TRAEGERIC ACID AND OTHER METABOLITES OF ASPERGILLUS FLASCHENTRAEGERI

A Thesis presented by

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to

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SUMMARY

The remarkable transformation of the antifungal antibiotic traegeric acid, by means of copper sulphate in ethereal sulphuric acid, has been elucidated by inter-relation with the new nor-diterpene pigment flascherone, which is also produced by Aspergillus flaschentraegeri. This has allowed the structure of the antibiotic to be elaborated as an unusual diterpene pseudo-acid.

Esterification of the antibiotic led to a variety of products depending on the conditions. Formation of one product obtained with diazomethane was shown to involve attack at the enone system to give a cyclopropyl ketone grouping. Varied chemical transformations of this compound, including reductive studies and ozonolysis have established the relative configuration at 2 of the asymmetric centres, and afforded evidence concerning the remaining centres. Routes to heavy atom and mono-carbonyl derivatives for X-ray and ORD work, respectively, have been investigated.

The known anthraquinone physcion and a related pigment have been isolated from the fungus. The demonstration of nuclear overhauser effects in the n.m.r. spectrum of the latter has played a part in establishing its structure as the first C-prenylated anthraquinone. A phthalic acid metabolite has been characterised and synthetic studies on it carried out.

I should like to express my sincere thanks and appreciation to my supervisor, Dr. N.J.McCorkindale, for his constant guidance and advice during the course of this work and also in the presentation of this thesis.

I should also like to thank the Science Research Council for maintenance during the last three years and Professor R.A.Raphael, F.R.S., for providing the opportunity to carry out this research.

I am also indebted to Mrs.M.Tait, Miss M.McCartney and staff of the Mycology Department for technical assistance in the preparation and separation of fungal extracts. Thanks are also due to Mr. J.Cameron, B.Sc., and his staff (micro-analyses), to Mrs. F.Lawrie (infra-red spectra), Dr. J.Roberts and staff (mass spectra) and to Messrs J.Gall and A.Haetzman n.m.r. spectra). I am grateful to my laboratory colleagues for many helpful discussions.

Finally, I should like to acknowledge the assistance of my wife and my mother in the typing of this thesis.

CONTENTS

INTRODUCTION

The Fungi[1]

Since the work described in this thesis was carried out on the products of (secondary) fungal metabolism, a short introduction to these lower members of the plant kingdom is given below.

The fungi (Eumycetes) form a sub-division of the Thallophyta possessing eucaryotic structure, i.e. without cells walls or chlorophyll to promote photosynthesis. They are usually saprophytic with hydrolytic enzymes to break down dead animal or plant tissue or participate in a host-parasite relationship which can be mutually beneficial: the lichens are symbiotic associations of algae and fungi. Absorption of nutriment occurs (in fungi) through the hyphae, minute tubes which form the visible mycelial stromata.

Fungi may be classified according to their mode of reproduction. The Fungi Imperfecti may produce asexual spores (conidia). The production of sexual spores allows division of the remaining fungi into Phycomycetes (oospores or zygospores), Ascomycetes (spores formed in sac-like asci) and Basidiomycetes (spores on basidia).

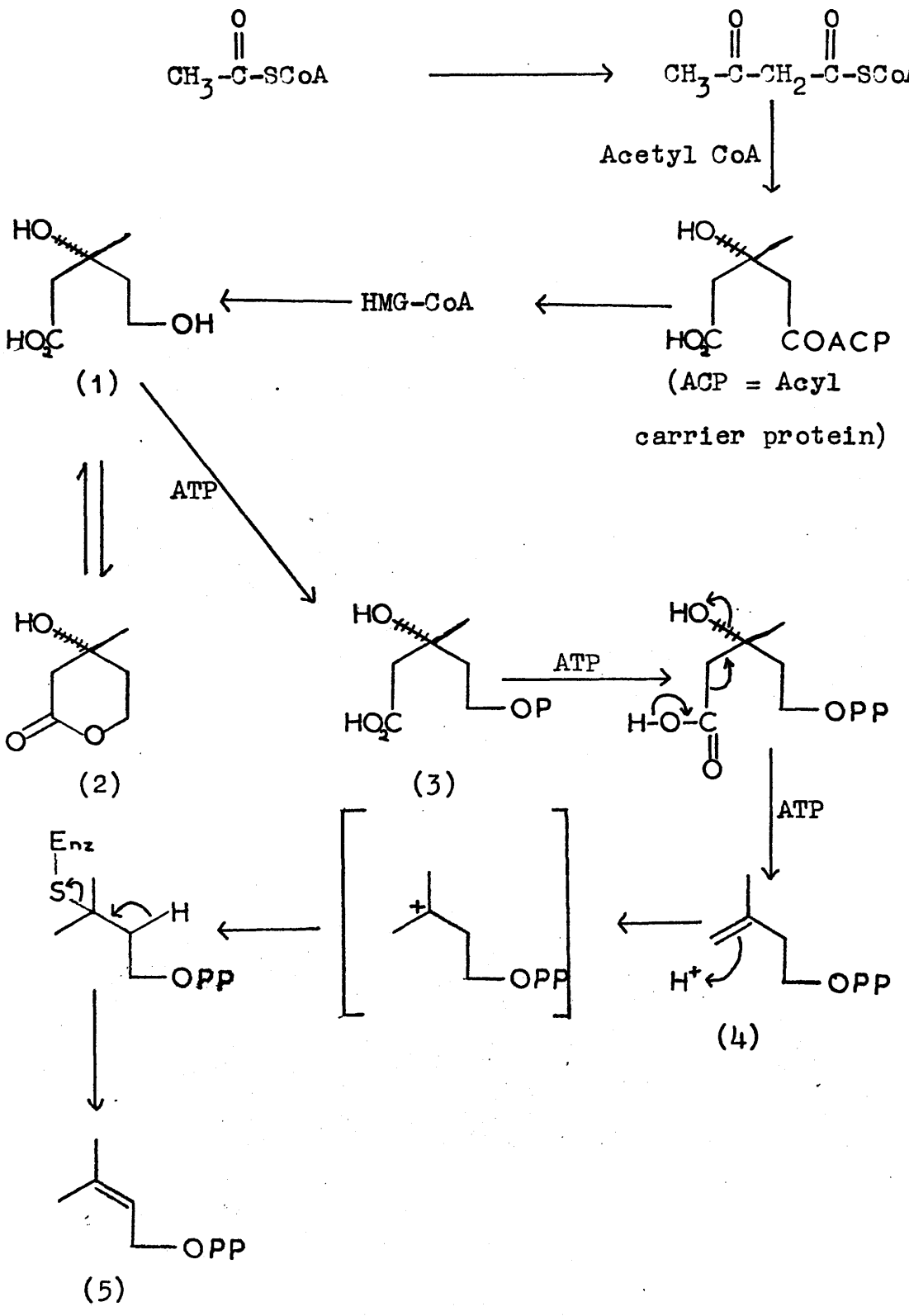
Many fungi have a destructive effect on human foodstuffs, e.g. Penicillium digitatum and expansum on apples. However, much useful work is done in nature by e.g. soil fungi which

metabolise dead tissue in the early stagesof breakdown to humus. They may be divided into 'inhabitants' of wide distribution and 'invaders' whose occurence as e.g. root parasites is linked to that of the host plant.

Utilisation of fungi is a very long established human activity: the manufacture of bread and cheese and the fermentation of alcoholic beverages are well-known. A number of fungi are edible and the production of some (e.g. truffles) is highly lucrative. Acute toxicity to humans is exhibited by (e.g.) the genus Amanita. Fungi have an industrial application in the large-scale production of a wide range of metabolites whose in vitro synthesis is difficult or costly, e.g. citric acid ex Aspergillus and Penicillium sp.

An important development in the study of fungi has been the isolation from them of antibiotic compounds, e.g. streptomycin and the penicillins. The present work was carried out on the mould Aspergillus flaschentraegeri whose broth extract exhibited antifungal activity.

1. Dictionary of the Fungi, G.C.Ainsworth, C.M.I., Kew, 1961.

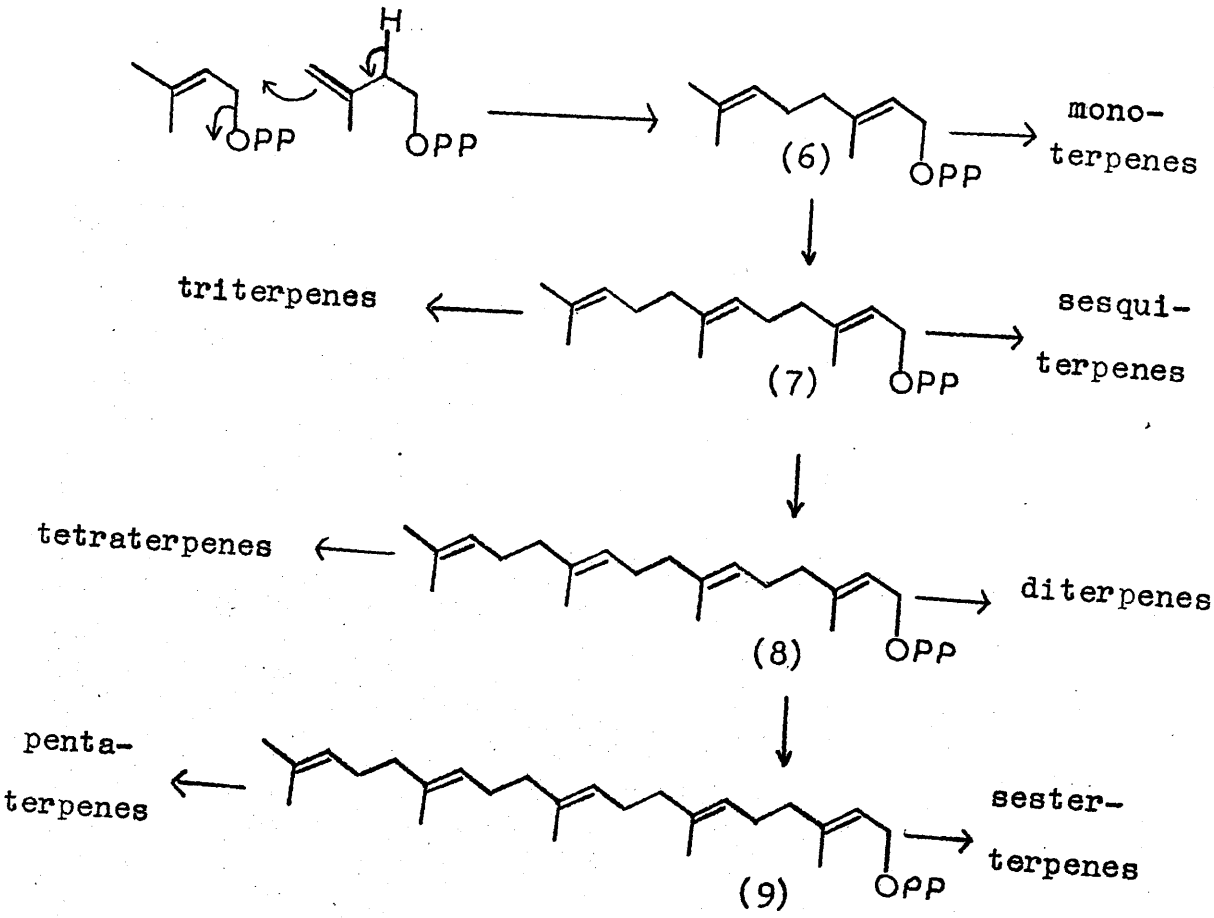

CH3-C-SCoA
CH3-C-CH2-C-SCoA
Acetyl CoA
HO
HO2C
OH
HMG-CoA
COACP
(ACP = Acyl carrier protein)
(1)
ATP
(2)
OP
(3)
ATP
OPP
H-O-C
ATP
(4)
Enz
S
H
OPP
H+
(5)

Fungal Diterpenes and their Biosynthesis.[1]

i) General.

The biosynthesis of diterpenes, as of all terpenoid natural products, is governed by the Biogenetic Isoprene Rule[2,3] which states that 'terpenes are compounds formed by combination of isoprene units to aliphatic substances such as geraniol, farnesol, geranylgeraniol, squalene and others of a similar kind, and can be derived from these aliphatic precursors by accepted cyclisation and, in certain cases, rearrangement mechanisms.'[4]

When acetic acid had been shown to be the initial precursor of this class[5], it was necessary to find a C_6 compound which could give rise to terpenes via a C_5 or 'active isoprene' species.[6] The hypothesis[7] that mevalonic acid (MVA, 1) is an intermediate on this route was based on a high (43.4%) incorporation of mevalolactone (2) in sterol biosynthesis in cell-free rat liver homogenates. The biogenesis of MVA from acetyl CoA and acetoacetyl CoA via hydroxymethylglutaryl (HMG) CoA[8] is outlined opposite: the absolute configuration shown is that of the natural isomer.[9] Initial labelling experiments showed that mevalolactone (2) is incorporated into squalene without prior reversal to acetate and that the lactone C-1 atom is lost in the formation of the 'isoprene unit.'[10]


H
OPP
OPP
(6)
OPP
mono-
terpenes
triterpenes
(7)
OPP
sesqui-
terpenes
tetraterpenes
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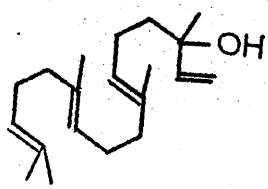
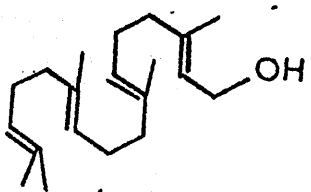
OPP
diterpenes
penta-
terpenes
(9)
OPP
sester-
terpenes


OH

(10)


OH

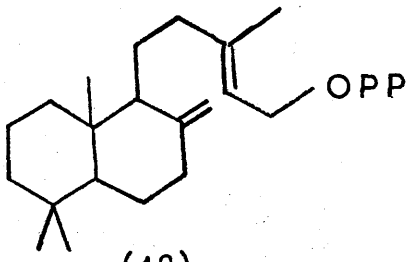
(11)

The involvement of ATP in isoprene polymerisation[11] prompted a search for a phosphorylated MVA derivative: Tchen[12] isolated a phosphate ester co-gener whose structure was proved[13] by synthesis to be 5-monophosphomevalonic acid (3). The route to isopentenyl PP* (4), the 'active isoprene,' has been investigated enzymatically[14]: it has been proposed that isomerisation of isopentenyl PP to 3,3-dimethylallyl PP (5) is necessary before condensation[15] of these to form geranyl PP (6). Repetition of this last step gives rise by similar sequences to farnesyl PP (7),[16,17] geranylgeranyl PP (8) and geranylfarnesyl PP (9).

The sequence (1)—(9) has in the above been outlined without reference to stereochemical ambiguities. Popjak and Cornforth, however, have demonstrated[18] fourteen such points in the biosynthesis of squalene from MVA and, employing specifically labelled precursors[19] have resolved all but one of these. The manner of tail-to-tail linking in squalene biosynthesis from two C_{15} precursors has received much recent attention.[20]

Geranyl-linalool (10), suggested by Ruzicka[2] as a probable diterpene precursor, occurs in nature,[21] as does its allylic isomer, geranylgeraniol (11).[22] An enzyme

* PP = pyrophosphate.



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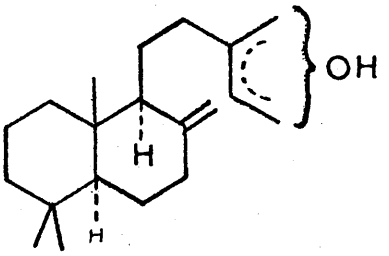

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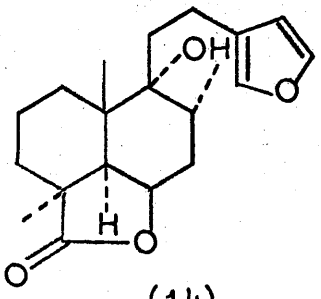

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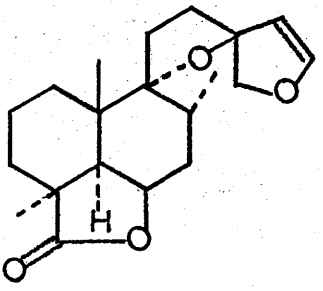

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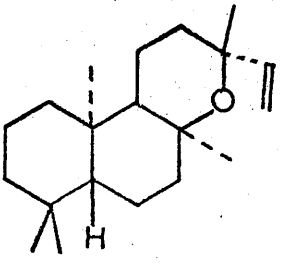

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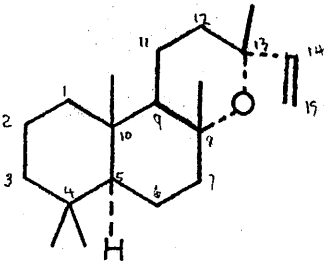


(17)

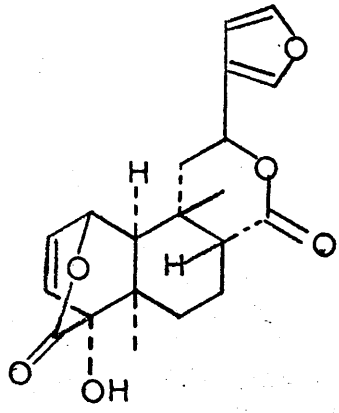
epi-13- (18)

system isolated[23] from Micrococcus lysodiekticus has been found to catalyse reactions between C_5, C_{10} and C_{15} allylpyrophosphates (5), (6) and (7) and isopentenyl PP (4). 53, 55 and 70% respectively of the labelled products from these reactions were identified as geranylgeraniol (11): smaller amounts of radioactivity were associated with geranyl-linalool (10) and less than 0.1% with C_{10} and C_{15} alcohols. Similar studies[24] have been carried out with enzyme systems from carrot root and pig liver.

ii) Bicyclic Diterpenes.

The first stage in geranylgeraniol PP cyclisation is thought to lead to the bicyclic intermediate, copalyl PP (12):[25] the revision of the stereochemistry of a number of diterpenes in the light of X-ray and circular dichroism (CD) evidence has upheld structure (13)[26] or its enantiomer as the common precursor of almost all skeleta. Bicyclic diterpenes known include the formerly problematic marrubiin (14):[27] the isolation of premarrubiin (15)[28] may offer a clue to the later stages of its biogenesis.

The mechanism of initial cyclisation is thought to be concerted and to result in trans-fused rings.[29] Induced by protonation of the terminal double bond, it may give rise to epimeric series of diterpenes (5α, 9α, 10β and 5β, 9β, 10α): 13-epimanoyl oxide (16), a fungal metabolite, and



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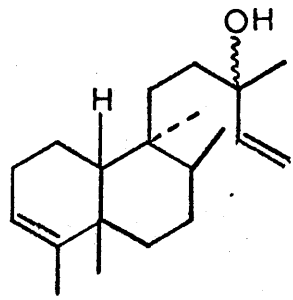

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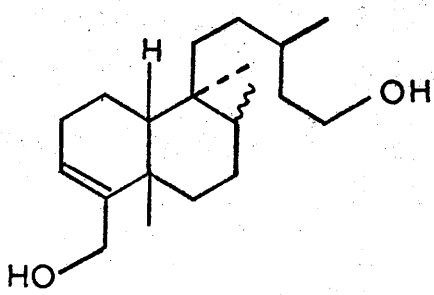

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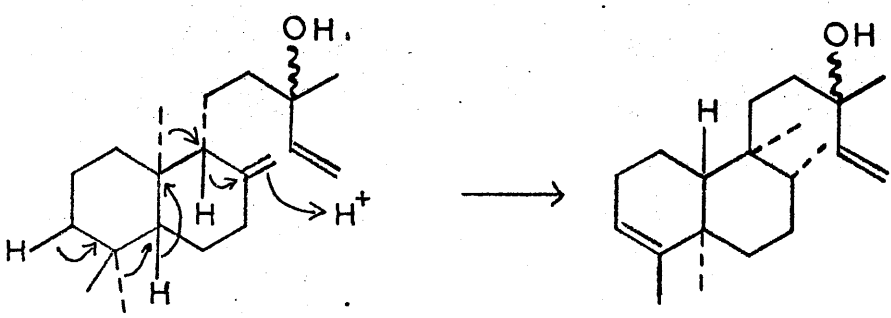

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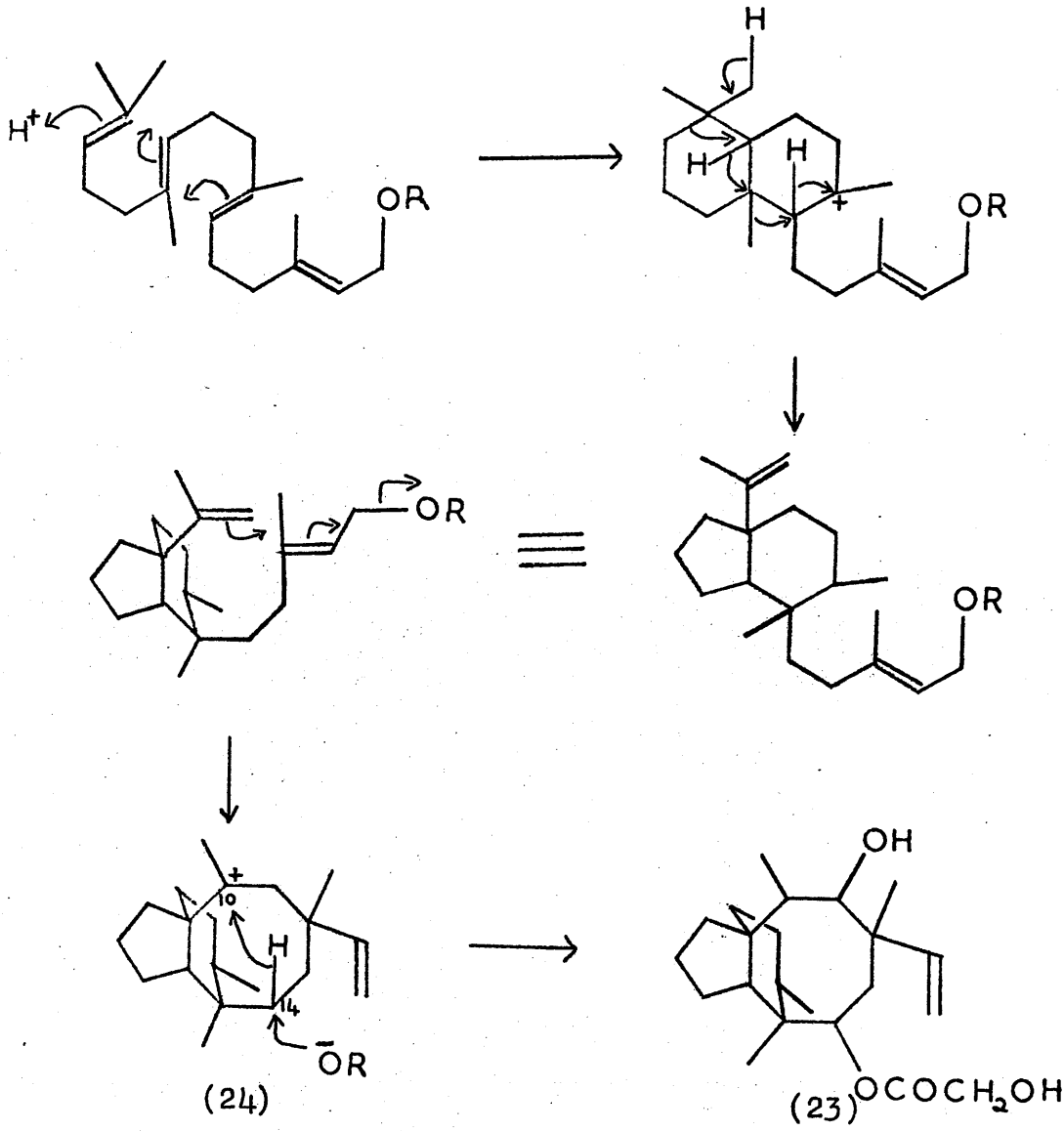

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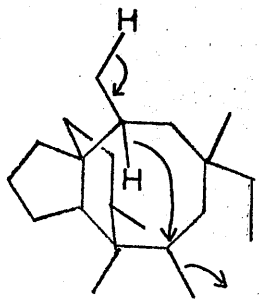

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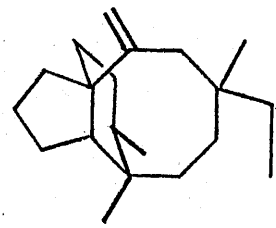

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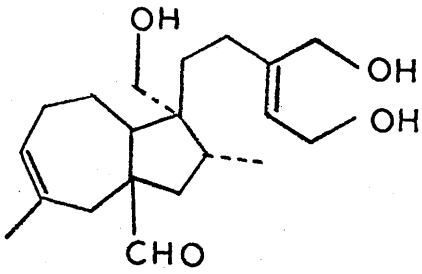
its antipode have been found. It has been suggested that inversion at C-13 may occur by replacement of a suitable leaving group (e.g. pyrophosphate) by a formal OH or H species: manoyl oxide (17)[31] should be compared with its C-13 epimer.

A recent hypothesis[32] envisages the participation of enzyme-surface nucleophiles (X-groups) in the stepwise build-up and cyclisation of isoprenoids. The isolation of intermediates in such a scheme would substantiate this theory and perhaps help elucidate the biosynthesis of the anomalous cis-fused bicyclic diterpenes. Columbin (19),[33,34] plathyterpol (20)[35] and cistodiol (21)[36] cannot readily arise from the product (13) of concerted cyclisation. The biogenesis of kolavelool(22)[37], an epimer of plathyterpol, is easily rationalised.

Perhaps the most interesting example of a rearranged bicyclic diterpene is pleuromutilin (23),[38] from Pleurotus and Drosophila species.[39] The transformation of carbonium ion (24) into (23) was proposed[40] by analogy with the in vitro conversion (25)→(26): the proximity of H-10 and H-14 was known from X-ray studies.

A less complex rearrangement of a labdane-type precursor is invoked for the biosynthesis of portulal (27):[41] the structure of this plant growth regulator has been



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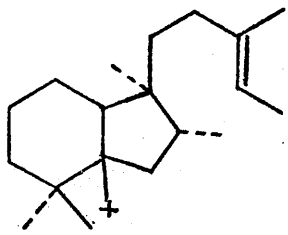

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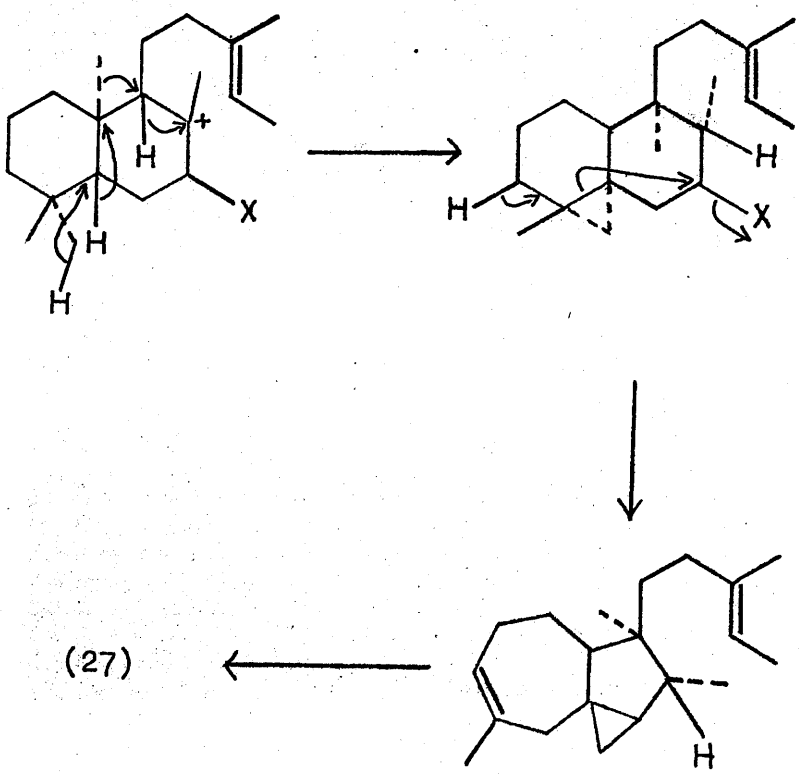

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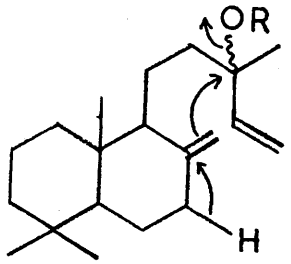

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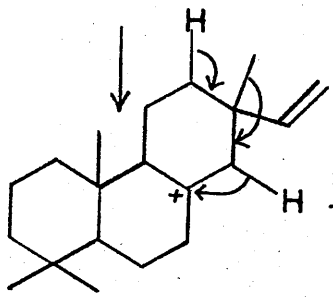

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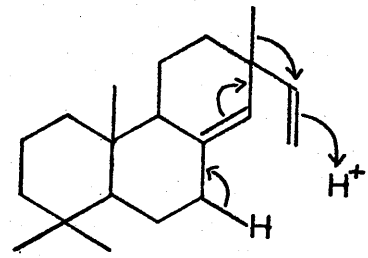

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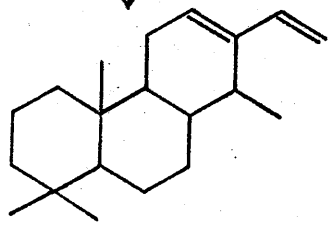

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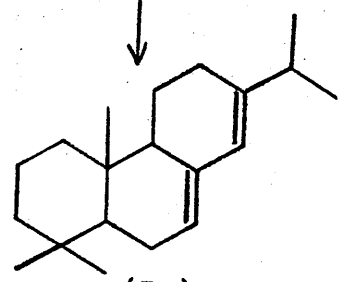

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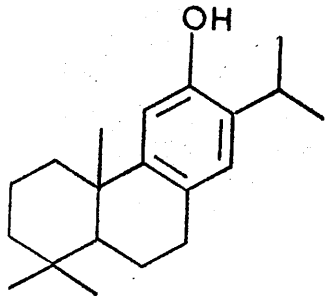

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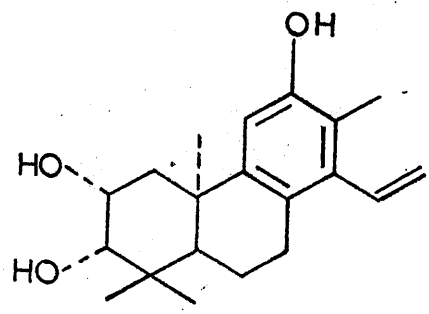

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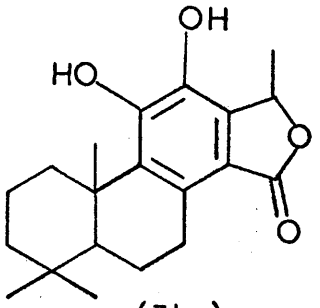

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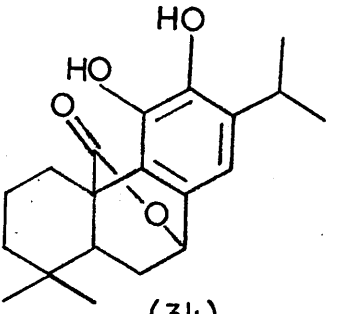

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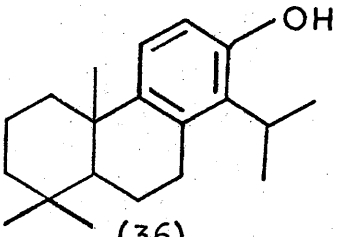

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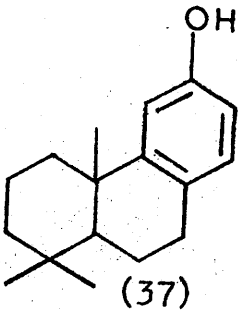

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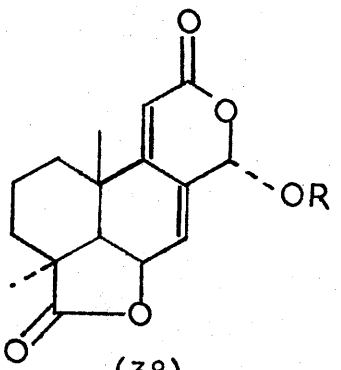

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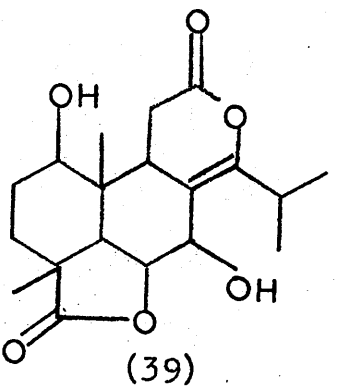

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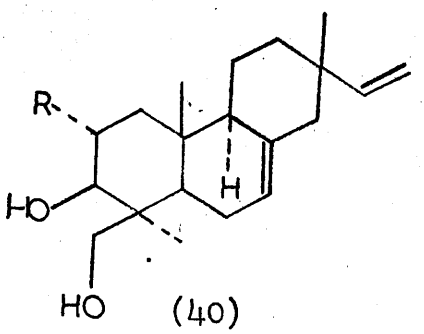


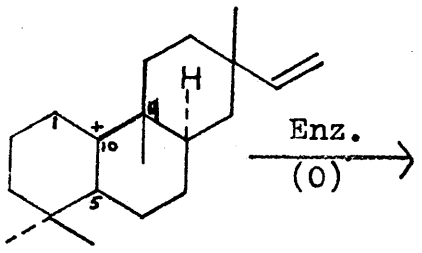
(40)

determined by X-ray analysis. A proposed biogenesis via primary carbonium ion (28) has been published: a more attractive alternative is suggested opposite.

iii) Tricyclic Diterpenes.

Further cyclisation of the bicyclic precursor (13) results in the general class of tricyclic diterpenes (e.g., pimaradiene, 30). Entry[29] into the abietane (31) and cassane (32) series from a pre-pimaradiene carbonium ion (29) gives structures with the facility to aromatise (in ring C) without loss of skeletal atoms, as in ferruginol (33).[42] Carnosol (picrosalvin), thought to be the first aromatic cassane derivative (34a)[43] has been shown to possess structure (34):[44] in cleistanthol (35),[45] presumably biosynthesised from (29) by a vinyl shift analogous to the rearrangement to (32), ring C is aromatic. In vitro rearrangements[46] have indicated possible modifications leading to totarol (36) and podocarpic acid (37). It has been suggested[47] that the fungal antibiotics LL-Z1271α (38, R=Me) and γ(38, R=H) are diterpenoid, by analogy with the nagilactone plant products (e.g., A, 39).[48] Other fungal tricyclic diterpenes are the virescenols,[49] A (40, R=OH) and B (40, R=H).

Rosenonolactone (41),[50-52] from Trichothecium roseum, has a rearranged pimaradiene skeleton into which acetic acid,



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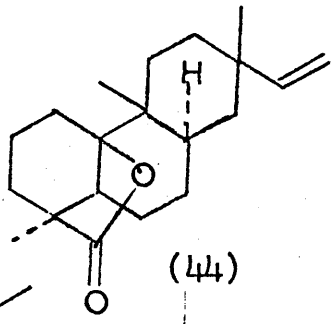

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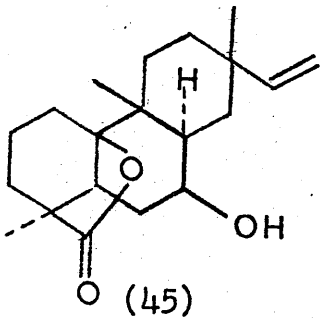

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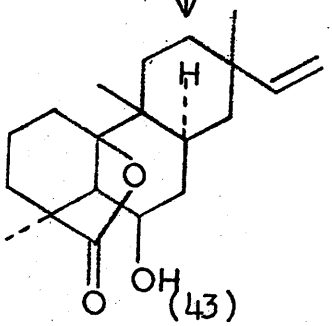

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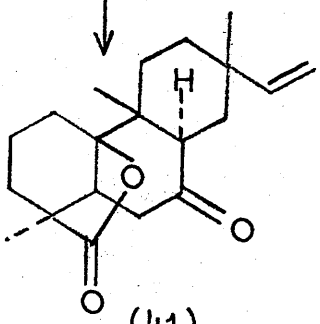

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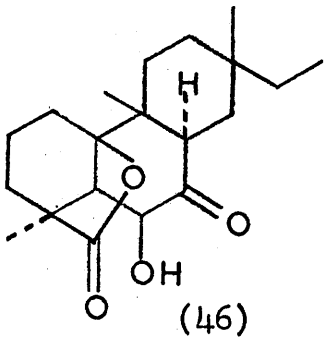

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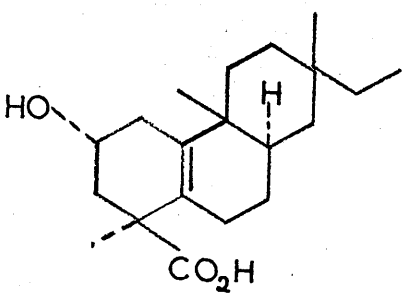

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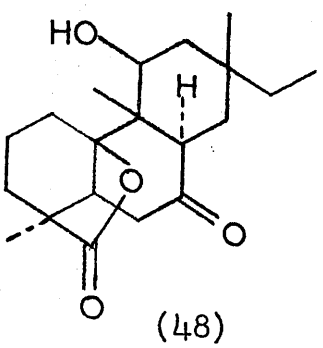


(48)

MVA,[53] geranyl and farnesyl pyrophosphates,[54] geranylgeran and labda-8(17), 13(14)-dien-15-ol (13)[54] are incorporated. Three stages in the biosynthesis are proposed,[55] viz:

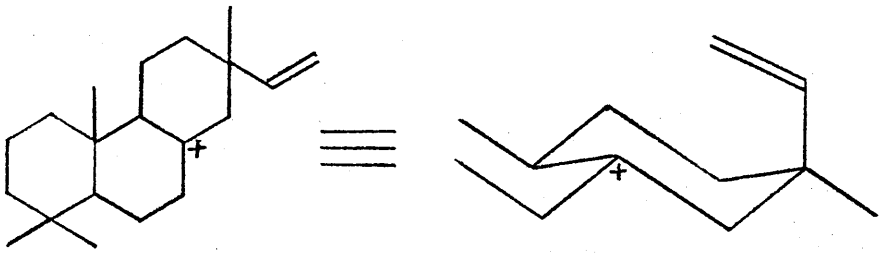
i) formation of the bicyclic precursor (13):

ii) cyclisation and rearrangement to the rosane skeleton:

iii) an oxidative sequence leading to individual products.

The incorporation of label from (2-^{14}C)-MVA into the 1-methyl but not the 1-carboxy position of gibberellic acid (66) and the corresponding position of rosenonolactone (41) was early proof of stereochemical integrity of the gem-dimethyl groups of the acyclic precursor. Other labelling experiments demonstrated the migration of the 10-methyl group to C-11 and of the proton at C-9 to C-8 in a concerted cyclisation. Since the departing C-10 methyl and the carboxyl group are both β, lactonisation must be a separate process. The carbonium ion (42) does not form a double bond ($\Delta^{1(10)}$ or $\Delta^{5(10)}$) by elimination and the formation of an α-C-OH or -C-Enzyme bond at C-10, displaced with inversion on lactonisation has been proposed.

An investigation[55] of the oxidative process at C-19 employing biclyclic precursors oxygenated at this position was hampered by low incorporation and scrambling of label. Oxidation of an enzyme-bound tricyclic intermediate may be taking place. Ring B oxidation in rosenonolactone (41) and rosololactone (43) was studied using deoxyrosenonolactone



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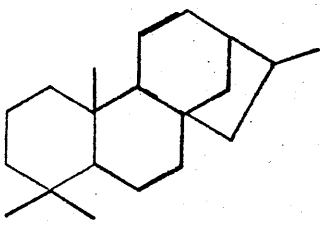

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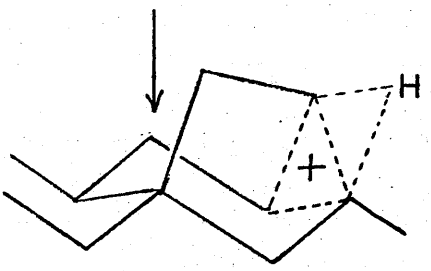

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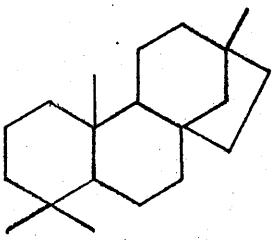

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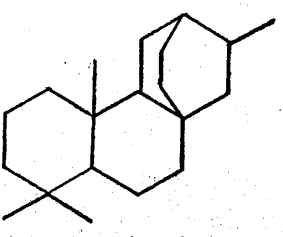

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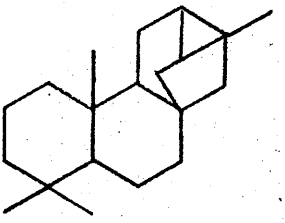

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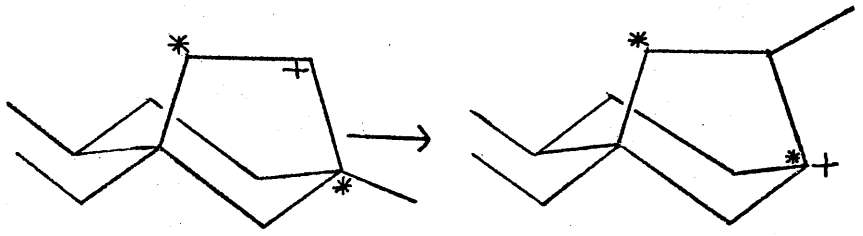

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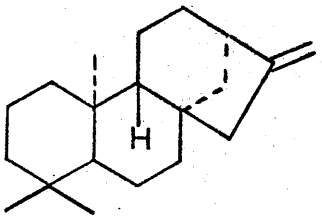

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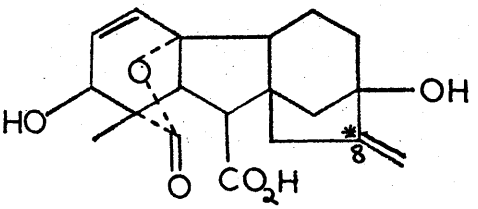

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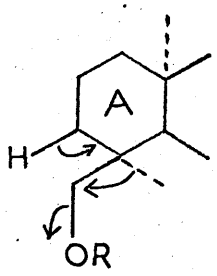

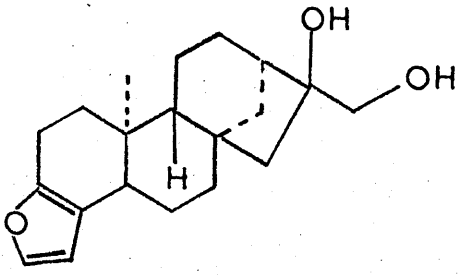

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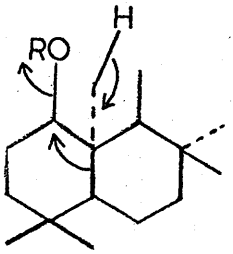

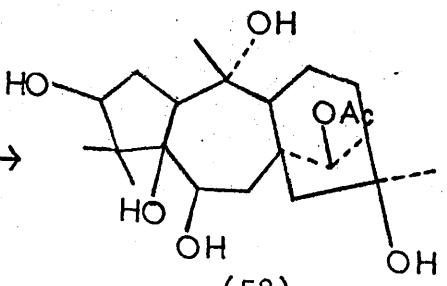

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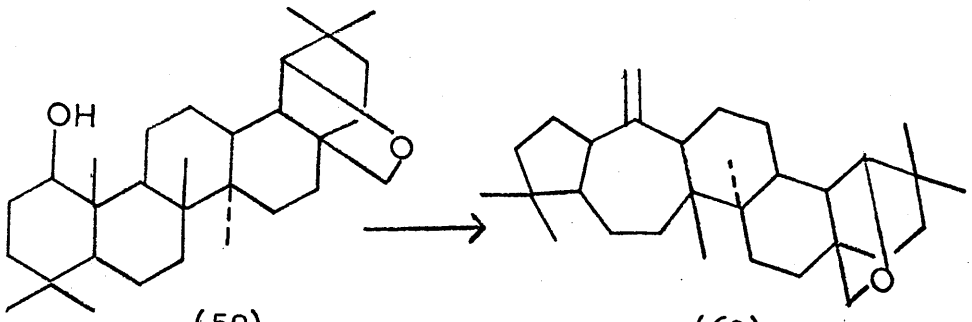

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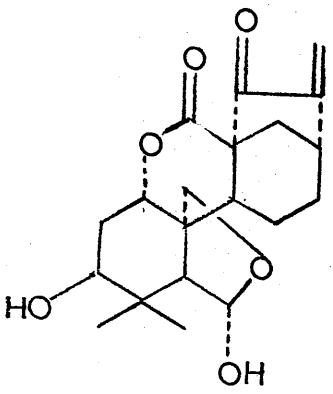

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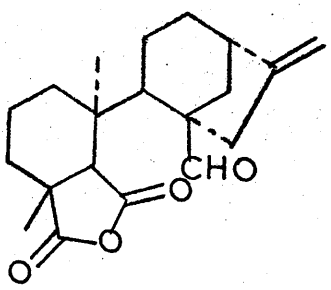

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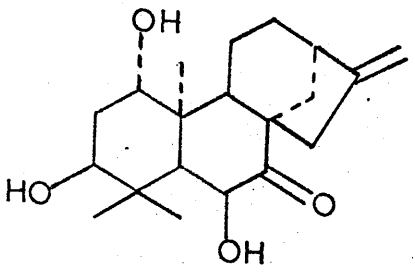

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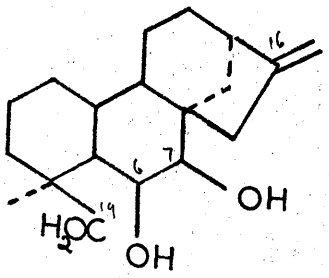

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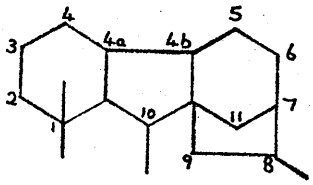

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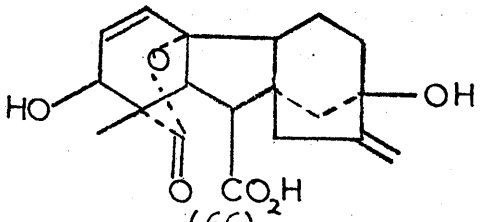


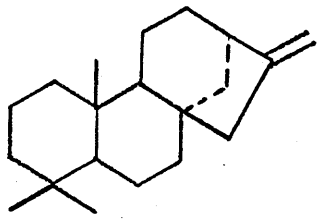
(66)

(44)[56] and rosenololactone (45)[57] labelled by feeding mevalonate to the fungus: the results are summarised opposite. 6 -hydroxyrosenonolactone (46),[58] isorosenolic acid (47)[59] and rosein III (48)[60] are related metabolites.

iv) Tetracyclic Diterpenes.[61]

The non-classical carbonium ion (49) derived from pre-pimaradiene (29) has been suggested[62] as the common precursor for this class: its collapse can result in the four known skeletal types, viz. kaurane (50), beyerane (51), atisane (52) and trachylobane (53), the latter being pentacyclic. The classical carbonium ion (54) has been ruled out[53] as a precursor for (e.g.) tetrahydrogibberellic acid (55) biosysthesised from (1-*C)-acetic acid: Kuhn-Roth oxidation allows isolation of C-8 (gibberellin numbering) as the carboxyl group of acetic acid which is labelled.

Some minor modifications of a (-)kaurene (56) precursor result in three new groups of diterpenes. Cafestol (57)[63] may arise through the rearrangement shown while a bond shift at the AB ring junction leads to the grayanotoxins[64,65] (e.g., G-I, 58). It is interesting, in this context, that C-10 invariably carries an exocyclic methylene group or tertiary hydroxyl function; also, in the triterpene field, a carbonium ion may be generated at C-1 under acid conditions, effecting a grayanotoxin-like synthesis: (59)- (60)[66]. The seco-ring B diterpenes, enmein (61)[67] and the fungal metabolite fugenal (62)[68] may have a



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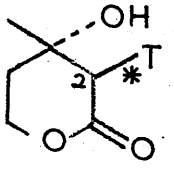

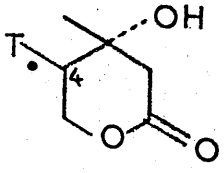

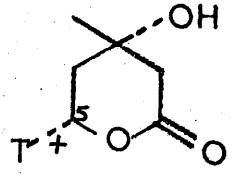

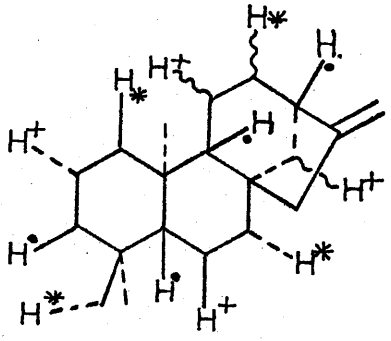

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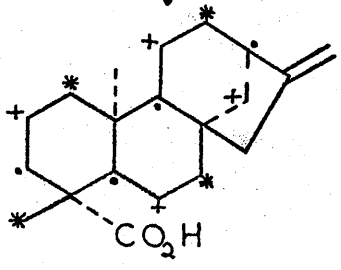
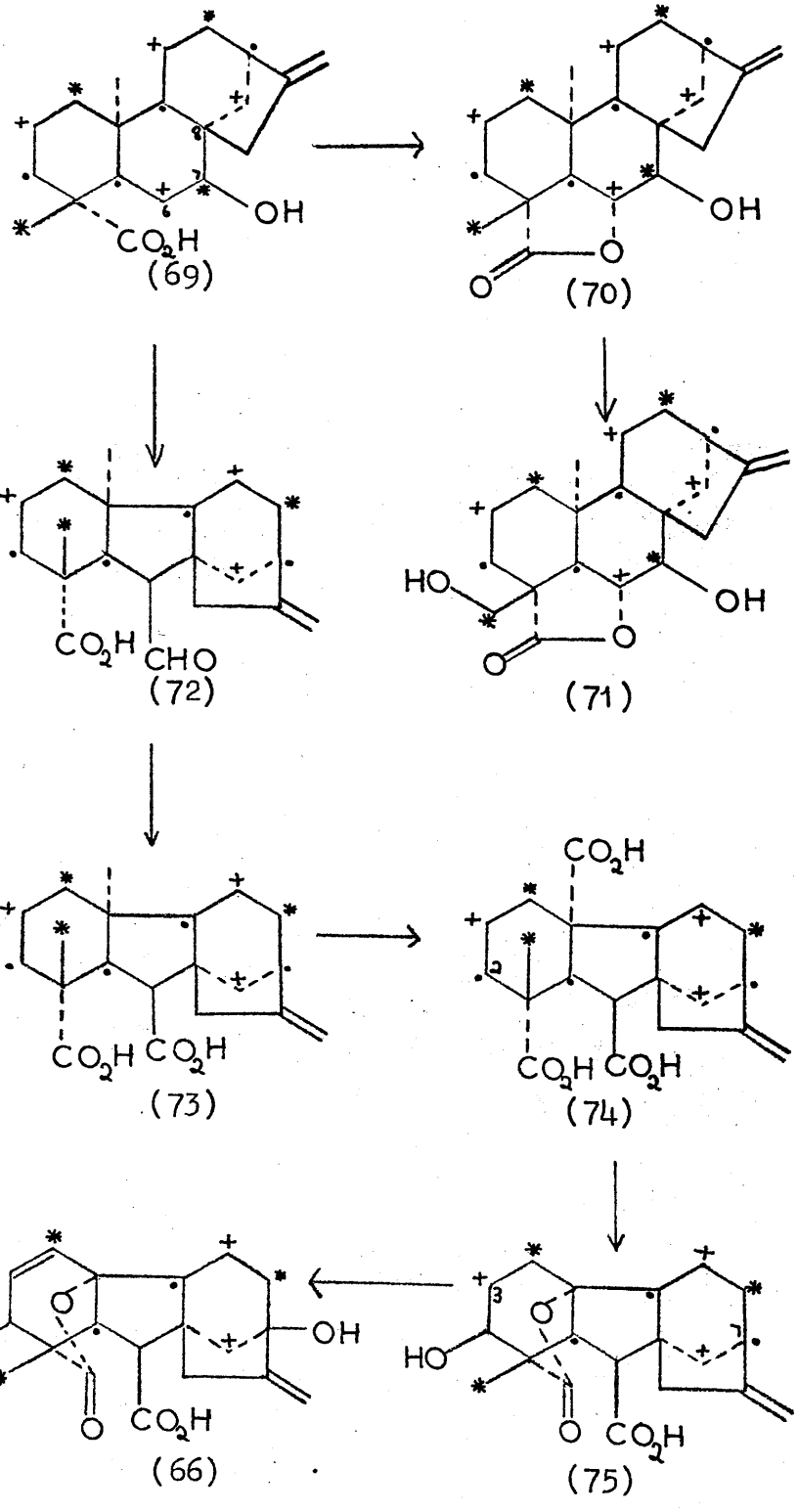


(64)

(69)

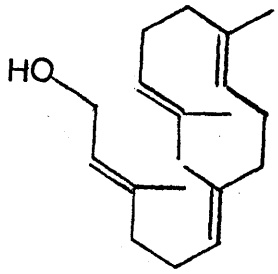

OH
CO2H
(69)
OH
O
O
(70)
CO2H
CHO
(72)
HO
OH
O
(71)
CO2H
CO2H
(73)
CO2H
CO2H
CO2H
(74)
HO
O
O
CO2H
(75)
HO
OH
O
CO2H
(66)

common precursor with the gibberellins; it has been postulated[69] that the opening of ring B in seco-kaurene biosynthesis and its contraction on the route to gibberellins proceed through structure (63). 6β,7β-dihydroxy-(-)-kaur-16-en-19-oic acid (64) has been shown[70] to be a precursor of fugenal (62).

The gibberellins, which are produced in quantity by the fungus Gibberella fujikuroi (the conidial form being Fusarium moniliforme), have important plant growth regulation properties[71] and have been isolated in small amounts from certain plants.[71a] The parent hydrocarbon, gibbane (65) is shown with 'gibberellin numbering' which will be used throughout.

Gibberellic acid (66)[71] biosynthesis from geranyl-geranyl PP occurs via (-)-labda-8,13-dien-15-ol PP (13), 8(14)-pimaradiene (30)[72] and (-)-kaurene (56).[73] Evidence from early stereochemical studies implying the intermediacy of (-)-phyllocladene(67)[74] has been refuted.[75]

The sequence has been studied in feeding experiments with 2-(R)-(2-^{3}H)-, 4-(R)-(4-^{3}H)- and 5-(R)-(5-^{3}H)-MVA whose labels were incorporated into (-)-kaurene in the pattern shown (68)[76]. (-)-Kaur-16-en-19-oic acid (64) is hydroxylated at the positions 6 and 7 with retention of configuration affording 6-hydroxy-(-)-kaur-16-en-19-oic acid (69) and kaurenolides (70) and (71). During ring B contraction of (69) to form



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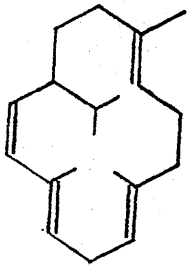

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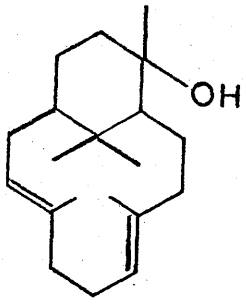

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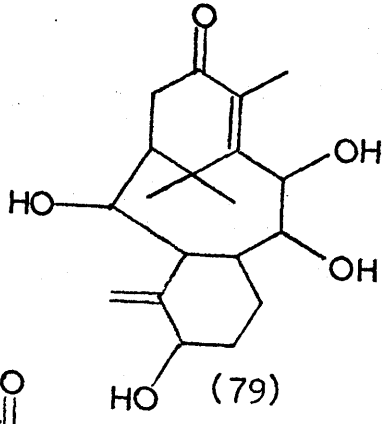

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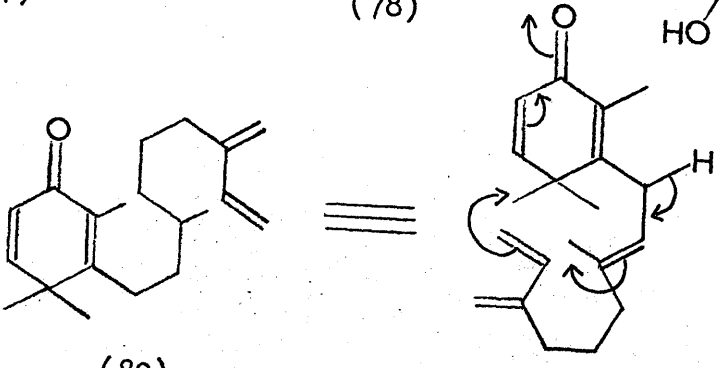

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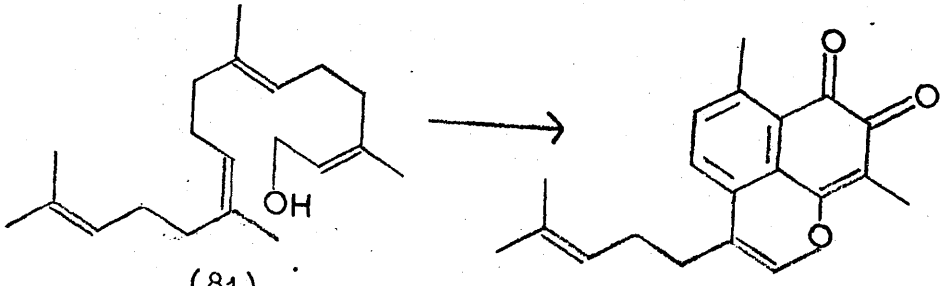

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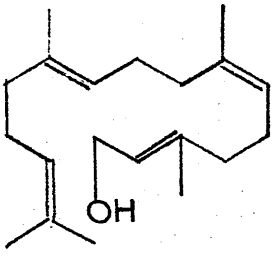

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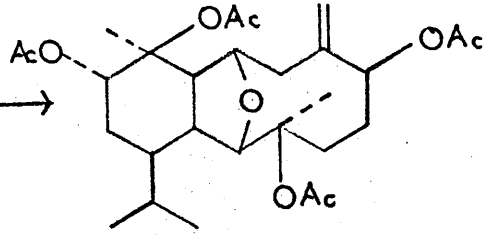

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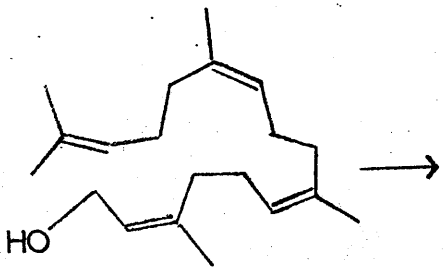

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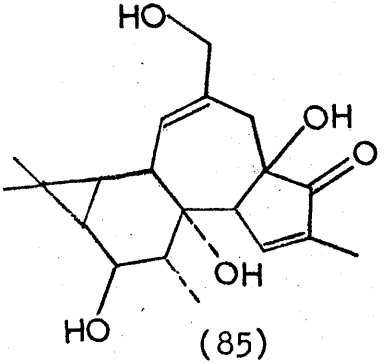

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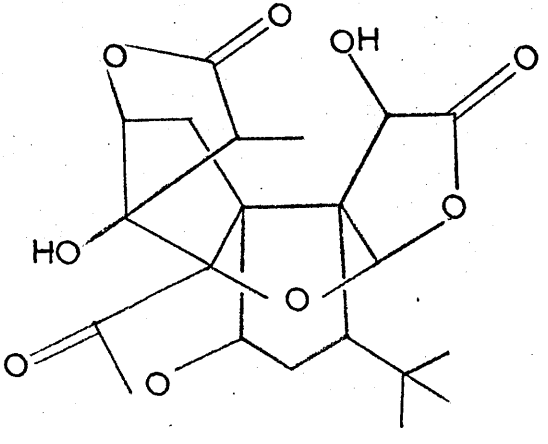


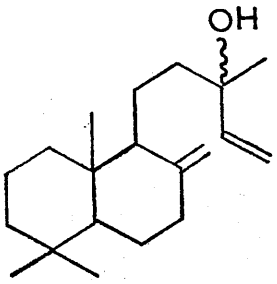
(87)

gibbane aldehyde (72), a hydride shift from C-6 to C-7 (which is extruded) occurs[77] with migration of the C-7 - C-8 bond. The biosynthesis proceeds through gibberellin A_{12} (73) to A_{13} (74)[78] whose decarboxylation does not involve an unsaturated acid. Hydroxylation at C-2 to give A_4 (75)[72] occurs with retention of configuration while formation of the Δ^3 double bond involves cis-dehydrogenation from the α-side, excluding hydroperoxidation and trans-elimination of the peroxide.[79] Hydroxylation at C-7 completes the biosynthesis of gibberellic acid (66).

The sites of gibberellin biosynthesis in F. moniliforme have been investigated;[80] it appears that only the early cyclisation stages occur inside the mycelial cell, while skeletal rearrangements and oxidative processes are performed by enzymic systems on the outer side of the cell walls.

v) Other Diterpenes.

The bicyclic intermediate (13) is not the precursor for all diterpenes. Erdtmann has proposed[81] that geranylgeraniol in conformation (76) is the starting point for a number of macrocyclic and other natural products: cembrene (77),[82] verticillol (78)[81] and the taxanes (e.g., taxicin II, 79)[83] may be thus derived. It would not then be necessary to invoke the seco-labdane structure (80)[84] in the latter's biosynthesis. Similarly, the forms (81), (82) and (83) of



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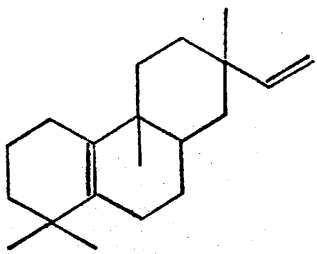

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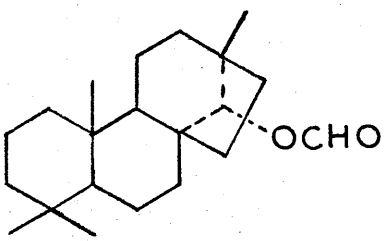

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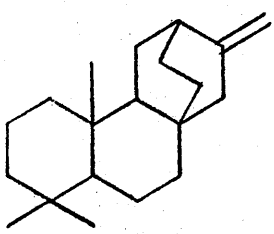

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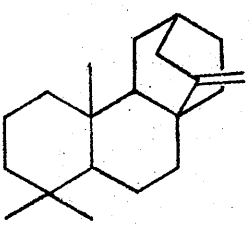

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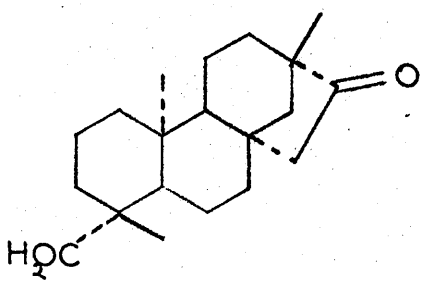


(93)

geranylgeraniol may give rise, respectively, to biflorin (84),[85] eunicellin (85)[86] and phorbol (86).[87] The ginkgolides (e.g., A, 87)[88] are not so readily arrived at.

In vitro studies[29] related to the biosynthesis of diterpenes have centred mainly on acid-catalysed rearrangements for comparison with *in vivo* cyclisations. Manool (88) and related compounds have been cyclised[89] to pimaradienes (30), rosadienes (89) and to hibol formate (90)[90]: rosenonolactone (41) has been synthesised[89] from a bicyclic precursor. In the tetracyclic group, (-)-kaurene (56) has afforded[91] a mixture of (-)-phyllocladene (67), (-)-atisirene (91) and (-)-neoatisirene (92); a kaurane (50) has been rearranged to a beyerane (51)[92] and an isosteviol (93) derivative has led[93] to the partial synthesis of pentacyclic trachylobane (53).

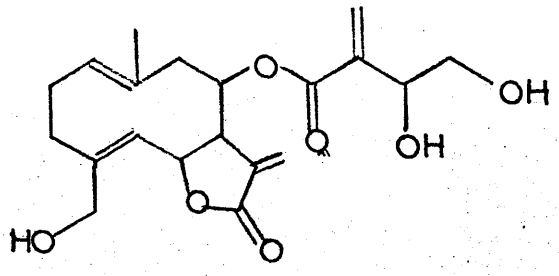
DISCUSSION

CHAPTER 1.

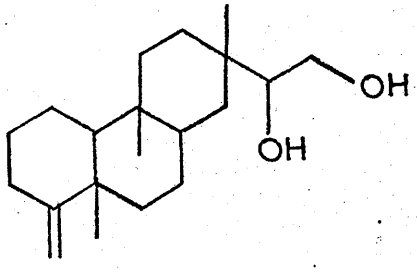
Preliminary Studies on Traegeric Acid.

Culture filtrates of the fungus Aspergillus flaschen-traegeri are known to be active against the fungus Botrytus alii. Repeated chromatography of material extracted from the fungal broth yielded[94] the crystalline compound traegeric acid (formerly referred to as'traegerin'), $C_{20}H_{26}O_5$, m.p. 160°C, responsible for this. It also had a bactericidal effect on Bacillus megatherium and Staphylococcus aureus. The incorporation of (2-^{14}C)-mevalolactone into the antibiotic indicated a terpenoid character: formulation as a diterpene was attractive though, for example, a more novel C_{15}-C_5 aggregate, cf. cninin (94)[95], could not be ruled out.

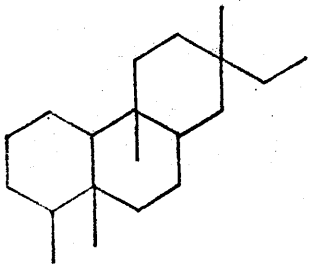
The n.m.r. spectrum of traegeric acid showed a number of features consistent with a diterpene skeleton. Three olefinic protons (4.32, 5.01 and 5.14τ) formed a typical ABX pattern, indicating the presence of a tertiary vinyl group, further substantiated by absorptions in the i.r. spectrum at 1627, 986 and 906cm^{-1}. Three tertiary methyl groups (singlets at 8.9τ(6H) and 9.0τ(3H)) could readily be accommodated in a gem-dimethyl grouping (ν_{C-C} 1390 and 1368 cm^{-1}) and a geminal methyl-vinyl system; the latter is common in tricyclic diterpenes, e.g., rosenonolactone (41)[51] and pimaradiene (30)[29] .



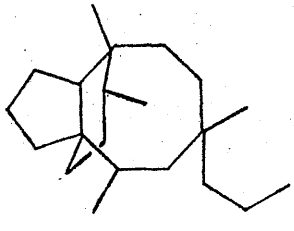
(94)



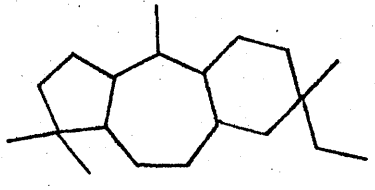
(95)



(A)



(B)

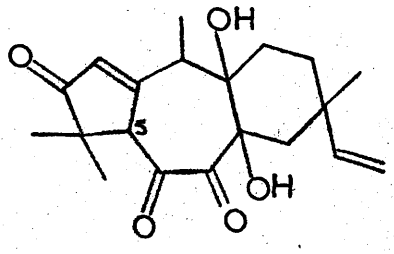


(C)

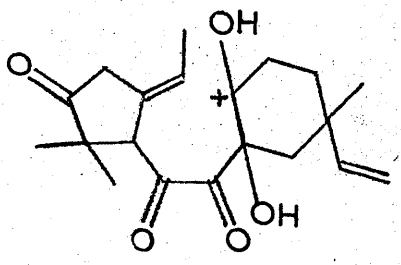
Four further angular positions seemed to be a structural requirement from the presence of singlet resonances at 3.32 and 7.40τ assigned, respectively, to an olefinic proton on a trisubstituted double bond and to one α to carbonyl, together with signals at 4.83 and 7.83τ, which disappeared on deuteration and were ascribed to tertiary hydroxyl functions in the absence of resonances appropriate to protons geminal with oxygen.

At this stage, evidence erroneously suggesting the presence of a secondary methyl group in the antibiotic was obtained. Solvent-induced shifts of resonances in carbonyl compounds have been extensively studied[96] and a plane rule devised.[97] In the spectrum of traegeric acid in benzene, two methyl signals moved to lower field revealing a signal which could be attributable to a secondary methyl group (8.91τ,d, J=6Hz.). This is an unusual feature for diterpene skeleta, found only in the rearranged pimarane, erythoxydiol Y (95),[98] pleuromutilin (23)[38] and the grayanotoxins (e.g., G-I, 58).[64,65] Among the tricyclic hydrocarbons (A), (B) and (C), only the last has the requisite number of angular positions and a gem-dimethyl group.

The olefinic resonance (3.32τ) and the u.v. spectrum of traegeric acid (λ_{max}242 nm.) might suggest the presence of an enone function, presumably in a 5-membered ring since no



(96)

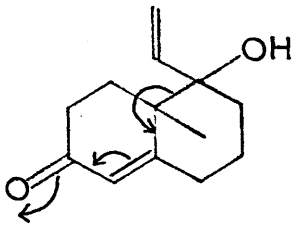


(100)

carbonyl absorption appeared below 1714 cm^{-1}. Hydrogenation of the antibiotic afforded 'deoxytetrahydrotraegeric acid', which gave a positive reaction with ferric chloride and showed the u.v. spectrum of a diosphenol (λ_{max} 280nm., with a base-induced reversible bathochromic shift of 50nm.); this implied the presence of the unusual α-diketone function in traegeric acid, whose slow solubility in aqueous sodium bicarbonate apparently indicated that enolisation was possible if not facile.

Although no structure seemed to fully satisfy the above data, formulation (96) was under consideration at the initiation of the present studies. It may be noted that in this structure, the value 7.47 would be abnormally high for a proton (H-5) α to one carbonyl group and vinylogously α to another. Since this might be due to unspecified shielding effects, (96) could not be immediately dismissed.

Apparent support for the presence of a secondary methyl group was obtained by subjecting the antibiotic to dehydrating conditions (anhydrous copper sulphate and sulphuric acid in ether). The only stable product (obtained in 10% yield) showed a well-defined sceondary methyl group (8.45τ, d, J= 5.5Hz.) which was coupled to a 1H quartet at 4.59τ. The acid-ctalysed transformation of the δ-hydroxy-α,β-enone (97) into (98) and (99) has been suggested to occur by the



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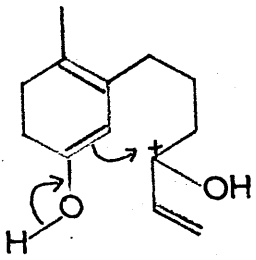
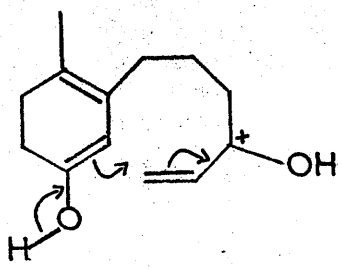
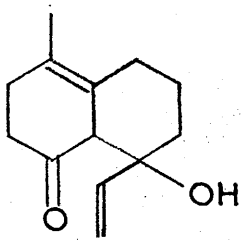
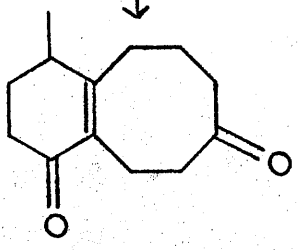
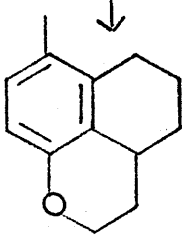








(99)



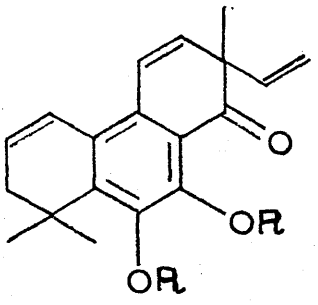
(98)

mechanism shown.[99] It was thought that comparable reaction of (96) might give products derived by collapse of the intermediate ion (100).

However, the structure of the isolated acid transformation product was unknown at this stage and formulations for the antibiotic involving a secondary methyl group remained in considerable doubt.

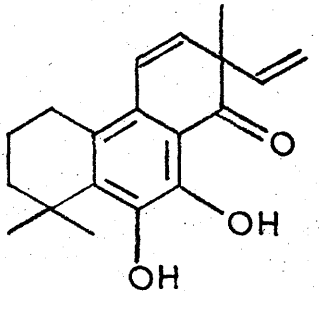
DISCUSSION

CHAPTER 2.

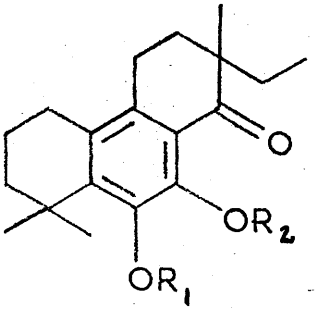


(101): R = H.

(102): R = Ac.



(103)



(104): $R_1 = R_2 = H$.

(105): $R_1 = R_2 = Me$.

(106): $R_1 = Me$, $R_2 = H$.

Flascherone and Dihydroflascherone.

In the chromatographic separation of the metabolites of A.flaschentraegeri, a series of fractions, obtained from the column immediately after elution of the fatty non-polar oils, contained a mixture of two co-distilling closely related pigments. Owing to their similar polarity (Rf = 0.50 and 0.55 in 100% benzene), repeated chromatography was required to effect separation.

The major pigment, flascherone (101), a bright yellow oil (b.p. 100°C/0.03mm.) gave an intense black colouration with ferric chloride and showed a parent ion at $^{m}/_{e}$ 296 corresponding to $C_{19}H_{20}O_3$, though an analytical sample was contaminated with the dihydro compound (H_{found} 7.32%; $H_{required}$ 6.80%). Two hydroxyl signals (-2.70 and 3.93τ) were evident in the complex n.m.r. spectrum; one of these was strongly chelated, possibly to an aryl ketone ($\nu_{C=O}$ 1640cm^{-1}).

The intense stain observed with ferric chloride was held to indicate the presence of a catechol grouping, further verification for which came from colorations on chromatographic paper with ethylene diamine-sodium carbonate[100] and ammonium molybdate-sulphuric acid reagents.[101] The reluctance of this system to form cyclic derivatives with (e.g.) methylene chloride[102] and dichlorodiphenylmethane[103]

was attributed to the strong intramolecular hydrogen bonding of the 7-hydroxyl group: chemical confirmation of the 1,2-diphenol system was not possible.

The pigment did however yield, on treatment with acetic anhydride in pyridine, the diacetate (102, 3H singlets at 7.68 and 7.70τ, parent ion $^{m}/_{e}$ 380 showing two successive losses of 42 mass units, i.e. ketene), an almost colourless oil which decomposed on attempted distillation. Its polarity (Rf = 0.45 in 100% chloroform) was considerably increased compared with the diol (101, Rf = 1.0 in 100% chloroform), a common effect in chelated hydroxy-ketones in which the true polarity of both carbonyl and hydroxyl groups is masked until the hydrogen bonding is broken by, e.g., acetate formation. This was borne out by the higher frequency of the aryl ketone grouping which now appeared at 1685cm^{-1}. Acetylation of the phenolic hydroxyl groups was also reflected in the u.v. spectrum of the diacetate which showed a single maximum at a shorter wavelength (245nm.) than the parent pigment. The reduced electron density of the aromatic ring also caused shifts in the resonances of the olefinic protons which clarified the splitting patterns. Thus the olefinic resonances consisted of 1H doublets (J = 10.5Hz.) at 3.10 and 3.93τ forming an AB system together with broad 1H doublets (J = 10.5Hz.) at 3.16 and 3.91τ coupled to a 2H multiplet at 7.72τ in an ABX_2 system. In addition, the vinyl

group gave rise to well-defined 1H multiplets at 4.17, 4.87 and 4.93τ (J_{AX} = 17.5, J_{BX} = 11 and J_{AB} = 1.0Hz.) forming an ABX system as verified by double irradiation. Three tertiary methyl groups were evidenced by singlet resonances at 8.62τ(3H) and 8.67τ(6H). These data confirm the tentative assignment of structure (101) to flascherone.

An examination of the very similar n.m.r. spectrum of the minor pigment (parent ion at $^{m}/_{e}$ 298) led to structure (103), 1.2-dihydroflascherone ($C_{19}H_{22}O_3$). The olefinic region showed the presence of the vinyl group and of only two olefinic protons (3.15 and 4.07τ) in an AB system. A 2H multiplet at 7.10τ (J_d = 6Hz., J_t = 3Hz.) was attributed to the methylene group at benzylic position 1.

The catalytic hydrogenation of flascherone over 10% palladium-charcoal afforded only hexahydroflascherone (104). Though no pure samples of intermediate di- and tetra-hydro products were obtained, it was shown by monitoring the reduction with n.m.r. that, as might be expected on electronic and steric grounds, ease of reduction was in the order: $\Delta^{15(16)} > \Delta^{1(2)} > \Delta^{11(12)}$. The hexahydro derivative (parent ion at $^{m}/_{e}$ 302) was readily purified since any dihydro impurity (103) in flascherone would afford the same product. The i.r. spectrum of the hexahydro product (104) was similar to that of flascherone, the carbonyl band (1645cm^{-1}) being again due to

Table 1. The E.T. band in 7,8-dioxygenated 1-tetralones.

Parent chromophore PhCOR	246nm.
Ring residues o, m and p	16nm.
Hydroxyl or methoxyl o and m	14nm.
Calculated λ_{max}	276nm.

Table 2. U.v. data on hexahydroflascherone (104) and related compounds.

Hexahydroflascherone (104)		275	314	397
	OH^-	283	320	420
6,7-O,O'-Dimethylhexahydro-flascherone (105).		278	360	
o-Vanillin[113]	H^+	266	350	
	OH^-	285	395	
5-Ethyl-o-vanillin		269	351	
4-Bromo-6-methoxy-7-hydroxy-indan-1-one[112]		262	343	
	OH^-	267	384	
5-Methoxy-8-hydroxy-1-tetralone[111]		260	356	
5-Hydroxy-8-methoxy-1-tetralone[111]		259	342	
5,8-Dimethoxy-1-tetralone[111]		253	338	

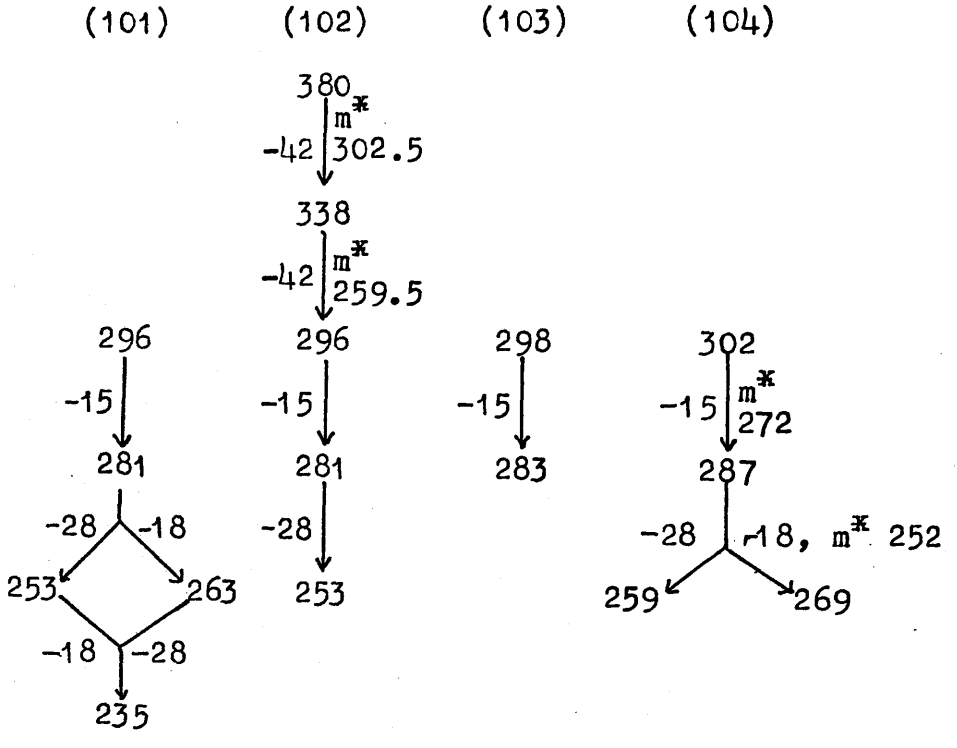
the substituted ortho-hydroxyacetophenone system (hydroxyl resonance at -2.82τ). A 4H multiplet at ca. 7.7τ was attributed to the benzylic C-1 and C-11 methylene protons while a 3H triplet (9.28τ, J = 7Hz.) was assigned to the methyl part of the ethyl group.

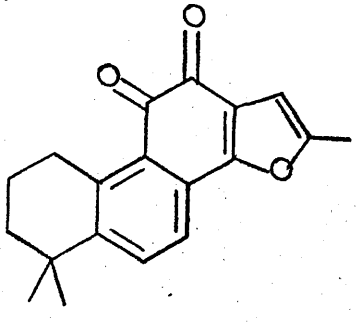
The action of methyl iodide or dimethyl sulphate with anhydrous potassium carbonate on hexahydroflascherone (104) afforded the dimethyl ether (105,* weak parent ion at $^{m}/_{e}$ 330, 3H singlets at 6.10 and 6.21τ), distillation of which caused considerable breakdown to a monomethyl ether (parent ion $^{m}/_{e}$ 316). The dimethyl compound displayed effects of breaking the 7-hydroxyl hydrogen bonding (Rf = 0.25 in 100% chloroform, $\nu_{C=O}$ 1690cm^{-1}) similar to those observed for the diacetate (102).

Hexahydroflascherone (104) and 6,7-O,O'-dimethylhexahydroflascherone (105) have a 7,8-dioxygenated 1-tetralone chromophore. The absorptions at 275 and 278nm. respectively were assigned to the E.T. band whose value calculated by Scott's rules[104] is 276nm. (Table 1), though the same result may be arrived at for 5,8-dioxygenated 1-tetralones whose

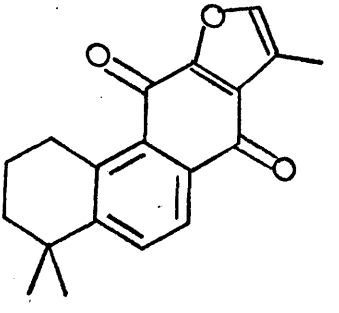
* It was shown by monitoring the reaction with t.l.c. that the 6-hydroxyl group did not react fast enough to permit isolation of a monomethyl ether (106), a mixture of the two ethers and starting material invariably being present at intermediate stages.

Table 3. Mass spectral breakdown of flascherone (101) and related compounds.





(107)



(108)

spectral data, with those of other relevant compounds, is shown in Table 2.

The close relationship between flascherone (101), dihydroflascherone (103) and the derivatives described in this chapter is reflected by the similar breakdown patterns observed in their mass spectra (Table 3).

The pigment flascherone (101) is thus a 20-norditerpene, the only previous examples of these being in the tanshinone group, involving 8 o- and p-naphthaquinones from Salvia miltirrhiza (e.g., T-II, 107)[105]. Some of these have a nor-abietane skeleton in which the isopropyl group is incorporated in a furanoid function (e.g., 108, an isotanshinone).[106]

DISCUSSION

CHAPTER 3.

Some Reactions of Traegeric Acid under Strongly Acid Conditions.

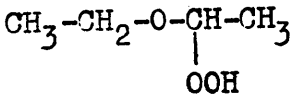
As indicated earlier, evidence for a secondary methyl group ~~in~~ appeared to be provided by its remarkable reaction with copper sulphate in ethereal sulphuric acid, affording a less polar and apparently neutral compound with a well-defined methyl doublet (8.45τ J = 5.5Hz.) and associated 1H quartet (4.59τ) in its n.m.r. spectrum. Since the moiety MeCH=, MeCH-OR or MeCH$\begin{matrix}O\\O\end{matrix}$ might account for these signals, it was thought that loss of acetaldehyde, e.g. by a retro-aldol process, might be partly responsible for the low yield. The reaction was therefore repeated with the addition of acetaldehyde to the mixture, with a gratifying improvement in the yield of the same compound as the only product. The transformation appeared to involve loss of carbon dioxide and water since an apparent molecular ion at $^{m}/_{e}$ 284 ($C_{19}H_{24}O_3$) was observed. However no carbonyl absorption was apparent in the i.r. and difficulty was experienced in interpreting this reaction in terms of structure (96).

At this stage, the important observation was made that, if the reaction was carried out in the presence of various aldehydes in place of acetaldehyde, analogous products were obtained. With benzaldehyde, for example, a product was

isolated whose n.m.r. showed a 5H multiplet at ca. 2.60τ and a 1H singlet at 3.77τ in place of the signals attributed to the secondary methyl group. Since these compounds all had almost the same mass spectrum (apparent molecular ion at $^{m}/_{e}$ 284), it was evident that they were acetals which were able to undergo facile mass spectrometric loss of the appropriate aldehyde. Peaks in the spectra of the benzaldehyde ($^{m}/_{e}$ 116 and 115) and m-bromobenzaldhyde ($^{m}/_{e}$ 184/6 and 183/5) products were assigned to the aldehyde parent ion and the corresponding acylium ion.

It follows that, irrespective of the source of the acetaldehyde in the first reaction carried out, the product (actual m.w. 328) is the acetal of a compound derived via a net loss of carbon dioxide only from traegeric acid which therefore does not have a secondary methyl group.

Accumulation of peroxides is a well-known hazard in storing aliphatic ethers and quantitative peroxide determinations have been recommended.[107] A sample of diethyl ether used in this reaction was found to contain $> 0.005\%$ peroxides, i.e. $> 10^{-4}$ mole in 50ml. Structures (109 and 110) for these compounds show their close relationship with hemiacetals and acetals; they might be expected to exhibit a similar acid lability, though the conditions used were strongly dehydrating. They could however take part in a



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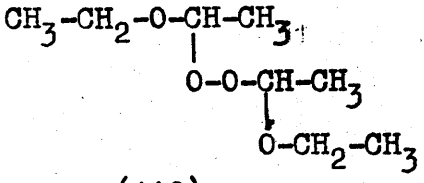

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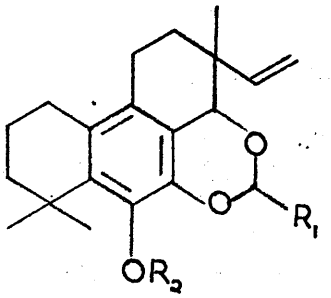


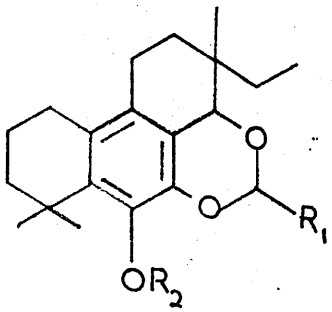
(111): R_1 = Pr, R_2 = H.

(112): R_1 = Me, R_2 = H.

(115): R_1 = Me, R_2 = Ac.

(116): R_1 = Ph, R_2 = H.

(116a): R_1 = m-Br-Ph, R_2 = H.



(113): R_1 = Me, R_2 = H.

(114): R_1 = R_2 = Me.

transacetalisation type of reaction (to give the observed yield of transformation product). When the reaction was carried out in di-n-propyl ether, the very poor yield of propylidene compound (111) isolated could be attributed to the fact that a freshly opened bottle of the ether was used and that this was relatively peroxide-free.

The n.m.r. spectrum of the ethylidene derivative (112) showed certain features in common with traegeric acid. A gem-dimethyl grouping (6H,s, 8.60τ) and geminal methyl and vinyl groups were present, though the latter grouping appeared in the n.m.r. as duplicated signals. This showed that (112), although apparently homogeneous (t.l.c. and sharp m.p.) was actually an inseparable mixture of C-14 epimers. The configuration of the secondary oxygen function at this centre, which was evidenced bytwo broad signals (5.36 and 5.42τ, total 1H), would be expected to influence the resonances of adjacent groupings. A broad 4H multiplet (ca. 7.67) was attributed to protons at the benzylic C-1 and C-11.

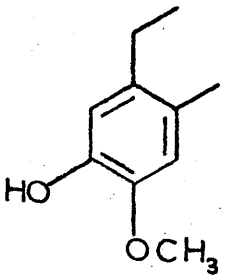
Catalytic reduction of (112) afforded the product (113) whose n.m.r. spectrum showed, in place of the resonances characteristic of a vinyl group, those of an ethyl group, the methyl part appearing as a 3H triplet at 9.08τ(J = 7Hz.). An exchangeable signal (4.60τ) and the colour reaction given by the compounds with ferric chloride were taken as evidence of a phenolic function. With reference to the pigment,

Table 4. U.v. data on 7,14-O-ethylidenetetrahydroflascherol and related compounds.

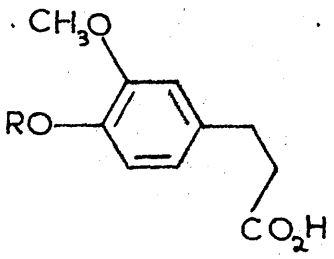
Compound (112)	λ_{max}	283, 291 nm.
(113)		284, 291 nm.
(114)		280, 288 nm.
(115)		279, 287 nm.
(116)		283, 291 nm.

These compounds may be compared with

5-Ethylcreosol[114]	λ_{max}	284 nm.



5-Ethylcreosol



(117): R = H.

(118): R = Ac.

flascherone (101) described previously, the ethylidene compound (112) can be designated 7,14-O-ethylidene-tetrahydro-flascherol.

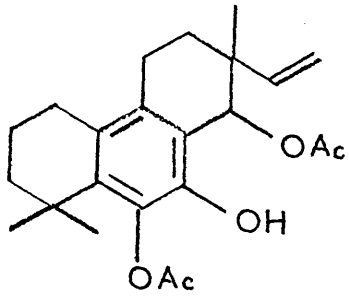
The treatment of 7.14-O-ethylidenehexahydroflascherol (113) with dimethyl sulphate and anhydrous potassium carbonate yielded the methyl ether (114, 3H singlet at 6.13τ) whose mass spectrum showed the true parent ion ($^{m}/_{e}$ 344). The acetate (115) was prepared from 7,14-O-ethylidenetetrahydroflascherol (112) by reaction with acetic anhydride in warm dry pyridine. The phenolic ester showed i.r. absorption at 1775cm^{-1} while a 3H singlet (7.67τ) in its n.m.r. spectrum was attributed to the acetate protons. In the mass spectral breakdown, the parent ion ($^{m}/_{e}$ 370) showed successive losses of acetaldehyde (44 units) and ketene (42 units).

The u.v. spectra of the compounds (112-116), Table 4, reflect their close relationship. The small effect ($\Delta\lambda_{max}$ = -4nm.) observed on acetylation of (112) is similar to that (λ_{max} 281→273nm.) which occurs when dihydroferulic acid[108] (117) is acetylated (118)[109]. The benzylidene compound (116) showed a reversible bathochromic shift in base while increased pH had no effect on the spectra of those incorporating acetaldehyde, (112) and (113). It is possible that, due to the inductive effect (+I) of the methyl group, the 7-oxygen lone pairs are more available for hydrogen bonding with the

phenolic hydroxyl in the acetaldehyde compounds which are therefore less acidic. This influence is reflected in the base strengths of ethylamine and benzylamine (pK_b 3.37 and 4.64 respectively).[110]

7,14-C-Ethylidenetetrahydroflascherol (112) also afforded, as a minor product in the acetylation reaction, the diacetate (119, 3H singlets at 7.76 and 7.81τ) whose i.r. spectrum showed absorptions corresponding to aryl (1780cm^{-1}) and aliphatic (1740cm^{-1}) ester functions. Signals attributable to an acetal grouping were absent and the resonance of the proton associated with the secondary oxygen function (4.05 and 4.22τ) showed a downfield shift of more than 1p.p.m. compatible with acetylation at that position. No evidence for the phenolic character of this compound was obtained from its i.r. and n.m.r. spectra, or from its behaviour with ferric chloride and the u.v. absorption was unaffected by addition of base.

The resonances attributed to H-14 in the n.m.r. spectra of the ethylidene compound (112) and the diacetate (119) and of their dihydro derivatives (113) and (120) are shown in Table 5. All these compounds are inseparable mixtures of C-14 epimers but duplicated signals are observed for the vinyl compounds (112) and (119) while only one signal appears in the dihydro derivatives at the higher of the two values.



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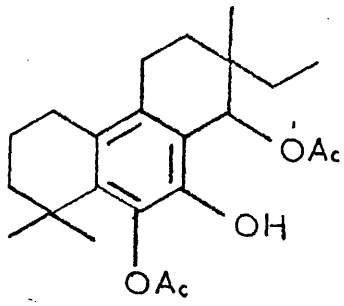
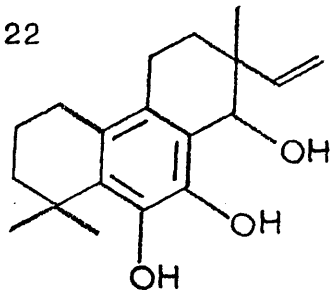


(120)

Table 5. N.m.r signals of H-14 (τ).

(112)	5.36 and 5.42
(119)	4.05 and 4.20
(113)	5.45
(120)	4.22



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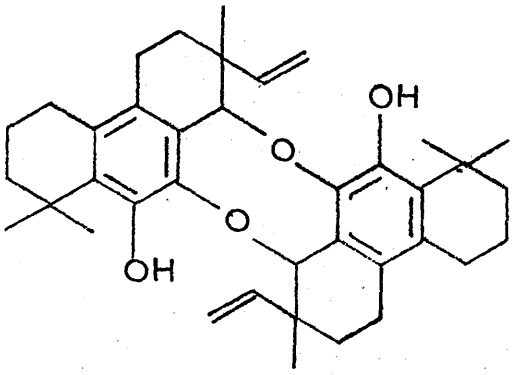

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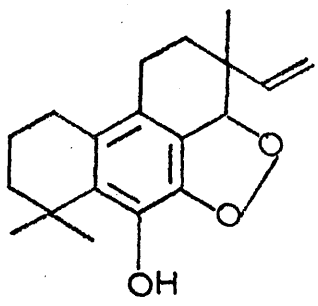


(123)

This may be attributed to the near-equivalence in electronic terms of the ethyl and methyl groups compared with the geminal methyl and vinyl groups at C-13.

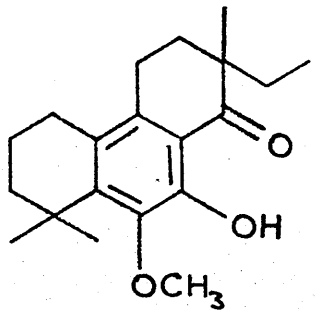
A transformation of traegeric acid related to that leading to 7,14-O-ethylidenetetrahydroflascherol (112) occurred when the antibiotic was refluxed in benzene with anhydrous copper sulphate. The i.r. spectrum (3550, 1640 and 1615cm^{-1}) was very simliar to that of (112) while its n.m.r. spectrum showed duplicated signals for the vinyl function and for one tertiary methyl group (8.57 and 9.09τ). One exchangeable proton (4.57τ) and the proton of a secondary oxygen function (5.39 and 5.48τ in the two epimers, total 1H) were present. Acetal formation in the absence of ether peroxides (and sulphuric acid) was not possible but the triol (121) was not an attractive structure for this non-polar compound. Dimerisation (122) or cyclic peroxide formation (123) may have taken place. However, this reaction proved to be irreproducible. Evidently some factor in the reaction is critical, e.g. the degree of hydration of the 'anhydrous' copper sulphate used. This remained to be determined.

In order to confirm the structure of the acetal derived by transformation of traegeric acid (7,14-O-ethylidenetetrahydroflascherol), interconversion with flascherone (101) was carried out. 6,7-O,O'-Dimethylhexahydroflascherone (105),

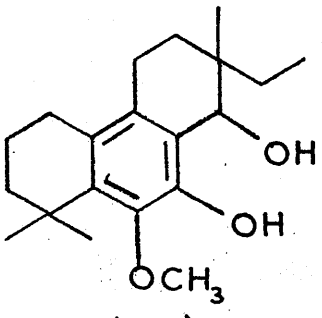
prepared from the pigment by catalytic reduction and treatment with methyl iodide, was subjected to a selective demethylation reaction with magnesium iodide etherate which cleaves only those methyl ethers giving rise to chelated hydroxyl groups.[115] The product, obtained in 45% yield was the considerably less polar monomethyl ether (124) whose 7-hydroxyl resonance (-2.88τ) and i.r. absorption (2950cm^{-1}, very broad) reflected strong hydrogen bonding to the carbonyl function ($\nu_{C=O}$ 1630cm^{-1}).

Reduction of 6-O-methylhexahydroflascherone (124) with sodium borohydride in ethanol yielded the secondary alcohol (125) whose hydroxyl protons had resonances at 2.61 and 3.10τ and which absorbed at 3520 and 3320cm^{-1} in the i.r. Since borohydride ion could approach the C-14 carbonyl site of reaction from above or below the molecule, a mixture of compounds epimeric at that position would be expected. As noted in the other reduced compounds (113) and (120) of this general structure, H-14 appeared as one signal (5.34τ).

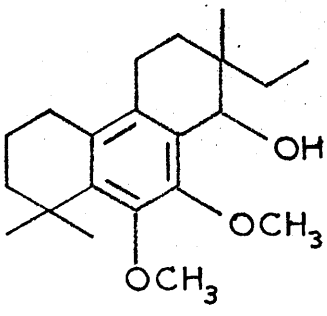
6-O-Methylhexahydroflascherol (125), exposed to normal acetalisation conditions with acetaldehyde, afforded the ethylidene compound (114), in an overall yield from flascherone of 25%, identical (t.l.c., i.r., n.m.r. and m.s.) with 6-O-methyl-7.14-O-ethylidenehexahydroflascherol prepared via (112) from traegeric acid. The n.m.r. spectrum of the



(124)



(125)



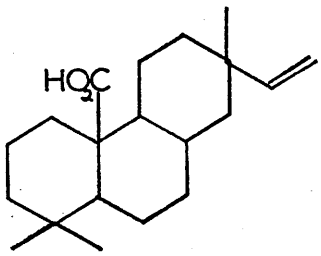
(126)

flascherone-derived compound showed two signals (8.90 and 9.11τ, total 3H) for the C-13 methyl group.

A series of reactions converting traegeric acid into a flascherone derivative was also carried out. The dihydro methyl ether (114) was prepared as previously recorded and the acetal hydrolysed in refluxing methanolic hydrochloric acid. The diol (125) was obtained in 35% yield after chromatography and had similar t.l.c. and spectral properties to (125) derived from flascherone. Treatment with methyl iodide and potassium carbonate afforded the dimethyl ether (126) whose n.m.r. spectrum showed 3H singlets at 6.13 and 6.15τ and a secondary hydroxyl function (7.60τ: H-14 at 5.47τ). A mild oxidation of this group was attempted with manganese dioxide in dry acetone for some weeks; a very slow reaction, which appeared from t.l.c. and i.r. monitoring to stop after 50% conversion, took place. The oxidation was completed by treatment with Jones reagent at0°C for 1 minute. 6,7-O,O'-Dimethylhexahydroflascherone (105) was obtained in 85% yield (15% overall from traegeric acid) and proved identical (t.l.c., i.r. and n.m.r. spectra) with the flascherone-derived compound.

DISCUSSION

CHAPTER 4.



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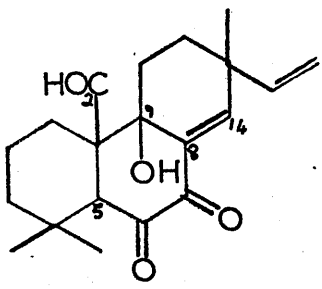

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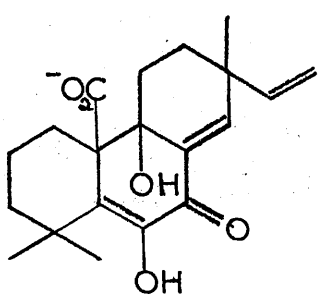


(129)

Table 6. The i.r. spectrum of traegeric acid (132).

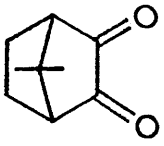
	Chloroform solution.		
(KBr)	(1.15mM)	(1.73mM)	(3.92mM)
3430(cm^{-1})	3616	3600	3600
3320	3458	3445	3445
1770	1775(ε467)	1772	1775
1745			
	1714(ε398)	1711	1713
1725			
1627	1623(ε225)	1621	1622

Traegeric Acid and its Reactions

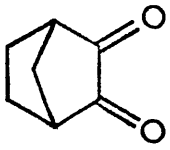
Determination of the structure of the transformation product (112) of traegeric acid cast new light on the structure of the latter. This transformation could be rationalised as involving decarboxylation of a pimaren-20-oic acid (127) to give the intermediate nor-diterpene triol (121) whose acetal was isolated. The 6,7-dihydroxy grouping in (121) corresponded to the α-diketone moiety in traegeric acid.

The structure (128) of the antibiotic could now be deduced from the n.m.r. and u.v. data. A trisubstituted double bond (olefinic proton at 3.32τ) incorporated in an enone system (λ_{max} 242nm.) could be assigned only as $\Delta^{8(14)}$. A 1H singlet (7.40τ) was attributed to H-5 and the exchangeable signal at 7.83τ to a hydroxyl group located at the remaining tertiary position, namely C-9.

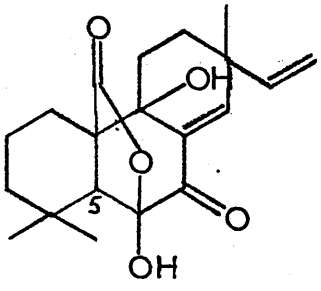
Further evidence for structure (128) came from the slow solubility of the antibiotic in aqueous sodium bicarbonate (without effervescence) and its u.v. spectrum in this medium which showed only a diosphenol absorption (species 129, λ_{max} 267nm.). However, i.r. data (Table 6)were not compatible with this formulation. Firstly, the hydroxyl band in a carboxylic acid would reflect intermolecular hydrogen bonding in the solid state (cf. deoxytetrahydrotraegeric acid);



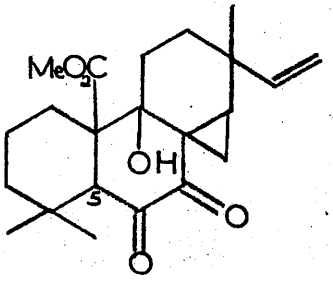
(130)



(131)



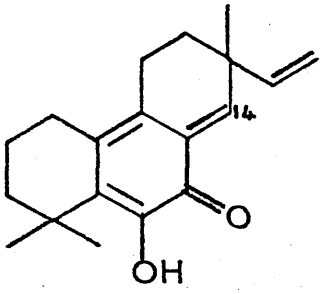
(132)



(133)

similarly, changes with concentration in the solution spectrum would be expected. The strong absorption (ca.1770cm^{-1}) can not be readily explained by consideration of (128). The i.r. spectra of α-diketones (130) and (131)[116] have carbonyl absorption at 1776 and1760, and at 1771 and 1760cm^{-1} respectively reflecting a maximum interaction between the carbonyl groups in these rigid systems.[117] However, absorptions at 1726 and 1743cm^{-1} have been recorded[118] for an 11,12-diketosteroid. The structure (132) was proposed for traegeric acid, the pseudo -acid hydroxyl proton appearing at 4.83 . H-5 (singlet at 7.40τ) is then β to two carbonyl groups and α to a carbon atom (C-6) bearing two oxygen functions. An analogous situation is met in (112) in which the acetal methyl resonance appears at 8.45τ.

An indication that the pseudo-acid formulation (132) was correct came from the n.m.r. spectrum of a product formed by treatment of traegeric acid with diazomethane,[119] assigned structure (133), i.e. methyl 8,14-methylenetraegerate (Chapter5). A 1H singlet resonance at 6.80τ was attributed to H-5 since this and the corresponding signal in traegeric acid (at 7.40τ) were enhanced (>25%) by irradiation at the 4,4-dimethyl group proton resonances.[130] The observed downfield shift in this signal (0.6p.p.m.) reflected generation of a carbonyl group at C-6 and was not adequately explained by the greater



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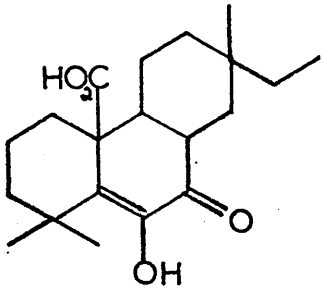

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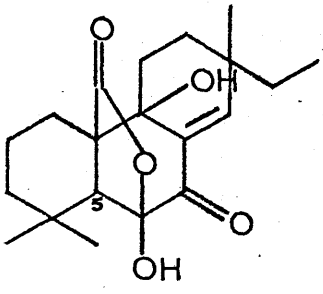

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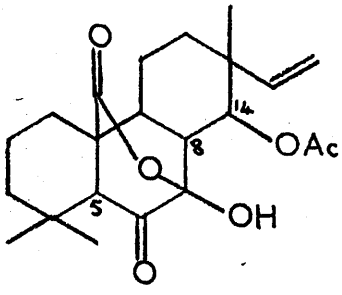


(137)

deshielding effect of a C-10 carboxylate group after esterification.

The transformation of traegeric acid (132) into (112) may proceed through (134). Aromatisation of ring B could now occur following 1,4-addition of water across the enone system. By analogy with reduction of (124) with sodium borohydride, a mixture of compounds epimeric at C-14 would be expected.

Further evidence for the presence of a potential carboxylic acid was furnished by a study of reduction of the antibiotic over palladium-charcoal, reported[94] to yield 'deoxytetrahydrotraegeric acid' (135, $C_{20}H_{30}O_4$, parent ion $^m/_e$ 344) which gave a colour reaction with ferric chloride. Its u.v. spectrum exhibited the bathochromic shift in base (λ_{max} 280- 330nm.) characteristic of a diosphenol group ($\nu_{C=O}$ 1660cm^{-1}) and showed no evidence of an enone function: the calculated maximum (Table 8) was in good agreement. A broad hydroxyl absorption (3200-2500cm^{-1}) in the solid state i.r. spectrum indicated the presence of a carboxylic acid ($\nu_{C=O}$ 1697cm^{-1} in solution). Reduction of the vinyl group was reflected in the appearance of a 3H triplet resonance (9.05τ, J = 7Hz.). The use of a less active catalyst (1% instead of 5% palladium-charcoal) effected selective reduction of the vinyl group (methyl triplet at 9.19τ, J = 7Hz.) and yielded 15,16-dihydrotraegeric acid (136, $C_{20}H_{28}O_5$) very similar to

the antibiotic ($\nu_{C=O}$ 1785cm^{-1}: pseudo-acid proton at 4.77τ, H-5 at 7.37τ).

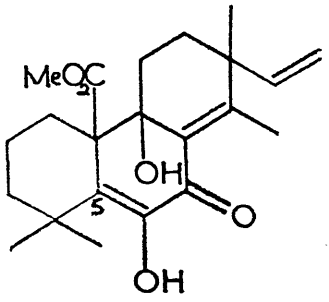
Interaction between the carboxylate function and the oxygen functions at C-6 and C-7 was observed in a number of products derived from traegeric acid. An attempted reductive acetylation with zinc and sodium acetate in acetic anhydride[120] afforded a monoacetate ($C_{22}H_{30}O_6$, parent ion $^m/_e$ 390, showing a loss of ketene; 3H singlet at 7.85τ) whose u.v. spectrum showed no absorption above 220nm; a diosphenolic chromophore was generated in base (λ_{max} 335nm., shifting to 286nm. on acidification). In the absence from the solid state i.r. spectrum of characteristic absorptions of carboxylic acid (ν_{O-H} 3200-2500cm^{-1}) and γ-lactone ($\nu_{C=O}$ ca.1770cm^{-1}) functions, this compound was assigned the pseudo-acid structure (137, hydroxylic proton at 3.50τ) incorporating a δ-lactone whose carbonyl absorption ($\nu_{C=O}$ 1725cm^{-1}) coincided with that of the acetate group. A 1H singlet resonance at 7.32τ was ascribed to H-5 and broad 1H signals at 4.39 and 4.66τ were attributed to H-14 and H-8 respectively. It was proposed that the very low value for the latter proton was due to the deshielding influence of oxygen lone pairs on the δ-lactone function to which it was held in close proximity in a rigid system. A similar though less effective influence may have been exerted by the 14-acetoxyl group. Inspection of a model of (137) vindicated this explanation and showed that

only a small coupling could exist between H-8, H-9 and H-14.

Alternative formulations, notably involving cleavage of ring C and generation of an exocyclic methylene group at C-8, offered inferior explanations of the data available. Further work on this compound, possibly including aromatisation of ring B, is required.

DISCUSSION

CHAPTER 5.



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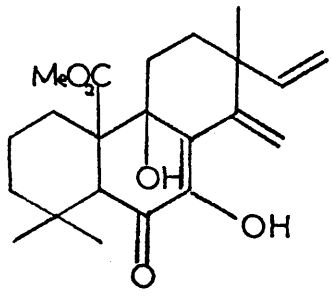
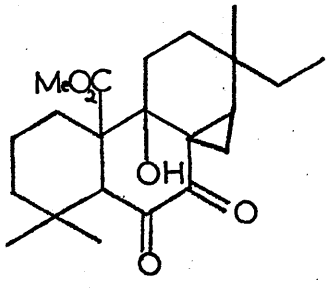


(138a)



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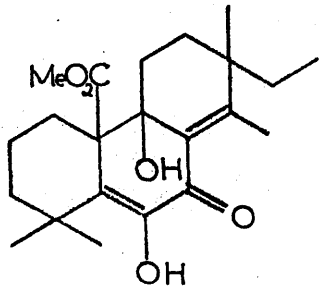


(140)

Part I: Esterification of Traegeric Acid.

Treatment of traegeric acid (132) with diazomethane[119] did not give a simple ester. Instead, two isomeric esters were obtained in which the enone system had also been attacked. The n.m.r. spectrum of the major product, methyl 8,14-methylenetraegerate (133, $C_{22}H_{30}O_5$, parent ion $^m/_e$ 374) showed a 3H singlet (6.27τ) assigned to a carbomethoxy group rather than a diosphenol O-methyl ether on u.v. spectral evidence (λ_{max} 273nm., with a bathochromic shift in base). Signals attributable to cyclopropyl protons (ca.9.0τ) were not discernible but the olefinic resonance observed in the spectrum of traegeric acid (H-14, 3.32τ) was absent.

The minor isomer, methyl 14-methyltraegerate (138, $C_{22}H_{30}O_5$, parent ion $^m/_e$ 374) showed in its n.m.r. spectrum a methoxyl signal (6.44τ) and four methyl singlets of which one (8.00τ) was assigned to a vinyl methyl group. The absence of a 1H singlet attributable to H-5 led to formulation (138) in which the lower of two hydroxyl resonances (2.82 and 7.63τ) was ascribed to the diosphenol grouping shown. However, the large discrepancy between the observed (λ_{max}^{obs} 319nm.) and calculated (λ_{max}^{calc} 279nm., Table 8) u.v. maxima suggested the possibility that the tautomeric form (138a) could exist in ethanolic solution. This is borne out by the high value (λ_{max} 373nm.) found in base. The dihydro derivatives (139) and

Table 7. Mass spectra of the esters (133) and (138)-(140).

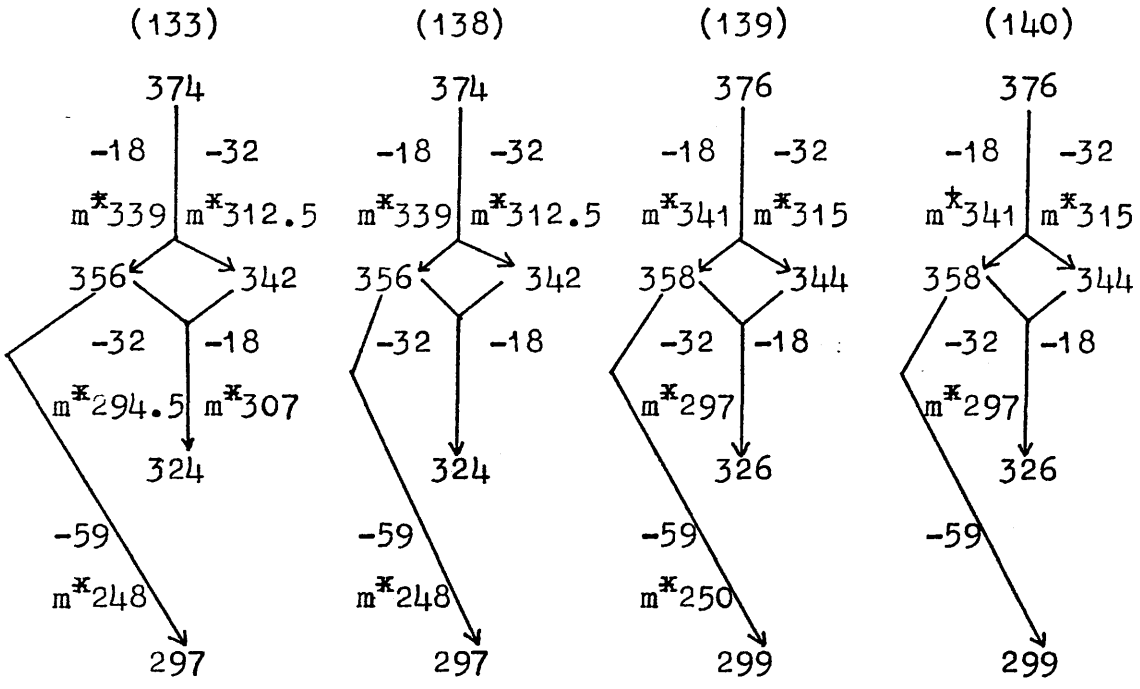
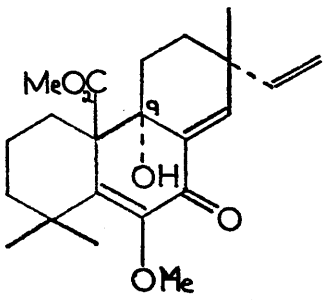


Table 8. Calculation of λ_{max} for (138)

Parent enone value	215nm.
α-hydroxyl group	35nm.
2 β-alkyl substituents	24nm.
exocyclic double bond	5nm.
λ_{max}^{calc}	279 nm.

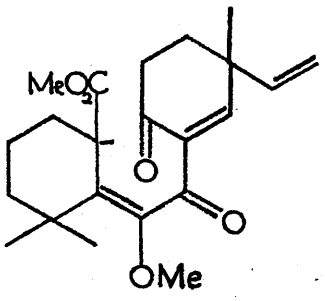
(140), $C_{22}H_{32}O_5$, parent ions $^m/_e$ 376, were prepared by catalytic hydrogenation, the newly formed primary methyl group being clearly evident in the n.m.r. spectrum of the latter as a 3H triplet at 9.20τ(J = 7Hz.). Mass spectral data of the compounds (133) and (138)-(140), Table 7, reflect their similarity in structure. There was no evidence for intermediate formation in interrupted diazomethane methylation reactions.

Attempted methylation of traegeric acid with methyl iodide and potassium carbonate led[94] to breakdown of the antibiotic. Methyl iodide and barium oxide in dimethylformamide have been used to prepare dimethyl ketal derivatives of non-enolisable α-diketone compounds.[121] Subjected to these conditions, traegeric acid (132) afforded, as a major product, a dimethoxy compound (141, $C_{22}H_{30}O_5$, parent ion $^m/_e$ 374, 3H singlets at 6.44 and 6.60τ) whose u.v. spectrum (λ_{max} 266 and 293nm.) was unaffected by base. Since a resonance attributable to H-5 was absent from the n.m.r. spectrum, one methoxyl was assigned to a diosphenol system. The u.v. absorption of this chromophore could not be predicted, the carbonyl group being also in conjugation with a $\Delta^{8(14)}$ double bond. The second methoxyl group was assigned to a ester group ($\nu_{C=O}$ 1730cm^{-1}) and only one exchangeable signal (7.55τ) was observed in the n.m.r. spectrum. Resonances attributed to the vinyl group appeared at 4.26, 4.89 and 4.92τ.

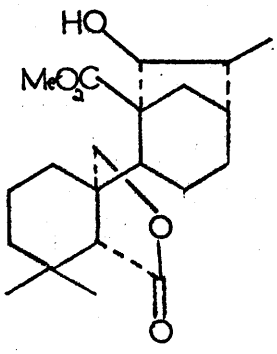


(141)

(141a): 9-epi.



(142)

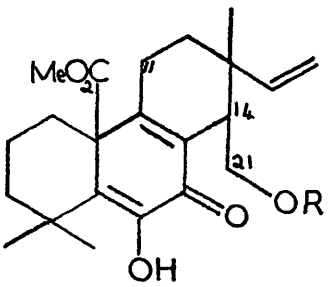


(143)

The second product of this reaction (3H singlets at 6.30 and 6.50τ) was evidently an isomer of (141), having an identical mass spectrum and i.r., u.v. and n.m.r. spectra which were very similar. The vinyl signals of this minor product (4.43, 5.04 and 5.33τ) were shown by subsequent studies on traegeric acid derivatives to be abnormally high and this compound was assigned the structure methyl 6-O-methyl-9-epitraegerate (141a). Epimerisation at C-9 may arise by retro-aldol reaction through carbanion (142); a similar result has been observed[122] on treatment of (143) with base. A later reaction (Chapter 5-II) involving ozonolysis of the $\Delta^{15(16)}$ double bond and internal acetal formation by the resulting aldehyde function with the 9-hydroxyl group will show that in the natural configuration, these groups are <u>cis</u>. The effect of inverting C-9 configuration, namely the shifting to higher field of the vinyl resonances, is in keeping with through-space deshielding of these protons by the oxygen lone pairs in the natural series.

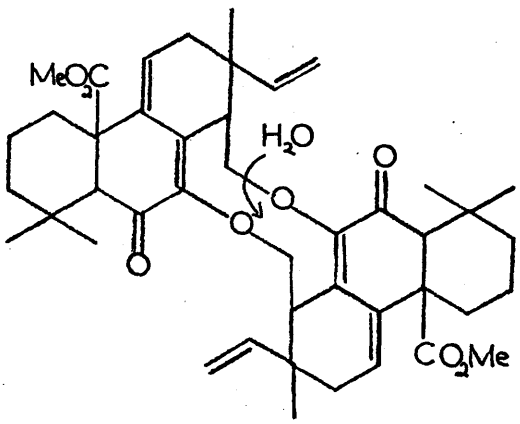
Part II: Reactions of methyl 8,14-methylenetraegerate and methyl 14-methyltraegerate.

It was proposed to test the ease of dehydration of the tertiary alcohol (133) which might be significant with respect to the stereochemistry at C-9 and at the same time afford a product whose n.m.r. spectrum would contain well-defined signals for protons at positions 11 and 12. However, treatment of methyl 8,14-methylenetraegerate (133) with p-toluenesulphonic acid in refluxing dry benzene yielded three products, in all of which the cyclopropyl grouping had been cleaved and the ester and vinyl groups remained intact. The first compound was a primary tosylate assigned the structure (144, $C_{29}H_{36}O_7S$, parent ion $^m/_e$ 528). This gave a colour reaction with ferric chloride and one exchangeable signal (3.28τ) in its n.m.r. spectrum was assigned to the diosphenolic hydroxyl group. The complex u.v. spectrum (λ_{max} 262 and 307nm.)disappeared on addition of base; the higher wavelength absorption may indicate the presence of a tautomeric form similar to (138a). N.m.r. evidence for the tosyloxy group (2H doublets at 2.42 and 2.76τ, J = 9Hz., 3H singlet at 7.62τ) disappeared when the compound was refluxed with sodium acetate in dry ethanol, affording the primary alcohol (145, $C_{22}H_{30}O_5$, parent ion $^m/_e$ 374, exchangeable H at 3.03 and 7.25τ). An ABX system was formed by the

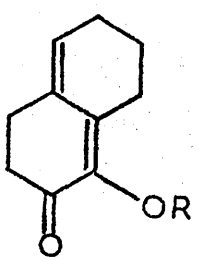


(144): R = Ts.

(145): R = H.



(146)



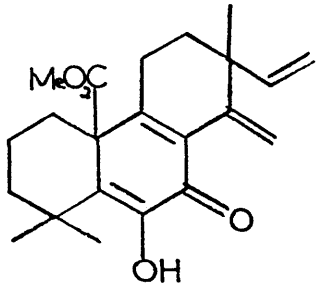
(147)

signals for the methylene group bearing the tosyloxy grouping (5.88 and 5.96τ, J_{AB} = 7Hz.) together with those for H-14 (7.28τ, J_{AX} and J_{BX} = 1Hz.). The former resonances moved to higher field (6.71 and 6.21τ, J = 11Hz.) in the n.m.r. spectrum of the alcohol (145) though 'twinning' was evident, signals also appearing at 6.72 and 6.40τ(J = 12Hz.). This could be explained by formation of a mixture of compounds diastereomeric at positions 8 and 14 during addition of diazomethane. A 2H multiplet (ca.7.3τ) in the spectrum of (144) was assigned to the C-11 methylene group whose protons were vinylogously α to carbonyl.

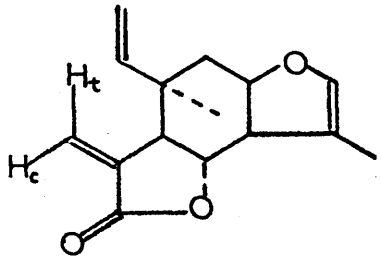
The second product from the reaction of methyl 8,14-methylenetraegerate(133) with p-toluenesulphonic acid had a complex u.v. spectrum (λ_{max} 258, 287 and 335nm.) though the highest wavelength absorption was in agreement with the value calculated (Table 9) for one of the possible chromophores (147, λ_{max}^{calc} 338nm.). The spectrum was unchanged by addition of base and the compound gave no reaction with ferric chloride. No monomeric formulation was in agreement with the spectral data and this compound was tentatively assigned the dimeric structure (146). In order to account for a hydroxyl function (ν_{O-H} 3520, exchangeable 1H signal at 3.92τ), this would have to be a monohydrate, perhaps involving a molecule of water trapped within the 10-membered heterocyclic ring linking the

monomer units. Attempted reduction of the vinyl group, to yield a product with a simplified low-field region in its n.m.r. spectrum, effected breakdown of the compound. Apart from signals due to the vinyl group derived from (133), a 1H multiplet (4.32τ) and two broad singlets (ca.4.8τ) were attributed respectively to H-11 (forming an ABX system with the resonances due to the C-12 methylene group) and the C-21 methylene group. Analytical and mass spectral data ($C_{22}H_{28}O_4$, parent ion $^m/_e$ 356) were not compatible with (146) but it was supposed that the trapped water molecule could be lost during crystallisation and vacuum-drying and that facile breakdown to monomer (148) could occur on introduction to the mass spectrometer.

The third product from the action of p-toluenesulphonic acid on (133) showed no evidence of a hydroxyl group. The u.v. absorption (λ_{max} 265 and 318.5nm.), though unchanged by addition of base, could not be attributed to any single chromophore and was similar (Table 10) to that of the primary tosylate (144). This compound was tentatively assigned the dimeric structure (149, $C_{44}H_{56}O_8$) which could undergo facile mass spectral cleavage to the monomer ($C_{22}H_{28}O_4$, parent ion $^m/_e$ 356). The CH-CH_2-O- grouping was indicated in the n.m.r. spectrum by 1H signals at 5.57 and 5.74τ(J_{AB} = 9Hz.) and 7.16τ(J_{AX} and J_{BX} ca. 5Hz.) forming an ABX system.



(148)



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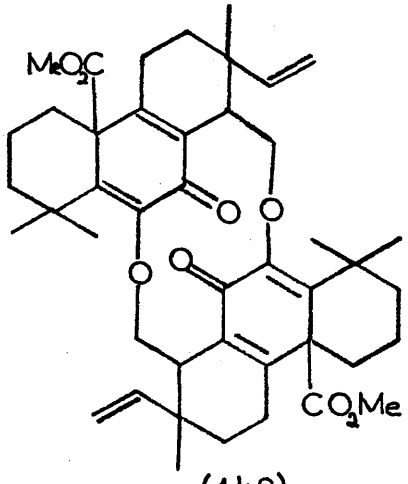

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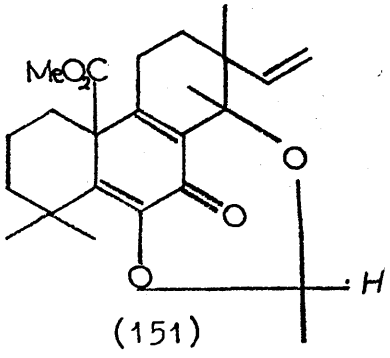
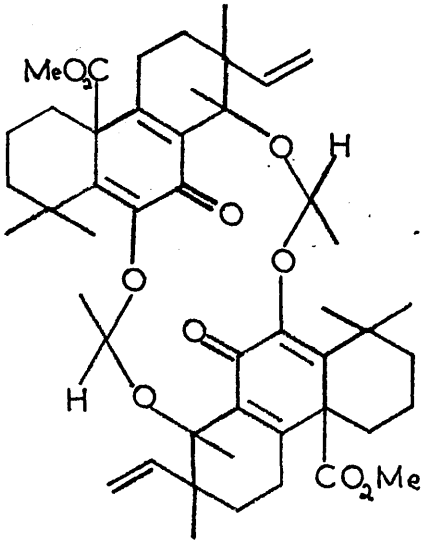


(151)



(152)

An apparently facile elimination of the 9-hydroxyl group occurred during formation of these three products though resonances attributable to H-11 and H-12 were discernible only in the n.m.r. spectrum of (146). Examination of the spectrum of a compound obtained from methyl 14-methylene-traegerate (164, p. 47) indicated more clearly the relationship between these protons. The structures assigned to the products of this reaction are sufficiently unusual to justify further investigation.

In order to prepare compounds related to (146) and (149), methyl 14-methyltraegerate (138) was subjected to the conditions used to effect the transformation of traegeric acid into (112) and two products isolated, to the major of which was assigned the structure methyl 8,14-dihydro-14-methylene-8,9-dehydrotraegerate (148, $C_{22}H_{28}O_4$, parent ion $^{m}/_{e}$ 356). I.r. ($\nu_{C=O}$ 1630cm^{-1}) and u.v.(λ_{max} 310nm.→ 386nm. in base) data were consistent with this formulation (cf. 138, Table 10) and the exocyclic methylene group protons appeared at 3.57 and 4.54τ with a typically small coupling (1.5Hz.). The non-equivalence of these protons was explained by the proximity of one to the 7-keto group. An analogous situation exists in isolinderalactone (150)[123] where the resonances of H_c and H_t are 3.70 and 4.36τ respectively.

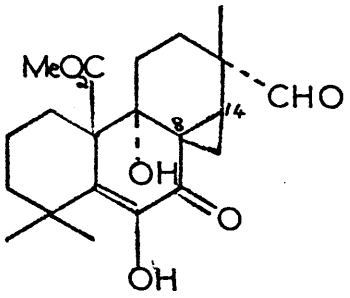
The second product of reaction of methyl 14-methyl-

Table 9. Calculation of λ_{max} for (147).[128]

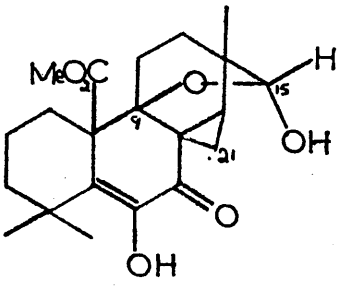
Parent enone value	215nm.
double bond extending conjugation	30nm.
α-hydroxyl group	35nm.
3 ring residues (β, γ and δ)	48nm.
2 exocyclic double bonds	10nm.
λ_{max}^{calc}	338nm.

Table 10. U.v. absorption of some traegeric acid derivatives.

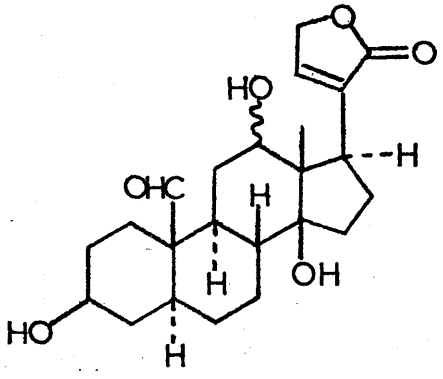
(138): λ_{max}	271 and 319nm., λ_{max}^{base}	373nm.
(144):	262 and 307nm.,	spectrum destroyed in base.
(145):	265 and 310nm.,	374nm.
(148):	288 and 310nm.,	386nm.
(149):	265 and 318.5nm.,	unchanged in base.
(151/152):	261 and 292nm.,	unchanged in base.



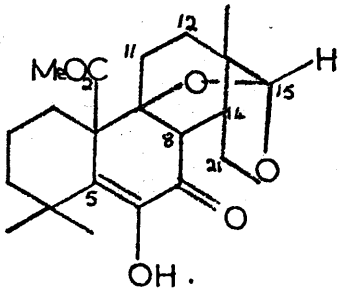
(153)



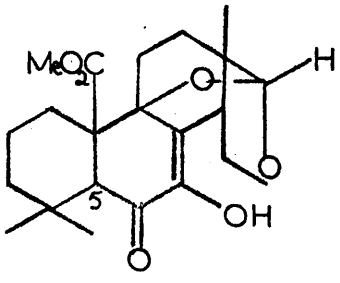
(156)



(154)



(155)

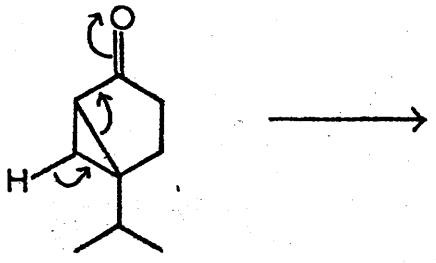


(155a)

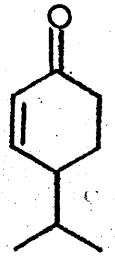
-traegerate (138) with acetaldehyde was an acetal (1H quartet at 4.83τ, 3H doublet at 8.50τ, J = 5.5Hz.) whose n.m.r. spectrum showed the presence of 4 tertiary methyl groups, resonances appearing at 8.72τ(9H) and 8.88τ(3H). These data are accommodated by the structure (151, $C_{24}H_{32}O_5$, parent ion $^{m}/_{e}$ 400). This however would be rather strained and a dimeric structure (152) giving rise to (151) in the mass spectrometer might also be possible. Table (10) shows the u.v. absorptions of (144), (145), (149) and (151/152) whose ring B substitution patterns are very similar.

The relative stereochemistry of the substituents in ring C of traegeric acid was established by ozonolysis of the derived methyl 8,14-methylenetraegerate (133). This afforded two products, the first of which was the aldehyde (ν_{C-H} 2710cm^{-1}, $\nu_{C=O}$ 1715cm^{-1}, 1H singlet at 0.41τ) with the expected structure 153 ($C_{21}H_{28}O_6$, parent ion $^{m}/_{e}$ 376). The deshielding effect of the formyl group was evident in the position of the resonances corresponding to H-12 (a 2H multiplet at 7.87τ). I.r. and n.m.r. spectra showed no evidence of hemiacetal formation between the hydroxyl and the formyl groups as might be expected from their 1,4-relationship (cf. calotropagenin, 154[124]). Conclusions about the relative stereochemistry of these groupings could have been drawn from the ease of lactonisation of the corresponding hydroxy-acid. The aldehyde, however, was obtained only in low yield and the desired

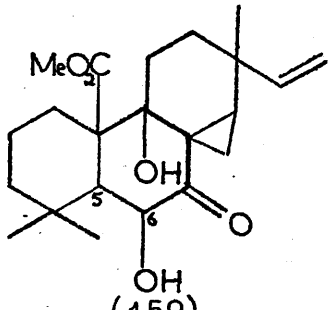
information was obtained from the unexpected structure of the second product of ozonolysis which was assigned the acetal structure (155, $C_{21}H_{28}O_6$). In the n.m.r. spectrum, H-15 appeared as multiplets at 4.50 and 4.75τ(total 1H) with complex though small couplings to the C-21 methylene protons (6.73 and 6.94τ, respectively) and (both) to protons at C-12 and C-14 (multiplet at 7.97), the latter forming an A_2B_2 system (J = 5Hz.) with a multiplet (7.27 and 7.15τ, total 2H) assigned to the C-11 methylene protons. Inspection of a model of (155) showed that, in the rigid fused-ring structure, H-14 and H-15 adopt a 'W'-configuration promoting a considerable degree of coupling[125] between these remote protons. The model indicated that attack by the hemiacetal group (156) at C-21 on the adjacent cyclopropyl ketone system had certain stereochemical requirements. Only one epimer of (156), namely that in which the cyclopropyl grouping lay on the same side of ring C as the hemiacetal function and in which the hydroxyl group of the latter was sufficiently close to C-21, could afford the observed acetal (155). In view of the stringent stereochemical requirements outlined above, (155) could not be a mixture of epimeric compounds and an alternative explanation of the duplication of n.m.r. signals was sought. The 6,7-diketone system had two α-methine positions, viz. C-5 and C-8, and could enolise in two ways, forming (155) or (155a), which would be expected to possess the same u.v.



(157)



(158)



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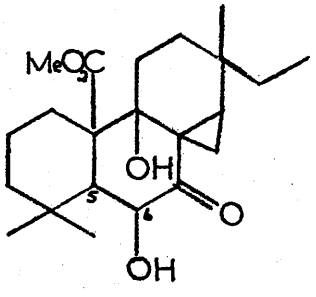
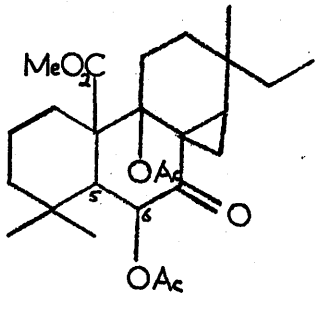


(160)



(161)

absorption (λ_{max} 312nm., with a reversible bathochromic shift of 70nm. in base) and i.r. spectrum ($\nu_{C=O}$ 1635cm^{-1}). It is reasonable to propose that quantitative conversion of the appropriate diastereoisomer of (153) into the acetal (155) had occurred during the work-up of the ozonolysis reaction and it is therefore not surprising that the hydroxy-aldehyde (153) isolated, comprising the opposite 8,14-diastereoisomer, could not be induced to form (155).

With a view to determining the absolute stereochemistry of traegeric acid by optical rotatory dispersion (ORD), a number of routes to monocarbonyl compounds were investigated. The use of metal hydrides gave many products which could not be separated; catalytic reduction of traegeric acid yielded a dihydro derivative (136) and deoxytetrahydrotraegeric acid (135). Reduction of (133) afforded the dihydro derivative (139) and, as shown later, a small quantity of the tetrahydro compound (159). Treatment of methyl 8,14-methylenetraegerate (133) with zinc and acetic acid at room temperature afforded a secondary alcohol ($C_{22}H_{32}O_5$, parent ion $^{m}/_{e}$ 376) whose formation was initially thought to involve reduction following cyclopropane cleavage analogous to the transformation: sabinaketone (157)→ enone (158).[126] However, its u.v. spectrum, and that of the dihydro derivative (160) prepared by catalytic hydrogenation, showed no absorption above 220nm. in ethanolic solution or on addition of deoxygenated base; passage of air

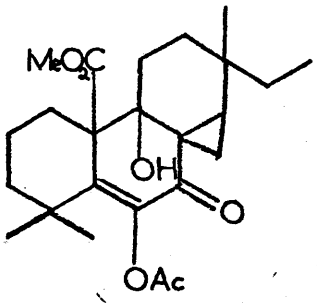
through the basic solution caused the appearance of a band at 335nm., moving on acidification to ca.285nm., which indicated that a diosphenolic chromophore could be readily generated by oxidation. The structure (159) was assigned to this compound whose n.m.r. spectrum showed two exchangeable proton signals at 6.63 and 8.27τ. The former was evidently part of a secondary hydroxyl function whose methine proton signal (H-6, 5.68τ) sharpened when the hydroxyl proton was exchanged and which was coupled in an AB system (J = 11Hz.) to a proton (H-5) which appeared as a sharp doublet at 7.39τ. The large coupling constant indicated that <u>trans</u> addition to the diosphenol had occurred. It was supposed that the AB ring junction was <u>trans</u> so that H-6 was on the same side of the molecule as the carbomethoxyl group. Hydrogen bonding in the α-ketol was reflected in i.r. absorption attributed to the 7-keto group ($\nu_{C=O}$ 1690cm^{-1}). Further evidence for structure (159) came from the reaction of methyl 8,14-methylenetraegerate (133) with zinc and d_4-acetic acid which furnished a product (parent ion $^{m}/_{e}$ 378) whose n.m.r. spectrum showed no resonances attributable to H-5 and H-6.

In order to fully substantiate this formulation, oxidation of (160) with chromic acid was carried out. This afforded a product shown by comparison of t.l.c. and spectral properties to be identical with a sample of methyl 8,14-methylene-15,16-dihydrotraegerate(139) obtained by catalytic hydrogenation of

(133): the same product was obtained from (139) under conditions of ozonolysis. Individual and mixed melting points (ca.165°C) of powdered samples of these compounds demonstrated their identity and established structure (159) for the product of zinc-acetic acid reduction.

Facile oxidation of the α-ketol grouping in (160) was demonstrated in its reaction with acetic anhydride in warm pyridine which afforded the corresponding diacetate (161, $C_{26}H_{38}O_7$, 3H singlets at 7.92 and 8.07τ) only as the minor product. Its mass spectrum showed an apparent parent ion at $^{m}/_{e}$ 402 (corresponding to loss of acetic acid) with subsequent loss of ketene (42 mass units). Strong i.r. absorption at 1730cm^{-1} was attributed to two alkyl acetate groups and the carbomethoxyl function (3H singlet at 6.46τ). Acetylation at C-6 caused a downfield shift in the geminal proton resonance (to 4.93τ) which formed an AB system (J = 10Hz.) with that of H-5 at 7.54τ.

The major product isolated from the acetylation reaction was the monoacetate (162, $C_{24}H_{34}O_6$, apparent parent ion $^{m}/_{e}$ 376 by a loss of ketene; 3H singlet at 7.82τ). The presence of a diosphenol acetate group was reflected in the appearance of a high carbonyl band in the i.r. spectrum (1765cm^{-1} and absorption at 250nm. (λ_{max}^{calc}250nm.) in the u.v. At present, the possibility that autoxidation of (160) to the diosphenol prior to acetylation cannot be ruled out.



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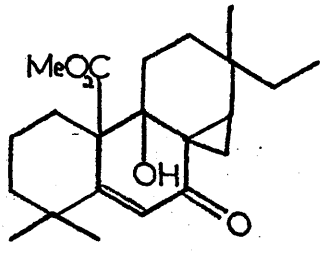

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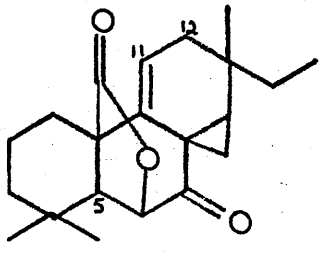


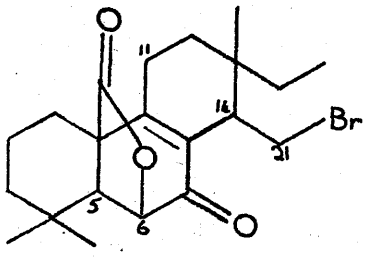
(164)

Treatment of (160) with p-toluenesulphonic acid did not effect dehydration to give enone (163) but afforded the γ-lactone (164, $\nu_{C=O}$ 1795cm^{-1}) whose n.m.r. spectrum showed no methoxyl resonance. Signals attributable to protons in ring C were evident, H-11 appearing as a triplet at 4.51τ(J = 5Hz.) coupled to H-12 (8.32τ): singlet resonances at 7.75 and 5.56τ were attributed to H-5 and H-6 respectively. The transformation could occur by protonation of the 6-hydroxyl group and attack by methoxyl lone pair at that position. The γ-lactone thus formed is 1,3-diaxial with respect to ring B, so that the H-C-H angle at C-5 and C-6 was nearly 90° and the coupling constant of this AB system was very small. The resonance of H-5 in this compound (7.75τ) may be compared with that in traegeric acid (132, 7.40τ) which possesses a pseudo-acid structure involving a similar γ-lactone linkage ($\nu_{C=O}$ 1775cm^{-1}).

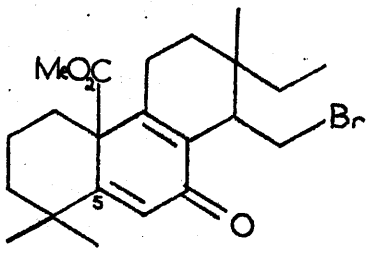
The γ-lactone function in (164) introduced to the molecule a degree of rigidity not present in the diacetate (161) This was reflected in the ketone carbonyl absorption of these compounds; in (164), the ketone is fully saturated ($\nu_{C=O}$ 1720cm^{-1}) because the cyclopropyl group cannot take up a planar conformation for conjugation. In the diacetate (161), however, the carbonyl frequency is 1685cm^{-1}, implying that such overlap may be achieved in the less rigid structure. These conclusions were upheld by examination of the appropriate models. An effect of this type was observed in the series of ketones shown in

Table 11. I.r. data of cyclopropyl ketones.[127]

Di-n-propyl ketone	1722cm^{-1}.
n-Propyl cyclopropyl ketone	1702cm^{-1}.
Di-cyclopropyl ketone	1694cm^{-1}.



(165)



(166)

Table 11.[127]

Attempts to prepare a heavy atom derivative of traegeric acid for X-ray studies proved fruitless. Catalytic reduction of methyl 8,14-methylenetraegerate (133) and subsequent treatment with bromoacetyl bromide and N,N-dimethylaniline in dry chloroform afforded, in very poor yield, three products of which the least abundant (product II, parent ion $^{m}/_{e}$ 374) contained no bromine and whose spectral properties could not be assigned to any structure. Formulations proposed for the other products could be readily derived from (160), the presence of which (as noted before, p. 44) was revealed by inspection of the n.m.r. spectrum of crude hydrogenated material from (133), doublets at 5.71 and 7.41τ(J = 10Hz.) and an exchangeable signal at 6.66τbeing in evidence.

Product I (165, $C_{21}H_{29}O_3Br$, parent ion $^{m}/_{e}$ 408/10) showed no evidence of a methoxyl or hydroxyl group in its i.r. and n.m.r. spectra. Similarity in structure with (164) was reflected in γ-lactone absorption ($\nu_{C=O}$ 1785cm^{-1}) and 1H singlet resonances at 5.44 and 7.62τ. Signals forming an ABX system (6.28, 6.57 and 7.32τ) were attributed to the protons of the C-21 methylene group (J_{AB} = 11Hz.) and H-14 (J_{AX} = 4.5Hz., J_{BX} = 3.5Hz.), the latter showing a small homoallylic coupling (ca.1Hz.) to the C-11 methylene group (ca.7.64τ). The u.v. absorption (λ_{max} 269nm.) could not be assigned to a definite chromophore, but the spectrum in base or acid was

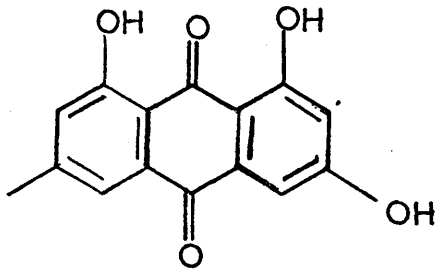
identical with that of (164), possibly indicating formation of the latter in base.

Product III (166, $C_{22}H_{30}O_3Br$) was a methyl ester ($\nu_{C=O}$ 1740cm^{-1}, 3H singlet at 6.48τ) whose u.v. spectrum (λ_{max} 251nm.) indicated the presence of a ketone ($\nu_{C=O}$ 1660cm^{-1}) in conjugation with a fully substituted double bond and with the $\Delta^{5(6)}$ double bond whose olefinic proton appeared at 3.62τ. Signals forming an ABX system were again present (6.25, 6.45 and 6.92τ) with homoallylic coupling between H-14 and one proton (7.15τ) of the C-11 methylene group, the geminal coupling being 10 Hz.

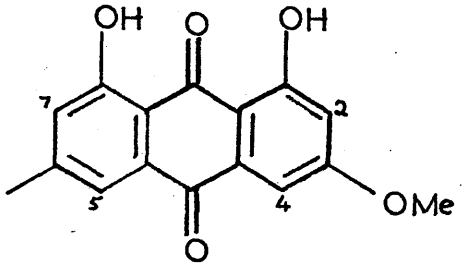
Insufficient material and time were available to investigate crystallographic and chemical work on these or other compounds.

DISCUSSION

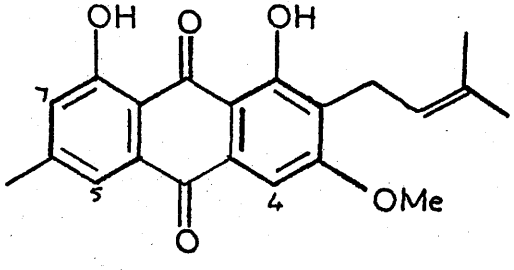
CHAPTER 6.



(167)



(168)



(169)

Table 12. U.v. spectra of the quinones (167)-(169)

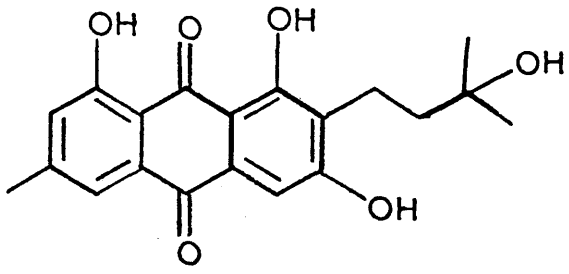
(167)	(168)	(169)
222nm.	218nm.	215nm.
252		
265		269
289	282	310
437	435, 456	438
520-530		

Table 13. Aromatic proton resonances of (168) and (169)

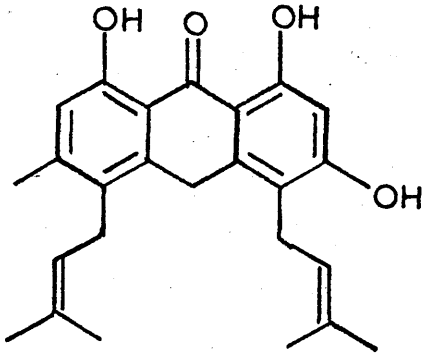
	(168)	(169)
H-5	2.41 τ	2.42
	$J_{5,7}$ = 2Hz.	$J_{5,7}$ = 1.5Hz.
H-7	2.92	2.98
H-4	2.63	2.65
	$J_{2,4}$ = 3Hz.	
H-2	3.32	

Physcion and a Related Metabolite.

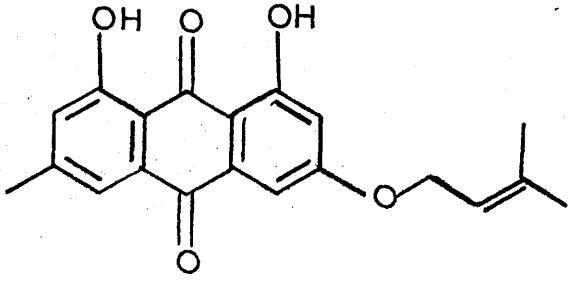
Two further pigments were obtained after elution of flascherone from a column of the metabolites of Aspergillus flaschentraegeri. The anthraquinonoid nature of these compounds was illustrated by their u.v. spectra, compared in Table 12 with that of emodin (167).[128] 2 exchangeable signals (-2.29 and -2.09τ)in the n.m.r. spectrum of the more abundant pigment (168, $C_{16}H_{12}O_5$, parent ion $^m/_e$ 284) were assigned to the protons of two chelated hydroxyl groups at positions 1 and 8, as indicated by i.r. absorption (1670 and 1625cm^{-1}) characteristic of this substitution pattern.[129] An aromatic methyl group (broad 3H singlet at 7.58τ) and a methoxyl function (3H singlet at 6.08τ) were positioned to allow the observed meta coupling between two pairs of aromatic protons. Broad 1H doublets (J = 2Hz.) at 2.41 and 2.92τ, which sharpened on irradiation at the aromatic methyl group, were attributed to H-5 and H-7 respectively, i.e. the lower value to the proton ortho to a quinonoid carbonyl group. Similarly, doublets at 2.63 and 3.32τ(J = 3Hz.) were assigned to H-4 and H-2. 1.8-Dihydroxy-3-methoxy-6-methylanthraquinone (physcion, 168) melted at 209-10°C and showed no depression of melting point when mixed with an authentic sample.



(170)



(171)



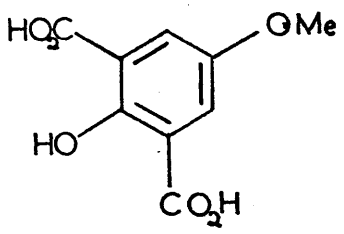
(172)

Similar i.r. absorption ($\nu_{C=O}$ 1681 and 1633cm^{-1}), hydroxyl(-2.34 and -2.06τ) and methyl (6.04 and 7.62τ) resonances and u.v. data (Table 12) showed that the minor pigment (169, $C_{21}H_{20}O_5$, parent ion $^m/_e$ 352) was closely related to physcion (168). Comparison of the aromatic proton resonances of these compounds (Table 13) indicated that the former was substituted at position 2. A 1H triplet at 4.83τ was assigned to the olefinic proton of a double bond substituted by two methyl groups (broad 3H singlets at 8.24 and 8.35τ) and by a methylene group (2H doublet at 6.63τ, J = 7Hz.) attached to the anthraquinone nucleus at C-2. Further evidence for the positioning of the dimethylallyl grouping came from a nuclear overhauser effect (NOE)[130] observed in the intensity of the H-4 resonance (25%) on irradiation at 6.04τ.

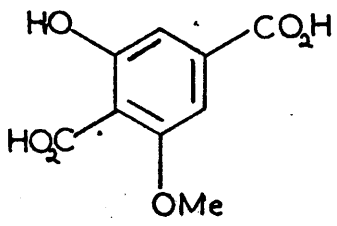
γ,γ-Dimethylallylphyscion (169) and the substituted emodin (170) isolated[131] from mycelial extracts of this fungus are the first C-prenylated anthraquinones obtained from nature. The C-prenylated anthrone (171) and an O-prenylated anthraquinone (madagascin, 172) have been obtained from a tree bark.[132]

DISCUSSION

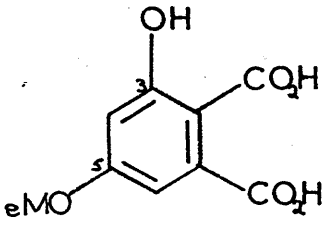
CHAPTER 7.



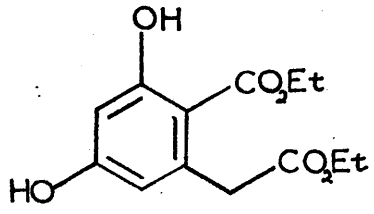
(173)



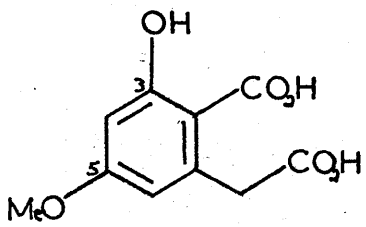
(174)



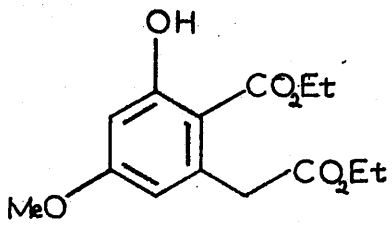
(175)



(176)



(178)



(177)

A Phthalic Acid Metabolite

Elution of a column of A.flaschentraegeri metabolites with ethyl acetate-chloroform mixtures afforded an aromatic diacid ($C_9H_8O_6$, parent ion $^m/_e$ 212) insoluble in the latter. Carboxyl group absorption ($\nu_{C=O}$ 1650 and 1700cm^{-1}) and a band ascribed to a chelated phenolic hydroxyl (ν_{O-H} 3000cm^{-1}) were evident in the i.r. spectrum; the compound gave a colour reaction with ferric chloride. 1H doublet resonances at 3.12 and 3.23τ(J = 3Hz.) were assigned to meta-coupled aromatic protons, neither of which was flanked by two carboxyl groups. A 3H singlet (6.02τ) was attributed to an aryl methoxyl group. A chelated hydroxyl group could not be accommodated between the unsubstituted positions,and structures (173) and (174) would not permit the observed non-equivalence of the aromatic protons. Formulation (175) was therefore adopted for this compound, i.e. 3-hydroxy-5-methoxyphthalic acid, whose mass spectral breakdown pattern is shown in Table 14.

In connection with these studies, 3-hydroxy-5-methoxy-homophthalic acid (178) was prepared from diethyl 3,5-dihydroxy-homophthalate (176) by methylation with diazomethane, followed by hydrolysis of the resulting monomethyl ether (177, 3H singlet at 6.18τ) to give the acid (178, $C_{10}H_{10}O_6$), whose methylene group appeared at 6.16τ. The i.r. spectrum showed

Table 14. Mass spectral breakdown of (175).

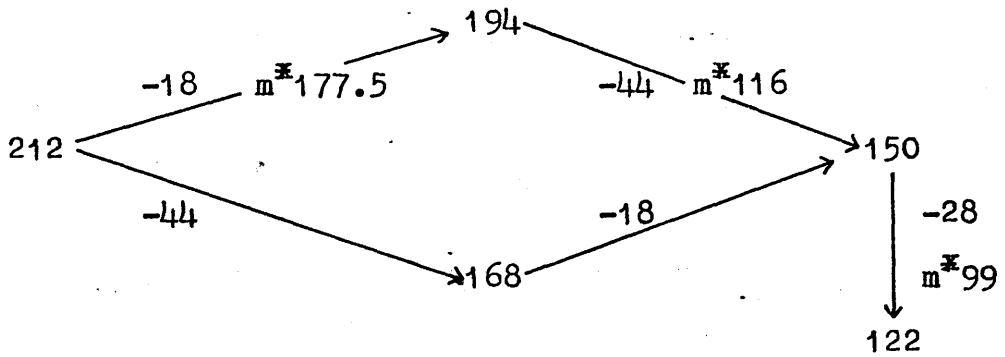
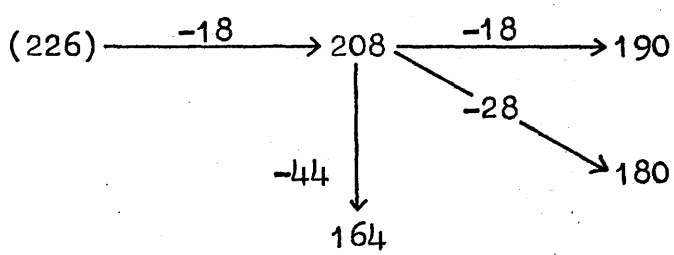


Table 15. Mass spectral breakdown of (178).



a broad hydroxyl band (3000cm^{-1}) and carbonyl absorption at 1685 and 1625cm^{-1}. An apparent parent ion ($^{m}/_{e}$ 208) appeared in the mass spectrum which is summarised in Table 15. A proposed oxidation to (175) was not, however carried out because of the low yields obtained in the preceding steps.[133]

EXPERIMENTAL

Instrumentation.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were obtained on a Unicam SP 800 recording spectrophotometer. Infra-red spectra were maesured with a Perkin-Elmer spectrometer. Nuclear magnetic resonance spectra were recorded with Varian T-60 and HA-100MHz. spectrometers. Unless otherwise stated, all values quoted were determined in deuterochloroform with tetramethylsilane as internal standard. Mass spectra were obtained with an AEI MS-12 mass spectrometer.

Thin Layer Chromatography.

Rf values were determined from elution on 0.25mm. layers of Kieselgel G, the compounds being located by spraying with ferric chloride (5% in methanol) and with ceric ammonium nitrare-sulphuric acid (1% in 10%) and heating; the results of the first spray and the colours developed by the latter spray are recorded thus:

T.l.c. Rf =0.45(5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: olive with grey halo.

(Numerical values are quoted as indications of relative polarity, not as quantitative reproducible results). Preparative thin layer chromatography (p.l.c.)

was carried out on 1mm. layers of Kieselgel Hf_{254}, bands being located by irradiation with ultraviolet light (λ= 254nm.).

General.

Diazomethane was prepared by the method of Moore and Reed from bis-(N-methyl-N-nitroso)-terephthalamide.[119] Hydrogenation experiments were carried out at 15°C and under 1 atmosphere of hydrogen. Ozone-enriched oxygen (6%) was used in ozonolyses, the reactions being done at -78°C (acetone-solid carbon dioxide bath). "Jones reagent" refers to an aqueous solution containing chromium trioxide (266mg./ /ml.) and concentrated sulphuric acid (405mg./ml.). All organic extracts were washed with a saturated brine solution and dried over magnesium sulphate; solvents were removed using a rotary film evaporator. "Light petroleum", unless otherwise stated, refers to light petroleum, b.p. 60-80°C.

The following abbreviations are used in reporting spectral data: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; and infl, point of inflection; also (in infra-red spectra): s, strong; m, medium; and w, weak.

In the following typical description of n.m.r. data: 3.9(1H,q, irr 8.4→ s,H-6); , irradiation at 8.4τ has resulted in collapse of a quartet at 3.9τ to a singlet: H-6 refers to all protons attached to C-6 unless denoted (e.g.) H-6α.

All assignments of n.m.r. signals to protons attached to oxygen were confirmed by exchange with D_2O.

EXPERIMENTAL

CHAPTER 1.

Growth and Extraction of the Mould.

Aspergillus flaschentraegeri (Commonwealth Mycological Institute No. 101,651) was subcultured onto 2% malt agar slants and seed grown on the same medium in Roux bottles (15 x 9cm.). A spore suspension prepared from 12 of these bottles and distilled water (2l.) was used to innoculate 100 Roux bottles which had previously been sterilised and contained 200 ml. each of the medium described opposite. The mould was grown at 75% humidity and 25°C and the cultures were harvested after 10 days.

The broth, its pH raised from 4 to 7, was adsorbed on activated animal charcoal (10g./l.), filtered and air dried. The adsorbed material was extracted with chloroform in a Soxhlet apparatus for 24 hours and the solvent evaporated to yield the crude broth extract (5g.).

Activity Assays.

I) Antifungal Assays.

Initial assays for antifungal activity using the serial dilution spore germination test on the conidia of Botrytus alii were carried out on replicate cultures of the fungus. After growth periods for A. flaschentraegeri of 5, 7, 10 and 12 days, each broth was filtered through

Culture Medium for Aspergillus Flaschentraegeri.

Glucose	1000g.
Ammonium tartrate	56g.
Potassium phosphate	100g.
Magnesium sulphate	20g.
Sodium chloride	20g.
Difco yeast extract	10g.
Malt extract concentrate	20ml.
Distilled water	to 20l.

The resulting fluid medium has pH = 5.

Table 16.

Initial Activity Assays on the Culture Filtrate of A.flaschentraegeri.

No. of days	5	7	10	12
Assay results	2/11	3/11	5/10	5/11

The table defines the dilution factor in powers of 2 at which a fungistatic effect (i.e. no germination, or germination less than 1%) / stunting effect is observed.

sterile No. 1 Whatman paper and the crude filtrates used for the assays (Table 16). From these data, the optimum growth time for the mould appeared to be 10 days and future growth for large scale production of the antibiotic was carried out for that period.

II) Antibacterial assays.

Traegeric acid, the antifungal antibiotic isolated from broth extracts of A. flaschentraegeri was assayed against cultures of Staphylococcus aureus and Bacillus megathereum, using the cup-plate method.

The antibiotic (1mg. in 5ml. 10% methanol - water; and 5 mg. in 1.9 ml. saturated sodium bicarbonate solution with 3.1ml. water) was compared in activity with suitable blank solutions. A bactericidal effect was observed for both samples of traegeric acid on the two micro-organisms tested.

Chromatographic Fractionation of the Metabolites of A. flschentraegeri.

Crude dry chloroform extract (15g.) of the broth (60l.) in ethyl acetate (50ml.) was adsorbed on silica (25g.), dried under reduced pressure, finely powdered and introduced on to a column of silica (600g., 120 x 5cm.) made up in light petroleum (1.5 l.). Separation was achieved by elution with 20, 30, 40 etc. % chloroform - light petroleum mixtures, using one column volume of each successively.

Table 17.

Summary of Chromatographic Separation of the A.flaschen-traegeri Metabolites.

Fractions	Eluting Solvent	Weight	Constituents
1-4	20% chloroform-light petroleum	0.5g.	Non-polar oils
5-17	20-50% chloroform-light petroleum	0.1g.	Dihydroflascherone, flascherone, physcion, dimethylallylphyscion
18-25	50-70% chloroform-light petroleum	0.1g.	Unidentified minor metabolites
26-41	80-90% chloroform-light petroleum; 100% chloroform	4.5g.	Traegeric acid, unidentified minor metabolites

Fractions (300ml.) were collected and monitored by thin layer chromatography.

Fractions (5-17) were combined and further separated by p.l.c., eluting with 8% ethyl acetate-light petroleum. A mixture of dihydroflascherone and flascherone (70mg.) and pure samples of physcion (10mg.) and dimethylallylphyscion (5mg.) were thus obtained; further chromatography using multiple elution with 5% ethyl acetate-light petroleum allowed, with difficulty, isolation of dihydroflascherone (10mg.) and flascherone (55mg.).

Bulked fractions (26-41) were further separated by p.l.c. Kieselgel H plates (50x20x0.1cm.) eluting overnight with chloroform. The active band (Rf 0.2-0.4) was removed and extracted with hot ethyl acetate, yielding a brown gum which crystallised on addition of benzene. Traegeric acid (1.5g.) crystallised from benzene or benzene-light petroleum as off-white plates, m.p. 155-7°C.

Column elution was continued with 5, 10, 20 and 50% ethyl acetate-chloroform; this latter elution yielded the chloroform-insoluble 3-hydroxy-5-methoxyphthalic acid (10mg.) and an amorphous solid.

EXPERIMENTAL

CHAPTER 2.

Dihydroflascherone(103).

The compound was isolated as described earlier, as a yellow gum.

T.l.c. Rf = 0.55 (100% benzene); Fe^{3+}: positive; Ce^{4+}: blue with pale brown halo.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3540(s), 1645(s), 1615(s).

u.v. λ_{max}(EtOH)nm: 247(ε23000), 271 infl(7600), 312 infl (4100), 412(3000).

(EtOH-NaOH)nm: 243(4300), 292(1800), 452(900); reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: -2.70(1H,s, OH); 3.15(1H,d, J=10, irr 4.07 → s, H-11); 3.93(1H,s, OH); 4.03(1H,m, $J_{15,16c}$= 9.5, $J_{15,16t}$= 19, irr 4.87→ s, H-15); 4.07(1H,d, J=10, irr 3.15 → s, H-12); 4.85(1H,m, $J_{15,16t}$= 19, irr 3.15→ d, J=1.5, H-16t); 4.79(1H,m, $J_{15,16c}$= 9.5, irr 3.15→ d, J= 1.5, H-16c); 7.10 (2H,dt, J_t= 6, J_d= 2, H-1); ca.8.15(4H,m, H-2, H-3); 8.53 (6H,s, H-18, H-19); 8.57(3H,s, H-17).

m.s. M^+ at $^m/_e$ = 298(1.0); also 283(0.66) and 229(0.58).

$C_{19}H_{22}O_3$ requires m.w. = 298.

Flascherone(101).

This compound was isolated as described earlier, as a yellow oil, b.p. 100°C/0.03mm.

T.l.c. Rf = 0.50 (100% benzene); Fe^{3+}: positive; Ce^{4+}: brown with purple halo.

i.r. ν_{max}(film)cm^{-1}: 3580(s), 1640(s), 1610(s).

u.v. λ_{max}(EtOH)nm: 288(13200), 425(2000).

(EtOH-NaOH)nm: 266(8600), 301(8800), 435(2800).

n.m.r. ($CDCl_3$)τ: -2.98(1H,s, OH); 3.03(1H,d, J=8, irr ca. 4.0→ s, H-11); 3.22(1H,br d, irr ca. 4.0→ br s, J=1.5, irr 7.70→ d, J=10, H-1); 3.78(1H,br s, OH); 3.9 - 4.2(3H, complex, changes effected by irradiation at 3.03, 3.22, 4.84 and 7.70; H-2, H-12, H-15); 4.81(1H,m, $J_{15,16t}$= 17, irr ca.4.0→ d, J=0.5, H-16t); 4.87(1H,m, $J_{15,16c}$= 11, irr ca.4.0→ d, J=0.5, H-16c); 7.70(2H,dd, $J_{2,3}$= 1.5, irr ca. 4.0→ d, J=5, H-3); 8.46(9H,s, H-17, H-18, H-19).

m.s. M^+ at $^m/_e$ = 296(1.0); also 281(0.65), 263(0.26), 253(0.38) and 115(0.18).

Analysis. Found: C 76.86, H 7.32%; $C_{19}H_{20}O_3$ requires C 77.00, H 6.80%; m.w. = 296.

Chromatography stains for identification of catechols.

The following solutions were made up for use on flascherone run on chromatographic paper; each was tested on catechol and a positive result obtained.

i) Vanillin (1g.) and p-toluenesulphonic acid (0.5g.) were dissolved in ethanol (50ml.) and a chromatogram sprayed. The paper was heated at 100°C for ten minutes: a pink to violet-red colour is indicated of a catechol. This test was negative for flascherone.

ii) Ethylene diamine (10% v/v in water) was mixed with an

equal volume of 1N sodium carbonate and a chromatogram sprayed. The The paper was heated at 100°C for 5 minutes. A positive result (deep brown colouration) was observed for flascherone.

iii) A mixture of ammonium molybdate tetrahydrate (20g.) and 2.5N sulphuric acid (2ml.) was made up to 2l. with distilled water. The resulting solution (pH4.9) gave a positive colouration (brown) for flascherone on silica.

Hexahydroflascherone(104).

Flascherone (100mg.) in ethyl acetate (20ml.) was hydrogenated overnight over 10% palladium-charcoal (25mg.). Filtration and evaporation of solvent yielded hexahydroflascherone (104,90mg.,90%) as a yellow oil, b.p.120°C/0.04mm. T.l.c. Rf = 0.55 (100% benzene); Fe^{3+}: positive; Ce^{4+}: red-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3520(m), 1645(m), 1615(m), 1590(w).

u.v. λ_{max}(EtOH)nm: 245(ε9600), 275(4000),315(780), 397(600).

(EtOH-NaOH)nm: 239(6100), 283(2000), 320(600), 420(1000).

n.m.r.($CDCl_3$)τ: -2.82(1H,s,OH);4.07(1H,s,OH);7.7(4H,m,H-1,H-11); 8.40(6H,s,H-18,H-19); 9.00(3H,s,H-17); 9.28(3H,t,J=7,H-16).

m.s. M^+ at m/e = 302(1.0); also 287(0.96), 269(0.94), 259 (0.45), 245(0.56), 230(0.23), 215(0.23), 204(0.23), 189 (0.94), 175(0.25), 128(0.23), 115(0.23), and 91(0.23).

m^* at m/e = 272, 254, 252, 240, 222, 191, and 124.5.

Analysis. Found: C 75.66, H 8.76%; $C_{19}H_{26}O_3$ requires C 75.46, H 8.67%; m.w.=302.

6,7-O,O'-Diacetylflascherone(102).

Flascherone(100mg.) in acetic anhydride(10ml.) was left overnight in the presence of dry pyridine(a few drops). The solution was poured into chilled dilute hydrochloric acid (50ml.) and ethyl acetate(50ml.) added. The organic layer was washed once with dilute hydrochloric acid, with water till neutral and dried. The resulting yellow gum(110mg.) was purified by p.l.c.(100%benzene) and yielded the diacetate (102,80mg.,75%) as a pale yellow oil which decomposed on distillation.

T.l.c. Rf=0.45(100%chloroform); Fe^{3+}: negative; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1780(vs), 1740(m), 1685(s), 1630(m).

u.v. λ_{max}(EtOH)nm: 245(ε 7100): unchanged by addition of acid or base.

n.m.r.($CDCl_3$)τ: 3.10(1H,d,J=10.5, irr 3.93→ s,H-11); 3.16 (1H,br d,J=10.5, irr 3.91→ br s,H-1); 3.91(1H,br d,J=10.5, irr 3.16→ br s,H-2); 3.93(1H,d,J=10.5, irr 3.10→ s,H-12); 4.17(1H,m,$J_{15,16c}$=11,$J_{15,16t}$=17.5, irr 4.90→ s,H-15); 4.87 (1H,m,$J_{15,16t}$=17.5, irr 4.17→ d,J=1, H-16t); 4.93(1H,m, $J_{15,16c}$=11, irr 4.17→ d,J=1,H-16c); 7.68(3H,s,OAc); 7.70 (3H ,s,OAc); 7.72(2H,m,H-3); 8.62(3H,s,H-17); 8.67(6H,s, H-18,H-19).

m.s. M^+ at m/e =380(0.10); also: 338(0.25), 296(1.0), 281(0.17),

253(0.10), 229(0.10), 165(0.06) and 82(0.17).

m^* at $^m/_e$ =302.5, 269, 267, 261, 259.5, 246, 216, 176 and 174.5.

$C_{23}H_{24}O_5$ requires m.w.=380.

6,7-O,O'-Dimethylhexahydroflascherone(105).

i) Hexahydroflascherone(60mg.) in dry acetone(20ml.) was refluxed overnight under dry nitrogen with dimethyl sulphate (a few drops) and an excess of anhydrous potassium carbonate. The mixture was filtered, solvent evaporated and p.l.c. (5% ethyl acetate-light petroleum eluted twice) yielded the dimethyl ether (105,50mg.,80%) as a yellow oil, b.p. 120°C/ /0.1mm.

ii) Hexahydroflascherone(80mg.) in dry acetone (20ml.) was refluxed overnight under dry nitrogen with methyl iodide (a few drops) and an excess of anhydrous potassium carbonate. After the mixture was filtered, evaporation and p.l.c. (105,70mg., 85%) as a yellow oil identical (t.l.c.,i.r., and n.m.r. spectra) with the product from the previous reaction.

T.l.c. Rf=0.25(100% chlroform); Fe^{3+}:negative; Ce^{4+}:yellow.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1690(s).

u.v. λ_{max}(EtOH)nm: 278(ε17000), 360(3500); unchanged by addition of acid or base.

n.m.r.($CDCl_3$)τ: 6.10(3H,s,OMe); 6.21(3H,s,OMe); 7.4(4H,m, H-1,H-11); 8.60(6H,s,H-18,H-19); 8.83(3H,s,H-17); 9.13(3H,t, J=7,H-16).

m.s. M^+ at $^m/_e$ =330: considerably more abundant ion at $^m/_e$ = 316(0.08), corresponding to demethylation during distillation; also: 299(0.40), 270(0.22), 255(0.09), 199(0.09), 143(0.22), 129(0.08), 101(0.08), 97(0.09), 87(0.63), 83(0.12) and/

/and 74(1.0).

m^* at m/e =287, 218, 195, 191, 152, 147, 133 and 108.5.

$C_{21}H_{30}O_3$ requires m.w.=330.

EXPERIMENTAL

CHAPTER 3.

The Acid Rearrangement of Traegeric Acid (- 7,14-O-Ethylidene-tetrahydroflascherol(112)).

i) in ether.

Traegeric acid(132,100mg.) in dry ether(50ml.) was vigorously stirred overnight with anhydrous copper sulphate (500mg.) and concentrated sulphuric acid(1 drop). Solid sodium bicarbonate was added till no further effervescence was observed and the mixture filtered; evaporation of solvent gave the crude product mixture as a yellow gum(75mg.). T.l.c. (100%chloroform) showed four major and many minor products of which only the least polar, separated by p.l.c. (100% benzene) was stable. The ethylidene derivative(112,10mg.,12%) crystallised from methanol as colourless needles, m.p. 139-40°C.

ii) in ether with added acetaldehyde.

a) Traegeric acid(132,100mg.) in dry ether(40ml.) was vigorously stirred for 40 hours with anhydrous copper sulphate (500mg.), sulphuric acid(1 drop) and acetaldehyde (a large excess). The reaction was worked up as in the previous case and t.l.c. (100% benzene) showed partial reaction to a single less polar product, identical (t.l.c., i.r. and n.m.r. spectra) with the stable product formed in the absence of acetaldehyde. P.l.c. (100% benzene) yielded pure 7,14-O-ethylidenetetrahydroflascherol (112,55mg.,65%) and traegeric acid (35mg.).

b) Traegeric acid (100mg.) in acetaldehyde (6ml.) was kept overnight at room temperature with anhydrous zinc chloride (130mg.). Only part of the acetaldehyde could be removed by evaporation (20°C/10mm.) and the crude product was separated with difficulty by p.l.c. (100% benzene) to yield the ethylidene compound (112,60mg.,70%).

T.l.c. Rf =0.80(100% benzene); Fe^{3+}: positive; Ce^{4+}: olive with yellow halo.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3550(s), 3080(m), 1645(m), 1615(s).

u.v. λ_{max}(EtOH)nm: 283(ε2100), 291(2400); unchanged by addition of acid or base.

n.m.r.($CDCl_3$)τ: 3.96(4.13)(1H,m,H-15); 4.59(1H,q,J=5.5, irr 8.45→ s, acetal H); 4.60(1H,s,OH); ca.4.9(2H,m,H-16); 5.36 (5.42)(1H,br s,H-14); 7.56(4H,m,H-1,H-11); 8.45(3H,d,J=5.5, irr 4.59→ s, acetal Me); 8.73(9.05)(3H,s,H-17); 8.60(6H,s, H-18,H-19).

m.s. M^+(apparent) at $^m/_e$ =284(0.57); also 269(1.0).

Accurate M^+(apparent) at $^m/_e$ =284.1752; $C_{19}H_{24}O_2$ requires m.w.=284.1776.

Analysis. Found: C 76.63, H 8.60%; $C_{21}H_{28}O_3$ requires C 76.79, H 8.59%; m.w.=328.

7,14-O-Ethylidenehexahydroflascherol(113).

7,14-O-Ethylidenetetrahydroflascherol(112,80mg.) in ethyl acetate(15ml.) was hydrogenated overnight over 10% palladium-charcoal(20mg.). Filtration through glass paper and evaporation of solvent yielded the hexahydro compound(113,

(113,70mg.,90%) which crystallised from methanol in rods, m.p. 166-7°C.

T.l.c. Rf =0.80(100% chloroform); Fe^{3+}: positive; Ce^{4+}: brown.
i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3560(s), 1620(m).
u.v. λ_{max}(EtOH)nm: 284infl(ε1300), 291(1500); unchanged by addition of acid or base.
n.m.r. ($CDCl_3$)τ: 4.58(1H,s,OH); 4.58(1H,q,J=5,irr 8.48→ s, acetal H); 5.45(1H,br s,H-14); ca.7.6(4H,m,H-1,H-11); 8.48 (3H,d,J=5,irr 4.48→ s,acetal Me); 8.59(6H,s,H-18,H-19); 8.92(9.21)(3H,s,H-17); 9.08(3H,t,J=7,H-16).
m.s. M^+ at $^m/_e$ =330(0.02); also 286(1.0), 271(0.75) and 259(0.25).
Analysis. Found: C 76.40, H 9.15%; $C_{21}H_{30}O_3$ requires C 76.33, H 9.15%; m.w.=330.

6-O-Acetyl-7,14-O-ethylidenetetrahydroflascherol(115) and 6,14-O,O'-diacetyltetrahydroflascherol(119).

7,14-O-Ethylidenetetrahydroflascherol(112,110mg.) in acetic anhydride(10ml.) was allowed to stand overnight at 60°C with dry pyridine(0.5ml.). The cooled reaction mixture was poured into cold 2.5N hydrochloric acid(50ml.) and ether(50ml.) added. The organic layer was washed with aqueous sodium bicarbonate (three times), with water (twice) and dried. P.l.c. (100% chloroform) yielded the O-acetyl-ethylidene compound (115,65mg.,55%) and the diacetate(119,34mg., 30%) as colourless gums which did not crystallise.

6-O-Acetyl-7,14-O-ethylidenetetrahydroflascherol(115).

T.l.c. Rf =0.60(2% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: red-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3080(w), 1775(s), 1640(w).

u.v. λ_{max}(EtOH)nm: 279(ε2200), 287(2600); unchanged by addition of acid or base.

n.m.r.($CDCl_3$)τ: 3.91(4.10)(1H,m,H-15); 4.61(1H,q,J=5,irr8.52→ s,acetal H); ca.4.9(2H,m,H-16); 5.34(5.39)(1H,br s,H-14); ca.7.6(4H,m,H-1,H-11); 7.67(3H,s,OAc); 8.52(3H,d,J=5,irr 4.61→ s,acetal Me); 8.62(9.07)(3H,s,H-17); 8.71(6H,s,H-18,H-19).

m.s. M^+ at $^m/_e$ =370(0.03); also: 326(0.13), 284(1.0) and 269(1.0). m^* at $^m/_e$ =255 and 247.5.

$C_{23}H_{30}O_4$ requires m.w.=370.

6,14-O,O'-Diacetyltetrahydroflascherol(119).

T.l.c. Rf =0.25(2% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: red-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3090(w), 1780(vs), 1740(s).

u.v. λ_{max}(EtOH)nm: 252(ε730), 269(610), 280(550); unchanged by addition of acid or base.

n.m.r.($CDCl_3$)τ: 4.05(4.20)(1H,br s,H-14); ca.4.3(1H,m,H-15); ca.4.9(2H,m,H-16); ca.7.45(4H,m,H-1,H-11); 7.76(3H,s,OAc); 7.81(3H,s,OAc); 8.71,8.76(2x3H,s,H-18,H-19); 8.97(9.05) (3H,s,H-17).

m.s. M^+ (apparent) at $^m/_e$ =326(0.17); also: 284(1.0) and 269(0.48) m^* at $^m/_e$ =255 and 247.5.

$C_{23}H_{30}O_5$ requires m.w.=386.

6,14-O,O'-Diacetylhexahydroflascherol(120).

The tetrahydrodiacetate(119,40mg.) in ethyl acetate(10ml.) with 10% palladium-charcoal(10mg.) was hydrogenated for 15 hours. The hexahydrodiacetate(120,26mg.,65%) was obtained as a colourless gum.

T.l.c. Rf =0.30 (2% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1780(vs), 1740(s).

n.m.r. ($CDCl_3$)τ: 4.22(1H,br s,H-14); ca.7.45(4H,m,H-1,H-11); 7.73(3H,s,OAc); 7.79(3H,s,OAc); 8.70,8.74(2x3H,s,H-18,H-19); 9.05(3H,t,J=7,H-16); 9.12(9.21)(3H,s,H-17),

m.s. M^+(apparent) at $^m/_e$ =328(0.11); also 286(1.0) and 271(0.22). m^* at $^m/_e$ =257 and 249.5.

$C_{23}H_{32}O_5$ requires m.w. = 388.

Tetrahydroflascherol dimer or peroxide (122 or 123).

Traegeric acid(132,100mg.) in dry benzene(30ml.) was stirred with anhydrous copper sulphate(10g.) under reflux for 20 hours. Filtration and evaporation of solvent afforded the crude product(80mg.) which was purified by p.l.c. (100% chloroform). Tetrahydroflascherol dimer or peroxide (122, 123, 40mg.,50%) was obtained as a colourless gum which readily decomposed at room temperature.

T.l.c. Rf =0.75 (100% benzene); Fe^{3+}: positive; Ce^{4+}: yellow-green.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3550(s), 1640(m), 1615(m).

n.m.r. ($CDCl_3$)τ: ca.4.00(1H,m,H-15); 4.57(1H,s,OH); ca.4.90 (2H,m,H-16); 5.39(5.48)(1H,br s,H-14); 7.50(4H,m,H-1,H-11); 8.57(6H,s,H-18,H-19); 8.67(9.09)(3H,s,H-17).

7,14-O-Benzylidenetetrahydroflascherol(116).

Traegeric acid(132,200mg.) in dry ether(40ml.) with benzaldehyde (an excess) and concentrated sulphuric acid (2 drops) was vigorously stirred for one week. Solid sodium bicarbonate was added until no further effervescence was observed and the mixture filtered. P.l.c. (eluting twice with 10% ethyl acetate-light petroleum) yielded the benzylidene compound (116,110mg.,50%) as a yellow gum which crystallised from methanol as colourless needles, m.p. 85-7°C.

T.l.c. Rf = 0.70(10% ethyl acetate-light petroleum);
Fe^{3+}: positive; Ce^{4+}: olive with grey halo.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3560(s), 1640(m), 1615(m).

u.v. λ_{max}(EtOH)nm: 283(ε2340), 291(2610).

(EtOH-NaOH)nm: 256infl(4400), 283(2750), 292(3080), 308(2480); reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: ca.2.60(5H,m,Ar-H); 4.00(1H,m,H-15); 4.60(1H,s,OH); 4.95(2H,m,H-16); 5.13(5.21)(1H,br s,H-14); ca.7.5(4H,m,H-1,H-11); 8.57(6H,s,H-18,H-19); 8.73(9.01)(3H,s,H-17).

m.s. M^+(apparent) at $^m/_e$ =284(0.33); also 269(1.0), 106(0.60), and 105(0.60).

$C_{26}H_{30}O_3$ requires m.w.=390.

7,14-O-m-Bromobenzylidenetetrahydroflascherol (116a).

Traegeric acid (132, 35mg.) was treated with m-bromobenzaldehyde (an excess) under the conditions previously used for benzaldehyde. The bromobenzylidene compound (10mg., 21%) was isolated by p.l.c. (50% benzene-light petroleum) and crystallised from benzene-light petroleum as needles, m.p. 162-5°C.

T.l.c. Rf = 0.90 (75% benzene-light petroleum): Fe^{3+}: positive: Ce^{4+}: deep yellow.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3540(s), 1640(m), 1615(m), 1590(m).

n.m.r. ($CDCl_3$)τ: ca.2.5 (4H,m, Ar-H): 4.00 (1H,m, H-15): 4.67 (1H,br s, OH): ca.4.8 (2H,m, H-16): 5.13 (1H,br s, H-14): 7.6 (4H,m, H-1, H-11): 8.56 (6H,s, H-18, H-19): 8.67 (9.02) (3H,s, H-17).

m.s. Apparent M^+ at m/e = 284(0.51): also 269(1.0), 184/6(0.14) and 183/5(0.20).

m^* at m/e = 255.

1,3-Dibromoacetone.

Bromine (5.67ml.) was added dropwise to a chilled mixture of acetone (7.34ml.) and 48% aqueous hydrogen bromide (10ml.). Water (40ml.) was added and the mixture fractionally distilled under reduced pressure. 1,3-Dibromoacetone (4.69g., 21%) was obtained as a colourless highly lacrimarory liquid, b.p. 108-10°C/10mm.

n.m.r. ($CDCl_3$)τ: 5.80(s).

Reaction of Traegeric Acid with Dibromoacetone in Ether.

Traegeric acid(132,100mg.) was reacted with 1,3-dibromoacetone under normal ketalisation conditions. The product (13mg.,17%) crystallised from methanol as colourless needles and was identical (t.l.c.,m.p., i.r., u.v. and n.m.r. spectra) with 7,14-O-ethylidenetetrahydroflascherol(112).

7,14-O-Propylidenetetrahydroflascherol(111).

Traegeric acid(132,100mg.) in di-n-propyl ether(40ml.) was stirred with concentrated sulphuric acid (1 drop) and an excess of anhydrous copper sulphate for 6 days. Solid sodium bicarbonate was added and the mixture filtered. P.l.c. (eluting twice with 10% ethyl acetate-light petroleum) yielded the propylidene compound (111,10mg.,10%) as a colourless oil.

T.l.c. Rf =0.80(100% chloroform); Fe^{3+}: positive; Ce^{4+}: purple-grey.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3540(s), 1635(m), 1610(m).

n.m.r. ($CDCl_3$)τ: 4.00(1H,m,H-15); 4.58(1H,s,OH); ca.4.8 (3H,m,acetal H, H-16); 5.35(5.42)(1H,br s,H-14); 7.58(4H,m, H-1,H-11); 8.61(6H,s,H-18,H-19); 8.74(3H,s,H-17).

m.s. M^+(apparent) at m/e = 284(0.53): also 269(1.0).

m^* at m/e = 255.

Estimation of Peroxides in Diethyl ether.

Ether(9ml.) was shaken with 1ml. of a saturated solution of potassium iodide(10g./7ml. distilled water); a pale yellow colouration in the aqueous layer indicated the presence of $>0.005\%$ peroxides in the ether sample. In a standard rearrangement reaction of traegeric acid(100mg.), ether(50ml.) contains $>10^{-4}$mole peroxides.

6-O-Methyl-7,14-O-ethylidenehexahydroflascherol(114).

7,14-O-Ethylidenehexahydroflascherol(113,50mg.) in dry acetone(20ml.) was refluxed overnight with dimethyl sulphate (a few drops) and anhydrous potassium carbonate under dry nitrogen. The mixture was cooled, filtered and solvent evaporated: crystallisation from methanol yielded the methyl ether (114,40mg.,90%) as colourless needles, m.p. 102-3°C.

T.l.c. Rf =0.65(100% benzene); Fe^{3+}: negative; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1625(m), 1595(m).

u.v. λ_{max}(EtOH)nm: 280(ε 680), 288(680); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 4.59(1H,q,J=5,irr8.46→ s,acetal H); 5.43(1H,s, H-14); 6.13(3H,s,OMe); 7.6(4H,m,H-1,H-11); 8.46(3H,d,J=5, irr4.59→ s,acetal Me); 8.62(6H,s,H-18,H-19); 8.75(8.91)(3H,s, H-17); 9.10(3H,t,J=7,H-16).

m.s. M^+ at $^m/_e$ =344(0.03); also 298(1.0), 285(0.72), 271(0.15) and 229(0.08).

m^* at $^m/_e$ =271, 261.5, 245, 209 and 184.

Analysis. Found: C 76.83, H 9.42; $C_{22}H_{32}O_3$ requires C 76.70, H 9.36%; m.w. =344.

6-O-Methylhexahydroflascherol(125).

The acetal(114,300mg.) in hot methanol(40ml.) was refluxed with 5N hydrochloric acid(0.5ml.) overnight under nitrogen. After evaporation of the methanol, ether(50ml.) and brine (50ml.) were added. The aqueous layer was extracted with ether (25ml.) and the combined organic extracts washed with brine until neutral, dried and evaporated. P.l.c.(5% ethyl acetate-light petroleum) of the resulting gum(300mg.) gave the monomethyl ether (125,115mg.,35%) as a pale yellow oil which decomposed on attempted distillation.

T.l.c. Rf =0.20 (10% ethyl acetate-light petroleum); Fe^{3+}: positive; Ce^{4+}: olive with pink halo.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3590(m), 3560(m), 3460(w,br), 3350(m,br).

n.m.r. ($CDCl_3$)τ: 2.50(2.93)(1H,s,OH); 5.39(1H,br,H-14); 6.15(3H,s,OMe); 7.45(1H,br s,OH); 7.51(4H,m,H-1,H-11); 8.63(6H,s,H-18,H-19); 9.05(9.12)(3H,s,H-17).

m.s. M^+ (apparent) at $^m/_e$ = 300(0.79): also 285(1.0).

6,7-O,O'-Dimethylhexahydroflascherol(126).

The monomethyl ether(125,80mg.) in dry acetone(20ml.) with methyl iodide (a few drops) and an excess of anhydrous potassium carbonate was refluxed overnight with stirring under dry nitrogen. The cooled mixture was filtered and solvent evaporated to yield the dimethyl ether (126,50mg.,60%) as a colourless gum.

T.l.c. Rf =0.35(10% ethyl acetate-light petroleum); Fe^{3+}: negative; Ce^{4+}: grey-green.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3620(s), 3530(m,br).

n.m.r. ($CDCl_3$)τ: 5.47(1H,br,H-14); 6.13(3H,s,OMe); 6.15(3H,s, OMe); ca.7.5(4H,m,H-1,H-11); 7.60(1H,br s,OH); 8.63(6H,s, H-18,H-19); 8.96(9.22)(3H,s,H-17).

m.s. M^+ at $^m/_e$ = 332(0.63): also 314(0.29), 299(0.42), 285(0.45), 262(0.92), 248(1.0), 229(0.26) and 215(0.34).

6,7-O,O'-Dimethylhexahydroflascherone(105).

6,7-O,O'-Dimethylhexahydroflascherol(126,40mg.) in dry acetone(40ml.) was stirred for two weeks with activated manganese dioxide(800mg.) under dry nitrogen. The mixture was filtered and solvent evaporated; t.l.c.(100% chloroform) showed partial reaction to a single product.

The mixture(40mg.) in acetone(10ml.) at 0°C was treated with 3 drops of Jones reagent and allowed to stand for one minute. Water(25ml.) was added and the acetone evaporated. The aqueous layer was shaken with ether(40ml.) and the

organic layer washed with water(3x25ml.) and dried. The ketone (105,35mg.,85%) was obtained as a yellow oil.

T.l.c. Rf =0.30(100% chloroform); Fe^{3+}: negative; Ce^{4+}: deep yellow.

i.r. $\gamma_{max}(CCl_4)cm^{-1}$: 1690(s).

n.m.r. $(CDCl_3)\tau$: 6.12(3H,s,OMe); 6.20(3H,s,OMe); 7.40(4H,m, H-1,H-11); 8.58(6H,s,H-18,H-19); 8.86(3H,s,H-17); 9.12(3H,t, J=6,H-16).

m.s. M^+ at $^m/_e$ = 330(1.0); also 315(0.25), 302(0.35), 301(0.41), 262(0.38), 260(0.53), 247(0.44), 245(0.50) and 217(0.69).

6-O-Methylhexahydroflascherone(124).

Magnesium(0.4g.) and iodine(2g.) were dissolved in dry ether(2.5ml.) and dry benzene(5ml.). A portion(2ml.) of this solution was added to 6,7-O,O'-dimethylhexahydro-flascherone(105,70mg.) in dry benzene(20ml.) and refluxed under dry nitrogen for 5 hours. The mixture was filtered, washed with sodium bisulphite solution, water and dried. The monomethyl ether (124,35mg.,45%) was isolated as a yellow gum.

T.l.c. Rf =0.70(100% benzene); Fe^{3+}: positive; Ce^{4+}: grey with purple halo.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: ca.2950(m,v br), 1680(w), 1630(s).

n.m.r. $(CDCl_3)\tau$: -2.88(1H,s,OH); 6.05(3H,s,OMe); ca.7.4 (4H,m,H-1,H-11,); 8.56(6H,s,H-18,H-19); 8.78(3H,s,H-17); 9.07(3H,t,J=7,H-16).

6-O-Methylhexahydroflascherol(125).

The ketone(124,30mg.) in ethanol(5ml.) was stirred overnight under nitrogen with sodium borohydride(2mg.) and the solvent evaporated. Ether(20ml.) and dilute hydrochloric acid(20ml.) were added and the organic layer washed with water and dried. 6-O-Methylhexahydroflascherol(125,30mg.,95%) was obtained as a yellow gum.

T.l.c. Rf =0.15(10% ethyl acetate-light petroleum); Fe^{3+}: positive; Ce^{4+}: deep purple.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3520(m,br), 3320(m,br), 1630(m), 1595(m).

n.m.r. ($CDCl_3$)τ: 2.61(1H,br s,OH); 3.10(1H,br s,OH); 5.34 (1H,br s,H-14); 6.32(3H,s,OMe); ca.7.5(4H,m,H-1,H-11); 8.61(9H,s,H-17,H-18,H-19); 9.03(3H,t,J=6,H-16).

6-O-Methyl-7,14-O-ethylidenehexahydroflascherol(114).

6-O-Methylhexahydroflascherol(125,30mg.) in dry ether (20ml.) was stirred overnight with concentrated sulphuric acid (1 drop) and an excess of acetaldehyde. Solid sodium bicarbonate was added and the mixture filtered. P.l.c. (eluting twice with 10% ethyl acetate-light petroleum) yielded the acetal (114,25mg.,80%) as a colourless gum. A mixed t.l.c. with a sample of 6-O-methyl-7,14-O-ethylidenehexahydroflascherol prepared from traegeric acid (132) showed a single homogeneous spot.

T.l.c. Rf =0.65(100% benzene); Fe^{3+}: negative; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1625(w), 1590(w).

n.m.r. ($CDCl_3$)τ: 4.57(1H,q,J=5,irr8.43→ s,acetal H); 5.43 (1H,s,H-14); 6.15(3H,s,OMe); ca.7.5(4H,m,H-1,H-11); 8.43 (3H,d,J=5,irr4.57→ s,acetal Me); 8.62(6H,s,H-18,H-19); 8.90(9.11)(3H,s,H-17).

m.s. Spectrum identical with that of 6-O-methyl-7,14-O-ethylidenehexahydroflascherol prepared from traegeric acid.

EXPERIMENTAL

CHAPTER 4.

15,16-Dihydrotraegeric acid(136).

Traegeric acid(132,50mg.) in ethyl acetate(15ml.) was shaken with 1% palladium-charcoal(25mg.) for 20 hours under hydrogen. The reaction mixture was filtered through a short celite column and pale yellow crystalline material(54mg.) isolated. Crystallisation from ether-light petroleum,b.p. 40-60°C, yielded the dihydro acid (136,45mg.,90%) as colourless needles, m.p. 155-7°C.

T.l.c. Rf =0.35(2% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: olive with pink halo.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3620(m), 3455(m), 1785(s), 1750(m), 1720(s), 1665(w), 1630(s).

u.v. λ_{max}(EtOH)nm: 242(ε 6200), 290(7700).

(EtOH-NaOH)nm: 266(5000).

n.m.r. ($CDCl_3$)τ: 3.09(1H,br s,H-14); 4.77(1H,s,OH); 7.37 (1H,s,H-5); 7.80(1H,s,OH); 8.87(6H,s,H-18,H-19); 9.10(3H,s, H-17); 9.19(3H,t,J=7,H-16).

m.s. M^+ at m/e =348(0.26); also 330(0.07), 302(0.07), 260(0.19), 232(0.30), 166(0,37), 138(1.0) and 124(0.26).

m^* at m/e =312.5, 276.5, 207, 115 and 109.5.

Analysis. Found: C 69.20, H 8.03; $C_{20}H_{28}O_5$ requires C 68.94, H 8.10; m.w. = 348.

Methyl 6-O-methyltraegerate(141).

Traegeric acid(132,350mg.) in dimethylformamide(10ml.) with methyl iodide(0.4ml.) and barium oxide(600mg.) was stirred at room temperature for 50 hours. Chloroform(100ml.) was added and the organic layer washed with water until neutral, with aqueous sodium bisulphite, with water again and dried. The crude mixture was separated into two major components by p.l.c., eluting three times with 20% ethyl acetate-light petroleum. These products, stereo-isomers of methyl 6-O-methyltraegerate, were warmed with animal charcoal in chloroform solution to decolourise and isolated as colourless oils.

Methyl 6-O-methyltraegerate(141,145mg.,40%) remained as an oil.

T.l.c. Rf =0.45(20% ethyl acetate-light petroleum); Fe^{3+}: negative; Ce^{4+}: bright red.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3600(m), 3460(m,br), 3080(m), 1730(s), 1675(s), 1630(s).

u.v. λ_{max}(EtOH)nm: 266(ε5800), 293(7300); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 3.35(1H,s,H-14); 4.26(1H,m,$J_{15,16c}$=10, $J_{15,16t}$=17,irr4.90→ s,H-15); 4.89(1H,m,$J_{15,16t}$=17,irr4.26 → d,J=1,H-16t); 4.92(1H,m,$J_{15,16c}$=10, irr 4.26→ d,J=1,H-16c); 6.44(3H,s,OMe); 6.60(3H,s,OMe); 7.55(1H,br s,OH); 8.76(3H,s, H-17); 9.01,9.13(2x3H,s,H-18,H-19).

m.s. M^+ at $^m/_e$ =374(1.0); also 342(0.48), 327(0.38), 315(0.38), 310(0.34), 283(0.53), 211(0.34), 163(0.42), 135(0.29), 107(0.35) and 105(0.24).

m^{*-} at $^m/_e$ =313, 307, 298, 295, 281, 265.5, 234, 219.5, 215.5 and 196.

$C_{22}H_{30}O_5$ requires m.w. = 374.

Methyl 6-O-methyl-9-epitraegerate(144a,88mg.,25%) crystallised from ether-light petroleum (b.p.40-60°C) as colourless needles, m.p. 143-5°C.

T.l.c. Rf =0.30(20% ethyl acetate-light petroleum); Fe^{3+}: negative; Ce^{4+}: bright red.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3600(m), 3475(m,br), 3080(w), 1730(s), 1675(s), 1630(s).

u.v. λ_{max}(EtOH)nm: 269(ε4800), 290(4300); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 3.20(1H,s,H-14); 4.43(1H,m,$J_{15,16c}$=10.5, $J_{15,16t}$=17, irr 5.20→ s,H-15); 5.04(1H,m,$J_{15,16c}$=10.5, irr 4.43→ d,J=1.5,H-16c); 5.33(1H,m,$J_{15,16t}$=17, irr 4.43→ d, J=1.5,H-16t); 6.30(3H,s,OMe); 6.50(3H,s,OMe); 7.75(1H,s,OH); 8.66(3H,s,H-17); 8.86,8.90(2x3H,s,H-18,H-19).

m.s. M^+ at $^m/_e$ =374; spectrum identical with that of the previous compound.

Analysis. Found C 69.9; H 7.80%; $C_{22}H_{30}O_5$ requires C 70.5, H 8.07%; m.w. = 374.

14-Acetoxy-8,14-dihydrotraegeric acid(137).

Traegeric acid(132,200mg.) in acetic anhydride(4ml.) with zinc dust(200mg.) and sodium acetate(40mg.) was stirred for 6 hours, filtered and solvent evaporated. After washing with hot ether, crystallisation from ethyl acetate-light petroleum yielded the acetate (137,70mg.,30%) as colourless rods, m.p. 198-200°C.

T.l.c. Rf =0.15(1% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: deep green.

i.r. ν_{max}(KBr disc)cm^{-1}: 3550(m), 3525(s), 3005(w), 1725(m), 1695(s), 1682(s), 1634(w).

u.v. λ_{max}(EtOH)nm: No absorption above 220.

(EtOH-NaOH)nm: 230(ε5600), 335(3400).

(EtOH-HCl)nm: 286(2700).

n.m.r. (d_6-acetone)τ: 3.50(1H,s,OH); 4.21(1H,m,$J_{15,16c}$=10.5, $J_{15,16t}$=17.5, irr 5.13→ s,H-15); 4.39(1H,s,H-14); 4.66(1H,br s, H-8); 5.11(1H,m,$J_{15,16t}$=17.5, irr 4.21→ d,J=0.5,H-16t); 5.15(1H,m,$J_{15,16c}$=10.5, irr 4.21→ d,J=0.5,H-16c); 7.32(1H,br s,H-5); 7.85(3H,s,OAc); 8.85(3H,s,H-17); 8.94,9.10(2x3H,s,H-18,H-19).

m.s. M^+ at m/e =390(0.01); also 348(0.01), 330(0.04), 312(0.04), 302(0.05), 284(0.09), 269(0.07), 253(0.15), 165(1.0), 137(0.67), 133(0.44), 109(0.44) and 105(0.44).

m^* at m/e =295, 267, 239, 124.5,114 and 86.5.

Analysis. Found: C 67.45, H 7.98%;

$C_{22}H_{30}O_6$ requires C 67.67, H 7.74%; m.w. = 390.

EXPERIMENTAL

CHAPTER 5.

The action of diazomethane on traegeric acid.

Traegeric acid (132,500mg.) in ether (20mg.) was treated with an excess of diazomethane from nitrosan (2.25g.) and the mixture left overnight. Glacial acetic acid was added dropwise until no further effervescence was observed and the solution filtered through glass paper to remove polymethylene. P.l.c. (100% chloroform) of the resulting yellow oil yielded two products (410mg. and 50mg.) which crystallised from benzene-light petroleum. Methyl 8,14-methylenetraegerate (133,385mg., 70%) and methyl 14-methyltraegerate (138,40mg., 10%) were obtained as colourless needles, m.p. 156-8°C and 140-2°C respectively.

Methyl 8,14-methylenetraegerate(133).

T.l.c. Rf =0.55 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: grey-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3600(m), 3440(m), 3085(m), 1795(w), 1735(s), 1665(m), 1640(m).

u.v. λ_{max}(EtOH)nm: 273(ε3900).
(EtOH-NaOH)nm: 376(9600).
(EtOH-HCl)nm: 315(4200).

n.m.r. ($CDCl_3$)τ: 4.13(1H,m,$J_{15,16c}$=10, $J_{15,16t}$= 18, irr 5.00 → s, H-15); 4.96(1H,m,$J_{15,16t}$=18, irr 4.13→ d,J=1, H-16t); 5.03(1H,m, $J_{15,16c}$= 10, irr 4.13→ d, J=1, H-16c); 6.27(3H,s,OMe);

6.80(1H,s, H-5); 7.60(1H,s,OH); 8.61(3H,s,H-17); 8.98, 9,08 (2x3H,s, H-18, H-19).

Nuclear overhauser effect: irr 9.03- 24°5% enhancement of signal at 6.80 .

m.s. M^+ at m/e =374(0.20); also 356(0.74), 342(0.47), 324(0.27), 297(0.74), 181(0.43), 179(0.47), 177(0.40), 161(0.94), 135(0.54), 109(0.54), 107(0.74), 105(0.47), 93(0.80) and 91(1.0).

m^* at m/e =339, 312.5, 307, 294.5 and 248.

Analysis. Found: C 70.38, H 7.85%; $C_{22}H_{30}O_5$ requires C 70.56, H 8.07%; m.w. = 374.

Methyl 14-methyltraegerate(138).

T.l.c. Rf =0.65 (5% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3565(m), 3370(m,br), 3080(w), 1725(s), 1630(s), 1590(m).

u.v. λ_{max}(EtOH)nm: 271(ε2190), 319(3480).

(EtOH-NaOH)nm: 235(4800), 373(9420).

(EtOH-HCl) nm: 231(2520), 317(2520).

n.m.r. ($CDCl_3$)τ: 2.82(1H,s, OH); 4.24(1H,m, $J_{15,16c}$= 11, $J_{15,16t}$= 17, irr 4.92→ s, H-15); 4.86(1H,m, $J_{15,16c}$=11, irr 4.24→ d, J=1, H-16c); 4.98(1H,m, $J_{15,16t}$=17, irr 4.24→ d, J=1, H-16t); 6.44(3H,s, OMe); 7.63(1H,s, OH); 8.00(3H,s,H-21); 8.60(3H,s, H-17); 8.85,8.93(2x3H,s, H-18, H-19).

m.s. M^+ at m/e =374(0.16); also 356(0.35), 342(0.22), 324(0.29),

309(0.22), 297(1.0), 274(0.88), 229(0.77) and 177(0.22).

m^* at $^m/_e$ =339, 312.5, 307, 248, 245, 219.5 and 176.5.

Analysis. Found: C 70.32, H 7.96%; $C_{22}H_{30}O_5$ requires C 70.56, H 8.07%; m.w. = 374.

Methyl 8,14-methylene-15,16-dihydrotraegerate(139).

Methyl 8,14-methylenetraegerate (133,170mg.) in ethyl acetate (20ml.) was shaken over 10% palladium-charcoal (150mg.) under hydrogen for 3 hours. Filtration through a short celite column yielded the dihydroester (139,164mg., 95%) which crystallised from benzene-light petroleum as colourless needles, m.p. 191-3°C.

T.l.c. Rf =0.55 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3475(m), 1730(s), 1685(m), 1665(m), 1615(w).

u.v. λ_{max}(EtOH)nm: 243(ε4100), 289(1900).

(EtOH-NaOH)nm: 234(4900), 337(6500).

(EtOH-HCl)nm: 233(4900), 285(3700).

n.m.r. ($CDCl_3$)τ: 3.93(1H,s,OH); 6.40(3H,s, OMe); 6.83(1H,s, H-5); 8.74(3H,s,H-17); 9.17,9.24(2x3H,s, H-18, H-19).

m.s. M^+ at $^m/_e$ =376(0.15); also 358(0.80), 344(0.50), 326(0.53), 299(1.0), 229(0.48), 181(0.60), 179(0.55), 109(0.53) and 91(0.60).

m^* at $^m/_e$ = 341, 315, 297, 264, 250, and 221.

Analysis. Found C 70.43, H 8.40%; $C_{22}H_{30}O_5$ requires C 70.19, H 8.57%; m.w. = 376.

Methyl 14-methyl-15,16-dihydrotraegerate(140).

Methyl 14-methyltraegerate (138,30mg.) in ethyl acetate (10ml.) was hydrogenated for 20 hours over 10% palladium-charcoal (10mg.). Filtration through glass paper and evaporation of solvent yielded a yellow oil (34mg.) which crystallised from ether-light petroleum (b.p. 40-60°C) to give the dihydroester (140,25mg.,80%) as colourless needles, m.p. 149-50°C.

The dihydroester (140,30mg.) in ethanol (15ml.) was hydrogenated overnight over 5% palladium-barium sulphate (5mg.). The product (25mg.,80%) was identical (t.l.c. and spectral properties) with methyl 14-methyl-15,16-dihydrotraegerate.

T.l.c. Rf = 0.65 (5% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3590(m), 3460(m,br), 1790(w), 1730(s), 1650(m), 1630(s).

u.v. λ_{max}(EtOH)nm: 279(9300), 316(13000).
(EtOH-NaOH)nm: 238(22000), 368(37000), 377(39000).
(EtOH-HCl)nm: 238(20000), 316(23000).

n.m.r. ($CDCl_3$)τ: 2.80(1H,s, OH); 6.49(3H,s, OMe); 7.60(1H,s, OH); 8.07(3H,s,H-21); 8.60(3H,s, H-17); 8.85,9.10(2x3H,s, H-18,H-19); 9.20(3H,t, J=7, H-16).

m.s. M^+ at m/e = 376(0.32); also 358(0.36), 344(0.77), 326(0.61), 299(0.52), 297(0.77), 274(0.46), 229(0.31) and 179(1.0).

m^* at $^m/_e$ = 341, 315, 297, 270.5 and 175.

$C_{22}H_{32}O_5$ requires m.w. = 376.

The action of p-toluenesulphonic acid on methyl 8,14-methylenetraegerate.

Methyl 8,14-methylenetraegerate (133,200mg.) in dry benzene (50ml.) was refluxed overnight with p-tolenesulphonic acid (100mg.) with a Dean and Stark arrangement. P.l.c. of the product (190mg.), eluting three times with 10% ethyl acetate -light petroleum, yielded three components.

The primary tosylate (144,80mg.,30%) was isolated as a colourless oil.

T.l.c. Rf = 0.55 (100% chloroform); Fe^{3+}: positive; Ce^{4+}: red-brown.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3380(m), 1730(s), 1670(w), 1635(s), 1610(s).

u.v. λ_{max}(EtOH)nm: 262(ε4100), 307(2600); the addition of base destroys the spectrum.

n.m.r. ($CDCl_3$)τ: 3.28(1H,s, OH); 4.21(1H,m, $J_{15,16t}$=18, $J_{15,16c}$ =10.5, irr 5.03→ s, H-15); 5.01(1H,m, $J_{15,16c}$=10.5, irr 4.21 → d, J=1.5, H-16c); 5.05(1H,m, $J_{15,16t}$=18, irr 4.21→ d, J=1.5, H-16t); 5.88(1H,d, J=7, H-21); 5.96(1H,br d, irr 7.28→ d, J=7, H-21); 6.45(3H,s,OMe); 7.28(3H,m, irr 5.96→ sharpens, H-11,H-14); 8.57(3H,s,H-17); 8.78,9.26(2x3H,s, H-18,H-19). Tosyl: 2.42(2H,d,J=9); 2.76(2H,d,J=9); 7.62(3H,s).

m.s. M^+ at $^m/_e$ = 528(0.02); also 459(0.05), 356(0.10), 297(0.10), 269(0.10), 172(0.20), 107(0.19) and 91(0.62).

$C_{29}H_{36}O_7S$ requires m.w. = 528.

The dimeric ether compound(146, 48mg., 25%) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless needles, m.p. 110-2°C.

T.l.c. Rf = 0.75(100% chloroform); Fe^{3+}: negative; Ce^{4+}: red-brown with grey halo.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3520(s), 1720(s), 1640(w), 1605(m), 1580(m).

u.v. λ_{max}(EtOH)nm: 258(ε4300), 287(3250), 335(2000).

(EtOH-NaOH) nm: 250(5500), 290(3500), 330(2000); reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 3.92(1H,s, OH); 4.08(1H,m, $J_{15,16t}$=19, $J_{15,16c}$ = 12, H-15); 4.32(1H,m, H-11); ca.4.8(4H,m, H-16, H-21); 6.19(3H,s, OMe); ca.7.4(3H,m, H-5, H-12); 8.63 6H,s, H-18. H-19); 8.70(3H,s, H-17).

m.s. M^+ at $^m/_e$ = 356(0.53); also 324(0.24), 263(0.78), 262(1.0), 248(0.65), 231(0.24), 215(0.30), 203(0.27), 187(0.21), 159(0.18), 131(0.24), 129(0.24), 128(0.24), 115(0.41) and 91(0.65).

m^* at $^m/_e$ = 295, 233, 203, 187, 162.5 and 154.5.

Analysis. Found: C 73.88, H 7.90%; $C_{22}H_{28}O_4$ requires C 74.13, H 7.92%; m.w. = 356.

The dimeric ether (149, 30mg., 15%) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless needles, m.p. 150-2°C.

T.l.c. Rf = 0.40 (100% chloroform); Fe^{3+}: negative; Ce^{4+}: green.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3090(w), 1740(s), 1695(m), 1650(s), 1615(w).

u.v. λ_{max}(EtOH)nm: 265(ε6100), 318.5(3120); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 4.19(1H,m, $J_{15,16c}$ = 11, $J_{15,16t}$ = 17, irr 4.87→ s, H-15); 4.85(1H,m, $J_{15,16c}$ = 11, irr 4.20→ d, J=1, H-16c); 4.89(1H,m, $J_{15,16t}$ = 17, irr 4.20→ d, J=1, H-16t); 5.57(1H,m, irr 7.16→ d, J=7, H-21); 5.74(1H,m, irr 7.16→ d, J=7, H-21); 6.34(3H,s,OMe); 7.16(1H,m, irr 5.65→ s, H-14); 8.70(6H,s, H-18, H-19); 8.95(3H,s, H-17).

m.s. M^+ at $^m/_e$ = 356(0.11); also 328(0.02), 297(1.0), 269(0.09), 185(0.08), 178(0.20), 128(0.08). 115(0.08), 95(0.08) and 91(0.12).

m^* at $^m/_e$ = 247.5.

Analysis. Found: C 74.04, H 7.85%; $C_{22}H_{28}O_4$ requires C 74.13, H 7.92%; m.w. = 356.

The primary alcohol (145).

The tosylate (144, 80mg.) was refluxed in dry ethanol (20ml.) with sodium acetate (100mg.) for 5 days, the

mixture cooled, filtered and solvent evaporated. Ether (50ml.) and water (50ml.) were added and the organic layer washed with water (2x50ml.) and dried. P.l.c. (100% chloroform) yielded the primary alcohol (145, 15mg., 30%) as a yellow gum which crystallised from ether-light petroleum (b.p. 40-60°C) as off-white prisms, m.p. 142-3°C.

T.l.c. Rf = 0.15 (100% chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3500(m). 3080(w), 1735(s), 1665(w), 1630(s), 1610(s).

u.v. λ_{max}(EtOH)nm: 265(ε8900), 310(5400).

(EtOH-NaOH)nm: 248(14000), 374(4200); reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 3.03(1H,s, OH); 3.93(1H,m, $J_{15,16t}$ = 18, $J_{15,16c}$ = 10, irr 4.99→ s, H-15); 4.98(1H,m, $J_{15,16c}$ =10, irr 3.93→ d, J=1.5, H-16c); 5.00(1H,m, $J_{15,16t}$ = 18, irr 3.93→ d, J=1.5, H-16t); 6.38(3H,s, OMe); 6.71(6.21)(1H,br d, J=11, H-21); 6.72(6.40)(1H,br d, J=12, H-21); 7.25(1H,s,OH); 8.62(3H,s, H-17); 8.78,9.18(2x3H,s, H-18, H-19).

m.s. M^+ at m/e = 374(0.35); also 305(1.0), 2970.55), 285(0.28), 269(0.40), 255(0.43) and 227(0.35).

$C_{22}H_{30}O_5$ requires m.w. = 374.

Ozonolysis of methyl 8,14-methylenetraegerate.

Methyl 8,14-methylenetraegerate (133,275mg.) in ethyl acetate (20ml.) was ozonised until a distinct blue colouration

developed (ca. 15 minutes); nitrogen was passed through the solution for 10minutes and the system warmed to 15°C. 5% palladium-charcoal (30mg.) was added and the mixture hydrogenated at room temperature for 4 hours, filtered and the product, a colourless oil (300mg.), isolated. P.l.c. (100% chloroform) yielded the acetal (155/a,35mg., 13%) and the aldehyde (153 ,45mg., 17%).

The acetal (155/a) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless prisms, m.p. 165-6°C.

T.l.c. Rf = 0.85 (5% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3380(m), 1730(s), 1665(w), 1635(s), 1615(s).

u.v. λ_{max}(EtOH)nm: 237 infl(ε4800), 266(4100), 312(2500).

(EtOH-NaOH)nm: 248(6500), 383(1900): reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 3.02(3.00)(1H,s, OH); 4.50(½H,m, irr 6.73, 7.9 sharpens, H-15); 4.75(½H,m, irr 6.94, 7.9 sharpens, H-15); 6.42(6.48)(3H,s, OMe); 6.73(6.94)(2H,m, irr 4.7, 7.9 sharpens, H-21); 7.27(7.15)(2H,t, J=5, irr→ br s, H-11); 7.9(4H,m, irr 6.73, 6.94, 7.25 sharpens, H-8, H-12, H-14); 8.69,8.78(2x3H,s, H-18, H-19).

m.s. M^+(apparent) at m/e = 330(0.98); also 301(0.53), 298(0.43), 283(0.39), 271(1.0), 270(0.41), 262(0.71), 247(0.58), 215(0.33), 203(0.38) and 201(0.41).

m^* at $^m/_e$ = 274.5, 269, 236, 233, 222.5 and 187.

$C_{21}H_{28}O_6$ requires m.w. = 376.

The aldehyde (153) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless prisms, m.p. 165-6°C.

T.l.c. Rf = 0.50 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: red-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3480(m), 3410(m), 2710(m), 1735(s), 1715(s), 1660(m), 1635(w).

u.v. λ_{max}(EtOH)nm: 285(ε13000).

(EtOH-NaOH)nm: 295(9800), 341(11000): reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 0.41(1H,s, H-15); 6.34(3H,s, OMe); 6.84 (1H,s, H-5); ca.7.8(2H,m, H-12); 8.74(3H,s, H-17); 8.97, 9.18(2x3H,s, H-18, H-19).

m.s. M^+ at $^m/_e$ = 376(0.17); also 358(0.41), 344(0.74), 329(0.89), 299(0.77), 289(0.49), 271(0.46), 229(0.51), 181(0.58), 167(0.63), 109(1.0), 107(0.67), 95(0.63), 93(0.68) and 91(0.82).

m^* at $^m/_e$ =341, 314.5, 297, 283.5, 272, 269, 228.5, 167 and 156.5.

$C_{21}H_{28}O_6$ requires m.w. = 376.

The aldehyde (153,30mg.) in ethyl acetate (20ml.) was refluxed overnight: t.l.c. showed no change. Animal charcoal (20mg.) was added and the mixture stirred overnight at room

temperature: t.l.c. (5% methanol-chloroform) showed a complex mixture in which the acetal (155/a) was not predominant.

Zinc-acetic acid reduction product (159).

Methyl 8,14-methyltraegerate (133 ,150mg.) with zinc dust (150mg.) in glacial acetic acid (15ml.) was stirred overnight at room temperature, filtered and solvent evaporated under reduced pressure. P.l.c. (100% chloroform) yielded the reduction product (159, 120mg., 80%), which crystallised from benzene-light petroleum as colourless prisms, m.p. 198-200°C.

T.l.c. Rf = 0.55 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: brown-purple.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3620(m), 3480(m), 1730(s), 1690(s), 1640(w).

u.v. λ_{max}(EtOH)nm: 278(ε1100).

(EtOH-NaOH)nm: 255 infl(1300), 332(1000); reverting to EtOH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 4.18(1H,m, $J_{15,16t}$ = 18, $J_{15,16c}$ = 10.5; irr 5.00→ s, H-15); 4.96(1H,m, $J_{15,16t}$ = 18, irr 4.18→ d, J=1.5, H-16t); 5.03(1H,m, $J_{15,16c}$ = 10.5, irr 4.18→ d, J=1.5, H-16c); 5.68(1H,dd, exchange OH (6.63)→ d, J=11, irr 7.39→ s); 6.41(3H,s, OMe); 6.63(1H,d, J=3, OH); 7.39 (1H,d, J=9, irr 5.68→ s); 7.77(2H,m); 8.27(1H.s,OH); 8.79, 9.02, 9.07(3x3H,s, H-17, H-18, H-19).

m.s. M^+ at $^m/_e$ =376(0.07); also 358(0.11), 326(0.09), 299(0.14), 272(0.16), 257(0.12), 229(0.14), 208(0.56), 190(1.0), 169(0.67) and 109(0.67).

m^* at $^m/_e$ = 173.5, 161, 151 and 136.5.

Analysis. Found: C 69.65, H 8.28%; $C_{22}H_{32}O_5$ requires C 70.19, H 8.57%; m.w. = 376.

The above reaction was also carried out in d_4-acetic acid, and a deuterated product isolated.

n.m.r. ($CDCl_3$) : Signals at 5.68 and 7.39 were absent.

m.s. M^+ at $^m/_e$ = 378 ($C_{22}H_{30}D_2O_5$)

This product was stirred overnight with acetic acid and re-isolated unchanged.

Reduction of the zinc-acetic acid product (dihydro derivative, 160)

The zinc-acetic acid product (159, 215mg.) in ethyl acetate (30ml.) was stirred overnight over 10% palladium-charcoal (50mg.) under hydrogen. Filtration and evaporation of solvent yielded the dihydro derivative (160, 188mg., 88%) as a colourless oil which crystallised from benzene-light petroleum as prisms, m.p. 189-90°C.

T.l.c. Rf = 0.50 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: grey-blue.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3610(m), 3490(m,br), 1720(s), 1680(s).

u.v. λ_{max}(EtOH)nm: No absorption above 230.

(EtOH-NaOH)nm: 338(ε1100).

(EtOH-HCl)nm: 291(910).

n.m.r. ($CDCl_3$)τ: 5.68(1H,dd, exchange OH (6.65)→ d, irr 7.39→ s); 6.37(3H,s, OMe); 6.65(1H,d, J=2.5,OH); 7.39 (1H,d, J= 9.5, irr 5.68→ s); 7.77(2H,m); 8.13(1H,br s, OH); 8.73, 8.97, 9.15(3x3H,s, H-17, H-18, H-19).

m.s. M^+ at m/e = 378(0.02); also 360(0.17), 346(0.10), 328(0.23), 210(0.71), 192(1.0), 169(0.29), 163(0.33), 137(0.33) and 109(0.33).

m^* at m/e= 311, 299, 175.5, 138.5, 106.5 and 102.5.

Analysis. Found: C 69.36, H 8.81%; $C_{22}H_{34}O_5$ requires C 69.81, H 9.05%; m.w. =378.

Oxidation of the dihydro derivative (ketone 139).

a) The dihydro derivative (160, 70mg.) in ethyl acetate (15ml.) was ozonised at -78°C until a blue colouration was observed. Nitrogen was passed through the solution, 5% palladium-charcoal (10mg.) added, hydrogenated at room temperature for 2 hours, filtered and solvent evaporated. The crude products were separated by p.l.c. (100% chloroform) to give the ketone (139, 35mg., 50%) which crystallised from ether-light petroleum (b.p. 40-60°C) as colourless prisms, m.p. 157-9°C.

b) The dihydro derivative (160, 100mg.) in acetone (15ml.) at 0°C was treated with Jones reagent (0.4ml.) with stirring and left for 30 minutes at room temperature. The solvent was evaporated and ether (50ml.) and water (50ml.) added. The aqueous layer was extracted with ether (25ml.) and the combined organic extracts washed with water till neutral and dried. The crude product, a yellow oil (100mg.) was purified by p.l.c. (eluting with 2x 100% chloroform). The ketone thus obtained (139, 67mg., 65%) was identical (t.l.c., i.r. and n.m.r. spectra) with the product from the previous reaction.

T.l.c. Rf = 0.45 (5% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 3600(m), 3440(m,br), 1730(s), 1700(s).

u.v. ν_{max}(EtOH)nm: 240(ε2700), 283(1300).

(EtOH-NaOH)nm: 235infl(3500), 300(2600), 333(2600).

(EtOH-HCl)nm: 235infl(3500), 287(3100).

n.m.r. ($CDCl_3$)τ: 6.31(3H,s,OMe); 6.80(1H,s); ca.7.60(1H,br,OH); 8.71, 9.13, 9.17 (3x3H,s, H-17, H-18, H-19).

m.s. M^+ at m/e = 376(0.28); also 358(1.0), 344(0.79), 326(0.84), 299(0.72), 181(0.72), 179(0.67), 109(0.62) and 91(0.60).

m^* at m/e = 341, 315, 309, 297 and 250.

Analysis. Found: C 70.10, H 8.58%: $C_{22}H_{32}O_5$ requires C 70.19, H 8.57%: m.w. = 376.

Attempted acetylation of the dihydro derivative → monoacetate (162) and diacetate (161).

The dihydro derivative (160, 100mg.) in acetic anhydride (7ml.) with pyridine (0.3ml.) was allowed to stand overnight at 60°C. Ice-cold 5N hydrochloric acid (100ml.) was added and extracted with ethyl acetate (3x 50ml.), the organic extracts washed with water till neutral and dried. The crude products (100mg.) were separated by p.l.c. (eluting twice with 100% chloroform). The monoacetate (162, 67mg., 60%) and the diacetate (161, 10%, 8%) were obtained as colourless oils.

The monoacetate (162).

T.l.c. Rf = 0.45 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: yellow-brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3585(m), 3470(m,br), 1765(s), 1730(s), 1685(s).

u.v. $\lambda_{max}(EtOH)nm$: 235(ε9200), 250(10100); unchanged by addition of acid or base.

n.m.r. $(CDCl_3)\tau$: 6.42(3H,s,OMe); 7.48(1H, br s, OH); 7.82 (3H,s,OAc); 8.77, 8.97, 9.25(3x3H,s, H-17, H-18, H-19), 9.15(3H,t, J=7, H-16).

m.s. M^+(apparent) at $^m/_e$ = 376(0.05); also 358(1.0), 344(0.38), 326(0.48), 299(0.75), 181(0.20), 179(0.20) and 149(0.43).

m^* at $^m/_e$ = 341, 297, 250.

The diacetate (162).

T.l.c. Rf = 0.55 (5% methanol-chloroform); Fe^{3+}: negative; Ce^{4+}: green.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3465(s), 1730(s), 1685(m).

n.m.r. $(CDCl_3)\tau$: 4.93(1H,d, J=10); 6.46(3H,s,OMe); 7.54 (1H,d, J=10); 7.92(3H,s,OAc); 8.07(3H,s,OAc); 8.79, 9.18, 9.28(3x3H,s, H-17, H-18, H-19).

m.s. M^+ (apparent) at $^m/_e$ = 402(0.10); also 360(0.21), 328(0.94), 301(0.51), 210(0.85), 192(1.0), 179(1.0) and 164(0.85).

Attempted dehydration of the dihydro derivative (160).

To the dihydro derivative (160, 100mg.) in pyridine (5ml.) at 5°C was added phosphorus oxychloride (5 drops), the mixture left overnight at room temperature, poured into ice-cold 2N hydrochloric acid (50ml.) and ether (50ml.) added.

The organic layer was washed with water till neutral and dried. T.l.c. (2% methanol-chloroform) of the crude product showed many components.

The p-toluenesulphonic acid transformation product from the dihydro derivative (164).

The dihydro derivative (160, 200mg.) in benzene (50ml.) with p-toluenesulphonic acid (70mg.) was refluxed for 4 hours with a Dean and Stark arrangement. 5% sodium bicarbonate (50ml.) was added to the cooled reaction mixture and the organic layer washed with water and dried. The crude product (80mg.) crystallised from benzene-light petroleum to give the transformation product (164, 60mg., 34%) as colourless prisms, m.p. 156°C.

T.l.c. Rf = 0.75 (100% chloroform); Fe^{3+}: negative; Ce^{4+}: bright green.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1795(s), 1720(s), 1640(w).

u.v. λ_{max}(EtOH)nm: 235(ϵ5200), 260infl(1600); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 4.51(1H,t, J=5, irr 8.32→ s); 5.56(1H,s); ca.7.5(2H,m); 7.75(1H,s); 8.32(2H,m, irr 4.51→ sharpens); 9.04, 9.15, 9.31(3x3H,s, H-17, H-18, H-19).

m.s. M^+ at m/e = 328(1.0); also 284(0.27), 269(0.27), 255(0.34), 227(0.37), 215(0.86), 199(0.51), 185(0.58), 171(0.61), 159(0.55), 157(0.68) and 145(0.58).

Analysis Found: C 76.58, H 8.50; $C_{21}H_{28}O_3$ requires C 76.79, H 8.59%; m.w. = 328.

Attempted aromatisation of methyl 14-methyltraegerate (138).

Methyl 14-methyltraegerate (138,100mg.) in dry ether (40ml.) with acetaldehyde (0.2ml.) and concentrated sulphuric acid (2 drops) was stirred for 4 days with anhydrous copper sulphate (500mg.). Solid sodium bicarbonate was added until no further effervescence was observed, the mixture filtered and solvent evaporated. P.l.c. (15% ethyl acetate-light petroleum) yielded methyl 8,14-dihydro-14-methylenedehydrotraegerate (148, 30mg., 35%) and the acetal (151/2, 20mg., 25%) as colourless oils.

Methyl 8.14-dihydro-14-methylenedehydrotraegerate(148).

T.l.c. Rf = 0.40 (15% ethyl acetate-light petroleum); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3360(s), 1735(s), 1675(m), 1630(s), 920(s).

u.v. λ_{max}(EtOH)nm: 244(ε15000), 253(11000), 288(7680), 310(4800).

(EtOH-NaOH)nm: 255(12000), 267(11500), 386(3600); reverting to EtCH spectrum on acidification.

n.m.r. ($CDCl_3$)τ: 2.73(1H,s,OH); 3.57(1H,d, J=1.5, irr 4.54 → s, H-21); 4.22(1H,m, $J_{15,16c}$ = 10.5, $J_{15,16t}$ = 17.5, irr 5.12→ s, H-15); 4.54(1H,d, J= 1.5, irr 3.57→ s, H-21);

5.05(1H,m, $J_{15,16c}$ =10.5, irr 4.22→ d, J= 1.5, H-16c); 5.18 (1H,m, $J_{15,16t}$ =17.5, irr 4.22→ d, J= 1.5, H-16t); 6.35(3H,s, OMe); 8.60, 8.73, 8.81 (3x3H,s, H-17, H-18, H-19).

m.s. M^+ at m/e = 356(0.16); also 297(0.63) and 229(1.0).

m^* at m/e = 248 and 176.5.

$C_{22}H_{28}O_4$ requires m.w. = 356.

The acetal (151/2).

T.l.c. Rf = 0.10 (15% ethyl acetate-light petroleum); Fe^{3+}: negative; Ce^{4+}: olive.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1740(s), 1660(s), 1605(m).

u.v. λ_{max}(EtOH)nm: 261(ε7370), 292(3460); unchanged by addition of acid or base.

n.m.r. τ: 3.90(1H,m, $J_{15,16c}$ =10, $J_{15,16t}$ =18, H-15); 4.83 (1H,q, J =5.5, irr 8.50→ s, acetal H); 4.95(1H,m, $J_{15,16c}$ = 10, $J_{16c,16t}$ = 1.5, H-16c); 5.01(1H,m, $J_{15,16t}$ =18, $J_{16c,16t}$ = 1.5, H-16t); 6.40(3H,s, OMe); 7.50(1H,dm, H-11); 8.50(3H,d, J =5.5, irr 4.83→ s, acetal Me); 8.72(9H,s, H-18, H-19, H-21); 8.88(3H,s, H-17).

m.s. M^+ at m/e = 400(0.03); also 372(0.17), 357(1.0), 328(0.11), 297(0.20), 290(0.30), 287(0.26), 286(0.40), 271(0.35) and 269(0.23).

$C_{24}H_{32}O_5$ requires m.w. = 400.

The action of bromoacetyl bromide on methyl 8,14-methylene-15,16-dihydrotraegerate (133).

To methyl 8,14-methylene-15,16-dihydrotraegerate (133, 1.0g.) with N,N-dimethylaniline (1ml.) in dry chloroform (100ml.) was slowly added bromoacetyl bromide (1ml.) in dry chloroform (25ml.) and the mixture refluxed for 4 hours. 6N sulphuric acid (200ml.) and ether (300ml.) were added and the organic layer washed with 6N sulphuric acid (200ml.), water (2x200ml.), aqueous potassium carbonate (2x200ml.) and dried. Evaporation (35°C/10mm.) overnight yielded a yellow gum (1.15g.); extensive chromatography gave three products in poor yield.

Product I (165, 55mg., 5%) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless prisms, 151-2°C.

T.l.c. Rf = 0.80 (100% chloroform); Fe^{3+}: negative; Ce^{4+}: green.

i.r. $\nu_{max}(CHCl_3)cm^{-1}$: 1785(s), 1690(s), 1610(m).

u.v. λ_{max}(EtOH)nm: 234(ε2600), 269(5200).

(EtOH-NaOH)nm: 235(4200), 260(1200); unchanged by addition of acid.

n.m.r. ($CDCl_3$)τ: 5.44(1H,s, H-6); 6.28(1H,dd, J_1 = 11, J_2 = 4.5, irr 7.32- d, J = 11, H-21); 6.57(1H,dd, J_1 = 11, J_2 = 3.5, irr 7.32- d, J = 11, H-21); 7.32(1H,br m, irr 6.42- br s, H-14): 7.62(1H,s, H-5); 7.64(2H,m, H-11); 9.02, 9.12, 9.40 (3x3H,s, H-17, H-18, H-19); 9.30(3H,t, J = 7, H-16).

m.s. M^+ at m/e = 408/410(0.02); also 364/366(0.24), 295/297 (0.42), 285(0.33), 271(0.45), 215(1.0), 157(0.57), 145(0.63) and 91(0.15).
m^* at m/e = 241, 239, 223, 162, 149, 117.5 and 97.5.
Accurate M^+ at m/e = 408.1262/410.1226; $C_{21}H_{29}O_3Br$ requires m.w. = 408.1299/410.1279.

Product II (35mg., 3.5%) crystallised from ether-light petroleum (b.p. 40-60°C) as off-white prisms, m.p. 136-8°C.
T.l.c. Rf = 0.25 (100% chloroform); Ce^{4+} : brown.
i.r. $\nu_{max}(CCl_4)cm^{-1}$: 3430(br,m), 1740(s), 1710(s), 1645(s), 1610(m).
u.v. λ_{max}(EtOH)nm: 278(ε1200), 283(1200): unchanged by addition of acid or base.
n.m.r. ($CDCl_3$)τ: 6.42(3H,s,OMe); 7.47(1H,s); 8.79, 9.12, 9.15 (3x3H,s, H-17, H-18, H-19).
m.s. M^+ at m/e = 374(0.01); also 360(0.17), 301(1.0), 283(0.08), 273(0.15), 245(0.16), 231(0.42), 205(0.42), 175(0.17), 161(0.15), 107(0.24) and 91(0.24).
m^* at m/e = 266, 252, 177.5 and 139.5.

Product III (166, 84mg., 7.5%) crystallised from ether-light petroleum (b.p. 40-60°C) as colourless prisms, m.p. 147-8°C.
T.l.c. Rf = 0.75 (100% benzene); Fe^{3+}: negative; Ce^{4+}: orange.

i.r. $\nu_{max}(CCl_4)cm^{-1}$: 1740(s), 1660(s), 1635(s), 1615(m).

u.v. λ_{max}(EtOH)nm: 251(3800); unchanged by addition of acid or base.

n.m.r. ($CDCl_3$)τ: 3.62(1H,s, H-6); 6.25(1H,dd, J_1 = 10, J_2 = 4, irr 6.92- d, J = 10, H-21); 6.45(1H,dd, J_1 = 10, J_2 = 3, irr 6.92- d, J = 10, H-21); 6.48(3H,s, OMe); 6.92(1H,br m, irr 6.35- br m, H-14); 7.15(1H,dm, J =10, irr 8.60- m, H-11); 8.80, 9.01, 9.38 (3x3H,s, H-17, H-18, H-19); 9.11(3H,t, J = 7, H-16).

m.s. M^+ at $^m/_e$ > 420(0.28); also 363/365(1.0), 283(0.63), 273(0.39), 214(0.58), 213(0.58) and 199(0.50).

m^* at $^m/_e$ = 314, 312, 234.5, 160.5 and 151.

Analysis Found: C 62.55, H 7.38%; $C_{22}H_{30}O_3Br$ requires C 62.55, H 7.16%; m.w. = 422/424.

EXPERIMENTAL

CHAPTER 6.

2-(γ,γ-Dimethyl)-allylphyscion (1,8-dihydroxy-3-methoxy-2(γ,γ-dimethyl)-allyl-6-methylanthraquinone,169).

This pigment was isolated as described previously and crystallised from acetone-water as yellow plates, m.p.210-2°C.

T.l.c. Rf =0.42 (50% benzene-light petroleum); Fe^{3+}: positive; Ce^{4+}: red.

i.r. ν_{max}(mull)cm^{-1}: 1681(m), 1633(s), 1620(m), 1598(w), 1575(m).

u.v. λ_{max}(EtOH)nm: 269(ε33000), 310(10000), 438(7800).
(EtOH-NaOH)nm: 261(35000), 318(13000), 524(6800).

n.m.r. ($CDCl_3$)τ: -2.34(1H,s, OH); -2.06(1H,s, OH); 2.42 (1H,d, J=1.5, irr 2.98→ s, H-5); 2.65(1H,s, H-4); 2.98 (1H,d, J=1.5, irr 2.42→ s, H-7); 4.83(1H.t. J=7, irr 6.63 → s, H-β); 6.04(3H,s, OMe); 6.63(2H,d, J=7, irr 4.83→ s, H-α); 7.62(3H,s, Ar-Me); 8.24,8.35(2x3H, br s, γ,γdimethyl). Nuclear overhauser effect; irr 6.04→ 25% enhancement of signal at 2.65 .

m.s. M^+ at $^m/_e$ = 352(0.54); also 310(1.0) and 298(0.50). Accurate M^+ at $^m/_e$ = 352.13219: $C_{21}H_{20}O_5$ requires m.w.= 352.13106.

Analysis. Found: C 68.27, H 6.15%; $C_{21}H_{20}O_5.H_2O$ requires C 68.10, H 5.99%.

Physcion (1,8-dihydroxy-3-methoxy-6-methylanthraquinone, 168).

Physcion was isolated as described before; crystallisation from acetone yielded orange needles, m.p.209-210°C, mixed m.p.with an authentic sample, 205°C.

T.l.c. Rf = 0.60 (25% chloroform-benzene); Fe^{3+}: positive; Ce^{4+}: red.

i.r. ν_{max}(mull)cm^{-1}: 3600(w), 1670(w), 1625(s), 1612(m), 1565(w).

u.v. λ_{max}(EtOH)nm: 282(ε18000), 435(7000), 456(6500).

(EtOH-NaOH)nm: 313(8000), 518(6900).

n.m.r. ($CDCl_3$)τ: -2.29(1H,s, OH); -2.09(1H,s, OH); 2.41(1H,d, J=2, irr 2.92→ s, H-5); 2.63(1H,d, J=3, irr 3.32→ s, H-4); 2.92(1H,d, J=2, irr 2.41→ s,H-7); 3.32(1H,d, J=3, irr 2.63→ s, H-2); 6.08(3H,s,OMe); 7.58(3H,br s,Ar-Me).

Nuclear overhauser effect; irr 6.08→ no significant enhancement of any signal.

m.s. M^+ at $^m/_e$ = 284(1.0); also 255(0.39), 241(0.34), 227(0.22), 226(0.22), 213(0.28), 198(0.18), 185(0.20), 139(0.18) and 128(0.22).

m^* at $^m/_e$ = 227, 201 and 173.5.

Analysis. Found: C67.69, H 4.30%; $C_{16}H_{12}O_5$ requires C 67.60, H 4.25%; m.w. = 284.

EXPERIMENTAL

CHAPTER 7.

3-Hydroxy-5-methoxyphthalic acid (175).

This compound was isolated as described before and crystallised with difficulty from ethyl acetate-light petroleum as colourless plates, m.p. 135-40°C.

T.l.c. Rf = 0.50 (50% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: red-brown.

i.r. ν_{max}(mull)cm^{-1}: 3000(m,br) 1700(s), 1650(s), 1615(s).

u.v. λ_{max}(EtOH)nm: 258(ε5300), 308(3500).

(EtOH-NaOH)nm: 245(5600), 300(2200); reverting to EtOH spectrum on acidification.

n.m.r. (TFA)τ: 3.12(1H,d, J=3, H-6); 3.23(1H,d, J=3, H-4); 6.02(3H,s, OMe).

(d_6-DMSO)τ: ca.3.3(3H,v br, OH); 3.33(1H.d. J=2.5, H-6); 3.42(1H,d, J=2.5, H-4); 6.20(3H,s, OMe).

m.s. M^+ at m/e = 212(0.33); also 194(0.75), 178(0.25), 168(0.58), 150(1.0), 134(0.58), 122(1.0) and 106(0.42). Accurate M^+ at m/e = 212.03225; $C_9H_8O_6$ requires M^+ at m/e = 212.03208.

3-Hydroxy-5-methoxyhomophthalic acid (178).

Diethyl 3,5-dihydroxyhomophthalate (176,400mg.)[133] in ether (35ml.) was reacted overnight with diazomethane from nitrosan (2.73g.). P.l.c. (100% chloroform) gave the monomethyl ether (177,227mg., 55%), which crystallised from ether-light petroleum (b.p. 40-60°C) as colourless

plates, m.p. 63-4°C.

T.l.c. Rf = 0.80 (100% chloroform); Fe^{3+}: positive; Ce^{4+}: yellow.

i.r. ν_{max}(mull)cm^{-1}: 3170(m), 1730(s), 1650(m), 1600(w).

n.m.r. ($CDCl_3$)τ: -1.66(1H,br s, OH); 3.56(1H,d, J=2.5, H-4); 3.70(1H,d, J=2.5, H-6); 5.65(2H,q, J=7, OCH_2); 5.85(2H,q, J=7, OCH_2); 6.13(2H,s, Ar-CH_2); 6.18(3H,s, OMe); 8.63(3H,t, J=7, Me); 8.76(3H,t, J=7, Me).

The diester (177,100mg.) in 2N sodium hydroxide (10ml.) was refluxed for 15 minutes, the mixture acidified and the precipitated material (100mg.) distilled (b.p. 125°C/0.008mm.), yielding pure diacid (178), m.p. 150-5°C.

T.l.c. Rf = 0.10 (5% methanol-chloroform); Fe^{3+}: positive; Ce^{4+}: brown.

i.r. ν_{max}(mull)cm^{-1}: ca.3000(v br, m), 1685(s), 1625(s), 1615(w).

n.m.r. (d_6-DMSO)τ: -0.53(1H,br s, OH); 3.5(4H,m, 2xOH, H-4, H-6); 6.10(3H,s,OMe); 6.16(2H,s, CH_2).

m.s. $M^{=}$ (apparent) at $^{m}/_{e}$ = 208(0.44); also 190(0.26), 180(1.0), 164(0.47) and 151(0.26).

Analysis. Found: C 53.09, H 4.41%; $C_{10}H_{10}O_6$ requires C 53.10, H 4.46%; m.w. = 226.

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