation I (X=F).[3,5]



	"intra"	"free"	"inter"
cm.$^{-1}$	ca. 3520 (sharp)	ca. 3610 (sharp)	ca. 3330 (broad)
	VI	VII	VIII

Sufficient ether was employed to ensure that some phenol-ether hydrogen-bonding was present, and under these conditions three distinct hydroxyl absorption bands were possible -- "free," "intra" and "inter" bands. Table IX gives the frequencies, half-band widths and apparent molecular extinction coefficients for these three absorption arising from species VI, VII and VIII. Also shown is the percentage of phenol non-bonded to ether, (rounded to the nearest 5%), as calculated by dividing the molecular extinction coefficient for the "free" or the intramolecularly hydrogen-bonded absorption in pure carbon tetrachloride by the corresponding value for the ether - carbon tetrachloride mixture. The relative abundance of the various species within any one series of compounds may be interpreted in terms of the strength of the hydrogen bonds and steric effects. Also included in the Table are the values for phenol itself, 2-methylphenol, 2-t-butylphenol, 4-isopropylphenol and 4-t-butylphenol. The appearance of the absorption bands is illustrated in Figures 2, 7 and 8.

All three absorptions are present only in ortho-bromophenols lacking a substituent in the 6-position. Ortho-bromophenols possessing a 6-substituent lack a "free" band, and the alkyl phenols lack an "intra" band. The "free" and "intra" bands are at virtually the same frequencies as in pure carbon tetrachloride solution (cf. Tables I and II).

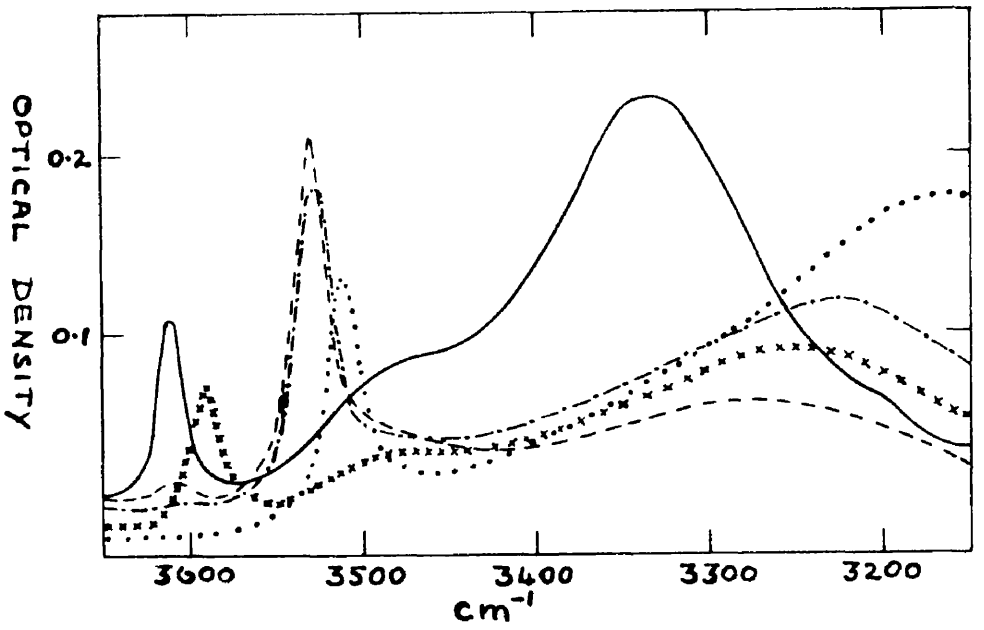


Figure 7. Hydroxyl absorptions of 2-bromophenol (- - -), 2,4-dibromophenol (-.-.-), 2-bromo-4-nitrophenol (....), 2-fluorophenol (×××) and phenol (——) in ether-carbon tetrachloride (Table IX, compounds 1, 9, 10, 39 and 42)(all 0.003M. in 0.474M. ether-CCl_4 in 5mm. cells).

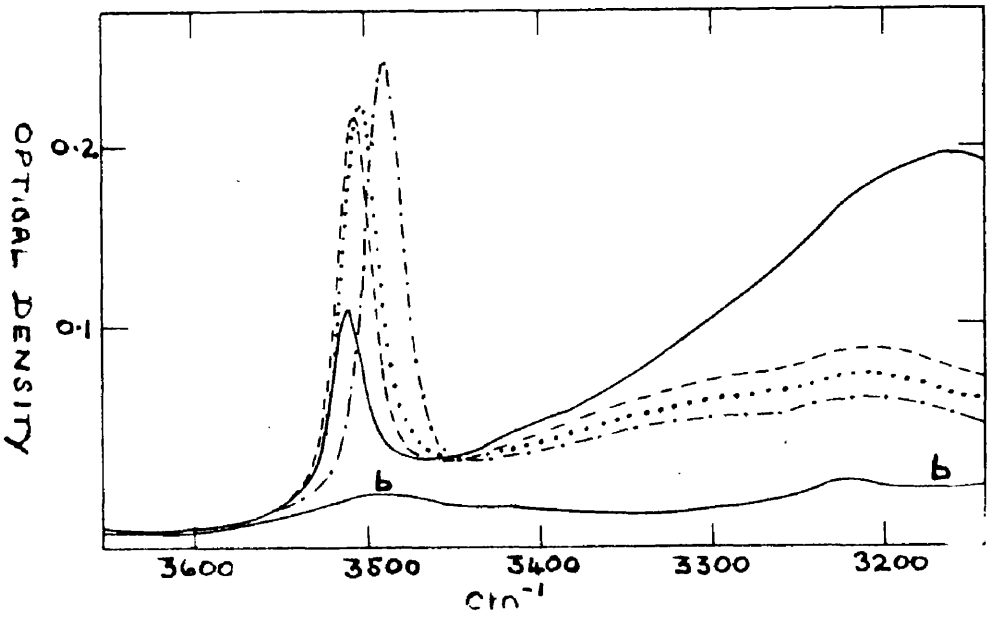
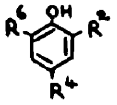


Figure 8. Hydroxyl absorptions of 6-alkyl substituted 2-bromo-4-nitrophenols (Table IX, compounds 10, 21, 23 and 25) in ether-carbon tetrachloride. b-Ether-CCl_4 background. Substituent in 6-position: H (——), methyl (- - -), isopropyl (....) and t-butyl (-.-.-)(all 0.003M. in 2.3M. ether-CCl_4 in 5mm. cells).

TABLE IX. HYDROXYL STRETCHING ABSORPTIONS of SUBSTITUTED PHENOLS in ETHER - CCl_4 SOLUTIONS.



Compound No.	R^2	R^4	R^6	"Free" ν	"Free" $\Delta\nu_{\frac{1}{2}a}$	"Free" ε_a	"Intra" ν	"Intra" $\Delta\nu_{\frac{1}{2}a}$	"Intra" ε_a	"Inter" ν	"Inter" $\Delta\nu_{\frac{1}{2}a}$	"Inter" ε_a	% Non-bonded to ether.
42 a	H	H	H	3613	21	60		——		3337	140†	150	30
47 a	H	iPr	H	3615	17	65		——		3349	135†	120	55
48 a	H	tBu	H	3614	17.	60		——		3345	140	120	50
43 a ⊕	Me	H	H	3613	19	60		——		3340	170	110	35
44 a ⊕	tBu	H	H	3648 3607	* 17	10 60		——		3338	150	115	
1 a	Br	H	H	3608	*	10	3530	22	130	(3275)	br	35	80
4 a	Br	iPr	H	(3603)	*	*	3535	22	100	(3280)	br	25	65
6 a	Br	tBu	H	3609	*	15	3533	26	135	(3285)	br	35	90
9 a	Br	Br	H		——		3529	25	110	(3230)	280†	65	65
11 a	Br	Br	Me		——		3527	20	160	(3292)	br	25	85
13 a	Br	Br	iPr		——		3523	20	155	(3300)	br	20	80
15 a	Br	Br	tBu		——		3509	19	185	(3315)	br	20	85
35 a	F	H	H		——		3592	21	40	(3257)	280†	50	20
41 a	F	NO_2	H		——		3570	30	20	(3134)	330†	135	10
10 a	Br	NO_2	H		——		3514	24	80	(3166)	270†	110	35
10 b	Br	NO_2	H		——		3512	22	65	(3164)‡	275†	125	25
21 b	Br	NO_2	Me		——		3509	22	135	(3210)‡	br	35	60
23 b	Br	NO_2	iPr		——		3504	24	150	(3210)	br	45	65
25 b	Br	NO_2	tBu		——		3489	24	170	(3210)	br	40	75
9 b	Br	Br	H		——		3528‡	28†	60	(3229)	240†	130	35
11 b	Br	Br	Me		——		3527	24	100	(3295)	br	55	55
13 b	Br	Br	iPr		——		3522	25	100	(3300)	br	45	55
15 b	Br	Br	tBu		——		3509	26	135	(3334)	br	35	60

Footnotes. a Examined in 0.47M. ether/CCl_4 solution.

b Examined in 2.3M. ether/CCl_4 solution.

⊕ 0.1M. phenol in 0.5M. ether/CCl_4 solution.

† Measured by band reflection. * Not measured.

‡ Unsymmetrical band. Values in parenthesis are approximate. br = broad.

All measurements on ether - CCl_4 solutions were run on the scale 4mm. = 10cm.$^{-1}$ The recorded band positions cannot be as accurate as those examined in pure carbon tetrachloride since the broad OH...O bands are difficult to place to ± 10 cm.$^{-1}$

The percentages have been rounded to the nearest five units.

% Unbound = $\frac{\varepsilon \text{ in } CCl_4}{\varepsilon \text{ in ether}/CCl_4} \times 100$ where ε is with respect to either "free" or "intra".

The intermolecular band appears in some cases to extend up to ca. 3600 cm.$^{-1}$

Solutions approximately 0.47M. (ca. 5% v/v) and 2.3M. (ca. 25% v/v) with respect to the ether were employed, but the equilibrium did not seem unduly sensitive to the ether molarity as 0.67M. ether solutions gave results closely similar to those obtained in 0.47M. ether solutions. This is to be expected since in either solution the ether:phenol molecular ratio is in excess of 100:1.

In the series 2-methylphenol, phenol, 2-bromophenol, 2,4-dibromophenol and 2-bromo-4-nitrophenol, (compounds 43, 42, 1, 9 and 10 respectively), the increasing acidity of the phenolic hydroxyl group as the series is ascended is evident from the progressive shift to lower frequencies of the intermolecularly-bonded band. Consideration of the apparent extinction coefficients and of the percentage of "free" or intermolecularly-bonded form present is, however, complicated by the presence of an intramolecular hydrogen bond in compounds 1, 9 and 10 which bear ortho-bromine atoms. In these three compounds there is competition between the bromine atom and the ether molecules for the right to hydrogen bond to the proton, but this competition is, of course, not present in compounds 42 and 43. A particularly good illustration of this effect is obtained by comparing compounds 43 and 1 since the methyl group (2.0 Å) and the bromine atom

(1.95 Å) are of almost exactly the same radius. Introduction of a 2-bromine atom into compound 42, i.e. to form compound 1, decreases drastically the amount of intermolecular-bonding despite the already noted acidifying effect of the bromine atom since the hydroxyl group shows great preference for the formation of an intramolecular hydrogen bond. Introduction of another bromine atom in the 4-position to form compound 9, however, while resulting in a further increase in the acidity of the hydroxyl group, now increases the amount of "inter-bonding" over compound 1. Replacement of this 4-bromine atom by a nitro group so increases the acidity of the hydroxyl group that compound 10 attains the same degree of "inter-bonding" as compound 43 despite the competition afforded by the 2-bromine atom.

A similar situation has been found to pertain between 4-nitrophenol and phenol.[117] Here measurements were made in ether - chloroform, and it was found that the more acidic nitro derivative gave a greater increase in intensity and a greater shift to lower frequency of the hydroxyl absorption due to the species associated with the ether. It would therefore seem that increased acidity of the hydroxyl group favours intermolecular hydrogen-bonding rather than intramolecular hydrogen-bonding. These effects are illustrated in Figure 7. A related situation has been reported in the salicylaldehyde series where pyridine was used to induce

competitive hydrogen-bonding.[118]

4-Alkyl substitution, which produces a slight decrease in acidity, has very little effect on the association with ether of either phenol or 2-bromophenol, but in the two principle series shown in Table IX (compounds 9, 11, 13 and 15 and compounds 10, 21, 23 and 25) a 6-alkyl group reduces considerably the extent of intermolecular association with ether, presumably by restricting access to the hydroxyl group and by virtue of the greater strength of the intramolecular hydrogen bond through steric compression of the OH...Br distance (cf. Table II). The effect of increasing bulk in the ortho-substituent on ether bonding in these series is illustrated in Figure 8.

In the para-bromo series (compounds 9, 11. 13 and 15) there is a rise in frequency of the associated band which parallels the fall in intensity consequent upon the increasing bulk of the 6-alkyl substituent, the order being H $\ll$ methyl $<$ isopropyl $<$ t-butyl. This is to be anticipated in view of the increased restriction offered to the approach of the ether molecule and is in marked contrast to the lowering in frequency of the intramolecular bond already discussed. The fall in intensity is perhaps the most sensitive measure of the 6-alkyl effect.

In several of the experiments a rather broad symmetrical band of variable intensity was sometimes observed near

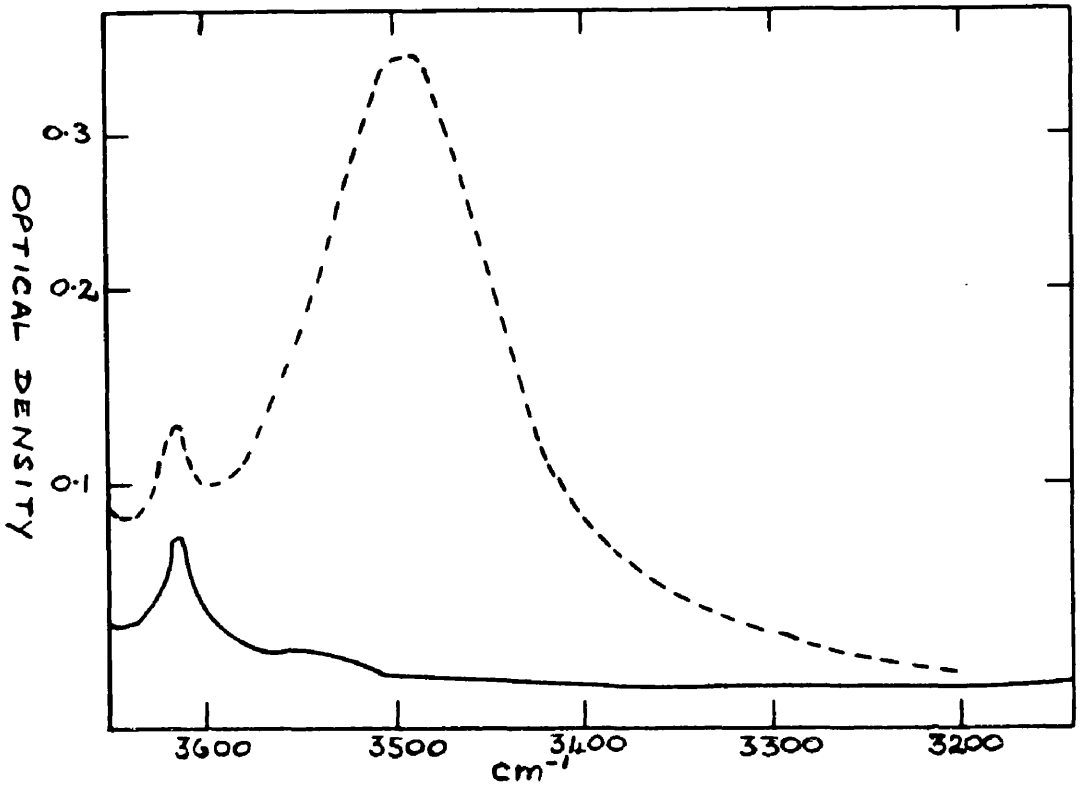


Figure 9. Hydroxyl absorption of water in CCl_4 and Ether-CCl_4 showing HO-H...OEt_2 association. (—) CCl_4 Water (Sat.)/CCl_4; (- - -) Et_2O (0.47M.) in CCl_4 water (Sat.)/ Et_2O (0.47M.) in CCl_4. (1cm. quartz.)

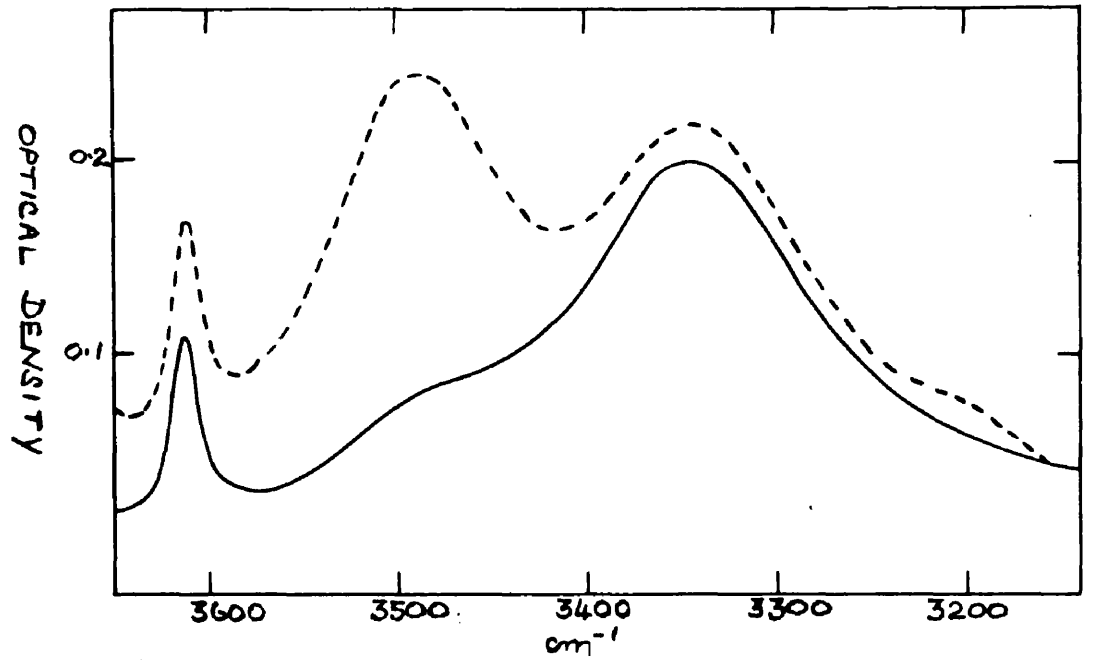


Figure 10. Effect of water contamination of mixed Ether-CCl_4 solutions. (——) p-t-butyl-phenol 0.00125M. in 0.47M. Et_2O-CCl_4; (- - -) as above, with excess water (Sat.) (1cm. quartz.)

3500 cm.$^{-1}$,[109] and its appearance is illustrated in Figure 10. This band was found to be due to the presence of dissolved water in association with ether molecules (H-O-H...OEt_2 or some similar complex). Water alone added to ether - carbon tetrachloride solution absorbs at 3614 cm.$^{-1}$ ($\Delta\nu_{\frac{1}{2}}$ = 30 cm.$^{-1}$) weak, and 3494 cm.$^{-1}$ ($\Delta\nu_{\frac{1}{2}}$ = 100cm.$^{-1}$) strong (Figure 9). It is difficult to exclude all traces of water during the preparation of solutions because both ether and some of the phenols are highly deliquescent and consequently the quantitative data recorded in Table IX may not be entirely accurate.

2-Fluorophenol.

Unlike the other ortho-halophenols which exhibit two hydroxyl stretching frequencies attributable to the cis and trans conformations I and II (X = Cl, Br or I),[1-5] 2-fluorophenol shows but a single hydroxyl absorption band in carbon tetrachloride solution at 3592 cm.$^{-1}$ (Table I). This unusual value has led to queries as to whether it represents a bonded or non-bonded hydroxyl absorption. Baker and Kaeding,[5] on the basis of a series of elegant competitive intramolecular hydrogen-bonding studies in unsymmetrical 2,6-dihalophenols, came to the conclusion that this single band in 2-fluorophenol was due to conformation I (X=F) -- the intramolecularly hydrogen-bonded conformation -- since 2,4-dibromo-6-fluorophenol absorbs at 3574 cm.$^{-1}$ and 3522 cm.$^{-1}$ in carbon

tetrachloride. The low frequency band at 3522 cm.$^{-1}$ being assignable to OH...Br bonding leaves the high frequency absorption for the conformation in which the OH is directed towards the fluorine atom, and the its value of 3574 cm.$^{-1}$ being very near their value of 3584 cm.$^{-1}$ for 2-fluorophenol strongly indicates that the latter is a bonded peak. Similarly 2,6-difluorophenol is known to absorb at 3586 cm.$^{-1}$ in tetrachlorethylene,[119] and since measurements in the two solvents are comparable -- for instance 2,4-dibromo-6-t-butylphenol absorbs at 3507.5 cm.$^{-1}$ in tetrachlorethylene and at 3509 cm.$^{-1}$ in carbon tetrachloride -- this observation provides further evidence for the "intra-bonded" conformation.

Since the hydrogen bond in 2-fluorophenol is weaker than that in 2-bromophenol and yet there is evidence of the "free" conformation in the latter, it would be expected that there would be even more "free" conformation (II) present in the former. Baker[3] suggests that if it is indeed present, its non-appearance is due to its occurrence on the high frequency side of the main band with a very small wave number separation. Since it has been shown here that the frequency of the "free" band is more sensitive to change in solvent than the "intra" band, it was initially reasoned that examination in hexane might reveal the presence of a "free" band by means of an increased separation. Hexane, however, is known to favour the less polar conformation, in this case the "intra-bonded"

form. On examination of 2-fluorophenol in n-hexane, only one band at 3603 cm.$^{-1}$ was observed with no evidence of a shoulder on the high frequency side.

Attention was therefore turned to a comparison of 2-fluorophenol (compound 39) and 2-fluoro-4-nitrophenol (compound 41) in ether - carbon tetrachloride solution since the acidifying effect of the 4-nitro group would be expected to produce effects similar to those observed with the ortho-bromophenols. As is evident from Table IX, both the fluoro compounds display a much greater degree of "inter-bonding" than their bromo counterparts, and the "inter" bands are at slightly lower frequencies. This latter fact might indicate that 2-fluorophenol is more acidic than 2-bromophenol, but in view of the opinion[5] that the reverse is probably true it is presumably a reflection of the difficulty in measuring the exact position of these broad bands. The increased amount of "inter-bonding" is probably largely due to the decreased strength of the OH...F bond relative to the OH...Br bond, the hydroxyl group thereby being more accessible to ether molecules.

This study seems to show that 2-fluorophenol in ether - carbon tetrachloride solution behaves in a qualitatively similar fashion to 2-bromophenol, though the hydroxyl group in the former is more freely able to bond to ether molecules. This is unlikely to be a steric effect since it has been

shown[109] that groups smaller in size than t-butyl have negligible effects in this connection. It may point to the decreased strength of the hydrogen bond (OH...F) or to the possibility that the hydroxyl group is simply oriented towards the highly polar fluorine atom without actually being bound to it.

E. The Effect of State on the Hydroxyl Stretching Frequency.

As a continuation of the competitive intermolecular hydrogen-bonding studies described in section D, all the bromophenols included in Tables I-IV were examined by infrared spectroscopy as films or Nujol mulls. The infrared frequencies of various functional groups are well known to change with the physical state of aggregation[120] due to hydrogen bridges or dipole interactions, and the investigations described in this section were undertaken with the object of determining what effect the availability of an intramolecular hydrogen bond of varying strength might have on the extent and type of the intermolecular hydrogen-bonding normally encountered between phenol molecules in the solid and liquid states.[121] This represents an extension of the situation in which competitive intermolecular hydrogen-bonding was induced by adding ether to carbon tetrachloride solutions of the phenols (Table IX). The present case is, however, more complex in that "inter-

bonding" may occur not only with the phenolic oxygen atom of another molecule, but also with the aromatic ring,[122] the bromine atoms or in some cases with a nitro group in the para position of the second molecule.

In the liquid state, all these interactions might be expected to occur simultaneously owing to the many possible orientations of the molecules with respect to one another, although phenols containing large groups in the ortho positions would tend to enter into dimeric, rather than polymeric, association.[109,123] In the solid state, on the other hand, the ordered arrangement of the crystal lattice would be expected to favour certain interactions to the exclusion of others, the strength of the intermolecular hydrogen bonds being related to the distance between the interacting groups.[124] When the phenols are present as dispersed solids in Nujol the possibility of solution in the paraffin must be considered. Such solutions would be expected to give rise to absorptions similar to those observed in hexane which in turn are known to differ little from those in carbon tetrachloride solution (cf. Table VI).

The measurements relating to the hydroxyl stretching absorptions are summarised in Table X. For discussion purposes, the spectra have been grouped into four distinct but overlapping sections in which the hydroxyl absorptions have been assigned to specific hydrogen bond types,

TABLE X. HYDROXYL STRETCHING ABSORPTIONS of SUBSTITUTED o-BROMOPHENOLS.

Compound	No.	State	"Intra"* ν	(Δν)	$\Delta\nu_{\frac{1}{2}}$	Strength	"Inter" OH...O ν	$\Delta\nu_{\frac{1}{2}}$	Strength	"Inter" OH..NO_2 ν	$\Delta\nu_{\frac{1}{2}}$	Strength
2,4-Dioromo-6-methylphenol	11	L	3515	(13)	50	s						
2,4-Dibromo-6-ethylphenol	12	L	3520	(7)	48	s						
2,4-Dibromo-6-isopropylphenol	13	L	3520	(4)	45	s						
2,4-Dibromo-6-s-butylphenol	14	L	3520	(4)	48	s						
2-Bromo-3,4,6-trimethylphenol	29	L	3515	(9)	50	s						
2,4-Dibromo-6-phenylphenol	17	N	3490	(26)	56	m						
2,4-Dibromo-3-methyl-6-t-butylphenol	20	N	3490	(0)	40	m						
2,4-Dibromo-3,5,6-trimethylphenol	28	N	3505	(12)	28	m						
2-Bromo-4,6-di-t-butyl-3-methylphenol	30	N	3500	(0)	-	m						
2-Bromo-4-cyclohexylphenol	7	N	3532	(2)	40	m						
2,4-Dibromo-6-t-butylphenol	15	N	3508 3482	(1) (27)	- -	m m						
6-Cyclohexyl-2,4-dibromophenol	16	N	3522 3470	(2) (52)	- -	w m						
2-Bromo-4-methylphenol	2	L	3505	(31)	175	m	Extensive low frequency "tail" ascribed to "Inter-bonding."					
2-Bromo-4-ethylphenol	3	L	3580	(17)	200	m						
2-Bromo-4-isopropylphenol	4	L	3520	(14)	150	s						
2-Bromo-4-s-butylphenol	5	L	3520	(16)	135	m						
2-Bromo-4-t-butylphenol	6	L	3515	(19)	145	m						
2,4-Dibromophenol	9	N	3530	(0)	30	w	3445	105	m			
2,4-Dibromo-6-methylphenol	11	C	3500	(28)	30	m	3420	160	m			
		N	3528 3502	(0) (26)	- -	m m	3420	-	m			
2,4-Dibromo-3,6-dimethylphenol	19	N	3515	(3)	-	w	3400	110	m			
2,4-Dibromo-5,6-dimethylphenol	27	N	3525	(2)	-	w	3390	120	m			
1-Bromo-2-hydroxy-5,6,7,8-tetrahydronaphthalene	31	N	3525	(0)	-	vw	3310	230	m			
1-Bromo-2-naphthol	32	N	3515	(3)	-	vw	3275	200†	s			
4-Bromo-5-hydroxy-3-nitrothionaphthen	35	N					3410	150	m			
3-Bromo-2-naphthol	33	N					3405	105	m			
2,4,6-Tribromophenol	18	N					3400	130	m			
2-Bromo-4-phenylphenol	8	N					3250	220	m			
1-Bromo-2-hydroxydibenzofuran	37	N					3270	160†	m			
4-Bromo-5-hydroxythionaphthen	34	N					3235	145	m			
2-Bromo-4-t-butylphenol	6	C					3395 3150	130† 190†	m m			
2-Bromo-4-nitrophenol	10	N								3390	55	m
2-Bromo-6-methyl-4-nitrophenol	21	N								3400	80	m
2-Bromo-6-ethyl-4-nitrophenol	22	N								3405	45	m
2-Bromo-6-isopropyl-4-nitrophenol	23	N								3420	50	m
2-Bromo-6-s-butyl-4-nitrophenol	24	N								3395	45	m
2-Bromo-6-t-butyl-4-nitrophenol	25	N					3335	190†	m	3420	40†	m
2-Bromo-6-cyclohexyl-4-nitrophenol	26	N								3380	50	s

Footnotes. L liquid film N Nujol mull C crystalline film

s strong m medium w weak vw very weak - not measured

† measured by band reflection

* The values in parenthesis (Δν) are for ν_{CCl_4} minus ν solid or liquid.

Many of the bands although reasonably sharp, are asymmetrical - wider on low frequency side.

although in certain cases the assignments cannot be made with absolute certainty.

The spectra were scanned from 3650-650 cm.$^{-1}$ and will be published as Documentation of Molecular Spectroscopy Index Cards nos. 10200-10235. Except in cases where the hydroxyl region was examined in more detail, the spectra were recorded at 180 cm.$^{-1}$/min. More detailed spectra were recorded at 90 cm.$^{-1}$/min.

"Intra" Bond (OH...Br): Compounds 2-7, 11-14, 15-17, 20 and 28-30, examined as mulls in Nujol or as liquid films, as seen from Table X, all exhibit a fairly sharp band of medium or strong intensity at ca. 3500 cm.$^{-1}$ which is assigned to the "intra" (OH...Br) hydrogen bond. This assignment is made on the grounds of the position and sharpness (small $\Delta\nu_{\frac{1}{2}}$) of the bands (cf. Tables I-IV)--quite unlike the breadth of typical "inter-bonded" hydroxyl bands. Compounds 2-6, (as liquid films), display quite sharp bands but at the same time possess large apparent half-band widths due to extensive "tailing" on the low frequency side. This is ascribed to the presence of a degree of "inter-bonding." Compounds 15 and 16, (as Nujol mulls), show split bands whose origin will be discussed in more detail later.

Mixture of "Intra" and "Inter" Bonds: The presence of both "intra" and "inter" hydrogen-bonding was observed with

compound 11 (as a crystalline film) and compounds 9, 11, 19, 27, 31 and 32 (as Nujol mulls). All exhibit two distinct bands, one weak at ca. 3500 cm.$^{-1}$ corresponding to the "intra-bonded" conformation, and the other broad and of medium or strong intensity in the region 3450-3250 cm.$^{-1}$ which is ascribed to the "inter" bond between the phenolic hydroxyl groups of different molecules. Compound 11 (as a Nujol mull) exhibits a split band which will be discussed later.

"Inter" Bond Only (OH...O): Crystals of compounds 8, 18, 33-35 and 37 (Table X) are presumed to contain only "inter" bonds since they exhibit a single broad band of medium or strong intensity in the region 3450-3250 cm.$^{-1}$ The apparent half-band widths vary considerably from compound to compound as do the general shapes of the bands. Compound 6 (as a crystalline film) involves a special situation and this will be discussed separately.

"Inter" (OH...NO_2) Bond: Compounds 10 and 21-26 (Table X) have been grouped together since they all display a sharp band of medium or strong intensity near 3400 cm.$^{-1}$ which is assigned to specific intermolecular hydrogen-bonding between the phenolic hydroxyl group of one molecule and the nitro group of an adjacent molecule. Compound 25 has a second broad band of medium intensity at 3335 cm.$^{-1}$ indicating that both OH...NO_2 and OH...O types are present. Compounds 10 and 21-24 exhibit "tailing" on the low frequency side of the

band which may also be indicative of the presence of some OH...O "inter-bonding." It is possible that compound 35 should be assigned to this section although the apparent half-band width (150 $cm.^{-1}$) would contra-indicate this.

In the course of the studies with the Nujol mulls of compounds 11, 15 and 16, what at first sight appeared to be inconsistent results were observed, but further investigation showed that the anomalies had their origin in the state of the compound in the mull. Examination under a microscope (magnification x100) showed that three distinct cases were to be discerned. These were:- i complete solution of the bromophenol in the Nujol; ii dispersal of the bromophenol throughout the mull as liquid droplets due to mixed m.p. depression; and iii dispersal of the bromophenol throughout the Nujol as fine solid particles. That a certain amount of the compound was concurrently in solution at equilibrium with the dispersed material in cases ii and iii was demonstrated in certain specific instances when it was found that progressive increase in the proportion of Nujol present led to complete solution. These effects are, therefore, distinct from those due to factors such as polymorphism,[125] orientation [126] or reflectivity and scattering effects.

A detailed study with compound 29 (m.p. 31.5-32°) showed that both the frequency of the hydroxyl absorption and the apparent half-band width varied according to the quantity of

Nujol used to make up the mull - the results being summarised below. With only a trace of Nujol present,

State	ν_{OH}	$\Delta\nu_{\frac{1}{2}}$
Liquid film	3515	50
Conc. mull in Nujol	3515	48
" " " "	3518	42
" " " "	3520	40
Dilute " " "	3522	20
Solution in hexane	3525.5	11

microscopic examination revealed that the compound was dispersed as fine droplets throughout the paraffin whilst the position of the hydroxyl absorption was at 3515 $cm.^{-1}$ with an apparent half-band width of 48 $cm.^{-1}$ The spectrum was in fact virtually superposable on that obtained from compound 29 as a liquid film. Progressive increase in the proportion of Nujol was found to increase the frequency of the band and decrease the half-band width until the absorption approached that observed in hexane solution. At the same time, examination of the mulls under the microscope showed progressive disappearance of the dispersed droplets until a homogeneous solution was obtained. The absorptions shown above for intermediate proportions of Nujol therefore

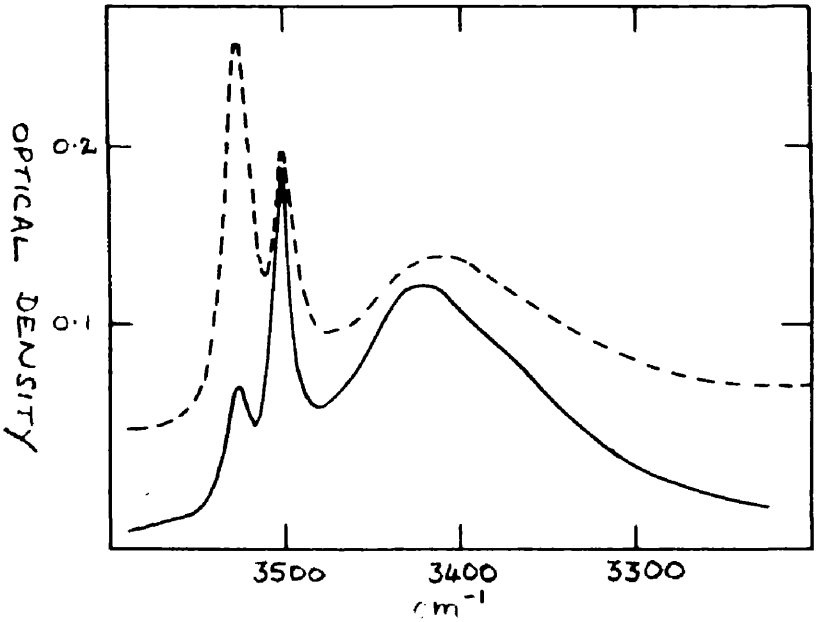


Figure 11. Effect of increasing amount of Nujol on the spectrum of dispersed 2,4-dibromo-6-methylphenol (compound 11). (—) small amount of Nujol: (---) large amount of Nujol.

represent double absorptions arising from the mixture of dispersed liquid and Nujol solution, but the two bands are not resolved because of their small frequency separation. The same situation can be expected to arise with other low-melting bromophenols whenever the solubility in Nujol is such that it can permit the co-existence of dispersed droplets, but no example of this situation other than with compound 29 was encountered in the present study.

On the other hand, compounds 11, 15 and 16 showing split bands in Nujol (Table X) were found to exist as a saturated solution in Nujol with an excess of finely dispersed solid particles also present. In these cases the higher frequency absorption, whose position is very close to that of the "intra-bonded" absorption in carbon tetrachloride solution (cf. Table II), can be assigned to "intra-bonding" in the material dissolved in the Nujol whilst the lower frequency absorptions represent "intra-bonded" absorptions in the crystalline state. This behaviour is illustrated in Figure 11 for compound 11. The effect of increasing the Nujol proportion is to increase the strength of the highest frequency band (3528 $cm.^{-1}$; "intra" in solution) relative to those of the two lower frequency bands at 3502 $cm.^{-1}$ ("intra" in crystal) and 3420 $cm.^{-1}$ ("inter" in crystal). Further increase in the proportion of Nujol led to the eventual disappearance of dispersed solid particles and also to the

disappearance of the two low frequency absorptions.

Compounds 17 and 28 when studied as Nujol mulls show absorptions whose frequencies are 26 cm.$^{-1}$ and 12 cm.$^{-1}$ lower respectively than the values observed in carbon tetrachloride solution (cf. Tables II and III). These compounds must therefore be assumed to have very low solubility in Nujol and the observed absorption arises from the solid state with its characteristic dipolar interactions. In contrast, compounds 7, 9, 19, 20, 27 and 30-32 which are fully dissolved in Nujol show "intra-bonded" bands at frequencies varying little in position from the values seen with carbon tetrachloride solutions (cf. Tables I-IV).

Solubility effects in Nujol were also observed with compounds 8, 18, 33-35 and 37 which at normal concentrations exhibited only "inter-bonded" bands arising from suspended solid. Increase in the proportion of Nujol led to progressive solution and at the same time to a progressively increasing "intra-bonded" absorption in the region of 3500 cm.$^{-1}$

Two compounds, viz. 6 and 11, were further studied as both liquid films and crystalline films (Table X). In the case of compound 6, it was found that crystallisation of the liquid film between the rock-salt plates occurred sufficiently slowly to permit a study of the changes in absorption during the gradual transition from the liquid to the solid state

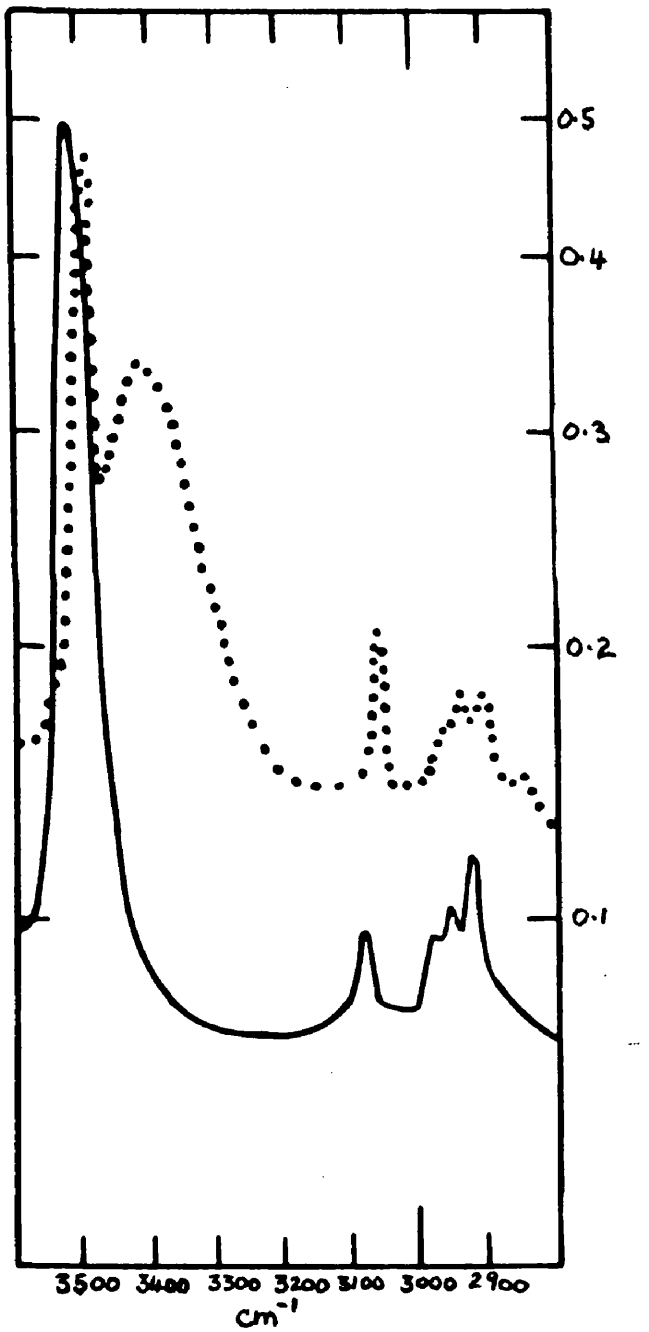


Figure 13. Effect of complete crystallisation on liquid film spectrum of 2,4-dibromo-6-methylphenol (compound 11) in the region 3600-2800 $cm.^{-1}$ (——) liquid film: (····) crystalline film.

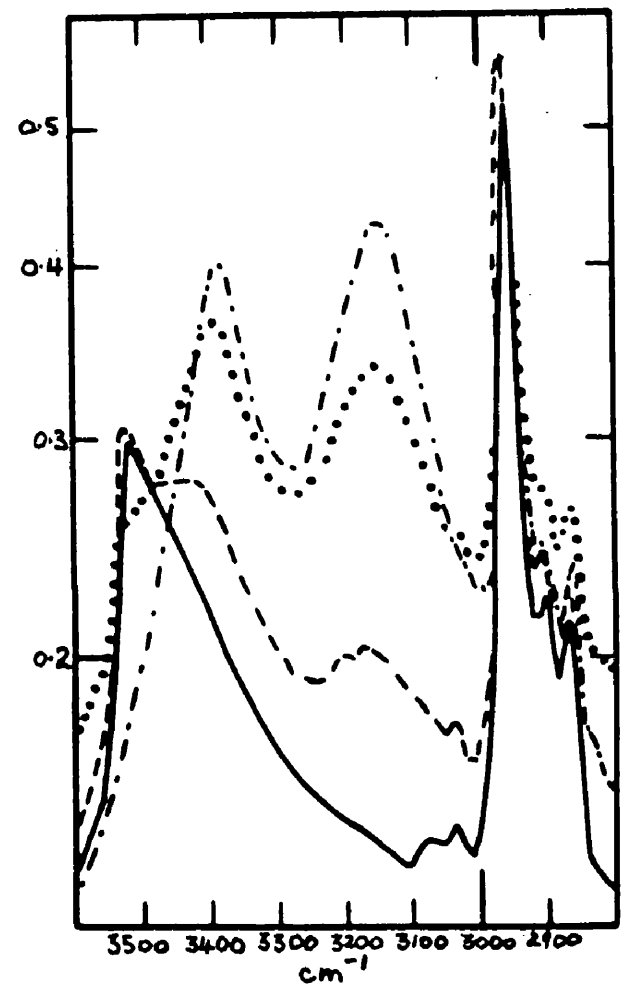


Figure 12. Effect of increasing degree of crystallinity on a liquid film spectrum of 2-bromo-4-t-butylphenol (compound 6) in the region 3600-2300 $cm.^{-1}$ (——) liquid film; (- - -) after 30 mins.: (····) after 16 hours: (-·-·) after 3 weeks.

and the results are illustrated in Figure 12. The extent of crystallinity was checked by viewing through crossed "Polaroid" sheets. The liquid film has a broad tailed band at 3515 $cm.^{-1}$ indicative of the formation of "intra" and "inter" bonds. As crystallisation proceeded this band gradually disappeared to be replaced by two bands at 3395 $cm.^{-1}$ and 3150 $cm.^{-1}$ which can only be assigned to some well-defined specific "inter-bonding" of the hydroxyl groups in the crystal lattice. The behaviour of compound 11 on complete crystallisation from the melt is illustrated in Figure 13, both "intra" and "inter" bonds being present in the crystalline film. The behaviour of this compound in Nujol has already been discussed.

It is apparent from the above results that routine examination of infrared spectra recorded for "mulls" in Nujol might profitably be accompanied by a careful check as to the actual state of the compound in the mull. Solid state measurements when carefully interpreted could be useful in providing a method of rapid selection of crystals suitable for X-ray diffraction studies, for example in circumstances where instances of pure "intra" or "inter" hydrogen-bonding are required.

Nujol, being a commercial brand of liquid paraffin, can be likened in general physico-chemical character to the lipids which occur in living organisms, and both might

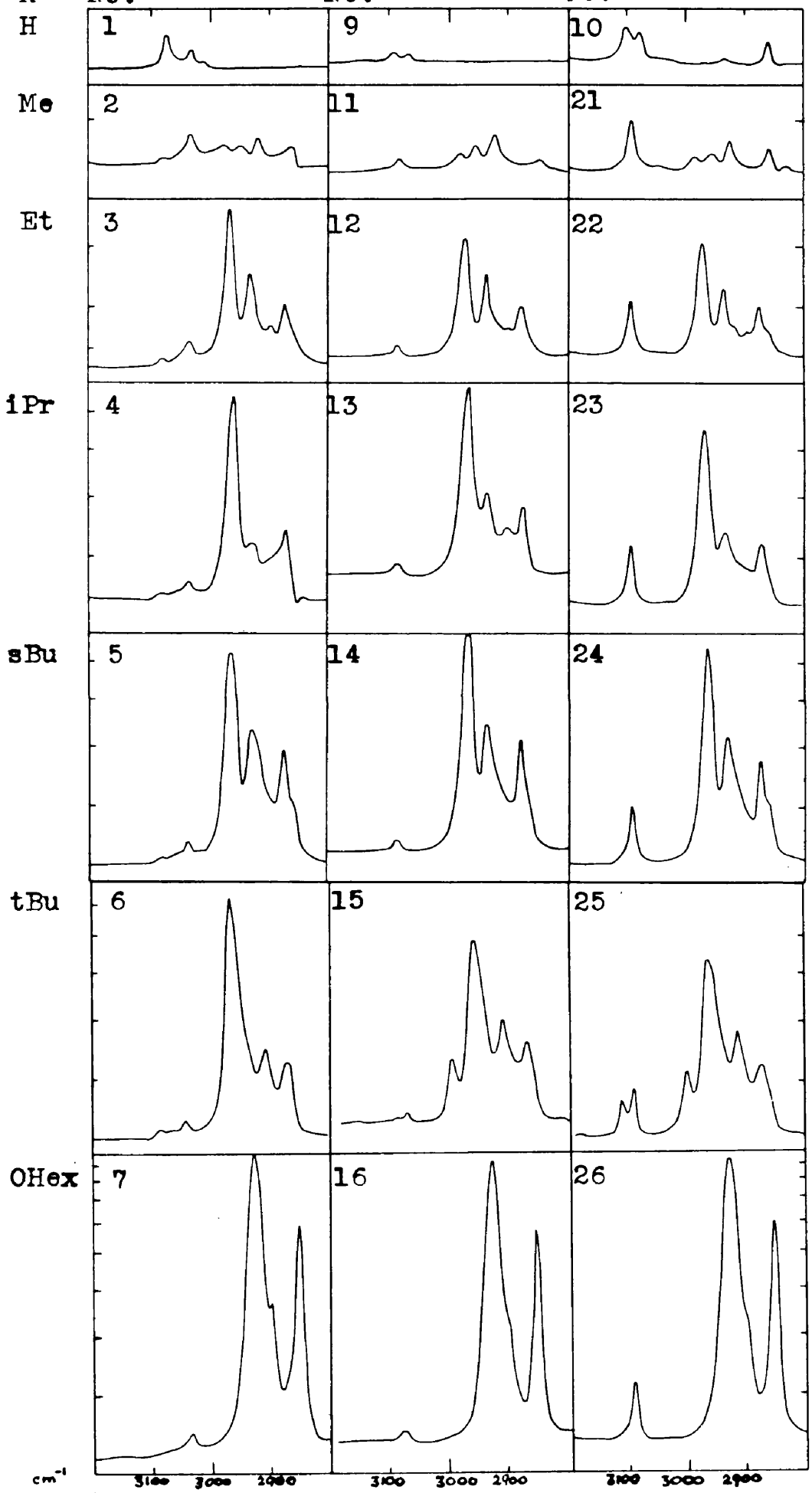


Figure 14. CH stretching absorptions of the three series 1-7; 9,11-16; 10,21-26; in CCl_4. (0.005M. in 5mm.cells)

therefore be expected to show a similar ability to dissolve organic compounds such as the bromophenols under discussion. The antibacterial activity of phenols has been related to their lipid solubility,[33-35] and therefore the behaviour of the bromophenols in Nujol which has been described could be taken as an indication of a similar behaviour at their site of action in the microorganism. The results obtained in carbon tetrachloride solutions (Tables I-IV), since they closely parallel those for bromophenols dissolved in Nujol, may be therefore directly applicable to the problem of predicting bactericidal activities.

F. CH Stretching Frequencies in CCl_4.

During the examination of the bromophenols in carbon tetrachloride solution, an opportunity was afforded to record the CH stretching absorptions. Figure 14 illustrates the regularity of the absorptions obtained for the three series compounds 1-7; 9, 11-16; 10, 21-26.

G. Predictions of Bactericidal Activities of o-Halophenols.

It was noted in section D that an increase in the acidity of the hydroxyl group of the ortho-bromophenols under discussion normally results in an increase in intermolecular hydrogen-bonding at the expense of intramolecular hydrogen-bonding, and that only when the hydroxyl group is

increasingly restricted by the bulk of the group in the 6-position is there any preferential increase in "intra-bonding."

For the compounds in Table I, therefore, where no substituent is present in the 6-position, the bactericidal efficiency would be expected to fall in the following sequences on the basis of the frequency of the free hydroxyl band, this being a measure of the acidity of the hydroxyl group.

$$38 > 1 > 40$$

$$10 > 9 > 8 > 7 \approx 6 \approx 5 \approx 4 \approx 3 \approx 2$$

provided the original premise that bactericidal potency depends upon the ability to enter into intermolecular association with constituents of the living organism is valid.

In table II, (compounds 11-18 and 21-26), there is a substituent present in the 6-position which controls the strength of the hydrogen bond. The biological activity would therefore be expected to be inversely proportional to the strength of this bond. Badger's rule[127] states that the strength of the hydrogen bond is proportional to ν_{OH}. On this basis, the following order of activity would be expected to hold true.

$$11 > 12 > 13 \approx 14 \approx 16 \approx 17 \approx 15$$

Compound 18 is not consistent with the rest of the series since the group in the 6-position is a bromine atom

and not an alkyl group. Compound 17 may similarly prove to be anomalous since intramolecular hydrogen-bonding is also taking place with the with the π electrons of the phenyl group.

Similarly, for the para-nitro series, the expected order of potency is

$$10 > 21 > 22 > 24 > 23 \approx 26 > 25$$

Table III. Restriction to intermolecular hydrogen-bonding by the hydroxyl group which is afforded by the 6-alkyl group is increased by the buttressing effect of substituents all round the ring. In this case, the order of antimicrobial activity would be expected to be

$$27 > 29 > 19 > 28 > 20 > 30$$

Ether Studies. The competition between "inter-bonding" and "intra-bonding" afforded by the presence of ether provides a direct method for measuring the ability of the phenol to form an intermolecular hydrogen bond. For the two principal series in this study, the expected order of potency therefore is

$9 > 11 > 13 > 15$ in 0.474M. ether

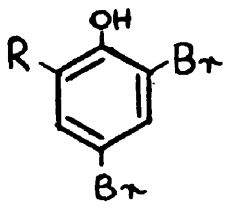
$9 > 11 \approx 13 > 15$ in 2.4M. ether

$10 > 21 > 23 > 25$ in 2.4M. ether

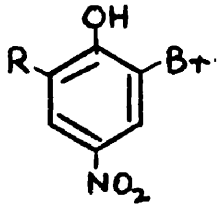
These results are summarised in Table XI.

The results obtained in section A have been published in Spectrochimica Acta, a reprint being included as Appendix 4. The results obtained in sections B, C and D have been accepted for publication in the same journal.

TABLE XI. PREDICTED ORDER of BACTERICIDAL ACTIVITIES of GROUPS of ortho-BROMOPHENOLS as derived from CCl_4 and CCl_4 - ETHER STUDIES.

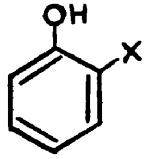


CCl_4	CCl_4-Ether 0.474M.	CCl_4-Ether 2.4M.
9,11	9	9
12	11	11,13
13,14,16	13	
17		
15	15	15

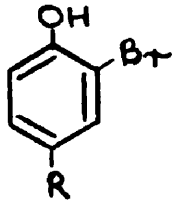


CCl_4	CCl_4-Ether
10	10
21	21
22	
24	
23,26	23
25	25

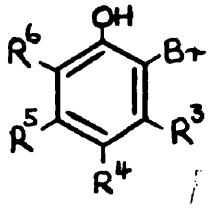
Increasing Activity ↑



CCl_4
38
1
40



CCl_4
10
9
8
7,6,5,4,3,2



CCl_4
27
29
19
28
20
30

Increasing Activity ↑

Experimental.

All spectra were measured with a Unicam S. P. 100 double-beam spectrophotometer equipped with an S. P. 130 sodium chloride prism-grating double monochromator operated under vacuum conditions. The general proceedure has been previously described.[109] Carbon tetrachloride (AnalaR), carbon disulphide (AnalaR) and n-hexane (spectroscopic grade) were used without purification. Chloroform (AnalaR) was freed from ethanol by two successive passages through blue silica gel immediately before use. The "M & B" anhydrous diethyl ether (sodium dried), chloroform and carbon tetrachloride solutions were examined immediately after preparation. Acetonitrile was purified by successive prolonged treatments with potassium hydroxide, calcium chloride and phosphorus pentoxide, followed by distillation. The solutions were scanned from 3650 $cm.^{-1}$ to 2600$cm.^{-1}$ and the films and Nujol mulls from 3650 $cm.^{-1}$ to 650 $cm.^{-1}$

Melting points were taken on a Kofler block. The light petroleum was of boiling point 60-80°. The simple ortho-halophenols (Table I, compounds 1, 38-40) were examined by gas – liquid chromatography on a 25% Apiezon L/Celite column. Only ortho-fluorophenol contained any resolved impurity, and the absence of even traces of the para-halo isomer as an impurity was verified by examining synthetic mixtures.

Compounds 1, 9, 18, 38-40, 42, 43 and 45-48 were purified from commercially available samples. The following compounds were prepared by literature proceedures; 10,[128] 31,[129] 32,[130] 33,[131] 34,[132] 35,[113] 36,[133] 37,[134] and 44.[135] Compounds 11-14, 16, 17, 19, 20, 27 and 28 were prepared by bromination of the corresponding phenol by the following general procedure. To the phenol (0.01 mole) and sodium acetate (0.015 mole) dissolved in cold glacial acetic acid (50 ml.) was added bromine (0.02 mole)as a 50% w/v solution in glacial acetic acid. After 10 minutes the reaction mixture was poured into cold water (250 ml.) and the product, if a solid, collected by filtration, washed with water, dried and crystallised from acetic acid. Where the product was liquid, it was extracted from the aqueous suspension with ether. After washing the ethereal extract with saturated sodium carbonate solution and water, it was dried over sodium sulphate. On removal of the ether, the product was distilled under reduced pressure.

Compounds 8, 29 and 30 were prepared as above, but employing only one mole of bromine.

Compounds 2-7 were prepared according to the method described for compound 6 by Dains and Rothrock.[136] Gas - liquid chromatographic examination of the products so obtained showed that compounds 3 and 5 were contaminated with starting material. They were, therefore, further

purified by preparative gas – liquid chrcmatography. Compounds 5 and 7 have not been previously described.

Compound 5, 2-bromo-4-s-butylphenol, was obtained as a colourless liquid, b. p. 76.5° at 0.8 mm. (Found: C, 52.4; H, 5.4. $C_{10}H_{13}BrO$ requires C, 52.4; H, 5.7%).

Compound 7, 2-bromo-4-cyclohexylphenol, separated as needles, m. p. 35-36° from glacial acetic acid. (Found: C, 56.6; H, 5.6. $C_{12}H_{15}BrO$ requires C, 56.3; H, 5.9%).

The remaining compounds were prepared as described below. Compounds 15, 22 and 24-26 have not been described previously.

Compound 15, 2,4-dibromo-6-t-butylphenol. p-Bromophenol was converted into 4-bromo-2-t-butylphenol by the method of Hart.[135] Treatment of the product with one mole of bromine in acetic acid gave 2,4-dibromo-6-t-butylphenol as needles from acetic acid, m. p. 54-55°. (Found: C, 39.3; H, 4.2. $C_{10}H_{12}Br_2O$ requires C, 39.0; H, 3.9%).

Compound 21, 2-bromo-6-methyl-4-nitrophenol. This compound has been previously described[137] but was conveniently prepared by the following alternative procedure. To o-cresol (8.8 g.) in glacial acetic acid (10 ml.) was added dropwise with cooling a 10% w/v solution of nitric acid in acetic acid (51.3 ml.). After pouring the mixture into cold water (250 ml.), the nitration product was extracted with ether and heated at 100° under reduced pressure to remove

o-nitrocresol. The residue on extraction with boiling water yielded needles of 2-methyl-4-nitrophenol (2.0 g.), m. p. 83-85°; (lit.,[138] m. p. 83-85°). Bromination with one mole of bromine in acetic acid in the presence of sodium acetate gave 2-bromo-6-methyl-4-nitrophenol as needles m. p. 121-121.5° from acetic acid; (lit.,[137] m. p. 120°).

Compound 22, 2-bromo-6-ethyl-4-nitrophenol. o-Ethylphenol was nitrated as described for o-cresol and the ethereal extract of the product washed with sodium carbonate solution followed by water. After removal of the solvent, the residual gum was extracted with hot light petroleum and the residue crystallised from formic acid giving needles of 2-ethyl-4-nitrophenol, m. p. 84-85°; (lit.,[139] m. p. 79-80°). Bromination with one mole of bromine in acetic acid gave 2-bromo-6-ethyl-4-nitrophenol as light yellow rhombs m. p. 101.5-102° from acetic acid. (Found: C, 39.0; H, 3.0. $C_8H_8BrNO_3$ requires C, 39.0; H, 3.3%).

Compound 23, 2-bromo-6-isopropyl-4-nitrophenol. This compound, although previously described,[140] was prepared by an alternative procedure. o-Isopropylphenol was nitrated by the general procedure and the oily product crystallised from light petroleum to give 2-isopropyl-4-nitrophenol as needles m. p. 86.5-87.5°; (lit.,[140] m. p. 86°). Bromination in acetic acid yielded 2-bromo-6-isopropyl-4-nitrophenol, m. p. 93°; (lit.,[140] m. p. 87-88°).

Compound 24, 2-bromo-6-s-butyl-4-nitrophenol. Mononitration of o-s-butylphenol furnished 2-s-butyl-4-nitrophenol as needles, m. p. 87-88° from acetic acid followed by recrystallisation from benzene - light petroleum. (Found: C, 61.9; H, 6.3. $C_{10}H_{13}NO_3$ requires C, 61.6; H, 6.7%). Bromination by the general procedure gave 2-bromo-6-s-butyl-4-nitrophenol as needles m. p. 91° from benzene - light petroleum. (Found: C, 43.6; H, 4.2. $C_{10}H_{12}BrNO_2$ requires C, 43.8; H, 4.4%).

Compound 25, 2-bromo-6-t-butyl-4-nitrophenol. Mononitration of of o-t-butylphenol gave the 4-nitro derivative, m. p. 143-144.5° from benzene - light petroleum; (lit.,[141] m. p. 138.5-139.5°). Monobromination of this compound in acetic acid gave 2-bromo-6-t-butyl-4-nitrophenol as needles, m. p. 81-81.5° from benzene - light petroleum; (Found: C, 43.8; H, 4.3. $C_{10}H_{12}BrNO_3$ requires C, 43.8; H, 4.4%).

Compound 26, 2-bromo-6-cyclohexyl-4-nitrophenol. Mononitration of o-cyclohexylphenol furnished 2-cyclohexyl-4-nitrophenol as needles, m. p. 158-159° from benzene; (Found; C, 65.3; H, 6.6. $C_{12}H_{15}NO_3$ requires C, 65.2; H, 6.6%). Bromination by the general procedure gave 2-bromo-6-cyclohexyl-4-nitrophenol as needles m. p. 155° from benzene - light petroleum; (Found: C, 48.2; H, 4.3. $C_{12}H_{14}BrNO_3$ requires C, 48.0; H, 4.7%).

Compound 42, 2-fluoro-4-nitrophenol. To o-fluorophenol (1.0 g.) in acetic acid (1.5 ml.) was added water (5 ml.) and to the resulting suspension was added sodium nitrite (0.6 g.) at -5° with swirling. After 10 minutes a further 0.15 g. of sodium nitrite was added and swirling continued for 20 minutes. After 12 hours the dark red precipitate was collected and a quantity (0.47 g.) was suspended in methylene dichloride.(20 ml.). Trifluoroacetic acid (5 ml.) and hydrogen peroxide (2 ml.; 90%) were added and the solution boiled for one hour. Removal of solvent followed by crystallisation from water gave 2-fluoro-4-nitrophenol as light yellow needles which after sublimation had m. p. 117-118.5°; (lit.,[142] 117-118°).

References.

1. Davies, Trans. Faraday Soc., 1940, 36, 333; Mecke and Rossmy, Z. Electrochem., 1955, 59, 866; Keussler and Rossmy, ibid., 1956, 60, 136.
2. Rossmy, Luttke and Mecke, J. Chem. Phys., 1953, 21, 1606.
3. Baker, J. Amer. Chem. Soc., 1958, 80, 3598.
4. Hoyer, Chem. Ber., 1956, 89, 146.
5. Baker and Kaeding, J. Amer. Chem. Soc., 1959, 81, 5904.
6. See inter alia; Wulf, Jones and Deming, J. Chem. Phys., 1940, 8, 753; Errera, Bull. Soc. ind. Mulhouse, 1937, 103, 177; Barchewitz and Freymann, Compt. rend., 1937, 204, 1729; Badger and Bauer, J. Chem. Phys., 1936, 4, 711.
7. Mecke, Z. Electrochem., 1944, 50, 57.
8. Pauling, J. Amer. Chem. Soc., 1936, 58, 94.
9. Sidgwick and Callow, J. Chem. Soc., 1924, 125, 527.
10. Lamson, Stoughton and Bass, J. Pharmacol., 1936, 56, 50, 60, 63.
11. Cooper and Woodhouse, Biochem. J., 1923, 17, 600.
12. Kuroda, Arch. exp. Path. Pharmak., 1926, 112, 60.
13. Klarmann, Shternov and Gates, J. Amer. Chem. Soc., 1933, 55, 2576; Klarmann, Gates and Shternov, ibid., p.4657; Klarmann, Shternov, Gates and Bloomfield, J. Lab. Clin. Med., 1934, 19, 835; Klarmann, Shternov and Gates, ibid., 1934, 20, 40.

14. Lockemann and Heiken, Zentr. Bakt., 1939, 145, 61; Tetumoto, J. Agric. Chem. Soc. Japan, 1940, Bull.16, 182.
15. Wolf and Westveer, Arch. Biochem. Biophys., 1952, 40, 306.
16. Mason, Brown and Minga, Phytopathology, 1951, 41, 164; Shirk, Corey and Poelma, Arch. Biochim. Biophys., 1951, 32, 392; Shirk and Corey, ibid., 1952, 38, 417.
17. Gersdorf and Smith, Amer. J. Pharm., 1940, 112, 197.
18. Bechold and Ehrlich, Z. physiol. Chem., 1906, 47, 33.
19. Blicke and Stockhaus, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1933, 22, 1090.
20. Pyman, Chimie et Industrie, 1941, 45, 280.
21. Ferguson, Proc. Roy. Soc., 1937, B127, 387.
22. Heiken, Angew. Chem., 1939, 52, 263.
23. Tiwari, Mukerji and Tripathi, J. Indian Chem. Soc., 1958, 35, 110.
24. Woodward, Kingery and Williams, J. Lab. Clin. Med., 1934, 19, 1216.
25. Schultz, M.S. Thesis, Northwestern University, 1939.
26. Suter, Lawson and Smith, J. Amer. Chem. Soc., 1939, 61, 161.
27. Dunker, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1959, 39, 437.
28. See for example:- Hugo, J. Pharm. Pharmacol.,1957, 9, 145; Bennett, Adv. Appl. Microbiol., 1959, 1, 123.
29. Cooper, Biochem. J.,1913, 7, 175.

30. Tetumoto, J. Agric. Chem. Soc. Japan, 1940, Bull.16, 189.

31. Roberts and Rahn, J. Bact., 1946, 52, 639.

32. Gale and Taylor, J. Gen. Microbiol., 1947, 1, 77.

33. Fogg and Lodge, Trans. Faraday Soc., 1945, 41, 359.

34. Hess and Speiser, Experentia, 1958, 14, 362.

35. Allawala and Riegelmann, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1954, 43, 93.

36. Ordal, Wilson and Borg, J. Bact., 1941, 42, 117; Ordal and Deromedi, ibid., 1943, 45, 293.

37. Alexander and Trim, Proc. Roy. Soc., 1946, B133, 220.

38. Ordal, Proc. Soc. Exp. Biol. Med., 1941, 47, 387.

39. Reichel, Deut. med. Woch., 1947, 72, 551.

40. Engelhardt, Biochem. Z., 1927, 190, 217.

41. Cooper, J. Soc. Chem. Ind., 1947, 66, 48.

42. Cooper and Haines, J. Hyg., 1928, 28,163.

43. Klarmann, Shternov and Von Wowern, J. Bact., 1929, 17, 423.

44. Evans and Fishburn, Chemist and Druggist, 1943, 140, 126.

45. McGowan, J. Soc. Chem. Ind., 1949, 68, 253.

46. Suter, Chem. Rev., 1941 28, 269.

47. Shirk, Poelma and Corey, Arch. Biochim. Biophys., 1951, 32, 386.

48. Chien, Chung and Tai, J. Chinese Chem. Soc., 1936, 4, 361; Chien and Yin, ibid., 1939, 7, 40.

48a. cf. Beckett, Patki and Robinson, J. Pharm. Pharmacol., 1959, 11, 421; and references therein.

49. Meyer and Hemmi, Biochem. Z., 1935, 277, 39.

50. Pauling, J. Chim. phys., 1962, 59, 1.

51. Wulf and Featherstone, Anesthesiology, 1957, 18, 97.

52. Albert, "Selective Toxicity," Methuen and Co. Ltd., London, 1st. Edition, 1951, p. 28; Ing, Farmaco, 1959, 14, 612.

53. Beckett and Casy, J. Pharm. Pharmacol., 1955, 7, 433; Beckett in "Progress in Drug Research," ed. E. Jucker, Birkhauser Verlag, Basel, 1959, Vol. I, p. 455.

54. Mullins, Chem. Rev., 1954, 54, 289.

55. Lamb and Harris, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1960, 49, 583.

56. Albert, Rubbo and Burvill, Brit. J. Exp. Path., 1949, 30, 159.

57. Veldstra, Ann. Rev. Plant Physiol., 1953, 4, 151.

58. Armstrong, Bradbury and Standen, Ann. Appl. Biol., 1951, 38, 555.

59. Veldstra and van de Westeringh, Rec. Trav. chim., 1951, 70, 1127; Haagen-Smit and Went, Proc. k. ned. Akad. Wetenschap., 1935, 38, 852.

60. Bessman and Doorenbos, Ann. Intern. Med., 1957, 47, 1036; Chenoweth, Pharmacol. Rev., 1956, 8, 57; Eichhorn, "Coordination Compounds in Natural Products," in "The Chemistry of Coordination Compounds," ed. J. C. Bailar, Reinhold Publ. Cor., New York, 1956.

61. Page and Robinson, Brit. J. Exp. Path., 1943, 24, 89.

62. Simon, Nature, 1950, 166, 343.

63. Brodie and Hogben, J. Pharm. Pharmacol., 1957, 9, 345.

64. Rall, Stabenau and Zubrod, J. Pharmacol., 1959, 125, 185.

65. Krahl and Clowes, J. Cell. Comp. Physiol., 1938, 11, 1, 21.

66. Ing in Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, 1953, Vol. III, p. 392.

67. Hoffman, Scweitzer and Dalby, J. Amer. Chem. Soc., 1940, 62, 988; Ind. Eng. Chem., 1941, 33, 749.

68. Bell and Roblin, J. Amer. Chem. Soc., 1942, 64, 2905; Krebs and Speakman, Brit. Med. J., 1946, 1, 50.

69. Shapiro, J. Theoretical Biol., 1961, 1, 289.

70. Trevan and Boock, Brit. J. Exp. Path., 1927, 8, 307; Krahl, Keltch and Clowes, J. Pharmacol., 1940, 68, 330; Skou, Acta pharmacol. toxicol., 1954, 10, 281

71. Kalow, J. Pharmacol., 1954, 110, 433.

72. Crane, J. Pharmacol., 1921, 18, 319.

73. Orloff and Berliner, J. Clin. Invest., 1956, 35, 223.

74. Lacassagne, Buu-Hoi, Daudel and Zajdela, Advances in Cancer Research, 1956, 4, 315.

75. Badger, Brit. J. Cancer, 1948, 2, 309; Coulson, Advances in Cancer Research, 1953, 1, 1; Pullman and Pullman, ibid., 1955, 3, 117.

76. Miller and Miller, Advances in Cancer Research, 1953, 1, 339; Badger, ibid., 1954, 2, 73.

77. Rideal, Trans. Faraday Soc., 1943, 39, 368; Davies, Chem. and Ind., 1953, 614.

78. Pimentel and McLellan, "The Hydrogen Bond," W. H. Freeman & Co., San Francisco, 1960, pp. 296 et seq.

79. Gero and Reese, Science, 1956, 123, 100.

80. Brownlee, Goss, Goodwin, Woodbine and Walls, Brit. J. Pharmacol., 1950, 5, 261.

81. Paulini, Rev. brasil. malariol. e doencas trop. Publ. avulsas, 1956, No. 7; Chem. Abs., 1958, 52, 5728g.

82. Rose and Spinks, Brit J. Pharmacol., 1947, 2, 65; Gorvin, J. Chem. Soc., 1949, 3304.

83. Haworth, Rose and Slinger, J. Chem. Soc., 1947, 820.

84. Belitser and Tsiperovitch, Ukrain. biokhim. Zhur., 1948, 20, 322; Chem. Abs., 1954, 48, 5895f.

85. Gero and Withrow, Nature, 1957, 180, 1354.

86. Pauling, Endeavour, 1948, 7, 43; Nisonoff and Pressman, J. Amer. Chem. Soc., 1957, 79, 5565.

87. Donnelly, Laskowski, Notley and Sheraga, Arch. Biochem. Biophys., 1955, 56, 369.

88. Allison, Biochem. J., 1957, 65, 212.

89. Singh and Singh, Proc. Indian Acad. Sci., 1954, 40B, 145; Morales and Botts, " Currents in Biochemical Research, 1956," Interscience Publishers Inc., New York, 1956; Saroff, Arch. Biochem. Biophys., 1957, 71, 194.

90. Bogaty, J. Soc. Cosmetic Chemists, 1960, 11, 333.

91. Sadler, Chem. Rev., 1960, 60, 575.

92. Ann. N. Y. Acad. Sci., 1957, 69, 1-254.

93. Wright and Serenius, J. Appl. Chem., 1954, 4, 615.

94. Fellman and Fujita, Biochem. Biophys. Acta, 1962, 56, 227.

95. Soldatova and Voroshin, Trudy Khar'kov. Farm. Inst., 1959, 1957 No. 1, 85; Chem. Abs., 1960, 54, 22838d.

96. Brooks, Eglinton and Morman, J. Chem. Soc., 1961, 661.

97. Reid in "Proceedings of the Symposium on Drugs Affecting Lipid Metabolism," editors Garattini and Paoletti, Elsevier, Amsterdam, 1961, p. 423; Andrews, Brit. J. Pharmacol., 1958, 13, 419; Biochem. J., 1960, 75, 298.

98. Davies, Trans. Faraday Soc., 1938, 34, 1427; Finch and Lippincott, J. Phys. Chem., 1957, 61, 894.

99. Zumwalt and Badger, J. Amer. Chem. Soc., 1940, 62, 305.

100. Jones and Watkinson, Chem. and Ind., 1960, 661.

101. Puttnam, J. Chem. Soc., 1960, 5100.

102. Goddu, Analyt. Chem., 1958, 30, 2009; J. Amer. Chem. Soc., 1960, 82, 4533.

103. Bellamy and Williams, Proc. Roy. Soc., 1960, A254, 119.

104. Ingraham, Corse, Bailey and Stitt, J. Amer. Chem. Soc., 1952, 74, 2297.

105. Szmant and Suld, J. Amer. Chem. Soc., 1956, 78, 3400.

106. See e. g. Stone and Thompson, Spectrochim. Acta, 1957, 10, 17; Flett, ibid., p. 21.

107. Oki and Hirota, Spectrochim. Acta, 1961, 17, 583.

108. Thompson and Steel, Trans. Faraday Soc., 1956, 52, 1451; Krueger and Thompson, Proc. Roy. Soc., 1957, A243, 143; Thompson, Needham and Jameson, Spectrochim. Acta, 1957, 9, 208; Baker and Shulgin, J. Amer. Chem. Soc., 1959, 81, 1523.

109. Bellamy, Eglinton and Morman, J. Chem. Soc., 1961, 4762.

110. cf. Ingold and Taylor, Canad. J. Chem., 1961, 39, 471.

111. cf. Baker and Shulgin, J. Amer. Chem. Soc., 1958, 80, 5358.

112. Beckering, J. Phys. Chem., 1961, 65, 206.

113. Martin-Smith and Reid, J. Chem. Soc., 1960, 938.

114. Ingold and Taylor, Canad. J. Chem., 1959, 39, 220.

115. Bellamy and Hallam, Trans. Faraday Soc., 1959, 55, 220.

116. Whetsel and Kagarise, Spectrochim. Acta, 1962, 18, 315, 329, 341.

117. G. Eglinton, personal communication.

118. Brooks and Morman, J. Chem. Soc., 1961, 3372.

119. J. G. Watkinson, personal communication to G. Eglinton.

120. Thompson, Nature, 1946, 158, 234; Richards and Thompson, J. Chem. Soc., 1947, 1248; Hartwell, Richards and Thompson, ibid., 1948, 1436; Richards

and Thompson, Proc. Roy. Soc., 1948, A195, 1; Halford and Schaeffer, J. Chem. Phys., 1946, 14, 141; Carpenter and Halford, ibid., 1947, 15, 99.

121. Pimentel and McLellan, "The Hydrogen Bond," W. H. Freeman & Co., San Fransisco, 1960.

122. Sears and Kitchen, J. Amer. Chem. Soc., 1949, 71, 4110.

123. Coggeshall and Saier, J. Amer. Chem. Soc., 1951, 73, 5414.

124. Reference 121, pp. 255-295.

125. Kendall, Analyt. Chem., 1953, 25, 382; Ebert and Gottlieb, J. Amer. Chem. Soc., 1952, 74, 2806.

126. Barnes, Gore, Williams, Linsley and Petersen, Analyt. Chem., 1947, 19, 620.

127. Badger and Bauer, J. Chem. Phys., 1937, 5, 839.

128. Van Erp, Rec. Trav. chim., 1910, 29, 187.

129. Schroeter, Annalen, 1922, 426, 83.

130. Smith, J. Chem. Soc., 1879, 35, 789.

131. Clemo and Spence, J. Chem. Soc., 1928, 2811.

132. Fries, Heering, Hemmecke and Siebert, Annalen, 1936, 527, 83.

133. Bordwell and Stange, J. Amer. Chem. Soc., 1955, 77, 5939.

134. Gilman and Van Ess, J. Amer. Chem. Soc., 1939, 61, 1365.

135. Hart, J. Amer. Chem. Soc., 1949, 71, 1966.

136. Dains and Rothrock, Amer. Chem. J. 1895, 17, 113; Beilstein, VI, 525.

137. Robertson, J. Chem. Soc., 1908, 93, 788.

138. Spiegel, Munblit and Kaufmann, Ber., 1906, 39, 3240.

139. Jones and Kenner, J. Chem. Soc., 1931, 1842.

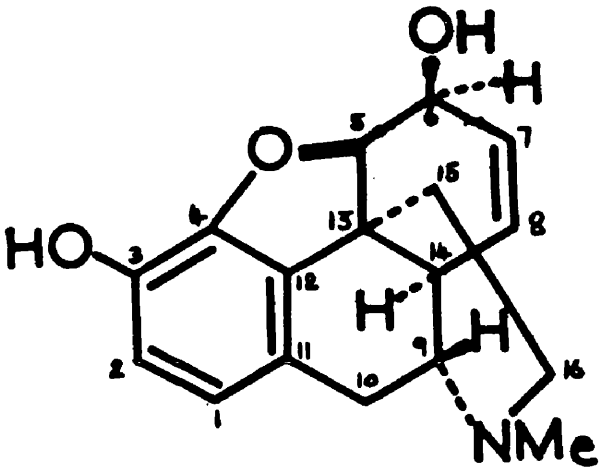
140. Fileti, Gazzetta, 1886, 16, 113.

141. Ipatieff, Pines and Friedmann, J. Amer. Chem. Soc., 1938, 60, 2495.

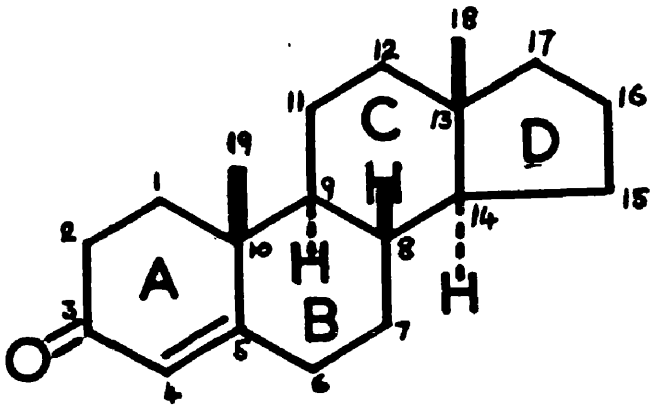
142. Berg and Newbery, J. Chem. Soc., 1949, 642.

PART II.

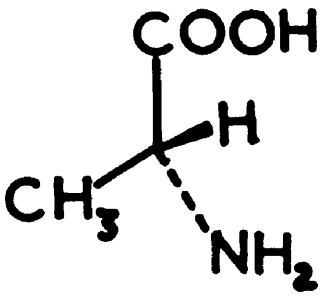
ATTEMPTED EPIMERISATION at $C_{(14)}$ in CODEINONE.


OH
H
O
HO
H
H
NMe

I


18
17
12
16
11
C
13
D
19
1
9
15
2
14
10
8
H
H
A
B
H
3
O
5
7
4
6

II

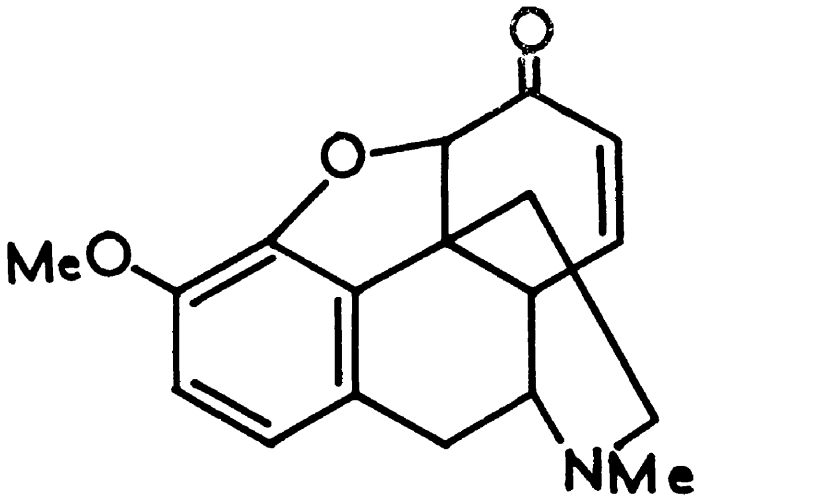

COOH
H
CH3
NH2

III

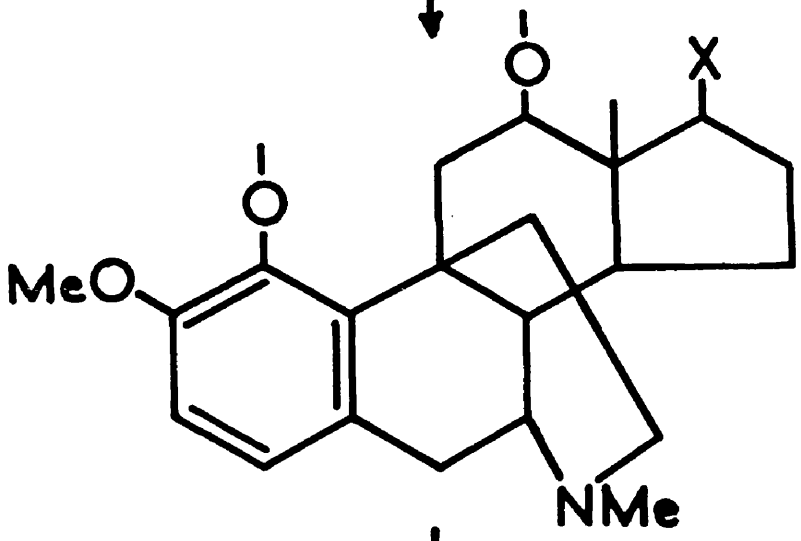
Introduction.

Two groups of compounds of particular interest to the medicinal chemist are characterised by carbon skeletons containing hydrophenanthrene ring systems, namely the morphine alkaloids and the steroids. In view of the many attempts to produce morphine-like analgesics free from addicting properties by modification of the morphine molecule (I)[1] on the one hand, and to increase the potency, improve the specificity or accentuate a secondary biological characteristic of the naturally-occurring steroid hormones by chemical changes in the steroid molecule (II)[2] on the other, the conversion of morphine-like structures into steroid-like structures offered an interesting project which might also be expected to produce many chemically and biologically interesting intermediate compounds.

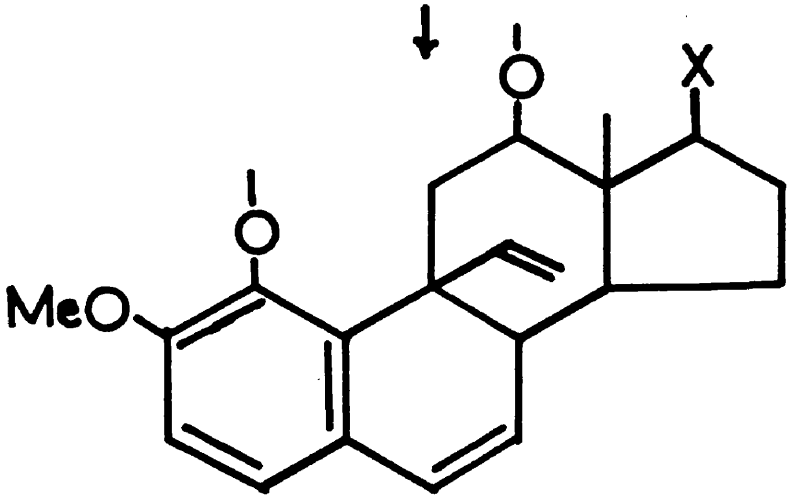
One of the most fruitful of the great number of structural modifications which have been performed in the steroid series has been the substitution of a halogen atom in the 9α-position of 11-oxygenated steroids.[3] This particular substitution has produced the most rewarding results where the halogen is fluorine,[4] yielding drugs possessing both increased potency and increased specificity. Thus several members of this class show high anabolic and androgenic activity[5] while the anti-inflammatory,[6,8] and



IV



V



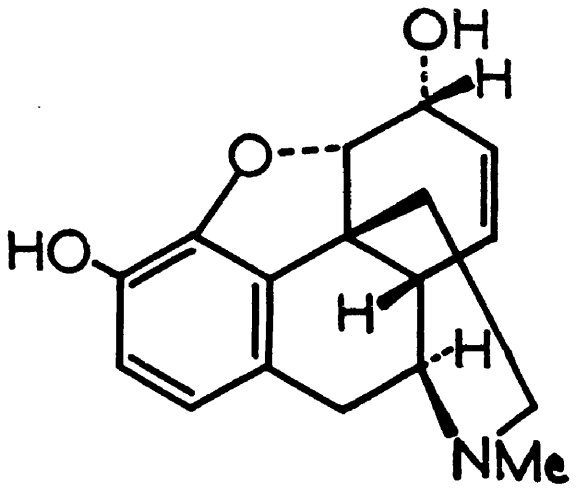
VI

scheme x

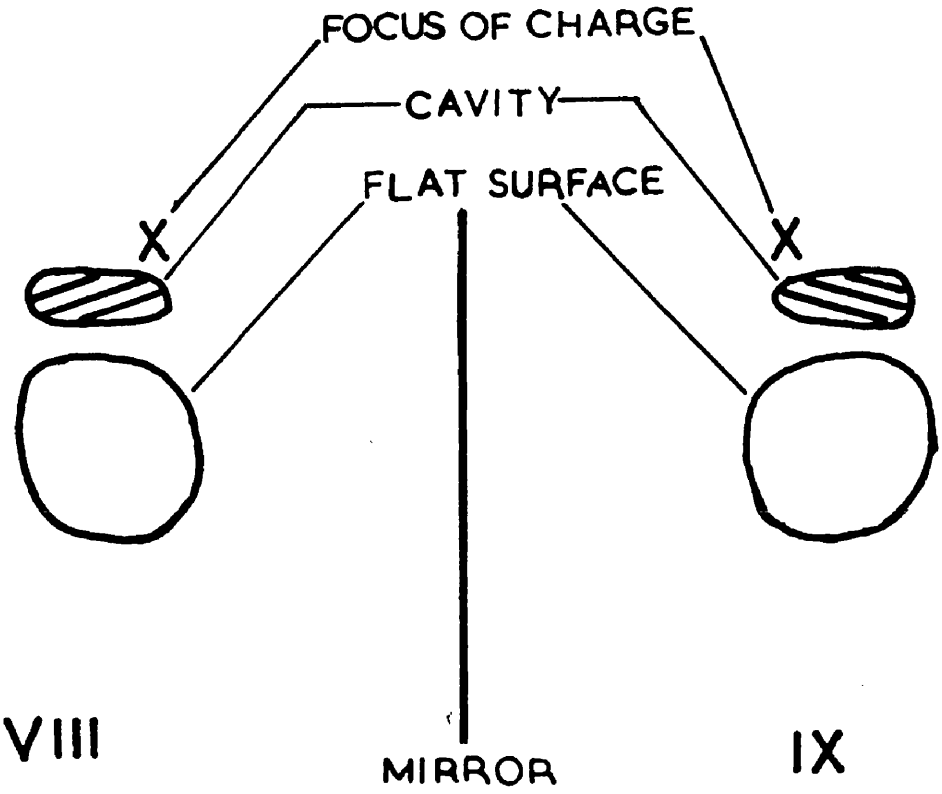
glucocorticoid[7,8] activities of a number of others are far in excess of those of hydrocortisone. At the same time, the mineralocorticoid effects of certain of these fluoro compounds are greatly superior to those of deoxycorticosterone.[7,9] The introduction of substituents other than fluorine at $C_{(9)}$, particularly electron-withdrawing groups,[3] would thus appear to offer interesting possibilities.

As can be seen from the hypothetical scheme ☪, which does not portray the stereochemistry involved, the conversion of codeinone (IV) into a derivative such as V would produce, after two successive Hofmann eliminations,[10] or thermal decomposition of the methine N-oxide after one Hofmann elimination,[11] new steroid types such as VI. In addition to possessing oxygen functions capable of being utilised as centres for further reactions and a 19-nor structure [12] (known to intensify progestational,[13] anabolic[14] and mineralocorticoid[15] properties), these derivatives would also retain a vinyl group at $C_{(9)}$ (steroid numbering) capable of conversion into various electron-withdrawing functions such as carboxylic acid, aldehyde etc.

Such a conversion as shown in scheme ☪ would not only be of biological interest, but also of considerable stereochemical significance. It is now well established that the absolute stereochemistry of natural (–)-morphine is that shown in I (both from chemical degradations[16] and from



VII



VIII

IX

X-ray crystallographic studies on morphine hydriodide dihydrate[17] and codeine hydrobromide[18]), although at one time evidence was advanced in favour of the mirror image (VII).[19] In adducing his pictorial model of the hypothetical morphine receptor site before the absolute stereochemistry of morphine was established with certainty, Beckett[20] arbitrarily assigned to it this mirror image configuration (VII). The receptor site, in terms of Beckett's hypothesis on which he based his deductions concerning other optically active analgesics, should thus be portrayed as VIII and not as IX.

Morphine, having the absolute configuration portrayed in I, is thus related to D-(−)-alanine (III) as indeed are (−)-N,N-dimethyl-1,2-diphenylethylamine[21] and the more analgesically active enantiomers of the methadone[22]* and thiambutene[23] types. Several examples are, however, known where the more analgesically active enantiomers are actually related to L-(+)-alanine.[24] Pethidine itself is, of course, optically inactive due to a plane of symmetry in the molecule and for optically active pethidine derivatives absolute configurations have not as yet been conclusively proven.[20] Unfortunately, Beckett has advanced arguments as to why the analgesically active antipodes in the methadone and thiambutene series (in their correct absolute stereochemistry) should give a better fit to IX than to VIII, and if his arguments are valid they afford disproof of, not support for,

* Configuration of (−)-isomethadone - Beckett, Kirk and Thomas, J. Chem. Soc., 1962, 1386.

his picture of the receptor since morphine cannot fit IX. On the other hand, it is possible that Beckett's arguments are invalid in which case the receptor could be something like that portrayed in VIII. That Beckett's arguments in support of the more analgesically active enantiomers of the methadone and thiambutene series could be false follows from the fact that these molecules, unlike morphine, are non-rigid and it was found necessary to invoke intramolecular interactions between the nitrogen atom and the carbonyl group in the case of the methadones[25] and between the nitrogen and sulphur atoms in the case of the thiambutenes[23] to ensure an appropriate differentiation of fit to IX between the D and L isomers. There is no evidence that such interactions are indeed playing a role at the receptor site. It is of some interest that relative configurations of certain analgesics have been determined by the method of stereoselective adsorbents.[26]

Dextrarotatory morphine (VII), the enantiomer of natural (−)-morphine, has been prepared both from sinomenine[27] and from (+)-dihydrothebainone,[28] and has been shown to be devoid of analgesic activity[29,30] although it exhibits a significant antitussive action.[30] In fact, (+)-morphine antagonises the analgesic activity of its optical antipode.[30]

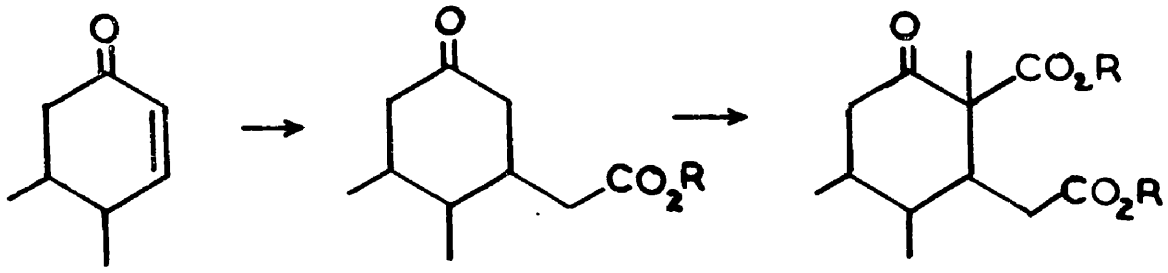
Interest has been maintained in the absolute stereochemistry of the morphine alkaloids for reasons other than

those connected with attempts to picture the analgesic receptor site. In a paper delivered at the Anniversary Meeting of the Chemical Society at Sheffield on April 4th., 1962, Professor A. R. Battersby stated that both radio-labelled (+)- and (−)-tetrahydropapaverine are incorporated into the morphine molecule in <u>in vivo</u> feeding experiments, and moreover that (−)-tetrahydropapaverine, which possesses the absolute configuration corresponding to VII and not to I,[31] is taken up to much the greater extent. In view of Professor Battersby's wish to repeat the feeding experiments, however, these observations have not been reported in print.[32] Unambiguous conversion of the morphine ring system into that of a known steroid possessing a rigidly established absolute stereochemistry might therefore be expected to throw some light on this apparent contradiction.

Both morphine[33] and codeine[34] are known to be N-demethylated <u>in vivo</u>, and normorphine has been shown to be a potent analgesic.[35] It has been postulated that such dealkylation represents an essential step in the onset of analgesia[36] although this hypothesis has not found favour elsewhere[37] on the basis that such N-demethylation has not been demonstrated to occur in the central nervous system, and that normorphine is if anything less potent than morphine. Development of tolerance to morphine has, however, been shown to accompany a reduction in activity of the N-demethylating

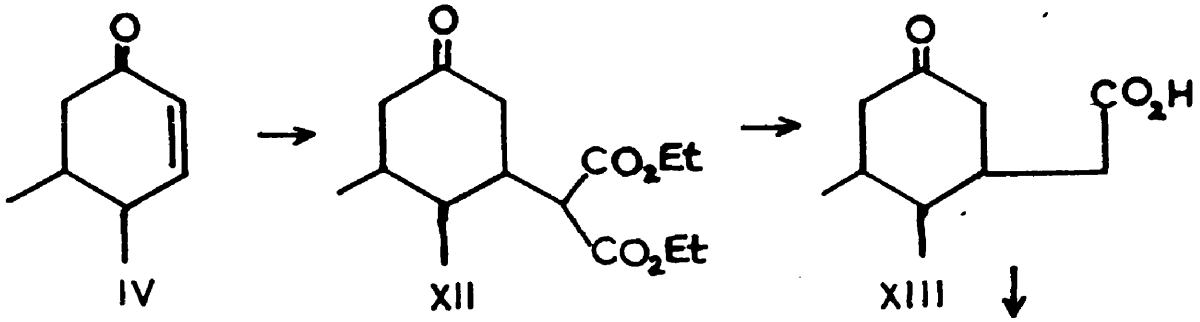
enzyme in the liver[38] although it has been suggested [39] that the similarity of receptors for narcotic and demethylating action is not as great as had been previously supposed. This does not necessarily invalidate the dealkylation hypothesis since stereospecificity may only be required after demethylation has taken place. There is, however, no reason to assume that if demethylation does occur in the central nervous system that it does so in the same fashion as in the liver.[39]

The steroid hormones having the absolute configuration shown in II,[40] it is apparent that the hydrogen atom at $C_{(14)}$ in morphine must be inverted in order to obtain the correct stereochemistry at $C_{(8)}$ in the steroid nucleus if a direct conversion from one series to the other is to be achieved. The stereochemistry present at $C_{(14)}$ in the naturally occurring morphine alkaloids (which possess the morphinan skeleton) is unfortunately more stable than the alternative B/C <u>trans</u> configuration (isomorphinan series), a fact used to advantage in going from the isomorphinan to the morphinan series in the first total synthesis of morphine.[41] Nevertheless the morphinan ⟶ isomorphinan conversion has been successfully carried out in the preparation of β-thebainone[42] and racemic β-Δ^6-dihydrodesoxycodeine methyl ether[43] and in the synthesis and resolution of 3-hydroxy-N-methylisomorphinan and its Δ^6-dehydro-derivative.[44]

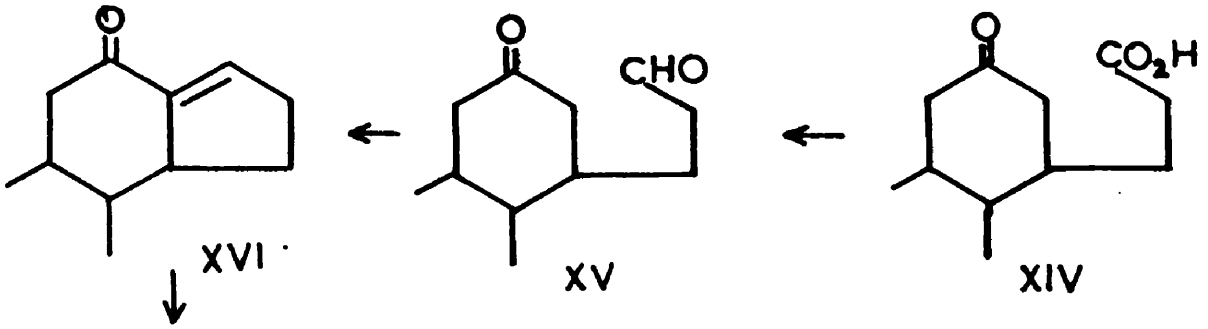


IV X XI

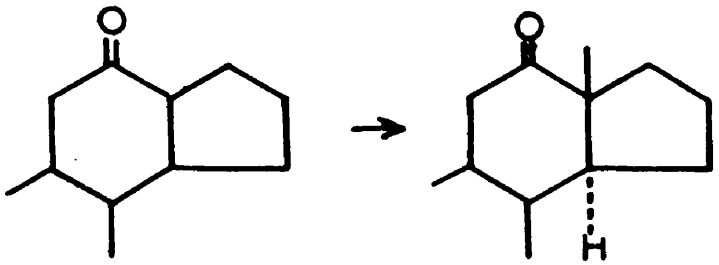
scheme y



IV XII XIII

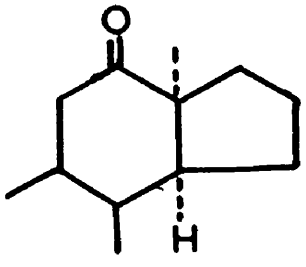


XVI XV XIV



XVII a XVIII

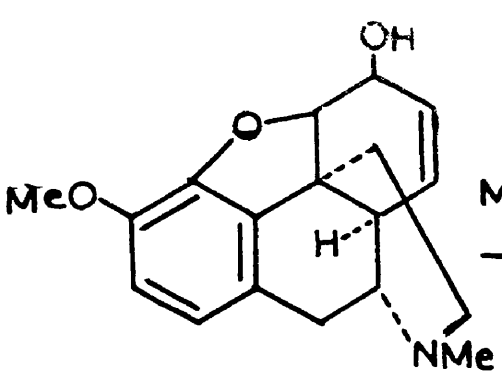
scheme z



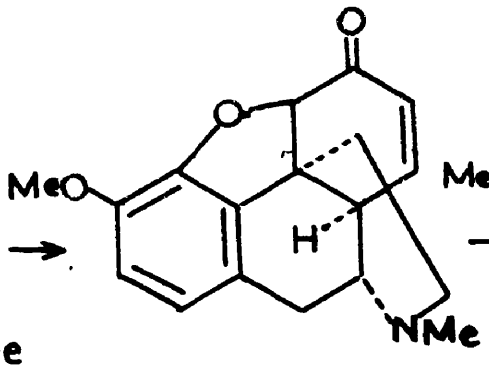
b

Assuming the epimerisation at $C_{(14)}$ to be successful, several methods can be envisaged for the elaboration of ring D. All would start with a Michael 1:4 addition to the α,β-unsaturated ketone IV, the conditions required by this reaction on the codeinone molecule having already been established.[45] Ring D could thus be added as illustrated in schemes *y* and *z*.

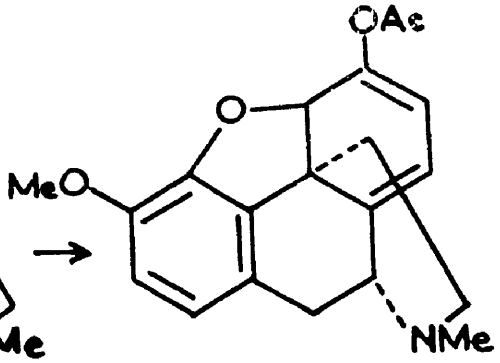
In scheme *y*, ring closure could be effected either as in Bachmann's syntheses of oestrone[46] and equilenin,[47] or by the acyloin condensation using lithium in liquid ammonia.[48] The latter method would, of course, also be expected to reduce the aromatic ring A, i.e. the Birch reduction,[49] which in itself could be advantageous. Scheme *z* would follow the steps indicated. In the event of ring closure followed by methylation at the C/D ring junction giving the unnatural <u>cis</u>-fused isomer XVIIIb, photochemical conversion to XVIIIa would be possible by analogy with previous work in the steroid field.[50] A method of stereochemical control of angular methylation of fused ring ketones has, however, been worked out.[51]



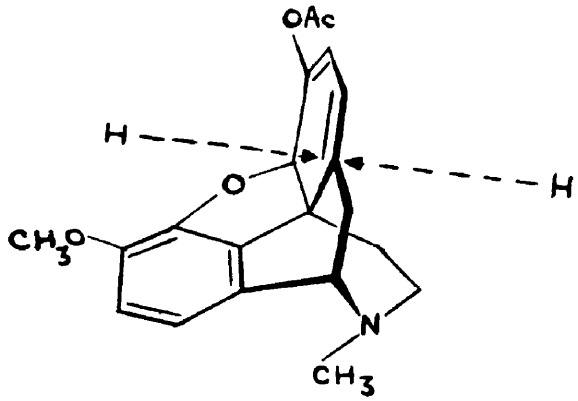
XIX



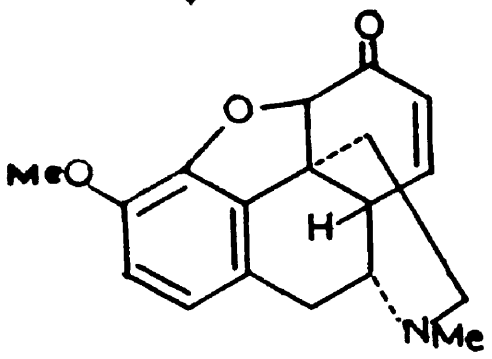
XX



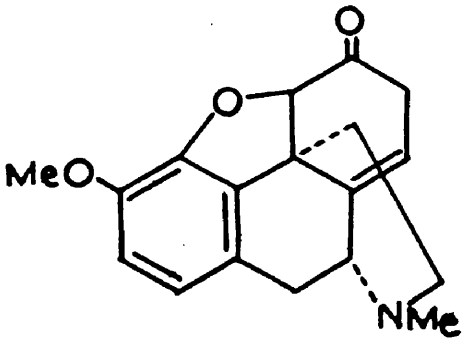
XXI



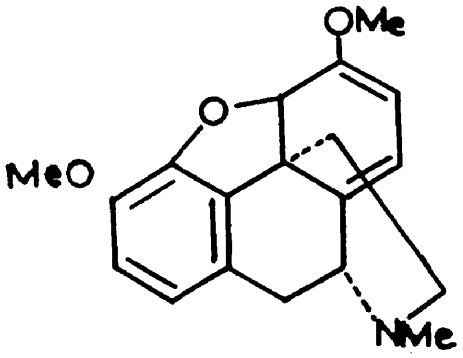
XXIII



XXII



XXIV

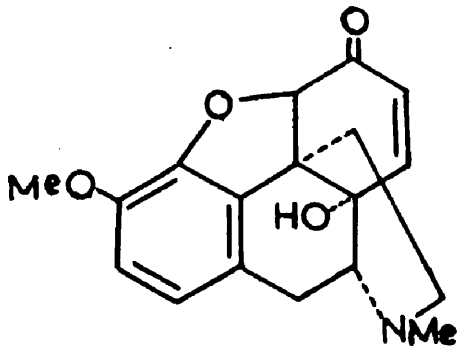


XXV

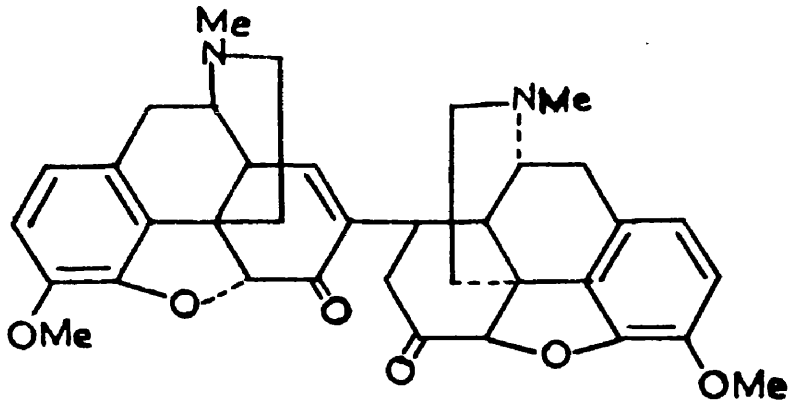
Discussion.

It has been explained in the Introduction that the crux of the projected synthesis was the epimerisation at $C_{(14)}$ in the morphine ring system. The compound actually employed in the investigation was codeine (XIX), the methyl ether of morphine, and it was hoped to carry out the inversion by the scheme XIX→XX→XXI→XXII, in which codeine is oxidised to codeinone (XX) and the removal of the 14α-hydrogen atom is effected by formation of the known enol acetate (XXI)[52] which contains an 8–14 double bond.

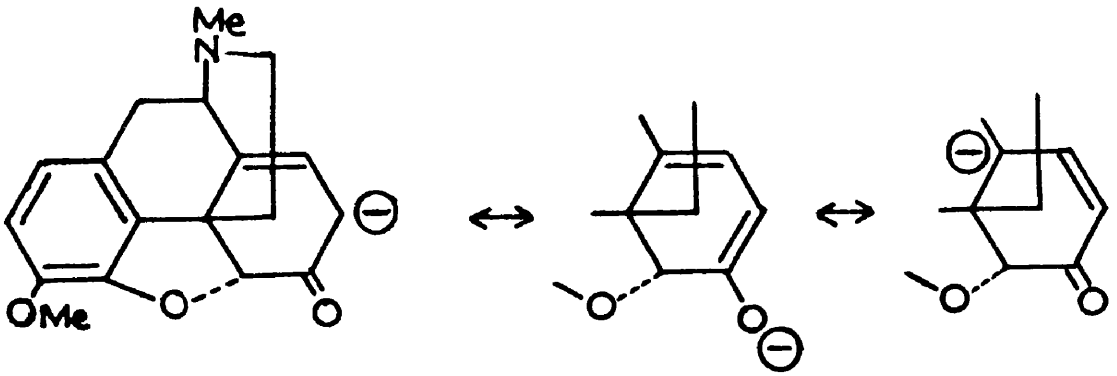
Examination of a model of codeinone enol acetate reveals that the $C_{(14)}$ carbon atom is probably even more accessible to the addition of a proton from above the plane than from below the plane of the molecule, as shown in XXIII. It might, therefore, be reasonably expected that mild hydrolysis of codeinone enol acetate (XXI) would yield both codeinone (XX) and the unknown 14-epicodeinone (XXII), the proton being added from below in the former case and from above in the latter. Although $C_{(14)}$ epimers have previously been prepared in the morphine series,[41-44] in only one case has this been achieved in a compound in which the 4–5 oxide bridge remains intact.[64] The isomerisation of neopinone (XXIV) with active charcoal, for example, produced only a 54% yield of codeinone,[53] and Bentley has suggested[54] that 14-epicodeinone (XXII) may well be produced in greater



XXVI



XXVII



XXVIII

quantities in the hydrolysis of thebaine (XXV) than is codeinone although it has not yet been isolated.

To test the feasibility of the reaction XXI→XXII, i.e. the formation of 14-epicodeinone, quantities of codeinone (XX) were required. The oxidation of codeine gives various products according to reagents and conditions,[45,55-57] and it was decided to utilise a method employing solid manganese dioxide in chloroform.[56] Use of a commercial grade of manganese dioxide[58] gave, in place of codeinone,[56] a 30% yield of 14-hydroxycodeinone (XXVI). This direct allylic oxidation has analogies in the conversion of cyclohexene into cyclohexenone,[59] of cholesteryl acetate into 7-hydroxy- and 7-oxo-cholesteryl acetates[59] and of vitamin A_1 into 3-hydroxy- and 3-oxo-retinene$_1$.[58] Use of "Attenburrow" manganese dioxide[60] was found to yield codeinone as previously reported.[56]

A sample of codeinone prepared by a different method was required for comparison purposes, and recourse was made to an oxidation proceedure using chromic oxide in acetic acid.[57] This gave, under certain conditions, not codeinone but the dimer XXVII known to result from the condensation of neopinone and codeinone and from solutions of the anion XXVIII, a resonance hybrid, which generates a mixture of neopinone and codeinone <u>in situ</u>.[53] The dimer was isolated only when an excess of base was employed in the

neutralisation of the acidic reaction mixture before isolation of the product. Since codeinone does not form a dimer on treatment with base, it may be that the codeinone produced exists in the reaction mixture as the dienol chromate ester which on treatment with base liberates the dienolate ion XVIIIb with consequent formation of the dimer.

Codeinone enol acetate (XXI) was prepared as previously described.[52] Mild alkaline hydrolysis with sodium carbonate yielded not codeinone or its 14-epimer but solely the dimer XXVII. The fact that the dimer arises from the anion XXVIII, which is a resonance hybrid incorporating a contribution XXVIIIb from the anion generated from codeinone enol acetate, provides a rational explanation of the dimer formation found in this case.

In view of the fact that simple epimerisation at $C_{(14)}$ was not readily achievable, coupled with the strong possibility that, even if successful, reversion of configuration at this centre would ensue on any base-catalysed condensation for the elaboration of steroidal ring D being carried out,[61] the project was discontinued.

These investigations on codeine have been reported in the Journal of the Chemical Society, a reprint being included as Appendix 2 to this thesis.

Experimental.

M. p.s were taken on a Kofler block.

14-Hydroxycodeinone (XXVI). Codeine (1 g.) in chloroform (100 ml.) was shaken at room temperature with manganese dioxide[58] for 24 hr. After filtration, the manganese dioxide was extracted several times with hot chloroform, and the residue (0.48 g.) from the combined filtrates was crystallised from ethyl acetate, giving material (0.3 g.) which on sublimation had m.p. and mixed m.p. 279.5–280.5° and $[\alpha]_D$ −116° (c 0.92 in 10% acetic acid) {lit.,[62,63] m.p. 275°, $[\alpha]_D$ −111° (in 10% acetic acid)}, having the correct infrared spectrum (KCl disc). The oxime had m.p. 278° (decomp.) (lit.,[62] 279–280°) (Found: C, 65.6; H, 5.9; N, 8.5. Calc. for $C_{18}H_{20}N_2O_4$: C, 65.9; H, 6.1; N, 8.5%).

Dimer (XXVII). (a) Codeine chromate (10 g.) was oxidised as previously reported[57] and the acid extract of the chilled ethereal solution (50 ml. of N-sulphuric acid) was poured into N-sodium hydroxide (165 ml.). The solution darkened and after removal of a small quantity of insoluble material (40 mg.) it was extracted with chloroform (25 ml.). Crystallisation of the oily residue therefrom yielded material (0.5 g.), m.p. 244° (from ethyl acetate), $[\alpha]_D$ −198.5° (c 0.9 in $CHCl_3$), $\lambda_{max.}$ 230, 281 mμ (ε 19,000, 3120) [lit.,[53] m.p. ca. 245° (decomp.), $\lambda_{max.}$ 230, 281 mμ (ε 18,100, 2860)] (Found: C, 72.4;

H, 6.4. Calc. for $C_{36}H_{38}O_6N_2$: C, 72.7; H, 6.4%).

(b) Codeinone enol acetate[52] (0.5 g.) in methanol (10 ml.) was treated with a saturated solution of sodium carbonate (8 ml.), ethanol (50 ml.) being added to keep the whole in solution. After 5 min. on the steam bath the solution was left for one hour at room temperature. Dilution with water followed by extraction of the red solution with chloroform gave a residue (0.47 g.) which, crystallised from ethyl acetate, had m.p. and mixed m.p. 244° (0.35 g.). The infrared spectra (KCl disc) confirmed the identity of the two specimens.

References.

1. Small, Eddy, Mosettig and Himmelsbach, "Studies on Drug Addiction," Suppl. 138 to the Public Health Reports, U. S. Govt. Printing Office, Washington, 1932; Beckett, J. Pharm. Pharmacol., 1952, 4, 425; Eddy, Halbach and Braenden, Bull. World Health Organisation, 1956, 14, 353; Leutner, Arzneimittel Forsch., 1960, 10, 505; Beckett and Casy in "Progress in Medicinal Chemistry, Vol. 2," ed. Ellis and West, Butterworth, London, 1962, pp. 43-87.

2. Fried and Bormann, Vitamins and Hormones, 1958, 16, 303; Ann. N. Y. Acad. Sci., 1959, 82, pp. 797-1014; Bayliss, Proc. Roy. Soc. Medicine, 1959, 52, 929.

3. Fried, Cancer, 1957, 10(4), 752; Pincus, Vitamins and Hormones, 1959, 17, 307; Zaffaroni, Acta Endocrinologica, 1960, 34 suppl. 50, 139.

4. Buu-Hoi in "Progress in Drug Research," ed. Jucker, Birkhauser, Basel, 1961, Vol. 3, p. 51.

5. Herr, Hogg and Levin, J. Amer. Chem. Soc., 1956, 78, 500.

6. Singer and Borman, Fed. Proc., 1955, 14, 281.

7. Hogg, Lincoln, Jackson and Schneider, J. Amer. Chem. Soc., 1955, 77, 6401.

8. Hirschmann, Miller, Beyler, Sarett and Tishler, J. Amer. Chem. Soc., 1955, 77, 3166.

9. Fried, Herz, Sabo, Borman, Singer and Numerof, J. Amer. Chem. Soc., 1955, 77, 1068.

10. Battersby, Binks and Harper, J. Chem. Soc., 1962, 3534.

11. Bentley, Ball and Ringe, J. Chem. Soc., 1956, 1963.

12. McGinty and Djerassi, Ann. N. Y. Acad. Sci., 1958, 71,500.

13. Djerassi, Miramontes and Rosenkranz, J. Amer. Chem. Soc., 1953, 75, 4440; Ringold, Rosenkranz and Sondheimer, ibid., 1956, 78, 2477.

14. Herschberger, Shipley and Meyer, Proc. Soc. Exp. Biol. Med., 1953, 83, 175; Camerino, Patelli and Vercellone, J. Amer. Chem. Soc., 1956, 78, 3540; Saunders and Drill, Endocrinology, 1956, 58, 567.

15. Sandoval, Thomas, Djerassi, Rosenkranz and Sondheimer, J. Amer. Chem. Soc., 1955, 77, 148.

16. Kalvoda, Buchschacher and Jeger, Helv. Chim. Acta, 1955, 38, 1847; Corrodi and Hardegger, ibid., p. 2038.

17. Mackay and Hodgkin, J. Chem. Soc., 1955, 3261.

18. Lindsey and Barnes, Acta Cryst., 1955, 8, 227.

19. Bick, Nature, 1952, 169, 755.

20. Beckett and Casy, J. Pharm. Pharmacol., 1954, 6, 986.

21. Nakazaki, Chem. and Ind., 1962, 1577.

22. Beckett and Casy, Nature, 1954, 173, 1231.

23. Beckett, Casy, Harper and Phillips, J. Pharm. Pharmacol., 1956, 8, 860.

24. Janssen, "Synthetic Analgesics, Part 1, Diphenylpropylamines," Pergamon Press, London, 1960, pp. 118-119, 147-148, 154.

25. Beckett, J. Pharm. Pharmacol., 1956, 8, 848.

26. Beckett and Anderson, J. Pharm. Pharmacol., 1960, 12, 228I.

27. Goto and Yamamoto, Proc. Japan Acad., 1957, 33, 477; Chem. Abs., 1958, 52, 5441a.

28. Goto and Yamamoto, Proc. Japan Acad., 1956, 32, 45; Chem. Abs., 1956, 50, 13961g.

29. Goto, Yamasaki, Yamamoto and Ohno, Proc. Japan Acad., 1957, 33, 660; Chem. Abs., 1958, 52, 9425h.

30. Takagi, Fukuda, Watanabe and Sato, Yakugaku Zasshi, 1960, 80, 1506; Chem. Abs., 1961, 55, 8648h.

31. Corrodi and Hardegger, Helv. Chim. Acta, 1956, 39, 889.

32. Proc. Chem. Soc., 1962, 207.

33. Misra, Mule and Woods, J. Pharmacol., 1961, 132, 316.

34. Adler, J. Pharmacol., 1952, 106, 371.

35. Lasagna and Kornfeld, J. Pharmacol., 1958, 124, 260; Lockett and Davis, J. Pharm. Pharmacol., 1958, 10, 80.

36. Beckett, Casy and Harper, J. Pharm. Pharmacol., 1956, 8, 874.

37. Horlington and Lockett, J. Pharm. Pharmacol., 1959, 11, 415; Miller and Anderson, J. Pharmacol., 1954, 112, 191; Lasagna and Kornfeld, ibid., 1958, 124, 262.

38. Cochin and Axelrod, J. Pharmacol., 1959, 125, 105; Mannering and Takemori, ibid., 1959, 127, 187.

39. Takemori and Mannering, J. Pharmacol., 1958, 123, 178.

40. Crowfoot, Vitamins and Hormones, 1944, 2, 409.

41. Gates and Tschudi, J. Amer. Chem. Soc., 1952, 74, 1109; 1956, 78, 1380.

42. Small and Browning, J. Org. Chem., 1938-39, 3, 618.

43. Gates and Tschudi, J. Amer. Chem. Soc., 1950, 72, 4839.

44. Gates and Webb, J. Amer. Chem. Soc., 1958, 80, 1186.

45. King, Penprase and Kloetzel, J. Org. Chem., 1961, 26, 3558.

46. Bachmann, Kushner and Stevenson, J. Amer. Chem. Soc., 1942, 64, 974.

47. Bachmann, Cole and Wilds, J. Amer. Chem. Soc., 1940, 62, 824.

48. Sheehan, Coderre and Cruickshank, J. Amer. Chem. Soc., 1953, 75, 6231; Adams, Patel, Petrow and Stuart-Webb, J. Chem. Soc., 1956, 297.

49. Birch and Smith, Quart. Revs., 1958, 12, 17.

50. Wehrli and Schaffner, Helv. Chim. Acta, 1962, 45, 385.

51. Johnson, Allen, Hindersinn, Sausen and Pappo, J. Amer. Chem. Soc., 1962, 84, 2181.

52. G. P. 902,257/1954; Chem. Abs., 1956, 50, 5043c.

53. Conroy, J. Amer. Chem. Soc., 1955, 77, 5960.

54. Bentley, "The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, 1954, p. 166.

55. See inter alia: Ach and Knorr, Ber., 1903, 36, 3067; Knorr and Horlein, Ber., 1907, 40, 4889; Holmes and

Lee, J. Amer. Chem. Soc., 1947, 69, 1996; Cahn and Robinson, J. Chem. Soc., 1926, 908; Dieterle and Dickens, Arch. Pharm., 1926, 264, 257; G. P. 408,870/1923; Rapoport and Reist, J. Amer. Chem. Soc., 1955, 77, 490.

56. Highet and Wildman, J. Amer. Chem. Soc., 1955, 77, 4399.
57. Findlay and Small, J. Amer. Chem. Soc., 1950, 72, 3247.
58. Henbest, Jones and Owen, J. Chem. Soc., 1957, 4909.
59. Meunier, Zwingelstein and Jouanneteau, Bull. Soc. Chim. biol., 1953, 35, 495.
60. Attenburrow, Cameron, Chapman, Evans, Hems, Jansen and Walker, J. Chem. Soc., 1952, 1094.
61. c.f. Gates and Helg, J. Amer. Chem. Soc., 1953, 75, 379.
62. Freund and Speyer, J. prakt. Chem., 1916, 94, 135.
63. Lutz and Small, J. Org. Chem., 1939, 4, 220.
64. Gates and Hughes, Chem. and Ind., 1956, 1506.

PART III.

CONSTITUENTS of *BACOPA MONNIERI* (L) PENNELL.

Introduction.

A cursory survey of the drugs commonly employed in pharmacology and medicine reveals that biological activity is frequently associated with the presence of nitrogen atoms in a molecular structure. Likewise, noradrenaline and acetylcholine, the established peripheral neurohormones of the mammalian organism, as well as the pharmacologically potent naturally occurring principles histamine, 5-hydroxytryptamine and γ-aminobutyric acid, are all nitrogenous derivatives as are many of the compounds which act by mimicking or opposing the action of these substances. It is therefore not surprising that the alkaloids -- a large class of substances which can be defined as naturally-occurring nitrogen-containing compounds of more or less complex structure -- exhibit a wide range of biological activity and are thereby of particular interest to the medicinal chemist. At the same time, continued botanical and chemical interest in the alkaloids has been ensured by the variety of their sources of origin, their irregular distribution within the different plant families and their great diversity of chemical structure.

The toxic nature of various alkaloid-bearing plants has been known since antiquity. The ancient Greeks administered crude preparations of the alkaloids of hemlock

in the execution of criminals, and a number of alkaloids were utilised by native tribes in the preparation of arrow poisons,[1] the South American Indians, for example, employing the now well-known pot, calabash and tube curares,[2] and the African bushmen those from the Hemanthus species.[3] In the Middle Ages, the toxic properties of ergot alkaloids caused widespread epidemics of poisoning through the consumption of rye bread prepared from contaminated grain, and even today, plants containing potentially lethal alkaloids may represent a hazard to livestock, e.g. the Senecio species.[4]

It was not only the toxic properties of alkaloids, however, which led native peoples to cultivate their use. The Chinese scholar Emperor Shen Nung (2760 B.C.) observed the antifebrile effects of preparations from the roots of Dichroa febrifuga Lour which is now known to contain anti-malarial alkaloids, and he also noted the diaphoretic and stimulant effects of the drug Ma Huang from which the alkaloid ephedrine has been isolated.[5] The South American Indians were familiar with the antimalarial properties of Cinchona bark, and the same natives enjoyed the stimulant and euphoric properties of cocaine obtained by chewing Erythroxylon coca leaves. Even to this day, the Indians of Mexico and the South-western United States, during their religious orgies, consume the mushroom Psilocybe mexicana Heim and the mescal discs of the peyote cactus in order to

experience the psychotropic effects of the constituent alkaloids psilocybin and mescaline.[6]

The world-wide popularity now enjoyed by tobacco, which first appeared in Europe in 1558 and was popularised by Jean Nicot, remains a tribute to the mental relaxation and tranquillity afforded by the alkaloid nicotine. The same substance has also played a role of great significance in the development of our understanding of the structure of the autonomic nervous system.[7] Several other alkaloids are of interest on account of their unusual biological properties. Thus mescaline, which produces hallucinations in the form of highly-coloured geometrical patterns,[8] has seen application in experimental psychology, and the anti-mitotic properties of colchicine have inspired research into the histology and chemotherapy of cancer. Bulbocapnine through its central actions, induces a peculiar state of catalepsy permitting experimental animals to be bent into bizarre shapes.[9]

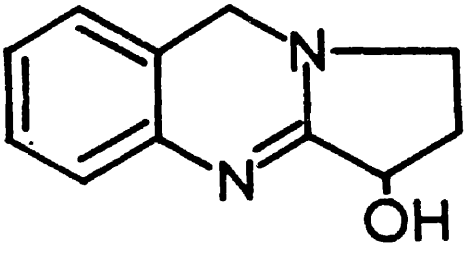
By far the greatest single factor in maintaining interest in the alkaloids, however, is the clinical importance of a select few of their number. Morphine, obtained from the seed capsule of the poppy plant Papaver somniferum, is still regarded by the majority of clinicians as the most useful analgesic in spite of its addictive properties,[10] and the vasoconstricter, sympathomimetic and

central nervous stimulant properties of ephedrine, a constituent of Ephedra equisetina and sinica, render it a valuable agent in the symptomatic treatment of asthma and bronchial disorders, and in the maintainance of blood pressure during spinal anaesthesia. Atropine, one of the belladonna alkaloids, is used preoperatively to suppress salivary, gastric and respiratory tract secretions, as a long-acting mydriatic, as an antispasmodic and in certain cases of Parkinson's disease. The related alkaloid hyoscine (syn. scopolamine) finds application in the prevention of motion sickness, while ergometrine, one of the ergot alkaloids, is widely used as an oxytocic in obstetrics. The advent of d-tubocurarine, the most important of the curare alkaloids,[11] whose skeletal muscle-relaxant properties greatly facilite operative manipulation, constitutes one of the most important advances in the field of surgery in the last ten years. The discovery of the tranquillising properties of reserpine was likewise a considerable stimulus to research into newer methods of treating abnormal mental states. Other alkaloids of medical significance include codeine which is used as an antitussive and analgesic, quinidine which has been employed as an antiarrythmic and antifibrillatory agent and colchicine which is of value in the treatment of gout. Physostigmine has been used to reduce intraoccular pressure in the

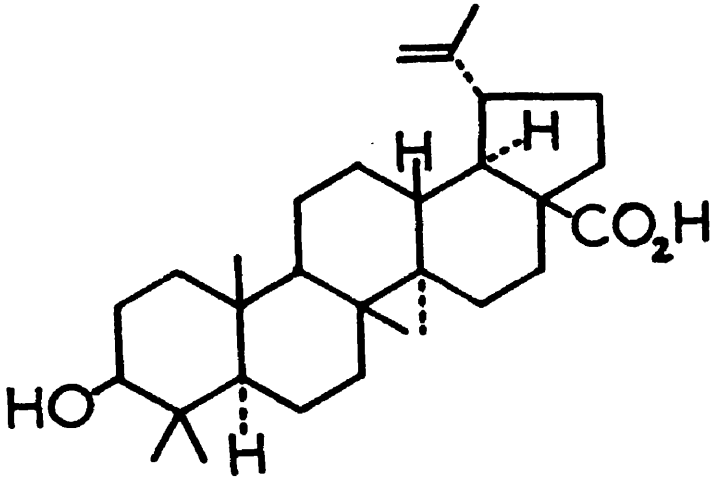
treatment of glaucoma.

The chemical characterisation of the tubocurarine molecule, [13] coupled with the recognition of the association between muscle relaxant activity and the presence within a drug molecule of two quaternary nitrogen atoms, led to the production of a number of chemically simpler and clinically useful neuromuscular blocking agents.[14] The physiological and pharmacological research subsequently carried out has greatly clarified our knowledge of the structure and function of the neuromuscular junction and of the mode of action of compounds acting at this site.

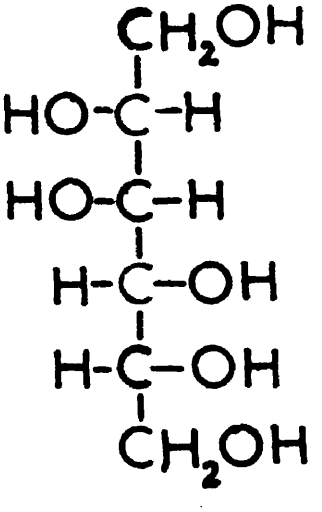
Much exploratory work has been done by means of phytochemical surveys to ascertain which plants do in fact contain alkaloids,[15] although R.H.F. Manske is of the opinion[16] that families which have not so far been found to contain alkaloids will only rarely yield such plants and that it may be safely supposed that the processes which gave rise to these genera or species involved mutations which are abnormal to the group as a whole. It would seem, however, that this opinion is based on little more than fragmentary evidence since only 97 families out of approximately 235 families recognised by botanists, representing about 2% of all the species involved, have been examined for alkaloidal content.[17] It has been pointed out[18] that greater numbers of organic compounds are



I



II



III

found in herbs than in trees although tree alkaloids are of larger molecular weight. The greatest possibility of finding new alkaloids would therefore seem to rest with the examination of herbaceous families.

Amoung the more widely distributed predominantly herbaceous families, the family Scrophulariaceae contains about 3,000 species, yet of these less than 1% have been tested for alkaloidal content.[17] A search of the literature has revealed that seven of these investigations have yielded positive results[19-32] and these have been listed in Table I. Only in the case of the Linaria species, however, has the alkaloid been identified[27,28] and it appears to be vasicine (syn. peganine, I), one of the Quinazoline group. In three other cases, the alkaloids were isolated as crystalline solids or as derivatives,[19-24,32] but no further identification or structural investigation appears to have been carried out.

This section of the thesis is concerned with an investigation of one of the more extensively scrutinised members of the family Scrophulariaceae, Bacopa monnieri (L) Pennell, as a source of pharmacologically-active alkaloids. The investigation was initiated since biologically-active alkaloids of unknown constitution had been reported to be present in this plant.[19-24] The fact that the structure of only one alkaloid in the family Scrophulariaceae had been

TABLE I. ALKALOID-CONTAINING MEMBERS of FAMILY SCROPHULARIACEAE.

Species	Habitat	Remarks	Reference
Bacopa monniera (L) Pennel	India	Three alkaloids isolated; crystalline derivatives prepared.	19-24
Bungea trifidia	Armenia	Positive alkaloid reaction; no specific identification.	25
Cordylanthus filifolius	California	Positive alkaloid reaction; no specific identification.	26
Linaria popovii	Russia	Crystalline alkaloid "linarine" (≡ vasicine) isolated.	27
Linaria vulgaris Mill	Russia	Alkaloid vasicine isolated.	28
Mimulus guttatus	New Zealand	0.1% Liquid alkaloid from dried tops; not investigated.	29
Pedicularis sp.	Armenia	Positive alkaloid reaction; no specific identification.	25
Scoparia dulcis	Australia	Positive alkaloid reaction; no specific identification.	30,31
	Argentina	Crystalline substance giving alkaloid reactions isolated.	32
Verbascum sp.	Armenia	Positive alkaloid reaction; no specific identification.	25
Verbascum virgatum	Australia	Questionable positive alkaloid reaction; no specific identification.	30

determined made it seem possible that the plant might contain alkaloids of some previously unknown structural type, thus providing further knowledge in the quest for the elucidation of the elusive relationship between chemical structure and biological activity.

Discussion.

Bacopa monnieri (L) Pennell (syn. Herpestis monniera, Monniera cuneifolia), a member of the family Scrophulariaceae, is a plant employed in Ayurvedic medicine under the name of "Brahmi". It has been used in India in the form of a syrup, as a liquid extract and in admixture with ghee, and has enjoyed a reputation as a powerful diuretic, aperient and nerve tonic. It has also been claimed to relieve hoarseness and improve memory.[21]

The plant was first investigated by Bose and Bose[19] who found it to contain a small amount of an uncharacterised alkaloid which they named "brahmine." The alkaloidal fraction was found to be highly toxic, frogs dying within ten minutes after administration of a dose of 0.5mg./100g. body weight, while rats and guinea-pigs succumbed within 24 hours at dose levels of 25mg./kg. Further investigations[21] revealed the presence of three alkaloids in the plant which were, however, only isolated in crystalline form as derivatives. It was observed[21] that the basic fraction of fresh samples of the plant consisted of predominantly one alkaloid (B_1), while samples stored for several years contained two other alkaloids (B_2 and B_3), but only small amounts of B_1. A sterol-like compound, m.p. 76°, was also isolated. A new alkaloid "herpestine" was

subsequently isolated in crystalline form[22] and several derivatives were prepared.

A further chemical examination of the plant by Malhotra and Das[23] revealed the presence of D-mannitol, a glycoside-saponin principle and potassium sulphate, nitrate and chloride. On pharmacological investigation of the plant, these workers found that the crude total alcoholic extract had cardiotonic, vasoconstrictor, sedative and neuromuscular blocking actions, while the glycoside-saponin had cardiotonic action in the normal and hypodynamic frog heart, sedative action in the rat and the guinea-pig, and smooth muscle spasmodic action on the rabbit and the guinea-pig ileum and on the rat uterus.

The identity of the dried plant was confirmed by Dr. F. Fish, Royal College of Science and Technology, Glasgow, and Dr. G. Taylor, Royal Botanic Gardens, Kew. The basic material, which was found to be best isolated by the method previously described,[21,22] amounted to only 0.002-0.003% of the dry weight of the plant. Since the quantity of alkaloids was so small, detailed chemical and pharmacological investigation was not possible. The presence of at least four components was, however, demonstrated by paper chromatography using water saturated with butanol-acetic acid (9:1) as solvent. The plant was then subjected to a detailed chemical examination.

Extraction with cold light petroleum gave a fraction which was separated into acidic and neutral components. Traces of a ketonic constituent were removed from the neutral material by treatment with 2,4-dinitrophenylhydrazine followed by chromatography over alumina, and the resulting solid was recrystallised from ethyl acetate. It corresponded in melting point and elemental composition to n-triacontane. Application of gas-liquid chromatography as described by Eglinton et al.[33], however, showed it to consist predominantly of C_{27}, C_{29} and C_{31} hydrocarbons with smaller amounts of C_{28}, C_{30} and C_{32} homologues. As gas-liquid chromatography cannot be relied upon to separate normal and iso-alkanes,[34] the mixture was subjected to mass-spectrographic analysis. The results indicated that no more than trace amounts of iso-alkanes could be present. A portion of the acidic component was methylated with diazomethane and upon application of gas-liquid chromatography, fourteen components were found to be present.

Cold chloroform extracted betulinic acid (II) which has previously been identified as a constituent of plants belonging to the family Scrophulariaceae.[35] Its identity was confirmed by conversion into betulin by reduction of the methyl ester, and comparison with an authentic specimen. Extraction with water or ethanol gave sodium chloride and a bitter glycosidic fraction from which no crystalline

components resulted after chromatography employing a variety of systems. No well-defined products resulted on hydrolysis with acid or base, or with the enzymes emulsin or β-glucosidase. Both infrared and ultraviolet spectra of the crude glycoside indicated the absence of steroid cardiac glycosides which are known to occur in some members of the family Scrophulariaceae, e.g. Digitalis spp. This fact is of some interest in view of the cardiotonic claims previously mentioned.[23]

Chromatography over alumina of a cold aqueous extract of the plant, using butanol saturated with water as solvent, yielded D-mannitol (III) identified by mixed melting point and infrared comparison with an authentic sample. We were unable to confirm the presence of a sterol-like compound reported by other workers.[21,22]

A note in the Journal of the Chemical Society summarising this work[36] is included as Appendix I to this thesis. Subsequent to this publication, the report of another chemical investigation of Bacopa monnieri appeared,[24] the results being very similar to those reported here. These workers, however, were in addition able to isolate a glycoside which on hydrolysis yielded glucose, arabinose and an aglycone. Only traces of alkaloid were found to be present.

Experimental.

Melting points were taken on a Kofler block. The light petroleum was of boiling point 60-80°.

Alkaloids. The crude basic material was best isolated by the proceedure previously described.[21,22] The yield ranged from 20 to 30 mg./kg. The gummy material was chromatographed on Whatman No. 1 filter paper sheet using water saturated with butanol-acetic acid (9:1) as solvent. Four spots were located by their white fluorescence under ultraviolet light,[37] their R_F values being 0.95, 0.85, 0.7 and 0.

Hydrocarbon fraction. The green residue resulting from the extraction of 20lb. of finely ground plant material with light petroleum (14l.) was exhaustively extracted with boiling ethanol to yield a white solid (2g.). This was shaken in light petroleum with 4N-sodium hydroxide. The precipitated sodium salts were removed by centrifugation and 0.8g. of neutral material reclaimed from the organic layer. This was treated with 2,4-dinitrophenylhydrazine hydrochloride in ethanol to remove traces of material showing infrared carbonyl absorption. The reaction mixture was taken to dryness, dissolved in light petroleum and chromatographed over alumina (Brockmann grade V). There resulted 0.1 g. material showing only paraffinic absorption and forming

plates m.p. 65.5-66.5°, from ethyl acetate. (Found: C, 85.4; H, 14.8. Calc. for $C_{30}H_{62}$: C, 85.3, H, 14.7%). Gas-liquid chromatography, with n-hexacosane as standard, showed it to be a mixture of heptacosane, octacosane, nonacosane, triacontane, hentriacontane and dotriacontane. Mass spectrographic analysis confirmed this result.

The sodium salts obtained above were reconverted into the acids with 6N-hydrochloric acid giving a wax of m.p. 69-71°. A portion was methylated with diazomethane; gas-liquid chromatography showed fourteen components.

Betulinic acid(II). A cold chloroform extract (3l.) of 500g. of the plant material previously exhausted with light petroleum was reduced in volume to 30ml. and shaken with 4N-sodium hydroxide. The precipitated sodium salt was collected and the acid regenerated. Crystallisation from ethanol followed by sublimation gave material (1.5g.) of m.p. 302-305°, $[\alpha]_D$ +7° (c 1.98 in pyridine) Robertson et al.[38] report for betulinic acid, m.p. 316-318°, $[\alpha]_D$ +7.9° (in pyridine) (Found: C, 79.3; H, 10.7. Calc. for $C_{31}H_{50}O_3$: C, 79.1; H, 10.6%).

Reduction of the ester by lithium aluminium hydride afforded betulin, identical with authentic material (infrared, mixed m.p.).

D-Mannitol (III). The residue (10g.) from an aqueous extract of finely ground plant material was filtered through

alumina (Brockmann grade V; 100g.) in butanol saturated with water. The initial 700ml. of eluant which contained glycosidic material was rejected. The next 900ml., on evaporation to dryness and after removal of sodium chloride, afforded D-mannitol (1.1g.), m.p. 166.5-167° (from ethanol) (lit.,[39] m.p. 166°)(Found: C, 39.8; H, 7.2. Calc. for $C_6H_{14}O_6$: C, 39.6; H, 7.7%). There was no mixed m.p. depression and the infrared spectrum was correct.

References.

1. Santesson, Skand. Arch. Physiol., 1936, 74, 239; and references therein.
2. McIntyre, "Curare, Its History, Nature and Clinical Use," University of Chicago Press, Chicago, 1947.
3. Nussbaum, Schweiz. med. Woch., 1913, 51, 133; Chem. Abs., 1913, 7, 2661[4].
4. de Waal, Farming in South Africa, 1941, 16, 69; Evans and Evans, Nature, 1949, 164, 30.
5. Nagai, Pharm. Ztg., 1887, 32, 700.
6. "Dispensatory of the United States of America," editors Osol and Farrar, J. B. Lippincott Co., Philadelphia, 25th Edn., 1955, p.1548; Heim, Compt. rend., 1956, 242, 965.
7. Langley, J. Physiol., 1898, 23, 240; 1905, 33, 374; 1911, 43, 125.
8. Mayer-Gross, Brit. Med. J., 1951, II, 317; Morselli, J. Psychologie normal et path., 1936, 33, 368.
9. Richter and Paterson, J. Pharmacol., 1931, 43, 677.
10. Bull. Narcotics, 1962, 14 No. 1, 53; Schaumann, Angew. Chem., 1954, 66, 765.
11. Griffith and Johnson, Anesthesiology, 1942, 3, 418.
12. Ann. N. Y. Acad. Sci., 1954, 59, 1-140.
13. King, J. Chem. Soc., 1935, 1381.

14. "Curare and Curare-like Agents," editors Bovet, Bovet-Nitti and Marini-Bettolo, Elsevier Publ. Co., Amsterdam, 1959; and references therein.

15. "Proceedings of the Symposium on Phytochemistry," Unesco Science Corporation Office for South-east Asia, Kuala Lumpur, 1957, pp. 1-119; Arthur and Cheung, J. Pharm. Pharmacol., 1960, 12, 567; and references therein.

16. Manske and Holmes, "The Alkaloids," Academic Press Inc., New York, 1950, 1st Edn., Vol. I, p. 1.

17. Willaman and Schubert, Economic Botany, 1955, 9, 141.

18. McNair, Amer. J. Bot., 1941, 28, 179.

19. Bose and Bose, J. Indian Med. Ass., 1931, 1, 60.

20. Steinmetz, "Codex Vegetabilis," Amsterdam, 1957; Chopra, "Indigenous Drugs of India," Dhur, Calcutta, 2nd Edn., 1958, p. 341.

21. Basu and Walia, Indian J. Pharm., 1944, 6, 84.

22. Basu and Pabrai, Quart. J. Pharm. Pharmacol., 1947, 137.

23. Malhotra and Das, Indian J. Med. Res., 1959, 47, 294.

24. Rastogi and Dhar, J. Sci. Ind. Res. (India), 1960, 19B, 455.

25. Zolonitskaya, Izvest. Akad. Nauk. Armyan S.S.R., Biol. i Sel'khoz Nauki, 1954, 7 No 5, 27; Chem. Abs., 1954, 48, 11727g.

26. Wall, Fenske, Garvin, Willaman, Jones, Schubert and Gentry, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1959, 48, 695.

27. Yunusov and Ismailov, Doklady Akad. Nauk S.S.S.R., 1956, 111, 25; Chem. Abs., 1961, 55, 10808e.

28. Men'shikov, Ban'kovskii and Frolova, J. Gen. Chem. U.S.S.R., 1959, 29, 3806; Chem. Abs., 1960, 54, 19744i.

29. E. P. White, personal communication.

30. Webb, "Australian Phytochemical Survey, Part I," Bulletin No. 241 of C.S.I.R.O., Melbourne, 1949.

31. Webb, "Australian Phytochemical Survey, Part II," Bulletin No. 268 of C.S.I.R.O., Melbourne, 1952.

32. Vaquero and Amengual, Arch. farm. y biochim. Tucuman, 1955, 7, 1; Chem. Abs., 1956, 50, 16033c.

33. Eglinton, Hamilton, Hodges and Raphael, Chem. and Ind., 1959, 955.

34. Carruthers and Johnstone, Nature, 1959, 184, 1131.

35. Retzlaff, Arch. Pharm., 1902, 240, 561; Maurer, Meier and Reiff, Ber., 1939, 72, 1870; Barton and Jones, J. Chem. Soc., 1944, 659.

36. Brown and Martin-Smith, J. Chem. Soc., 1960, 2783.

37. Munier and Macheboeuf, Bull. Soc. Chim. biol., 1949, 31, 1144.

38. Robertson, Soliman and Owen, J. Chem. Soc., 1939, 1267.

39. Fischer, Ber., 1890, 23, 383.

PART IV.

SYNTHETIC APPROACHES to THIONAPHTHEN ISOSTERES of HYDROXYTRYPTAMINES.

Introduction.

The lack of knowledge relating biological activity to chemical structure has often forced the medicinal chemist to adopt a speculative rather than a rational approach to the synthesis of new drugs. This has resulted all too frequently in an uninspired haphazard series of random structural modifications being made to a drug molecule of proven efficacy, or in the indiscriminate synthesis of large numbers of unrelated compounds for biological screening in the hope that at least one member might prove of some pharmacological interest or perhaps even of clinical value. Several theoretical interpretations of drug action have, nevertheless, been made, and these when taken in context do serve to rationalise and give a degree of coherence to certain approaches to the synthesis of new drugs.

Today, the effects of many (but not all) biologically-active substances are viewed in terms of the receptor theory of drug action (a concept first formulated by Ehrlich[1]) from which in turn have arisen the theory of metabolite displacement and the concept of bioisosterism, all of which have a direct bearing on the work to be discussed in this part of the thesis. Accordingly, a brief outline of these concepts is desirable in order to set this work in perspective.

The receptor theory of drug action postulates that drugs exert their effects through their combination with a hypothetical specific receptor site in the tissue of the organism to form a "drug - receptor complex." Despite intensive research, comparatively little is yet known of the precise chemical nature of these drug receptors and whether they even exist as discrete entities, but the theory has nevertheless proved an extremely valuable aid in so far as it stresses the frequent importance of the 3-dimensional geometry and the electronic distribution in the drug molecule. The drug - receptor complex can be visualised as originating from an interaction between complementary fields of force located both in the drug molecule and in the tissue.[2] The ability of the drug to evoke a positive biological response can then be considered to depend on two basic factors -- "affinity" and "intrinsic activity" -- both of which can be expressed in terms of mathematical constants. The affinity of a drug is then its ability to enter into complex formation with a given receptor and is thus inversely related to the dissociation constant of the drug - receptor complex.[3] The intrinsic activity, on the other hand, is a measure of the ability of the drug to evoke a biological response while complexed with the receptor,[3] and in turn relates the response to the number of occupied receptors. Since the binding forces involved are easily reversed at room

temperature by solvent extraction and dialysis and therefore involve energies of the order of that of heterogeneous catalysis, electrostatic, multiple van der Walls' and hydrogen bonds -- rather than covalent bonds -- must play the dominant role. Regions of high or low electron density in the drug molecule will contribute to the electrostatic components of the force field, whilst the stereochemistry of the molecule will to a large extent determine the van der Walls' bonding involved.

The physical nature of receptors is obscure although the acetylcholine receptor is thought to be a protein[4] or a mucopolysaccharide.[5] Indeed proteins believed to be the receptor substance have been isolated in a few instances.[6] A number of attempts have also been made to portray the shape and electrical charge distribution of certain receptors in a pictorial fashion. In the case of drugs which have been definitely established to act through disruption of normal enzymatic processes, the receptor can be regarded as involving enzymes, co-enzymes and the associated metallic ions.[7] In other cases, detailed pictorial representations have been presented of the receptors involved in analgesia,[8] in anticholinesterase activity[9] and in the muscarinic[10] and nicotinic[11] actions of acetylcholine based on information drawn from the molecular characteristics of the most potent drugs known to act in the particular manner in question.

Conclusions regarding the nature of receptors which have been deduced by consideration of non-rigid molecules can only be speculative since there is no reason to assume that the thermodynamically most stable conformation of the active molecule in solution is that actually adopted at the receptor site. The receptor itself, however, could conceivably be non-rigid and so alter its characteristics to suit the steric and electronic requirements of the drug molecule.[12] There is also the opposite hypothesis[13] that the receptors are relatively rigid, requiring a certain degree of flexibility in the drug molecule to ensure a statistically significant occupation of the former. Both these points of view tend to suggest that rigid molecules would not necessarily provide useful information concerning receptors.

The fact that the biological properties of some drugs are extremely sensitive to minor changes in stereochemistry, electron density and substitution pattern, as where individual optical antipodes exhibit different pharmacological characteristics[14] whilst in other cases, for example with the volatile general anaesthetics, compounds of widely differing chemical constitution show similar biological behaviour, has led several workers[15] to distinguish two types of biologically-active compound -- the structurally specific and the structurally non-specific -- although there is no hard and fast line of demarcation

between the two, one class merging into the other by way of compounds with intermediate properties.[7] There has been a tendency to regard structurally non-specific drugs as acting by a process of accumulation[16] although Mullins[17] has pointed out that there need be no fundamental distinction between the two types in terms of the receptor theory. That receptors themselves do display a considerable range of structural specificity is illustrated by the apparent existence of more than one one kind of receptor for 5-hydroxytryptamine[18] and in adrenergic transmission[19] on the one hand, and by the degree of chemical alteration permissible to the morphine-like analgesic molecule while still allowing an adequate fit to the same analgesic receptor[20] on the other. In the latter case it has been proposed[8] that analgesics do retain a considerable degree of specificity in that the more active enantiomers (in compounds possessing optical activity) have absolute configurations related to D-(−)-alanine, but the considerable number of exceptions which exist[21] would appear to render unreserved acceptance of this hypothesis difficult.

An extension to the idea of the existence of specific receptors is provided by the theory of metabolite displacement. Natural metabolites are believed to initiate certain fundamental processes at their receptor sites in a living organism. Where the ability to fit these receptors

is shared by a synthetic analogue of the metabolite, but the analogue is not able to produce a very pronounced positive effect, then it is likely to act as an antagonist of the metabolite. The intentional design of specific antagonists as a rational approach to the synthesis of new drugs has been described as "the revolution in pharmacology."[22] The idea was originally applied to drugs which had little or no activity of their own, but merely competed for the occupancy of the receptor sites, thereby reducing the activity of the natural metabolite. A natural extension to the concept has been made by including the situation where the analogue itself is able to elicit a positive biological response, its ability to do so being variously termed its "intrinsic activity"[3] or "efficacy."[23]

The experimental study of the physiological effects of antimetabolites might be expected to produce information regarding the sites of action or even to uncover some previously unsuspected role of the natural metabolite as well as delineating which parts of the naturally-occurring molecule are necessary for biological activity, thus providing further information for the design of potentially active structures. The identification of the exact site of action of the antimetabolite is of the utmost importance since the receptor site proper is only one of several sites which may be involved in the physiological history of a given

metabolite. Other binding sites include those of biogenesis, storage and biotransformation.[24] All four sites need not be discrete entities, however, since more than one process may proceed at any one centre. Where a multiplicity of binding sites does exist, the antimetabolite might be expected to exert its action in a different fashion at the different sites. For example, it is conceivable that it could mimic the action of the natural metabolite at the receptor, prevent its biotransformation and be without effect on its biogenesis.

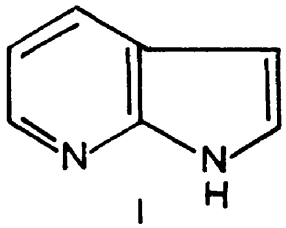
Antimetabolites can often be designed by suitable chemical alteration of a natural metabolite through such processes as substitution, homologation and isomerisation. One promising approach is provided by the application of the concept of bioisosterism. The concept of isosterism in its original form was introduced by Langmuir[25] who, as an extension to the grouping of elements in the Periodic Table, prepared a list of ions and diatomic molecules which, since they contain an identical number and arrangement of electrons, should have similar chemical properties. By Langmuir's definition, the groups were not necessarily isoelectric, a drawback to their biological application. These ideas were later expanded by Erlenmeyer and Leo[26] to include radicals related by Grimm's hydride displacement law.[27] These workers were the first to apply the concept of isosterism to

biological problems, redefining isosteres as "atoms, ions or molecules in which the peripheral layers of electrons can be considered to be identical." The concept of "bioisosterism" in its present form is due to Friedman[28] who coined the term to describe the relationship of isosteric compounds which display the same type of biological activity. Compounds in isosteric relationship may, in terms of receptor theory, possess either similar or antagonistic biological activity. The similarities in chemical and physical properties shown by the isosteres should ensure similar affinities for the same receptors, mimicry or antagonism then being determined by the intrinsic activities of the individual isosteres.

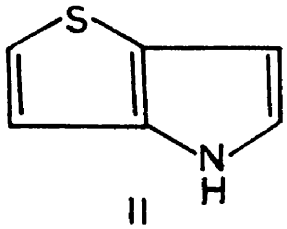
The scope and usefulness of bioisosteric replacement has been reviewed,[28,29] but a short account of representative types of substitution is not out of place here. The bioisosterism of ammonium and carbonium ions with regard to anionic sites has been postulated,[30] and even simple isosteric compounds show similar biological properties. Thus carbon dioxide and nitrous oxide have similar effects on slime mould[31] and the presence of the chlorate ion has been observed to reduce the rate of nitrite oxidation by Nitrobacter.[32] -C=C- and -S- have been described as "ring equivalents"[33] and this isosteric relationship has been utilised in a multitude of cases.[7]

The isosteric replacement of one halogen for another has seen considerable investigation, the resulting compounds often showing a stepwise alteration in potency as exemplified by certain antihistamines[34] and antitubercular compounds[35] and certain halogenated steroids.[36] The replacement of oxygen by sulphur in the carbonyl group has yielded particularly interesting results in the barbituric acids,[37] the hypnotics produced being short-acting as a result of rapid storage in body fat.[38] The thioester corresponding to procaine is likewise claimed to be a rapidly-acting anaesthetic.[39] The replacement of the sulphur atom in biotin produces a substance which is incorporated by certain organisms without conversion to the natural analogue,[40] and oxythiamine is found to reversibly displace thiamine, producing an accumulation of pyruvic and lactic acids in the blood of rats.[41]

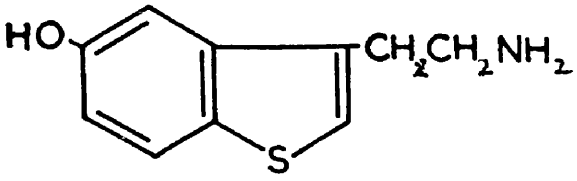
Despite extensive investigations on the replacement of thiophene and pyridine for benzene, comparatively little attention has been paid to the interchange of pyrrole and thiophene, although the effect of replacing the phenyl group by thienyl, furyl, pyrrole and pyridyl moieties has been studied in the case of antihistamines,[42] local anaesthetics[43] and analgesics.[44] The replacement of carbon by nitrogen has however seen wide application. Thus azahistidine is a specific antagonist of histidine in <u>E. coli</u>,[45] while



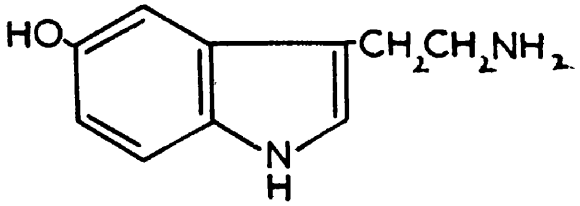
I



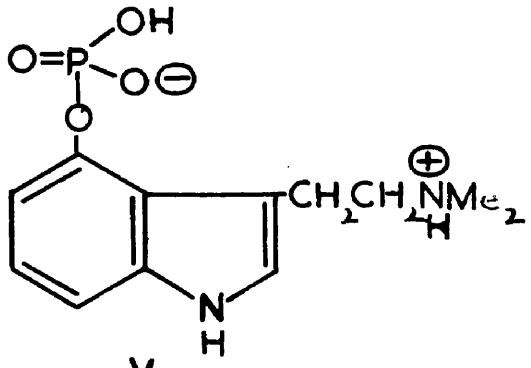
II



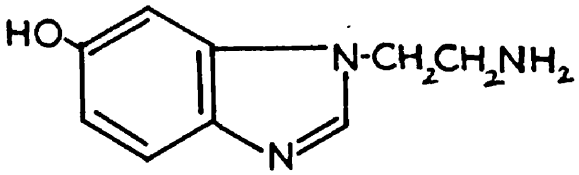
III



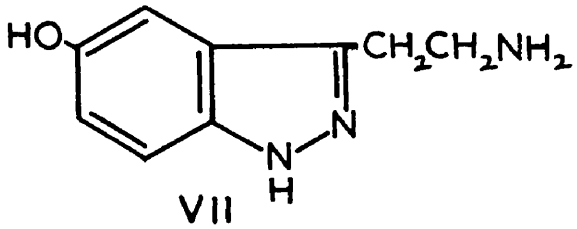
IV



V



VI



VII

azathymidine is more active than the parent pyrimidine as an inhibitor of the growth of several bacterial species.[46] The reverse replacement of carbon for nitrogen in guanine has produced an antagonist of this substance.[47]

Comparatively little attention has been paid to isosteres of indole derivatives which comprise a group of naturally-occurring substances of very considerable biological interest. Most attention has been focussed on the replacement of carbon by nitrogen to form such ring systems as 7-azaindole (I).[48] Thus the isomeric 2- and 7-azatryptophans are both antagonists of the parent amino acid[49] and the 7-aza isostere of α-methyltryptamine has been prepared for biological testing.[50] Investigations have also been made on the growth-promoting properties of thionaphthen acetic acids,[51] while the thionaphthen isostere of tryptophan has been shown to be an effective antagonist of the amino acid in Lactobacillus arabinosus[52] and to have bacteriostatic action against S. haemolyticus.[53] The recent synthesis of and investigations on the chemistry of the thieno-(3,2-b)-pyrrole ring system (II)[54] has made this isostere of indole available for conversion to derivatives of biological interest.

The preparation and pharmacological testing of thionaphthen isosteres of biologically-active indole derivatives had already been commenced in this

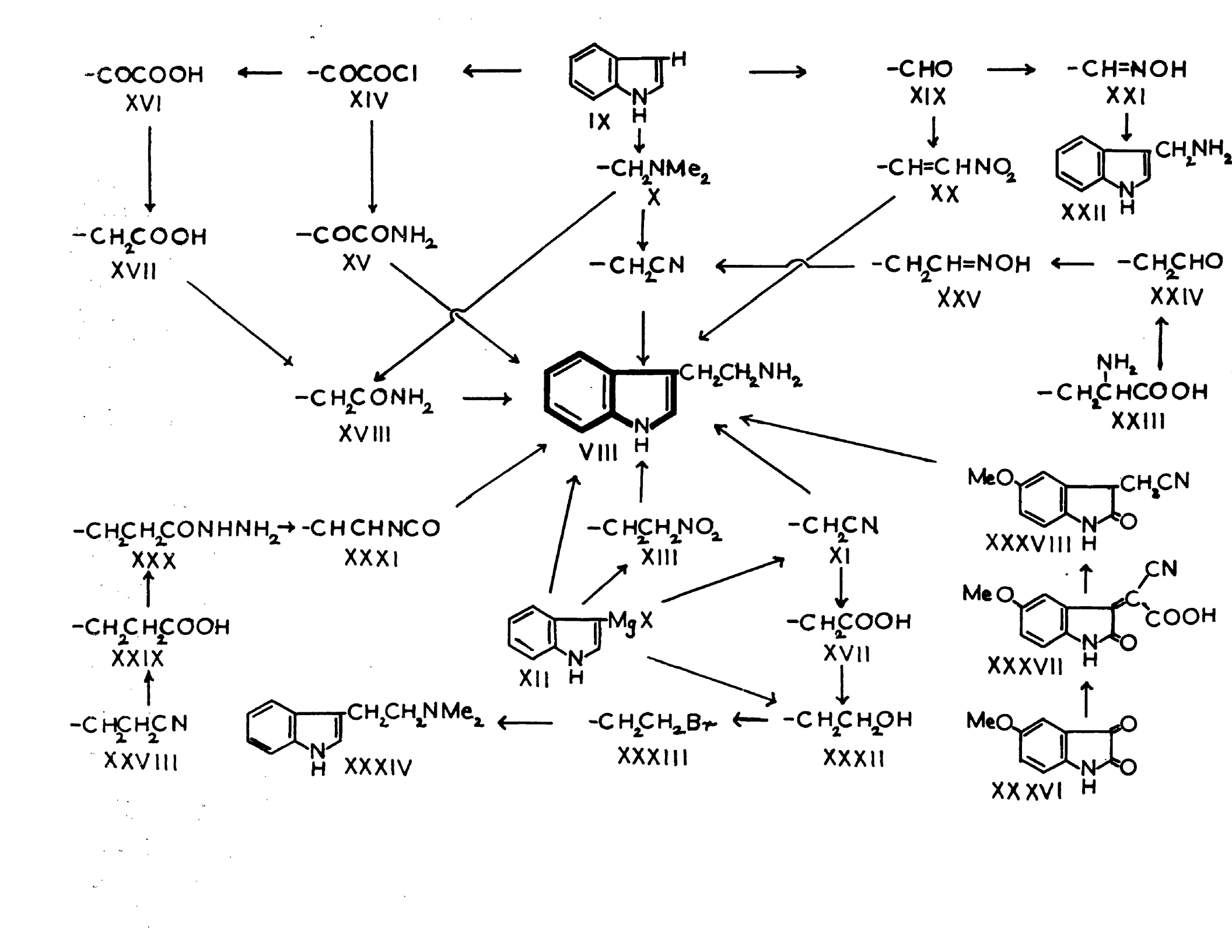
department.[55,56] The present work, a continuation of these studies, describes attempts to effect a synthesis of 3-(2'-aminoethyl)-5-hydroxythionaphthen (III), the thionaphthen isostere of 5-hydroxytryptamine, and investigations of a synthetic route to the thionaphthen isostere of 4-hydroxytryptamine.

5-Hydroxytryptamine (IV) is a natural metabolite which is widely distributed in the mammalian organism. Though its precise physiological significance has not been accurately defined, its varied pharmacological properties,[57] including its suggested transmitter role in the central nervous system, has focussed intensive interest on it and on related indole derivatives in recent years. Several investigations into the mode of action of 5-hydroxytryptamine have utilised substituted tryptamines as potential antagonists[58] with a view to illuminating the nature of the receptor system involved.

Pharmacological interest in 4-substituted tryptamines is of more recent origin, not only because of the pronounced activity of 4-hydroxytryptamine itself,[59] but also in view of the psychotic effects produced by the naturally-occurring compound psilocybin (V).[60]

The concept of bioisosterism has already been applied to the synthesis of analogues of the 5-hydroxytryptamine molecule in the preparation of 1-(2'-aminoethyl)-6-hydroxy-

benziminazole (VI)[61] and 3-(2'-aminoethyl)-5-hydroxy-indazole (VII)[62] and in the attempted preparation of 3-(2'-aminoethyl)-5-hydroxybenzofuran.[63] The further application of this concept to approaches to the synthesis of thionaphthen isosteres of hydroxytryptamines offered additional means of studying the nature of the receptor system concerned in the pharmacological action of these indole derivatives, and constitutes the work reported in this part of the thesis.


-COCOOH
XVI
-COCOCl
XIV
IX
-CHO
XIX
-CH=NOH
XXI
-CH2NMe2
X
-CH=CHNO2
XX
XXII
-CH2COOH
XVII
-COCONH2
XV
-CH2CN
-CH2CH=NOH
XXV
-CH2CHO
XXIV
-CH2CONH2
XVIII
VIII
NH2
-CH2CHCOOH
XXIII
-CH2CH2CONHNH2
XXX
-CHCHNCO
XXXI
-CH2CH2NO2
XIII
-CH2CN
XI
XXXVIII
-CH2CH2COOH
XXIX
MgX
XII
-CH2COOH
XVII
XXXVII
-CH2CH2CN
XXVIII
-CH2CH2NMe2
XXXIV
-CH2CH2Br
XXXIII
-CH2CH2OH
XXXII
XXXVI

Discussion.

As a result of earlier studies,[55,64-66] several nuclear-substituted thionaphthens were available as precursors for the synthesis of the thionaphthen isostere of 5-hydroxy-tryptamine, provided a suitable method for the elaboration of the β-ethylamine side chain could be found. Various methods for the introduction of this unit into aromatic compounds are known, and in the first instance it was decided to determine whether methods employed successfully in the synthesis of tryptamine and various substituted tryptamines could be adapted to the present needs.

The most widely employed method of introducing the side chain to form tryptamine (VIII) or its derivatives starts with the Mannich reaction on indole (IX) to give gramine (X).[67-70] Quaternisation of this base followed by a nucleophilic displacement of the quaternary ammonium function by cyanide ion then leads to 3-indolylacetonitrile (XI)[69,71-73] which in turn can be converted into the corresponding amide[68,70,73] or acid.[73,74] The cyanide or amide may be reduced with lithium aluminium hydride[68-70] to give the β-ethylamine side chain or the cyanide may be reduced with Raney nickel/hydrazine hydrate.[75]

The first synthesis of gramine did not in fact involve the Mannich reaction, but was effected by a reaction between

3-indolyl magnesium iodide (XII) and dimethylaminoacetonitrile.[76] A later extension of this approach involves the reaction between a 3-indolyl magnesium halide and chloracetonitrile to give the cyanide (XI).[77-80] Indole or its magnesium halide may also be nitroethylated to yield, after reduction of the product (XIII), either tryptamine itself or products substituted in the alkyl chain.[81] A further use of a 3-indolyl Grignard reagent has been developed in which ethyleneimine is used to give the tryptamine side chain in one step.[82]

A newer method of introducing the β-ethylamine side chain consists of the treatment of indole with oxalyl chloride to form 3-indolylglyoxalyl chloride (XIV).[72,83-85] This acid chloride may then be converted either into the corresponding amide (XV) and reduced to tryptamine with lithium aluminium hydride[72,84] or into the corresponding keto-acid (XVI) from which 3-indolylacetic acid (XVII) can be obtained.[85] This acid can then be converted into the amide (XVIII) via the acid chloride[86] or directly into the amide with urea,[87] reduction with lithium aluminium hydride then affording tryptamine.[87]

A further series of methods involves the use of 3-formylindole (XIX). This compound can be obtained by the action on indole of either carbon monoxide under pressure[88] or dimethylformamide in the presence of phosphorus

oxychloride,[72,89,90] or from the reaction of 3-indolyl magnesium iodide and ethyl formate in ether[91] or anisole.[92] The aldehyde has been condensed with nitromethane[72,90,93,94] in the presence of a variety of condensing agents, the resulting nitrovinyl compound (XX) being reduced to tryptamine with lithium aluminium hydride.[90] The oxime (XXI) of the aldehyde[91,95] has been reduced to 3-aminomethylindole (XXII) with sodium in alcohol[91] and the oxime (XXV) of 3-indolyl-acetaldehyde (XXIV) (which has been prepared from tryptophan (XXIII)[96] and from indole magnesium bromide (XII)[97]) can be dehydrated to 3-indolylacetonitrile with acetic anhydride.[98]

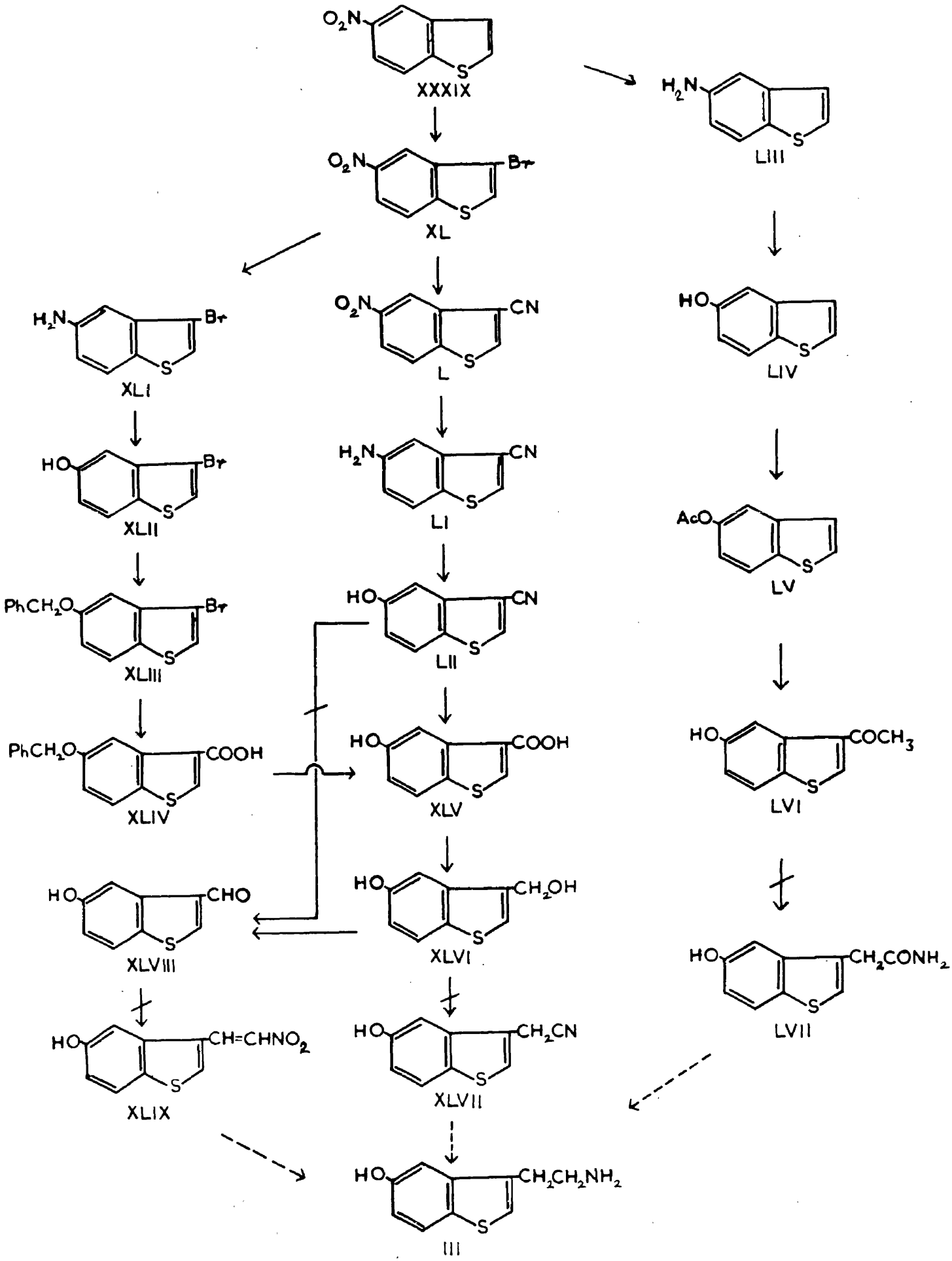
Two rather more involved routes to tryptamines are also available. In one, 5-methoxyisatin (XXXVI) is condensed with cyanoacetic acid and the product reduced and decarboxylated to yield the keto-cyanide (XXXVIII). This on reduction with sodium in isobutanol yields 5-methoxy-tryptamine.[99] In the other, indole is reacted with acrylonitrile to give 3-indolylpropionitrile (XXVIII) which on hydrolysis forms the corresponding acid (XXIX).[100] The hydrazide of this acid (XXX) has been converted to the isocyanide (XXXI)[101] which in turn forms tryptamine on reduction.

In addition, 3-(2'-dimethylaminoethyl)-indole derivatives are available through the following series of reactions. 3-Indolylacetonitrile (XI) has been hydrolysed

to the acid and thence reduced to the corresponding alcohol (XXXII).[74,79] This compound is also formed by the action of ethylene oxide on 3-indolyl magnesium bromide (XII).[102] The action of phosphorus pentabromide on the alcohol (XXXII) forms XXXIII which on treatment with dimethylamine yields 3-(2'-dimethylaminoethyl)-indole (XXXIV).[79]

The reactions which have been described refer to the synthesis of either tryptamine itself or of substituted tryptamines. Where the object of the work was the synthesis of 5-hydroxytryptamine, the phenolic hydroxyl group was without exception protected from the outset as the methyl[78,87,90,99] or benzyl[68,80,90,94] ether and the protecting group was removed during the last stage of the synthesis.

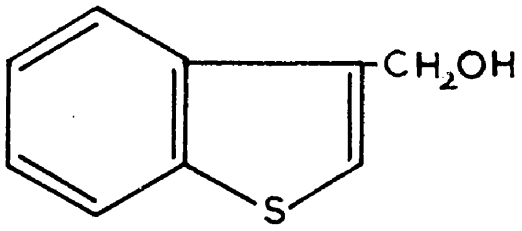
In a previous attempt to synthesise the thionaphthen isostere of 5-hydroxytryptamine,[55] the formation of 3-(2'-aminoethyl)-5-benzyloxythionaphthen was successfully achieved, but this compound resisted all attempts to remove the protecting benzyl group by either acid hydrolysis or catalytic hydrogenation. In the latter route, the catalyst was presumably being poisoned by the thionaphthen ring since sulphur-containing compounds are well known to do this. The methods which were initially attempted in the present investigation for the formation of the 5-hydroxytryptamine analogue were for the most part based on those used to


XXXIX
LIII
XL
L
LIV
XLI
LI
XLII
LV
XLIII
LII
XLIV
XLV
LVI
XLVIII
XLVI
LVII
XLIX
XLVII
III

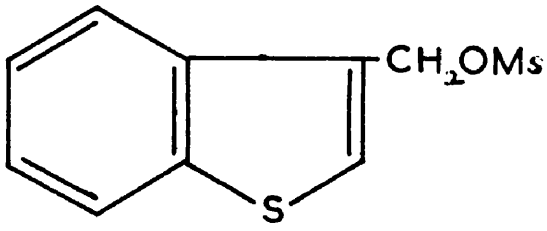
introduce the tryptamine side chain into indole which have just been discussed.

Thionaphthen does not undergo the Mannich reaction[103] so a direct conversion to a gramine-like analogue was not possible. It was therefore decided to adapt the general principle involved in the gramine methiodide to tryptamine conversion by utilising the known facile nucleophilic displacement of the methanesulphonyloxy group[104] rather than displacement of the quaternary ammonium function. The synthesis of the requisite methanesulphonate started from 5-nitrothionaphthen (XXXIX) which is available by a 4-stage synthesis from ortho-chlorbenzaldehyde.[105] From XXXIX, 5-hydroxy-3-hydroxymethylthionaphthen (XLVI) was prepared by a known route in seven stages.[55,105] An endeavour to reduce the number of stages involved by attempting a Grignard reaction on 3-bromo-5-nitrothionaphthen (XL) failed to yield any of the required 5-nitrothionaphthen-3-carboxylic acid. Similarly, a Grignard reaction on 3-bromo-5-hydroxy-thionaphthen (XLII) as expected failed to yield any 5-hydroxy-thionaphthen-3-carboxylic acid (XLV).

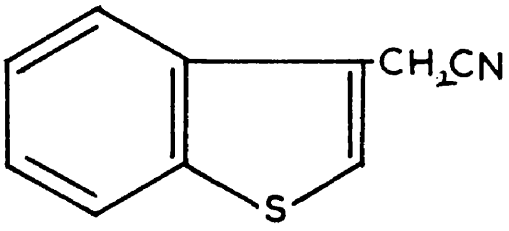
A study of the nucleophilic displacement of the methanesulphonyloxy group by cyanide ion was first made on the model compound, 3-methanesulphonyloxymethylthionaphthen (LIX) which was prepared from thionaphthen-3-carboxylic acid by reduction with lithium aluminium hydride[106] followed by



LVIII



LIX



LX

treatment of the resulting 3-hydroxymethyl compound (LVIII) with one mole of methanesulphonyl chloride in dry, ice-cold pyridine. It was initially found that, on working up the product under aqueous conditions, the alcohol was recovered indicating that the ester was hydrolysing extremely readily. Hence completely anhydrous conditions were employed throughout and indeed a satisfactory yield of the methanesulphonate resulted. The finding that hydrolysis of the sulphonate ester was so facile was encouraging in view of the fact that cyanide ion is known to be a more powerful nucleophile than hydroxide ion,[107] and so displacement by cyanide ion would be expected to occur even more readily than hydrolysis. The methanesulphonation was conducted in a pyridine-benzene mixture and after 24 hours the reaction mixture was decanted from the precipitated pyridine hydrochloride and taken to dryness on the oil pump. By extraction with ether, the ester was separated from the residue as a low-melting solid which showed typical methanesulphonate absorptions[108] at 1170 and 1365 $cm.^{-1}$ in the infrared. On treating this product with an excess of dry sodium cyanide[109] in dry dimethyl sulphoxide[109,110] at 100°, a product showing cyanide absorption at 2230 $cm.^{-1}$ was obtained.

The dimethanesulphonate ester of 5-hydroxy-3-hydroxymethylthionaphthen was prepared from this diol in a fashion similar to that just described for the preparation of

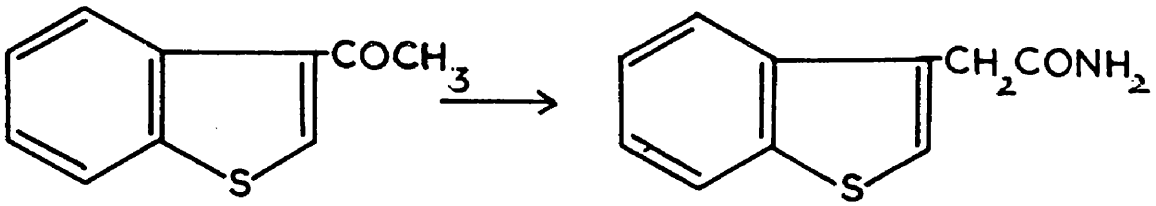
3-methanesulphonyloxymethylthionaphthen but employing two equivalents of methanesulphonyl chloride. The diester did not appear to be soluble in ether and so could not be completely separated from the pyridine hydrochloride. It would be expected that only the alcoholic methanesulphonate would be subject to nucleophilic attack by the cyanide ion, and that the phenolic hydroxyl group would be regenerated either during the aqueous work up or, if necessary, by subsequent alkaline hydrolysis. On treatment of the dimethanesulphonate with sodium cyanide in dimethyl sulphoxide, a product was obtained which, as well as showing hydroxyl absorption, also exhibited carbonyl absorption at 1700 $cm.^{-1}$ and split cyanide absorption at 2100 and 2150 $cm.^{-1}$ The presence of the two cyanide bands may be interpreted by analogy with the finding[71] that the reaction of N-methylgramine methiodide with sodium cyanide yields both the expected product, 3-cyanomethyl-N-methylindole, and a proportion of 2-cyano-1,3-dimethylindole. The split cyanide band was therefore attributed to the presence of a cyanide function in the 2-position (in conjugation with the aromatic system) and a second cyanide function not in such conjugation -- the required 3-cyanomethyl group. The presence of the carbonyl absorption may be accounted for in terms of the known reaction between cyanides and phenols to form phenolic ketones[111] (Hoesch synthesis).

A second approach to the synthesis of the thionaphthen analogue of 5-hydroxytryptamine was therefore attempted, the the starting material again being 5-hydroxy-3-hydroxymethyl-thionaphthen (XLVI). Oxidation of the benzylic alcohol function to the aldehyde group with "Attenburrow"[112] manganese dioxide appeared to proceed smoothly as evidenced by infrared analysis of the product (carbonyl absorption at 1680 $cm.^{-1}$) but no attempt was made to characterise the aldehyde (XLVIII) further in view of the possibility of autoxidation or the occurrence of a Cannizarro reaction. The crude product was instead treated with an excess of nitromethane in the presence of piperidine at 100°. Only high-melting relatively insoluble material could however be isolated from this reaction. The condensation of aldehydes with nitroalkanes[113,114] and reactive methylene groups[115] has been extensively studied and it has been pointed out[114] that generalisations as to the best condensing agent for any one reaction cannot be made in advance. In order to obtain an acceptable yield of the nitrostyrene, it is necessary to run a series of reactions with a variety of condensing agents, a distinct disadvantage where a rare aldehyde is concerned as in this case. Good yields of the desired product are obtained only if it is sufficiently insoluble (or is present in sufficient concentration) to precipitate out from the reaction mixture. If this does not occur,

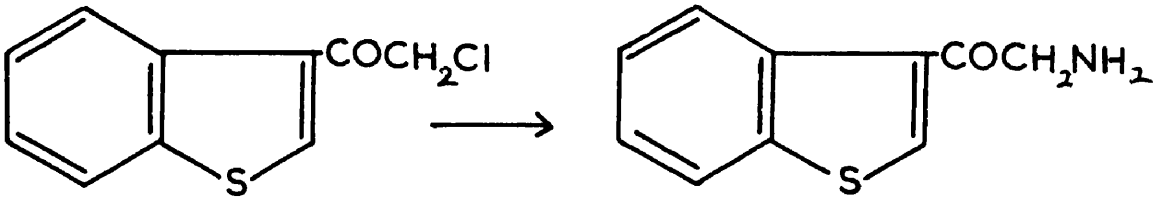
trimers are likely to result.[114,116] It therefore seemed very likely that polymerisation had occurred in the present case. Piperidine was chosen as condensing agent since it gave rise to the most rapid generation of colour in model experiments for the condensation of 3-acetylthionaphthen with nitromethane.

Attention was then turned to 3-cyano-5-nitrothionaphthen (LII) as a possibly suitable intermediate, this compound being available from 3-bromo-5-nitrothionaphthen (XL).[55] Reduction with sodium borohydride in the presence of palladised charcoal[117] yielded the amine (LI) which on diazotisation formed 3-cyano-5-hydroxythionaphthen (LII). This compound was hydrolysed in poor yield to the known 5-hydroxythionaphthen-3-carboxylic acid (XLV) by refluxing with concentrated sodium hydroxide for four days.

Several methods are available for the conversion of cyanides to aldehydes, the best known of which is the Stephen reaction.[118] Other methods involve the selective reduction of the cyanide to the imine which is then hydrolysed to the aldehyde.[119] This reduction is usually effected by one third mole of lithium aluminium hydride at -80°[120] or by milder reducing agents such as sodium triethoxyaluminohydride,[121] lithium triethoxyaluminohydride[122] and diethyl- and diisobutylaluminium hydride.[123] Other reduction procedures involve the use of sodium hypophosphite in the



LXI LXIV



LXII LXIII

presence of Raney nickel[124] or hydrogenation under pressure in the presence of phenylhydrazine.[125] The cyanide may also be converted to the ortho-ester[126] which can be reduced to the aldehyde with one quarter mole of lithium aluminium hydride.[127]

On application of the Stephen reaction to 3-cyano-5-hydroxythionaphthen (LII), only starting material was recovered. Attempted reduction of the cyano compound with one third mole of lithium aluminium hydride at -80° or in refluxing ether or tetrahydrofuran overnight similarly gave only a quantitative yield of starting material. It was therefore apparent that the 3-cyano group in the thionaphthen series is extremely unreactive since the comparatively powerful reducing properties of lithium aluminium hydride were unable to effect any reduction overnight at reflux temperatures. No recourse was therefore made to milder reducing agents such as sodium triethoxyaluminohydride.

Attention was then focussed on yet another potential method for the introduction of the β-aminoethyl side chain. 3-Acetylthionaphthen (available by a Friedel-Crafts acylation of thionaphthen[128]) was chlorinated by the method of Emerson[129] to give 3-chloroacetylthionaphthen (LXII) which on treatment with hexamine gave the hexamine complex. This compound on treatment with concentrated hydrochloric acid gave the amino-ketone (LXIII) in 10% overall yield

from 3-acetylthionaphthen. In view of the relatively small yields obtained in the above model experiments, a Willgerodt reduction[130] of 3-acetylthionaphthen was attempted as an alternative method of introducing the required side chain. By heating the ketone with ammonium polysulphide and pyrdine or dioxan in a sealed tube for four hours, a 37% yield of thionaphthen-3-acetamide (LXIV)[131] was obtained. A method of introducing the β-aminoethyl side chain in three stages was therefore available since reduction of the amide with lithium aluminium hydride would afford the corresponding amine.[87]

The acylation of 5-acetoxythionaphthen (LV) under Friedel-Crafts conditions was next investigated, 5-acetoxythionaphthen being available by a 3-stage synthesis from 5-nitrothionaphthen (XXXIX).[105] On acylation with acetyl chloride in carbon disulphide in the presence of aluminium chloride, a crystalline solid was obtained which showed both ketonic and ester absorptions in the infrared spectrum. Base hydrolysis of this product yielded both 5-hydroxythionaphthen (LIV) and a compound which analysed correctly for an acetyl 5-hydroxythionaphthen. The infrared spectrum of this compound in carbon tetrachloride solution showed two hydroxyl bands at 3602 and 3295 $cm.^{-1}$ and two carbonyl bands at 1673 and 1652 $cm.^{-1}$ indicating that hydrogen-bonding was occurring between these two groups. Although

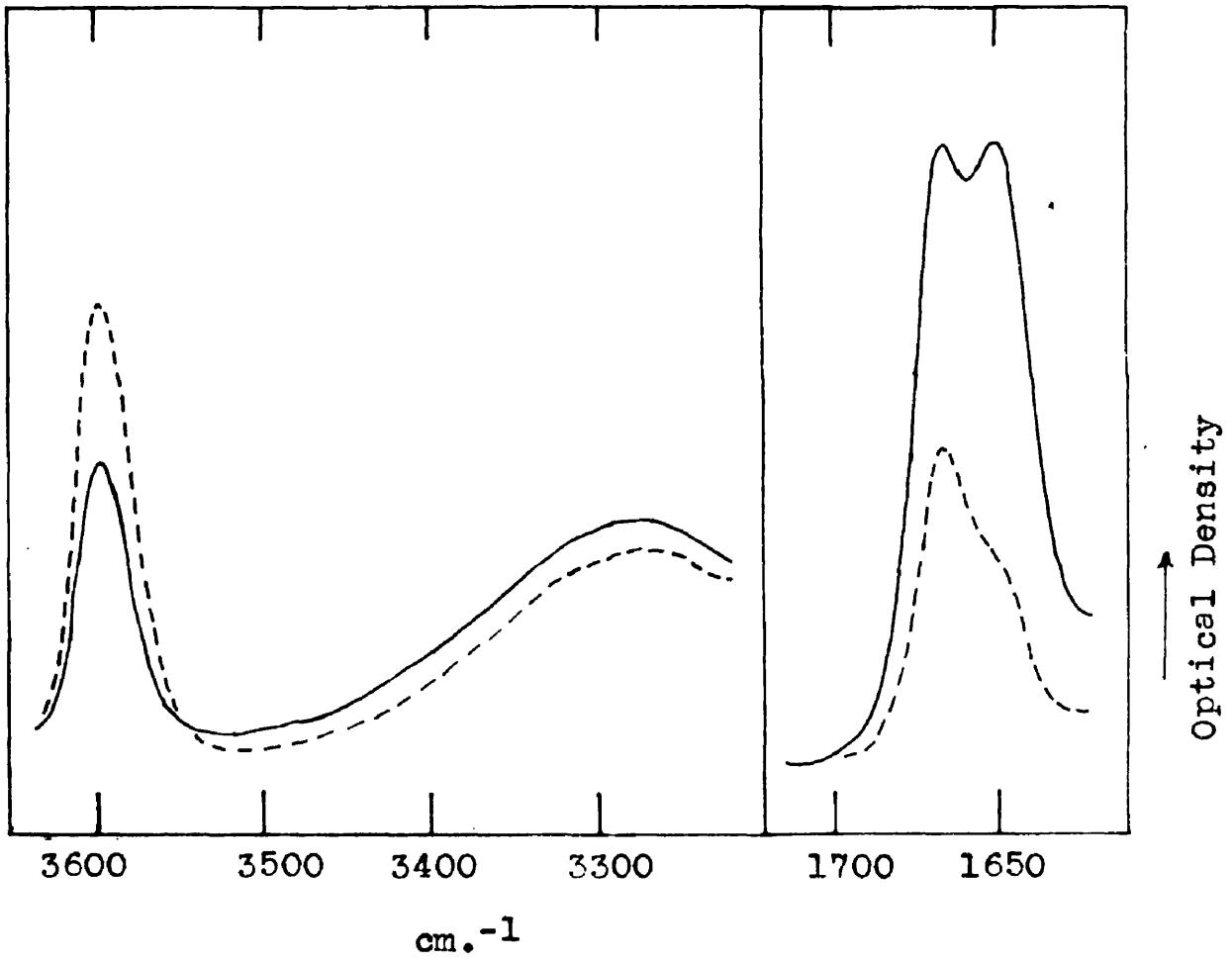


Figure 1. Hydroxyl and carbonyl absorptions of 3-acetyl-5-hydroxythionaphthen (1) in $CHCl_3$. —— saturated solution. - - - 1:4 dilution of saturated solution.

this type of hydrogen bond formation was initially observed with acetyl-5-hydroxythionaphthen (1) it was also seen with 3-methoxycarbonyl-5-hydroxythionaphthen (2), 3-cyano-5-hydroxythionaphthen (3) and to a lesser extent with 3-nitro-5-hydroxythionaphthen (4). The infrared data for cpds. 1-4 are given in Table I and illustrated in Figure 1.
In the case of compound 5, only the intramolecular OH...Br bond is observed at the concentration examined.[132] That hydrogen-bonding is occurring intermolecularly (by dimer formation) rather than intramolecularly is illustrated by the fact that the ratios of the extinction coefficients of non-bonded to bonded hydroxyl and non-bonded to bonded carbonyl are concentration dependent. The dimerisation still persists in chloroform although this solvent always tends to reduce the proportion of dimeric species in solution by preferential solvation of the more polar monomer.

This evidence is regarded as support for the existence of dimers such as LXV involving 16-membered rings whose association persists at dilutions in carbon tetrachloride well below those normally known to permit of intermolecular hydrogen-bonding (ca. 50 mM. for phenols). The existence in solution of stable dimers involving medium-sized rings closed by hydrogen bonds has been known for some time. For example, Bellamy and Rogasch[133] have discussed proton transfer in hydrogen-bonded dimers of the dimedone type (LXVI), and

similar observations of 12-membered ring formation have been made in the case of steroidal β-diketones.[134] A related instance of ring formation has been postulated for the anticoagulent drug dicoumarol[135] where the two enolised systems, both being part of the same molecule, can be held in close proximity by the methylene bridge, and an analogous situation pertains in certain heterocyclic phenols in the solid state.[136] There seems to be no obvious reason why larger rings should not be equally stable and in fact the known ring size has recently been increased to 14 with the finding that meta-nitrophenols and meta-methoxycarbonyl-phenols are capable of self-association in solution.[137] The present findings now increase the known ring size to 16.

The principal requirement for relatively stable dimer formation must be the presence of suitably disposed acidic and basic centres within the individual molecule. Ideally, the molecular framework should be such that there is no impediment to two molecules taking up the disposition shown in LXVII where the B...X separation is the normal hydrogen-bonding distance (eg. ca. 2.8 A for O...O), and the hydrogen atoms are oriented for favourable interaction with the lone pair electrons of the basic centre B. Although the correct orientation of very elongated molecules of type LXVII would be somewhat more difficult to achieve than that of their shorter counterparts, it is to be anticipated that

dimerisation will still persist, for example, in suitable diterminally-substituted aromatic systems, steroids, triterpenes and alkaloids. In many such cases, however, bulky substituents projecting from the face of the molecule might be expected to hinder dimer formation. Even in relatively flexible systems such as the di- and trinuclear novolaks, however, the existence of well-defined conformations where dimerisation permits closure of a ring of hydrogen bonds has been demonstrated.[138]

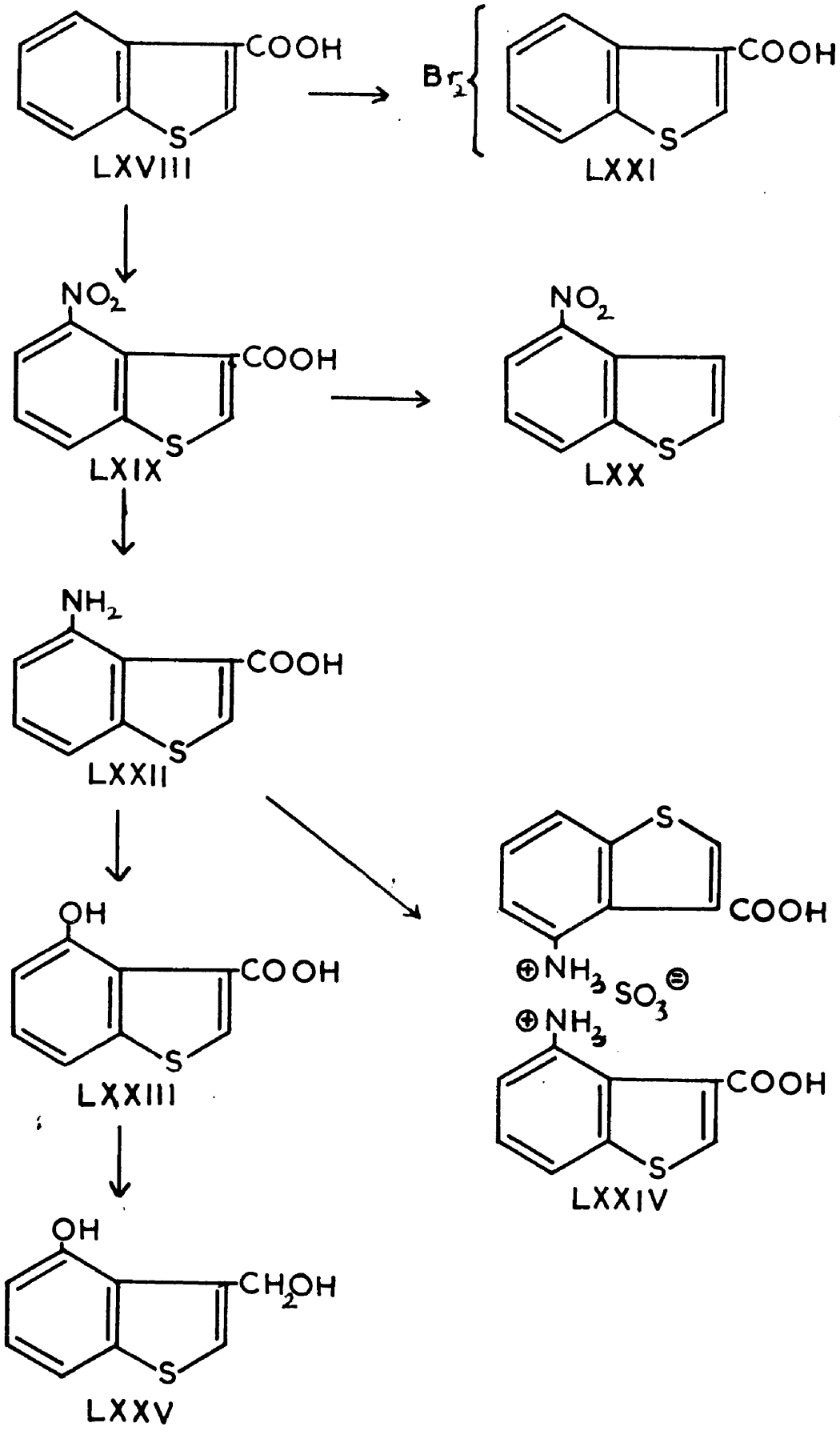
Although the position in polar media such as biological fluids is certainly different from that pertaining in organic solvents such as carbon tetrachloride and chloroform, the existence of stable 12-, 14- and 16-membered hydrogen-bonded dimers in the latter provides new avenues for the interpretation of drug-receptor interaction. It is conceivable that such macrocyclic hydrogen-bonded rings formed between a drug molecule and a tissue protein play an important role in drug action.

The acylation of 5-acetoxythionaphthen might conceivably occur in the 2-, 3-, 4-or 6-positions. In fact 5-acetoxythionaphthen is known to brominate in the 3-position[105] and thionaphthen itself acylates both in the 2- and 3-positions.[128,139] In the instance under discussion (acetyl-5-hydroxythionaphthen), the acetyl group cannot be in the 4- or 6-positions since this would enable it to bond

intramolecularly with the hydroxyl group. Its presence in the 2-position is likewise unlikely since it is difficult to see how dimer formation such as shown in LXVII could occur. It has been reported[140] that 2- and 3-acetylthionaphthens can be distinguished by the frequency of the carbonyl absorptions, the 3-acetyl compounds absorbing some 15 $cm.^{-1}$ higher than the 2-isomers due to a decreased degree of conjugation with the benzene ring. In the present work, however, it has been found that both 2- and 3-acetyl-thionaphthen absorb at 1675 $cm.^{-1}$

Application of the Willgerodt reaction to the mixture obtained from the Friedel-Crafts acylation yielded 3-acetyl-5-hydroxythionaphthen as the only water-soluble product. It since been found that the Willgerodt reaction also fails with 3-acetyl-5-hydroxybenzofuran.[141]

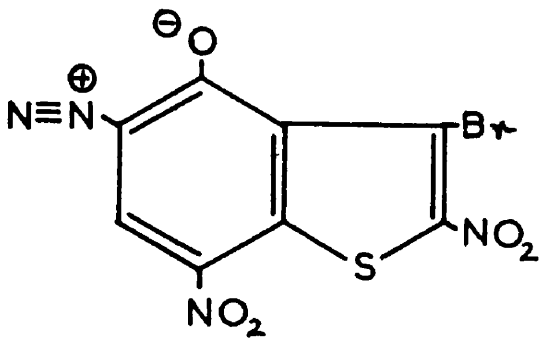
Several other methods to introduce the β-ethylamine side chain were attempted without success. Grignard reactions with ethylene oxide[102] on both 3-bromo-5-nitro-thionaphthen (XL) and 3-bromo-5-hydroxythionaphthen (XLII) failed to give any of the corresponding 3-(2'-hydroxyethyl) derivatives. An attempted nucleophilic displacement with cyanoacetic ester in the presence of potassium-t-butoxide on 3-bromo-5-nitrothionaphthen gave only intractable material. 5-Nitrothionaphthen neither chlormethylated nor did it form any 5-nitrothionaphthen-3-aldehyde on treatment with


COOH
S
LXVIII
Br2
COOH
S
LXXI
NO2
COOH
S
LXIX
NO2
S
LXX
NH2
COOH
S
LXXII
OH
COOH
S
LXXIII
OH
CH2OH
S
LXXV
S
COOH
⊕NH3 SO3⊖
⊕NH3
COOH
S
LXXIV

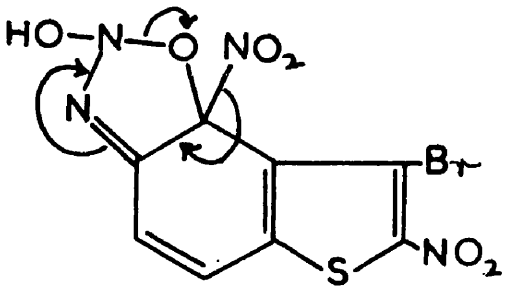
equimolar proportions of dimethylformamide and phosphorus oxychloride.

Of other methods theoretically available for introducing the required side chain into the thionaphthen ring system, it would seem that a Grignard reaction with chloracetonitrile[77-80] or ethyleneimine[82] on 3-bromo-5-hydroxythionaphthen (in which the hydroxyl group is protected as the methyl or benzyl ether) should be attempted in any further work to be undertaken in this field.

As an approach to the synthesis of the thionaphthen isostere of 4-hydroxytryptamine, the preparation of certain 3,4-disubstituted thionaphthens capable of serving as suitable intermediates was investigated. 3-Nitrothionaphthen is known to nitrate predominantly in the 4-position[142] so an investigation of the nitration product from thionaphthen-3-carboxylic acid was undertaken. Nitration of thionaphthen-3-carboxylic acid (LXVIII) with one mole of fuming nitric acid was found to give a mixture of products in which 4-nitrothionaphthen-3-carboxylic acid (LXIX) predominated. The structure of this compound was proved by decarboxylation to the known 4-nitrothionaphthen (LXX),[142] comparison being made with an authentic specimen. Monobromination of thionaphthen-3-carboxylic acid in acetic acid, on the other hand, was found to yield a mixture of a dibrominated derivative (LXXI) and unreacted starting



LXXVI



LXXVII

material. Bromination with an excess of bromine in the absence of solvent afforded the same dibromo derivative.

4-Nitrothionaphthen-3-carboxylic acid (LXIX) on reduction with stannous chloride yielded 4-aminothionaphthen-3-carboxylic acid (LXXII) which on diazotisation formed 4-hydroxythionaphthen-3-carboxylic acid (LXXIII). In view of the poor yields obtained in the diazotisation step, an attempt was made to form the phenolic acid by a Bucherer reaction[143] on the amine. The only compound isolated from the reaction mixture, however, proved to be the diamine sulphite (LXXIV). Reduction of 4-hydroxythionaphthen-3-carboxylic acid with lithium aluminium hydride gave 4-hydroxy-3-hydroxymethylthionaphthen (LXXV).

During previous work in this department,[55] the formation of a product of unknown constitution was observed during the nitration of 5-acetamido-3-bromothionaphthen with an excess of nitric acid in acetic acid. The compound was orange in colour and explosive and contained no acetyl group but showed strong infrared absorption at 2143 $cm.^{-1}$. A crystalline sample was supplied to Dr. G. A. Sim and Mr. C. C. Scott of the Department of Chemistry who, by means of X-ray crystallographic studies, showed the compound to be 3-bromo-2,7-dinitrothionaphthen-5-diazo-4-oxide (LXXVI). The third three-dimensional electron density distribution, shown in Figure 2 by means of superimposed contour sections

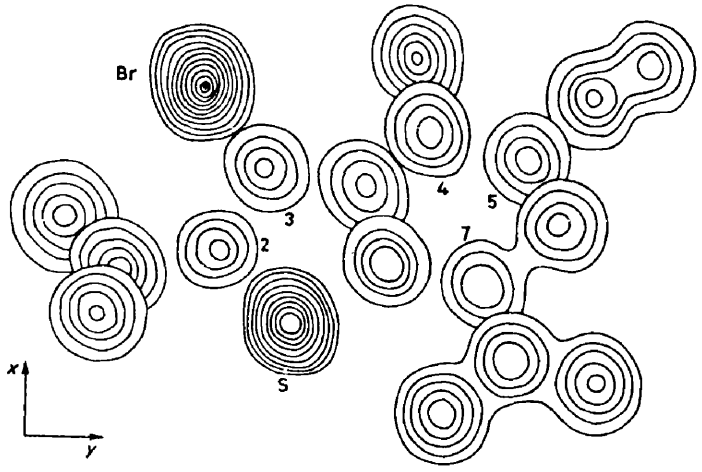


FIGURE. 2.
The third three-dimensional electron-density distribution for 3-bromo-2, 7-dinitrothionaphthen-5-diazo-4-oxide shown by means of superimposed contour sections drawn parallel to (001)*—contour levels arbitrary.*

drawn parallel to (001), shows clearly that the diazo nitrogen atoms are not involved in an oxadiazole ring, thus confirming deductions as to the structure of ortho diazo-oxides based on spectral evidence[144] and dipole moment studies.[145]
Despite the lack of precedent in the literature, ortho diazo-oxide formation through the direct action of nitric acid and without the intervention of nitrous acid may be a general phenomenon. The presence of acetic acid is not essential, and ortho diazo-oxide formation was observed on heating 5-acetamido-3-bromothionaphthen, 5-acetamido-3-bromo-4-nitrothionaphthen, 5-amino-3-bromo-4-nitrothionaphthen and 2-acetamido-1-nitronaphthalene in concentrated nitric acid, either alone or in the presence of an excess of urea. It was not observed, however, with acetanilide.

A suitable mechanism would involve intercession of a five membered cyclic intermediate (LXXVII) arising from the corresponding nitramine. Substitution at position 7 would follow the formation of 3-bromo-2-nitrothionaphthen-5-diazo-4-oxide from LXXVII in accordance with the known substitution characteristics of ortho diazo-oxides.[146]

This work on ortho diazo-oxide formation has been published in Chemistry and Industry, a reprint being included as Appendix 3.

Experimental.

3-(Methanesulphonyloxymethyl)-thionaphthen (LIX). 3-Hydroxymethylthionaphthen (0.56 g.) was dissolved in dry pyridine (1 ml.) and dry benzene (10 ml.) added. To this solution at 0 was added dropwise methanesulphonyl chloride (0.4 ml.; 1.5 mole) and after standing at 0° for 24 hours, the supernatant liquid was decanted from the precipitated pyridine hydrochloride and taken to dryness at 0° by means of an oil pump overnight. The residue was extracted with cold sodium-dried ether to give a low-melting solid (0.3 g.) showing methanesulphonate absorptions at 1170 and 1365 $cm.^{-1}$

This compound (0.3 g.), after heating with dry sodium cyanide (300 mg.) in dry dimethyl sulphoxide (10 ml.) on the steam bath for 1.5 hours and cooling gave a red oil (0.1 g.) showing cyanide absorption at 2220 $cm.^{-1}$ when the reaction mixture was poured into water and extracted with ethyl acetate.

3-(Methanesulphonyloxymethyl)-5-methanesulphonyloxy-thionaphthen. 5-Hydroxy-3-hydroxymethylthionaphthen (0.06 g.) in a pyrdine-benzene mixture was treated with two moles of methanesulphonyl chloride as above. The residue (0.1 g.) was not soluble in ether.

The diester-pyridine hydrochloride mixture (0.1 g.) was taken up in dry dimethyl sulphoxide and heated with dry

sodium cyanide (0.3 g.) on the steam bath for 1.5 hours. The reaction mixture was poured into water and extracted with ethyl acetate to yield a red oil (0.015 g.) showing carbonyl absorption at 1700 $cm.^{-1}$ and split cyanide absorption at 2100 and 2150 $cm.^{-1}$

5-Hydroxythionaphthen-3-aldehyde (XLVIII). 5-Hydroxy-3-hydroxymethylthionaphthen[55] (0.13 g.) in chloroform (150 ml.) was shaken for one hour with freshly ground "Attenburrow"[112] manganese dioxide. On filtering and taking the filtrate to dryness, a greenish-yellow solid (0.085 g.) showing carbonyl absorption at 1680 $cm.^{-1}$ was obtained.

Condensation with nitromethane. The above solid was dissolved in nitromethane (5 ml.) and piperidine (2 drops) added. After heating at 100 for one hour, the red solution was reduced in volume to 1 ml. and upon cooling, a solid (0.025 g.) was deposited. It was soluble in sodium hydroxide, nitromethane and dimethylformamide but insoluble in the common organic solvents.

5-Amino-3-cyanothionaphthen (LI). Sodium borohydride (0.35 g.) was dissolved in water and mixed with a suspension of 10% palladium on charcoal (0.1 g.) in water. To this mixture at room temperature was added 3-cyano-5-nitro-thionaphthen (0.873 g.) in tetrahydrofuran (30 ml.) over 0.5 hour with magnetic stirring. After this time, a further amount of sodium borohydride (0.1 g.) was added and the

mixture stirred for a further hour. The charcoal was removed by filtration and the clear filtrate acidified with 6N-hydrochloric acid and then neutralised with sodium carbonate. The neutral solution was extracted with ethyl acetate (25, 10 ml.) and the combined extracts washed with 6N-hydrochloric acid (15 ml.). The mixture was filtered to remove the precipitated amine hydrochloride and the layers were separated. The precipitated salt was added to the aqueous layer, 4N-sodium hydroxide (10 ml.) and ether (25 ml.) added, and the recovered amine extracted into the organic solvent. A sample of 5-amino-3-cyanothionaphthen crystallised from benzene as needles, m.p. 134.5-135°. The ether solution of the amine was reduced in volume to 5 ml., alcohol (10 ml.) and concentrated hydrochloric acid (3 ml.) added and 5-amino-3-cyanothionaphthen hydrochloride (0.717 g; 80%) collected by filtration. Recrystallised from water as feathery needles, m.p. 214-216°. (Found: C, 51.3; H, 3.7. $C_9H_7N_2SCl$ requires C, 51.3; H, 3.4%).

3-Cyano-5-hydroxythionaphthen (LII). 5-Amino-3-cyano-thionaphthen hydrochloride (0.5 g.) was dissolved in boiling water and the solution cooled rapidly to 5° to precipitate the hydrochloride as fine needles. Concentrated hydrochloric acid (5 ml.) was added and then a solution of sodium nitrite (0.164 g.) in water (20 ml.) dropwise with stirring over 0.5 hour. The suspension was stirred at 5°

for two hours and placed in the refrigerator overnight, after which time all the solid had dissolved. The solution of the diazonium salt was heated under nitrogen on the steam bath for two hours, filtered and the cooled red filtrate extracted with ethyl acetate (75, 25 ml.). The organic extract was washed with 4N-sodium hydroxide and the aqueous extract clarified with charcoal and acidified with concentrated hydrochloric acid to yield 3-cyano-5-hydroxythionaphthen (0.242 g.; 59%) which crystallised as needles from methanol/water and was sublimed at 130° for analysis. m.p. 196-198°. (Found: C, 61.5 ; H, 3.0 . C_9H_5NOS requires C, 62.4; H, 2.9%).

5-Hydroxythionaphthen-3-carboxylic acid (XLV). 3-Cyano-5-hydroxythionaphthen (0.05 g.) was refluxed with sodium hydroxide (5 g.) in water (10 ml.) for four days. The resulting solution was diluted, acidified with hydrochloric acid and extracted with ethyl acetate (15 ml.). The organic layer was washed with sodium carbonate, this extract being acidified with hydrochloric acid to give material (0.01 g.; 18%) identical with authentic 5-hydroxythionaphthen-3-carboxylic acid.[55] Starting material (0.016 g.) was recovered by extraction of the organic layer with sodium hydroxide.

3-Chloroacetylthionaphthen - hexamine complex. To a magnetically stirred solution of hexamine (1.62 g.) in

chloroform (10 ml.) at 50° was added 3-chloroacetylthionaphthen[129] (2.223 g.) in hot chloroform (15 ml.). After 15 minutes, the solution turned pink and a precipitate of white needles began to separate. The suspension was held at 50 for four hours, then chilled at −15° overnight. The complex (1.22 g.; 33%) was filtered off, washed with a little chloroform and dried.

3-Aminoacetylthionaphthen hydrochloride (LXIII). The above 3-chloroacetylthionaphthen - hexamine complex (0.83 g.) was suspended in ethanol (5 ml.) and concentrated hydrochloric acid (2.5 ml.) and stirred overnight at room temperature. The solid was filtered off and the cake suspended in water (5 ml.) into which a considerable amount dissolved leaving a grey-white residue. This solid was collected by filtration and recrystallised from water containing a little hydrochloric acid to give plates of 3-aminoacetylthionaphthen hydrochloride, (0.2 g.; 37%) m.p. 231-233°(d). (Found: C, 52.7; H, 4.7. $C_{10}H_{10}ClNSO$ requires C, 52.7; H, 4.4%.)

Thionaphthen-3-acetamide (LXIV). 3-Acetylthionaphthen (1.0 g.), sulphur(1.5 g.), pyridine (2.5 ml.) and concentrated (0.880) ammonia (2.5 ml.) were heated in a sealed tube at 163-165° for four hours. After allowing to cool, the tube was opened and the contents removed and taken to dryness. Extraction with boiling water afforded a buff-coloured residue of thionaphthen-3-acetamide (0.40 g.;

37%). Recrystallised from water to give plates, m.p. 171-174°. (lit.,[131] m.p. 171-173°).

3-Acetyl-5-hydroxythionaphthen (LVI). To 5-acetoxythionaphthen (0.40 g.) dissolved in anhydrous carbon disulphide (20 ml.) was added anhydrous aluminium chloride (0.28 g.) and redistilled acetyl chloride (0.148 ml.). The mixture was refluxed for two hours under anhydrous conditions, the aluminium chloride becoming gummy and fumes of hydrogen chloride being evolved. Water, 6N-hydrochloric acid and ether were then added, the layers separated and the organic layer washed with water and dried over sodium sulphate. On removal of the ether there remained an oil (0.406 g.) which crystallised on scratching. This product (0.5 g.) was heated with sodium hydroxide in aqueous methanol for 0.5 hour to give material (0.25 g.) of which 0.15 g. was soluble in petroleum ether and was identified as 5-hydroxythionaphthen. The insoluble residue was crystallised from dilute methanol as needles, m.p. 191-191.5°. (Found: C, 62.4; H, 4.2. $C_{10}H_8O_2S$ requires C, 62.4; H, 4.2%.).

4-Nitrothionaphthen-3-carboxylic acid (LXIX). To thionaphthen-3-carboxylic acid (1.4 g.) in glacial acetic acid (17.5 ml.) containing sulphuric acid (1.75 ml.) was added fuming nitric acid (0.385 ml.) and the mixture was heated to 60° for two hours. After cooling, the red solution was poured into water(250 ml.) and the yellow solid

was filtered off, washed with copious amounts of water and dried (1.2 g.). A specimen was repeatedly crystallised from acetic acid to give light-yellow needles of 4-nitrothionaphthen-3-carboxylic acid, m.p. 263-265° after sublimation at 200°. (Found: C, 48.4; H, 2.7. $C_9H_5NO_4S$ requires C, 48.4; H, 2.3%.).

4-Nitrothionaphthen (LXX). 4-Nitrothionaphthen-3-carboxylic acid (1.08 g.) and copper bronze (4 g.) in freshly distilled quinoline (30 ml.) were heated at 180 for one hour under nitrogen. After this time, no more effervescence was apparent and the mixture was allowed to cool under nitrogen and diluted with ether. After removal of the copper bronze and ether, the quinoline solution was poured into 6N-sulphuric acid (300 ml.). A solid was precipitated; from light petroleum it formed needles (0.6 g.; 69%), m.p. 84-85°. Mixed m.p. with an authentic sample 84-85.5°. The infrared spectra in KCl disc were identical.

4-Aminothionaphthen-3-carboxylic acid (LXXII). Finely powdered 4-nitrothionaphthen-3-carboxylic acid (1 g.) was added to a well-stirred solution of stannous chloride (6 g.) in concentrated hydrochloric acid (6 ml.) at 50°. After two hours stirring, the material was largely converted to colourless crystals of the tin double salt of the amine which, after cooling, were collected by filtration and

dissolved in boiling water (130 ml.), insoluble material being separated after cooling by centrifugation. The supernatant liquid was made alkaline with sodium hydroxide and acidified with acetic acid, the white flocculent precipitate which then appeared being removed by centrifugation. The supernatant liquid was extracted three times with ethyl acetate (20, 10, 10 ml.), the combined extracts taken to dryness and the residue extracted with boiling acetone. Addition of 6N-sulphuric acid to this acetone extract produced a flocculent precipitate of the amine sulphate (0.49 g.; 45%). A sample of the free amine, 4-aminothionaphthen-3-carboxylic acid, was recrystallised from acetic acid and sublimed as needles, m.p. 223-226°(d). (Found: C, 55.6; H, 3.5. $C_9H_7NO_2S$ requires C, 55.9; H, 3.7%.).

4-Hydroxythionaphthen-3-carboxylic acid (LXXIII). 4-Aminothionaphthen-3-carboxylic acid sulphate (3.0 g.) was dissolved in boiling water (800 ml.), the solution cooled rapidly to 5° to precipitate the sulphate as fine needles and concentrated sulphuric acid (5 ml.) added. To this suspension was added sodium nitrite (0.687 g.) in water (60 ml.) over one hour with stirring, and stirring was then continued for a further three hours. After standing in the refrigerator overnight, the suspension was heated on the steam bath under nitrogen for 1.5 hours and the precipitated

solid removed by filtration. The cooled filtrate was extracted with ethyl acetate (60, 20 ml.), the organic layer extracted with 4N-sodium hydroxide (15 ml.) and the alkaline solution charcoaled. After acidification with concentrated hydrochloric acid and cooling to 0°, the precipitate of 4-hydroxythionaphthen-3-carboxylic acid was removed by filtration (0.356 g.; 18%); recrystallised from acetic acid then water as needles; sublimed at 160° m.p. 206-208°. (Found: C, 55.9; H, 3.3. $C_9H_6O_3S$ requires C, 55.7; H, 3.1%.).

Attempted Bucherer reaction on 5-aminothionaphthen-3-carboxylic acid. 5-Aminothionaphthen-3-carboxylic acid (1 g.) was refluxed for 48 hours in 40% sodium bisulphite solution (100 ml.) with stirring to prevent bumping. The amino acid passed into solution after 3 hours but as the reaction proceeded, solid material separated. On completion of the reaction, the solution was diluted to 150 ml. with water and excess solid sodium hydroxide added. The solution was boiled for one hour with stirring to prevent bumping and at the end of this time, the smell of ammonia could no longer be detected. The cooled solution was filtered, acidified with 6N-hydrochloric acid, filtered again and to the filtrate was added concentrated hydrochloric acid (30 ml.). After standing for some time, the solution deposited fine needles (0.2 g.) which were quite soluble in

water and were recrystallised from dilute acetic acid and then sublimed. m.p. 270° (d). (Found: C, 48.1; H, 3.4; N, 6.3. $(C_9H_7NO_2S)_2H_2SO_3$ requires C, 46.1; H, 3.4; N, 6.0%). Base regenerated 5-aminothionaphthen-3-carboxylic acid.

4-Hydroxy-3-hydroxymethylthionaphthen (LXXV). To 5-hydroxythionaphthen-3-carboxylic acid (0.05 g.) in dry, redistilled tetrahydrofuran (25 ml.) was added lithium aluminium hydride (0.1 g.) and the suspension refluxed overnight. Excess of lithium aluminium hydride was decomposed by careful addition of water and after reducing the solution in volume, ethyl acetate and 6N-hydrochloric acid were added and the layers separated. The ethyl acetate was washed, dried over sodium sulphate and the solvent removed by distillation. The residue of 4-hydroxy-3-hydroxymethylthionaphthen was recrystallised from benzene - ethyl acetate as needles and then sublimed. (0.035 g.; 70%), m.p. 174.5-176°. (Found: C, 60.3; H, 4.8. $C_9H_8O_2S$ requires C, 60.0; H, 4.5%.).

Dibromothionaphthen-3-carboxylic acid (LXXI). To thionaphthen-3-carboxylic acid (0.5 g.) in a porcelain crucible was added bromine (1 ml.). After the HBr and excess bromine had evaporated off, the dibromo compound (0.55 g.) was recrystallised from alcohol and sublimed, m.p. 285-289°. (Found: C, 32.2; H, 1.2. $C_9H_4Br_2O_2S$ requires C, 32.3; H, 1.5%.).

3-Bromo-2,7-dinitrothionaphthen-5-diazo-4-oxide (LXXVI). To 5-acetamido-3-bromothionaphthen (0.5 g.) in acetic acid (1 ml.) was added concentrated nitric acid (1 ml.) and the solution was heated on the steam bath. Copious red fumes were evolved and after 0.5 hour crystals appeared. After standing overnight, the solid was filtered off, washed several times with water and dried. (0.4 g.; 62%) explodes at 160°. (Found: Br, 23.2; N, 16.3. $C_8HBrN_4O_5S$ requires Br, 23.1; N, 16.3%.).

With concentrated sulphuric acid the compound gave a deep crimson solution, deepening with heating. With concentrated hydrochloric acid and resorcinol, the compound gave a bright red solution which on standing precipitated a red solid.

The presence of acetic acid was not essential, and diazo-oxide formation was observed on heating 5-acetamido-3-bromothionaphthen, 5-acetamido-3-bromo-4-nitrothionaphthen, and 5-amino-3-bromo-4-nitrothionaphthen in concentrated nitric acid, either alone or in the presence of an excess of urea.

References.

1. Ehrlich and Morgenroth, "Studies in Immunity," John Wiley & Sons, New York, 1910.
2. van Rossum and Ariens, Experientia, 1957, 13, 161.
3. Ariens, van Rossum and Simonis, Pharmacol. Rev., 1957, 9, 218.
4. Nachmansohn, Science, 1961, 134, 1962.
5. Chagas in "Curare and Curare-like Agents," ed. Bovet, Bovet-Nitti and Marini-Bettolo, Elsevier Publ. Co., Amsterdam, 1959, p. 327.
6. Ehrenpreis, Science, 1959, 129, 1613; Fed. Proc., 1959, 18, 220; Biochim. Biophys. Acta, 1960, 44, 561; Hasson, ibid., 1962, 56, 275; Nistratova and Turpaev, Biokhimiya, 1961, 26, 952; Chem. Abs., 1962, 56, 1860e.
7. Martin-Smith and Reid, J. Medicin. Pharmaceut. Chem., 1959, 1, 507.
8. Beckett and Casy, J. Pharm. Pharmacol., 1954, 6, 986.
9. Nachmansohn and Wilson, Adv. Enzymol., 1951, 12, 259; Krupka and Laidler, J. Amer. Chem. Soc., 1961, 83, 1458.
10. Long, Luduena, Tullar and Lands, J. Pharmacol., 1956, 117, 29; Long and Lands, ibid., 1957, 120, 46; Beckett, Harper, Clitherow and Lesser, Nature, 1961, 189, 671.
11. Waser, J. Pharm. Pharmacol., 1960, 12, 577.
12. Koshland, Proc. Nat. Acad. Sci. U.S.A., 1958, 44, 98.

13. Gill, Proc. Roy. Soc., 1959, B150, 381.

14. Beckett in "Progress in Drug Research," ed. Jucker, Birkhauser Verlag, Basel, 1959, Vol. 1, p. 455.

15. Pfeiffer, Science, 1948, 107, 94; Ing, ibid., 1949, 109, 264; Schueler, Arch. intern. Pharmacodyn., 1953, 93, 417.

16. Ferguson, Proc. Roy. Soc., 1937, B127, 387.

17. Mullins in "Molecular Structure and Functional Activity of Nerve Cells," ed. Grenell and Mullins, Amer. Inst. Biol. Sci., Washington, D.C., 1956, pp. 123-166.

18. Gaddum and Hameed, Brit. J. Pharmacol., 1954, 9, 240; Gaddum and Picarelli, ibid., 1957, 12, 323.

19. Ahlquist, J. Amer. Pharmaceut. Assoc.,(Sci. Edn.), 1959 48, 647; Furchgott in "Symposium on Catechol Amines," ed. Krayer, Williams and Wilkins Co., Baltimore, 1959, p. 429.

20. Beckett, J. Pharm. Pharmacol., 1952, 4, 425.

21. Janssen, "Synthetic Analgesics, Part 1, Diphenylpropylamines," Pergamon Press, London, 1960, pp. 118-119, 147-148, 154.

22. Wooley in "Progress in Drug Research," ed. Jucker, Birkhauser Verlag, Basel, 1960, Vol. 2, p. 613.

23. Stephenson, Brit. J. Pharmacol., 1956, 11, 379.

24. Rappoport, Ann. N. Y. Acad. Sci., 1957, 66, 665.

25. Langmuir, J. Amer. Chem.Soc., 1919, 41, 1543.

26. Erlenmeyer and Leo, Helv. Chim. Acta., 1932, 15, 1171.

27. Grimm, Z. Electrochem., 1925, 31, 474; 1928, 34, 430; Angew. Chem., 1929, 42, 367; Naturwiss., 1929, 17, 535, 557.

28. Friedman in "Symposium on Chemical-Biological Correlation," Nat. Acad. Sci. - Nat. Research Council, publ. No. 206, Washington, D.C., 1951, p. 295.

29. Schatz in "Medicinal Chemistry," ed. Burger, Interscience Publishers Inc., Ney York, 1960, 2nd. Edn., p. 72.

30. Belleau in "Ciba Symposium on Adrenergic Mechanisms," ed. Vane, Wolstenholme and O'Connor, J. & A. Churchill Ltd., London, 1960, p. 223; Belleau and Triggle, J. Medicin. Pharmaceut. Chem., 1962, 5, 636.

31. Seifriz, Science, 1948, 107, 15.

32. Lees and Simpson, Biochem. J., 1955, 59, Proc i.

33. Hinsberg, J. prakt. Chem., 1916, 93, 302

34. Vaughan, Anderson, Clapp, Clark, English, Howard, Marson, Sutherland and Denton, J. Org. Chem., 1949, 14, 228.

35. Bloch, Brubacher, Erlenmeyer, and Suter, Helv. Chim. Acta, 1947, 30, 539.

36. Fried, Cancer, 1957, 10 (4), 752; Fried and Bormann, Vitamins and Hormones, 1958, 16, 303.

37. Tabern and Volwiler, J. Amer. Chem. Soc., 1935, 57, 1961.

38. Reference 29, p. 367.

39. Hausen and Fosdick, J. Amer, Chem. Soc., 1933, 55, 2872.

40. Hofmann and Winnick, J. Biol. Chem., 1945, 160, 449; Krueger and Peterson., J. Bact., 1948, 55, 693.

41. Frohman and Day, J. Biol. Chem., 1949, 180, 93.

42. Adamson, Chem. and Ind., 1951, 2.

43. Gilman and Pickens, J. Amer. Chem. Soc., 1925, 47, 245.

44. Beckett, Casy and Phillips, J. Medicin. Pharmaceut. Chem., 1960, 2, 245.

45. Jones and Ainsworth, J. Amer. Chem. Soc., 1955, 77, 1538.

46. Prusoff and Welch, J. Biol. Chem., 1956, 218, 929.

47. Kidder and Dewey, Arch. Biochem. Biophys., 1957, 66, 486.

48. Robison and Robison, J. Amer. Chem. Soc., 1956, 78, 1247.

49. Halvorson, Spiegelman and Hinman, Arch. Biochem. Biophys., 1955, 55, 512; Robison and Robison, J. Amer. Chem. Soc., 1955, 77, 457.

50. Williamson, J. Chem. Soc., 1962, 2833.

51. Crook and Davies, J. Chem. Soc., 1937, 1697; Crook, Davies and Smith, Nature, 1937, 139, 154; Kefford and Kelso, Austral. J. Biol. Sci., 1957, 10, 80.

52. Avakian, Moss and Martin, J. Amer. Chem. Soc., 1948, 70, 3075.

53. Elliot and Harington, J. Chem. Soc., 1949, 1374.

54. Tuite and Snyder, J. Amer. Chem. Soc., 1960, 82, 4364; and references therein.

55. Reid, Ph.D. Thesis, University of Glasgow, 1960.

56. Nanjappa, Ph.D. Thesis, University of Glasgow, 1962.

57. Erspamer, Pharmacol. Rev., 1954, 6, 425; Page, Physiol. Rev., 1954, 34, 563; 1958, 38, 277; "5-Hydroxytryptamine," ed. Lewis, Pergamon Press, London, 1958; Udenfriend, Vitamins and Hormones, 1959, 17, 33; Crossland, J. Pharm. Pharmacol., 1960, 12, 1; Maupin, Psychopharmacology, Service Centre Bulletin, U.S. Dept. of Health, Education and Welfare, Public Health Service, December, 1961, pp. 15-57; Erspamer in "Progress in Drug Research," ed. Jucker, Birkhauser Verlag, Basel, 1961, Vol. 3, p. 51.

58. Gyermek, Pharmacol. Rev., 1961, 13, 399.

59. Vane, Brit. J. Pharmacol., 1959, 14, 87; Erspamer, Glasser and Mantegazzini, Experientia, 1960, 16, 505.

60. Hollister, Arch. intern. Pharmacodyn., 1961, 130, 42.

61. Foster, Ing and Rodgers, J. Chem. Soc., 1957, 1671.

62. Ainsworth, J. Amer. Chem. Soc., 1957, 79, 5245.

63. Comer, Diss. Abs., 1962, 23, 66.

64. Hartough and Meisel, "Compounds with Condensed Thiophene Rings," Interscience publ., Inc., New York, 1954, pp. 17-166.

65. Martin-Smith and Gates, J. Amer. Chem. Soc., 1956, 78, 5351, 6177.

66. Martin-Smith and Reid, J. Chem. Soc., 1960, 938.

67. Kuhn and Stein, Ber., 1937, 70, 567; Bell and Lindwall,

J. Org. Chem., 1948, 13, 547.

68. Hamlin and Fischer, J. Amer. Chem. Soc., 1951, 73, 5007.

69. Woodward, Cava, Ollis, Hunger, Daenicker and Schenker, J. Amer. Chem. Soc., 1954, 76, 4749.

70. Ek and Witkop, J. Amer. Chem. Soc., 1954, 76, 5579.

71. Snyder and Eliel, J. Amer. Chem. Soc., 1948, 70, 1703, 1857.

72. Ames, Ames, Coyne, Grey, Lockhart and Ralph, J. Chem. Soc., 1959, 3388.

73. Thesing and Schulde, Ber., 1952, 85, 324.

74. Snyder and Pilgrim, J. Amer. Chem. Soc., 1948, 70, 3770.

75. Terent'ev, Preobrazhenskaya and Ge, Khim. Nauka i Prom., 1959, 4, 281; Chem. Abs., 1959, 53, 21879c.

76. Wieland and Hsing, Annalen, 1936, 526, 188.

77. Majima and Hoshino, Ber., 1925, 58, 2042.

78. Wieland, Konz and Mittasch, Annalen, 1934, 513, 1.

79. Hoshino and Shimodaira, Annalen, 1935, 520, 19.

80. Speeter, Heinzelmann and Weisblat, J. Amer. Chem. Soc., 1951, 73, 5514.

81. Noland and Hartman, J. Amer. Chem. Soc., 1954, 76, 3227; Noland, Cristensen, Sauer and Dutton, ibid., 1955, 77, 456; Noland and Lange, ibid., 1959, 81, 1203.

82. Bucourt and Vigneau, Bull. Soc. chim. France, 1961, 1190.

83. Kharasch, Kane and Brown, J. Amer. Chem. Soc., 1940, 62, 2242.

84. Speeter and Anthony, J. Amer. Chem. Soc., 1954, 76, 6208;

Pelchowitz, Kaluszyner and Bentov, J. Chem. Soc., 1961, 5418.

85. Nenitzescu and Raileanu, Acad. rep. populare Romine, Studii cercetari chim., 1959, 7, 243; Chem. Abs., 1960, 54, 7681d.

86. Shaw and Wooley, J. Biol Chem., 1953, 203, 979.

87. Shaw, J. Amer. Chem. Soc., 1955, 77, 4319.

88. Tyson and Shaw, J. Amer. Chem. Soc., 1952, 74, 2273.

89. Smith, J. Chem. Soc., 1954, 3842.

90. Young, J. Chem. Soc., 1958, 3493.

91. Putochin, Ber., 1926, 59, 1987.

92. Majima and Kotake, Ber., 1922, 55, 3859, 3865.

93. Seka, Ber., 1924, 57, 1868.

94. Ash and Wragg, J. Chem. Soc., 1958, 3887.

95. Shaw, McMillan, Gundmundson and Armstrong, J. Org. Chem., 1958, 23, 1171.

96. Gray, Arch. Biochem. Biophys., 1959, 81, 480.

97. Brown, Henbest and Jones, J. Chem. Soc., 1952, 3172.

98. Ahmad, Eelnurme and Spenser, Canad. J. Chem., 1960, 38, 2523.

99. Pietra, Farmaco, 1958, 13, 75; Chem. Abs., 1958, 52, 13704f.

100. Terent'ev, Kost and Smit, Zhur. obshchei Khim., 1956, 26, 557; Chem. Abs., 1956, 50, 13871h.

101. Justoni and Pessina, Farmaco, 1955, 10, 356; Chem. Abs.,

1955, 49, 13968d.

102. Oddo and Cambieri, Gazzetta, 1939, 69, 19.

103. Hansch and Lindwall, J. Org. Chem., 1945, 10, 381.

104. Hine, "Physical Organic Chemistry," McGraw Hill Book Co., Inc., London, 1956, p. 167.

105. Bordwell and Stange, J. Amer. Chem. Soc., 1955, 77, 5939.

106. Blicke and Sheets, J. Amer. Chem. Soc., 1949, 71, 2856.

107. Reference 104, p. 140.

108. Cross, "An Introduction to Practical Infra-red Spectroscopy," Butterworths Scientific Publications, London, 1960, p. 72.

109. Smiley and Arnold, J. Org. Chem., 1960, 25, 257.

110. Friedman and Shechter, J. Org. Chem., 1960, 25, 877.

111. Stephen, J. Chem. Soc., 1920, 117, 1529.

112. Attenburrow, Cameron, Chapman, Evans, Hems, Jansen and Walker, J. Chem. Soc., 1952, 1094.

113. Emerson, Chem. Rev., 1949, 45, 347; Ramirez and Burger, J. Amer. Chem. Soc., 1950, 72, 2781; Burger, Stein and Clements, J. Org. Chem., 1957, 22, 143.

114. Gairaud and Lappin, J. Org. Chem., 1953, 18, 1.

115, Kurian and Ittyerah, J. Indian Chem. Soc., 1961, 38, 959.

116. Jansen, Rec. Trav. chim., 1931, 50, 291.

117. Neilson, Wood and Wylie, J. Chem. Soc., 1962, 371.

118. Stephen and Stephen, J. Chem. Soc., 1956, 4695.

119. Garg, Diss. Abs., 1962, 23, 1933.

120. Smith and Rogier, J. Amer. Chem. Soc., 1951, 73, 4047.

121. Hesse and Schrodel, Angew. Chem., 1956, 68, 438; Annalen, 1957, 607, 24.

122. Brown, Shoaf and Garg, Tetrahedron Letters, 1959, No. 3, 9.

123. Miller, Biss and Schwartzman, J. Org. Chem., 1959, 24, 627.

124. Backeberg and Staskun, J. Chem. Soc., 1962, 3961.

125. Gaiffe and Pallaud, Compt. rend., 1962, 254, 496.

126. McElvain and Nelson, J. Amer. Chem. Soc., 1942, 64, 1825.

127. Claus and Morgenthau, J. Amer. Chem. Soc., 1951, 73, 5005.

128. Farrar and Levine, J. Amer. Chem. Soc., 1950, 72, 4433.

129. Emerson, J. Amer. Chem. Soc., 1951, 73, 1854.

130. Carmack and Spielman in "Organic Reactions," ed. Adams, John Wiley and Sons, Inc., New York, 1946, Vol. III, p.83.

131. Blicke and Sheets, J. Amer. Chem. Soc., 1948, 70, 3768.

132. Brown, Eglinton and Martin-Smith, Spectrochim. Acta, 1962, 18, 1593.

133. Bellamy and Rogasch, Proc. Roy. Soc., 1960, A257, 98.

134. Tamm and Albrecht, Helv. Chim. Acta, 1960, 43, 769.

135. Knobloch and Prochazka, Chem. Listy, 1953, 47, 1285; Coll. Czech. Chem. Comm., 1954, 19, 744.

136. Cairns-Smith, J. Chem. Soc., 1961, 182.

137. Anet and Muchowski, Proc. Chem. Soc., 1962, 219.

138. Cairns and Eglinton, Nature, 1962, 196, 535.

139. Bedell, Spaeth and Bobbitt, J. Org. Chem., 1962, 27, 2026; Cagniant, Cagniant and Faller, Bull. Soc. chim. France, 1962, 576.

140. Royer, Demerseman and Cheutin, Bull. Soc. chim. France, 1961, 1534.

141. I. M. Dennler, personal communication.

142. Fries, Heering, Hemmecke and Siebert, Annalen, 1937, 527, 83.

143. Bucherer, J. prakt. Chem., 1904, 69, 49.