THE CHEMISTRY OF FUNGAL METABOLITES

A THESIS PRESENTED BY

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CONTENTS.

Diagrams pertaining to any section are located at the end of that section.

I wish to record my thanks to my Supervisor, Dr. N.J. McCorkindale, for his encouragement and guidance during the course of this work. I am indebted to the Science Research Council for a maintenance grant, 1966-69, and to Professor R.A. Raphael, F.R.S., for providing the opportunity and facilities for research in this department.

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I also wish to thank my many friends in the Chemistry Department for their patience and advice during my stay here.

SUMMARY.

The major chemical constituents of the mould, Lenzites striata, have been separated chromatographically. From the broth have been isolated three isocoumarins, oospolactone, oosponol, and oospoglycol, known metabolites of another microorganism: an investigation of the biosynthesis of oospoglycol has shown that it is acetate derived.

The metabolites of the mycelium of L.striata have also been examined and shown to be acetyldehydroeburicoic acid, eburicoic acid and sulphurenic acid: a fourth triterpene metabolite of novel structure has been isolated and named "lenzitic acid."

The structures of two metabolites previously isolated from Daedalea quercina and Leptoporus stipticus have been revised and characterised as Δ^{1}-pyrazoline derivatives.

Several synthetic approaches to the ortho - terphenyl system have been investigated and the compounds thus prepared examined in the n.m.r. spectrometer: these results have assisted the elucidation of the structures of two related metabolites previously isolated from Aspergillus candidus, and shown to possess anti-keratinophilic activity. Nuclear Overhauser studies on both the model compounds and natural products have also been used in structure assignment.

GENERAL INTRODUCTION.

Although man's awareness of his natural surroundings stretches back beyond recorded time, his earliest writings bear proof of his curiosity in the world about him. Thus the extraction of metal-containing ores for their practical benefits, and of flowers, plants and insects to provide colouring matters for the beautification of his life, might all be widely viewed as man's first incursions into the field of natural product chemistry. Under this definition, albeit somewhat broad, must also be included the traditional use of certain plant and herbal extracts for the purposes of both healing and killing.

The first differentiation of organic and inorganic chemistry stemmed from the realisations that extracts from living sources were much more complex in their composition than those of inanimate origin. Thus, descriptions in early nineteenth century chemistry treatises of the constituents of certain plant and animal extracts marks the beginning of natural product chemistry as it is known today. The last century and a half has transformed this field of science from the listing of so-called "proximate principles" of vegetable and animal origin to the complete structural elucidation of

as many as fifty separate chemical entities from a single plant species. This transformation has been accelerated over the past twenty years through the revolution in physical instrumentation which not only facilitates purification of small quantities of natural product but allows the organic chemist to cull a wealth of information on its chemical structure, without significant loss of material.

The fungi provide an excellent example of how man has adapted his natural environment to his own advantage. Indeed, it would be no exaggeration to claim that in the use of fungi for his own ends, man has turned a natural enemy into an ally. Responsible for the destruction of much organic matter, the majority of plant diseases, and many diseases in animal and man, these organisms have also, for thousands of years, been used in the baking of bread and fermenting of wines; their use in the manufacture of antibiotics, of which the best known is surely penicillin, provides a more modern example of their pharmaceutical usefulness.

To the natural product chemist, the fungi represent almost ideal experimental material: requiring less space and less equipment for growth than the higher organisms,

they provide a cheap source of a large variety of metabolites, interesting in themselves and of potential biological use. Further advantages to the chemist are their ability to reproduce quickly, thus providing several generations in a shorter time than is required for higher plants and animals, and their low selectivity of permeability to substances in the external medium, convenient for the feeding of radioactive precursors in a biosynthetic study.

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The living cell obtains energy by the breakdown of sugars, a process which is also important as a source of matabolic intermediates. One of the important pathways for this catabolism of sugars is glycolysis, whereby hexose phosphate is split hydrolytically to give triose phosphate which, as the main outlet from the phosphorylated system, is oxidised by way of phosphoenol pyruvate and pyruvate to the thioester, acetyl-CoA.

This species performs three important roles: it is the main substrate of the tricarboxylic acid cycle, a key process in the production of phosphorylating and reducing agents, and in addition is the basic building unit for two main groups of secondary metabolites,

namely polyketides, via malonyl-CoA, and isoprenoids, via acetoacetyl-CoA.

Both of these classes of secondary metabolites have been encountered in the work to be described here (Sections 1 and 11); general examples quoted of the structural types are restricted in the main to those derived from fungal sources.

SECTION I.

FUNGAL POLYKETIDES.

INTRODUCTION.

The polyketide group of secondary metabolites is formally regarded as being derived from linear β-polyketone chains constructed in turn by head-to-tail linkage of "acetic acid" molecules. By such a mechanism a very large variety of structural types is obtained in nature, including the fatty acids, saturated and unsaturated, polyacetylenic compounds, and a host of cyclic structures, mainly aromatic, of varying degrees of complexity. Examples of all these classes are present in the fungi and attention in the following introduction has been mainly confined to the aromatic group of polyketides, in an attempt to demonstrate the considerable structural diversification in this class.

One of the simplest structures is that of triacetic acid lactone (1) isolated from Penicillium stipitatum,[1] obviously containing three "acetate" moieties, while the presence of four C_2 units is apparent in orsellinic (2) and 6-methyl salicylic acids (3), metabolites of several different moulds. The "homologue" (4) of orsellinic acid represents a simple example of a mould metabolite containing five acetate units and other examples in this category are citrinin (5), a constituent of several Penicillium species[2], and also the Curvularia metabolites[3,4], represented by the

structure (6). The numerous natural naphthalenes and naphthaquinones may be derived either from penta or hexa acetyl chains; chain folding of (7) results in structures of type (8), while (10) would derive from a hexa acetyl unit (9). In practice, it is difficult to distinguish the two routes; two examples of the quinones are flaviolin (11), from Aspergillus citricus[5] and 6-methyl -1,4 naphthaquinone (12), from Marasmius gramineum[6]. Monascorubin and rubropunctatin, both pigments of Monascus fungi[7,8] represent two examples of natural products with main carbon skeletons derivable from hexa-acetyl chains, (heavy lines in 13).

The seven-unit, i.e. hepta-acetyl chain gives rise to probably the greatest variety of secondary metabolites; the mould biphenyl, alternariol[9] (14), and griseophenone[10] (15) arise by the chain folding shown (Fig. 1), while griseofulvin (16), from P.griseofulvum Dierckx[11] is derived by the arrangement (Fig. 2). Some other elaborations of the hepta-acetyl chain and the mould metabolites, (17) through (23), arising from them, are indicated in Figure 3 [12].

Hardly less variety of structural types is shown by the octa-acetyl derived polyketides; sclerotiorin[13](24), from P.sclerotiorum[14] represents one such example and two others are the structurally analagous brefeldin A(25), isolated from

P.brefeldianum[15] and the large-ring lactone, curvularin, (26) a metabolite of a Curvularia species[16]. The most widespread example of this group, however, are the anthraquinones, with the same problem of origin as for the naphthaquinones, namely the choice of seven or eight acetate units, as indicated (Fig. 4). Rhodocladonic acid (29), a metabolite of various Cladonia species[17], is a bis alkylated example of the hepta-acetyl chain condensation, although this is less common than the octa-acetyl condensation of which endocrocin, isolated from Nephromopsis endocrocea[18], and emodin (28), a metabolite of the mould Cortinarius sanguineus[19], are characteristic examples. Structurally related to emodin, and co-occurring with it in Trichoderma viride[20], are pachybasin and chrysophanol (30). The presumed anthrone precursor (27) of the anthraquinones is known to occur with them in many moulds, such as Aspergillus fumigatus, which furnishes, as well as emodin, the first example (31) of a chlorine containing anthrone[21].

Both the anthraquinone and naphthaquinone-producing fungi show the common property of oxidative coupling of the monomeric natural products; thus, the mould, Daldinia concentrica, furnishes binaphthyl structure such as (32) as well as 1,8-dihydroxynaphthalene dimethyl ether[22] while the skyrins (33) from P. islandicum [23] are representative examples of the bi-anthraquinones.

This feature of oxidative coupling is also apparent in flavomannin (34), a metabolite of P.wortmanni[24], the monomeric unit of which is constructed from eight acetate units, and the structure of vioxanthin (35), from Trichophyton vidlaceum, has been proposed assuming dimerisation of two hepta-acetyl chains[25].

Nine acetate units comprise the chain which condenses to the quinones, nalgiovensin and nalgiolaxin (36), from P.nalgiovensis Laxa[26,27], and two further examples are the β-resorcylates, radicicol [28](37) (or monorden[29]), and zearelenone[30] (38), a metabolite of Gibberella zeae (Fusarium graminearum).

The two latter structures bear a resemblance to the supposed precursor (Fig. 5) of the tetracycline antibiotics from streptomyces; examples of condensation of the deca-acetyl chain, are the terramycins[31] (39) and also the "mycinone" antibiotics from the actinomycetes[32], an example of which is the rhodomycinone group (40), derived as indicated (Fig. 6). Yet another manner of folding of the ten unit chain (Fig. 7), gives rise to the metabolite, resistomycin (41), from Streptomyces resistomycificus[33]; despite recent controversy[34] concerning the position of the aromatic methyl group, position 9, rather than 8, seems the more "biogenetically correct"[35].

Apart from sequential assembly of acetate units, some structures have been shown to derive by condensation of two distinct polyketo chains: one such is citrimycetin (42)[36] for which two chains may condense in the sense $C_8 + C_6$ or $C_{10} + C_4$ (Fig. 8). The failure of the butyrate skeleton to be incorporated intact suggested the former mode was correct. It is suggested that sulochrin (43)[37] from Aspergillus terreus is another example, and the case of sclerin also in this category, will be discussed in some detail later.

ASPECTS OF THE BIOSYNTHESIS OF POLYKETIDES.

By far the largest body of evidence for the current theories of biogenesis of natural products is based on the incorporation *in vivo* of substrates specifically labelled with a convenient isotope, most usually carbon-14 or tritium. However, such evidence has always been preceded by an inspection of the natural product in question and of the metabolites produced by the same organism with a view to presenting an educated guess as to their biogenetic origins, be they from a common precursor or successive stages in the metabolism. The combination of these two approaches has proved a most powerful tool in the elucidation of the biosynthetic pathways (*vide infra*).

The acetate theory of biogenesis of the polyketides, originally conceived by Collie[38], and more recently independently restated[39], was first given a sound experimental basis by the now classical study on the mould, *Penicillium griseofulvum* Dierckx[40]: low sporing strains of this organism produce the metabolite, 6-methylsalicylic acid, which in a medium containing sodium acetate, isotopically labelled in the carboxyl

group, is labelled as indicated (44).

The actual C_2-units involved in the elaboration of this metabolite were later demonstrated to be one acetyl-CoA and three malonyl-CoA units, linearly assembled, reduced and cyclised. Studies on *P.urticae* Bainier[41], a mould producing both fatty acid and phenolic polyketides, in addition to proving that both classes of polyketide are derived in an analagous manner, showed that diethyl malonate(2-C^{14})is incorporated into 6-methylsalicylic acid in such a way as to give an (equal) distribution of label on atoms 3,5 and 7 only. This finding is explicable on the basis of repeated condensation of malonyl-CoA units with an acetyl-CoA "starter", the malonyl-CoA units themselves being derived by carboxylation of acetyl-CoA[42]. Conversely, feeding studies on *P. griseofulvum*, using sodium (1-C^{14}) acetate and unlabelled diethyl malonate, gave 6-methylsalicylic acid in which the isotope content at C-6 is 12-15% higher than that of the carboxyl group, indicating that unlabelled malonate is used in preference to labelled acetate for chain extension.

The formation of 6-methylsalicylic acid demonstrates ably the three processes distinguished by Bu'Lock[43]

in considering polyketide biogenesis. These are the assembly process for the linking of the C_2-units, the modification process i.e. reduction, cyclisation etc., and the various secondary reactions of the preformed polyketide derivatives. This latter process is apparent in the oxidation of 6-methylsalicylic acid via gentisyl derivatives to patulin (45), a metabolite of P. patulum: growth of this organism in a solution containing specifically labelled 6-methylsalicylic acid produces patulin labelled as indicated[44] (Fig. 9).

Apart from the use of specifically labelled substrates, much may be learned from comparison of the structural types occurring in the same organism, although such an approach has obvious possible pitfalls. An example stems from a recent examination of the constituents of the mould, Alternaria solani[45]; in addition to the classical anthraquinones (46) two new structural types, altersolanol A and B (47), were isolated suggesting an obvious biogenetic inter-relationship. This approach may be carried further by demonstrating experimentally the conversion in vivo of one metabolite to a related species; the formation of patulin from 6-methylsalicylic acid is one such example, while another is the demonstration that the

6-methyl ether of orsellinic acid (48) is the biochemical precursor of penicillic acid (49)[46].

A complementary approach to the solution of some biosynthetic problems in fungi is the induction of mutation in the organism either by ultraviolet irradiation or the use of chemical mutagens: from the new secondary metabolites thus formed, some inferences may be drawn concerning biosynthesis in the parent "wild" type. For example, the structure of averufin, a metabolite of Aspergillus versicolor, first suggested as (50) on the basis of its supposed biogenetic origin[47] from eight acetate residues and one molecule of acetoacetaldehyde, was revised to (51) in the light of the structures of mutant strain metabolites[48] (52), and also by comparison with averythrin and averantin (53), pigments produced by genotypically similar fungi[49]. As a further illustration of the usefulness of this method, the isolation of dihydro-citrinone (54) from a blocked mutant of Asp. terreus that was no longer able to produce citrinin (55) suggested that this lactone was an intermediate in the biosynthesis of citrinin[50]. The use of ethionine, as a C-methylation inhibitor, in cultures of P.stipitatum resulted in accumulation of tri

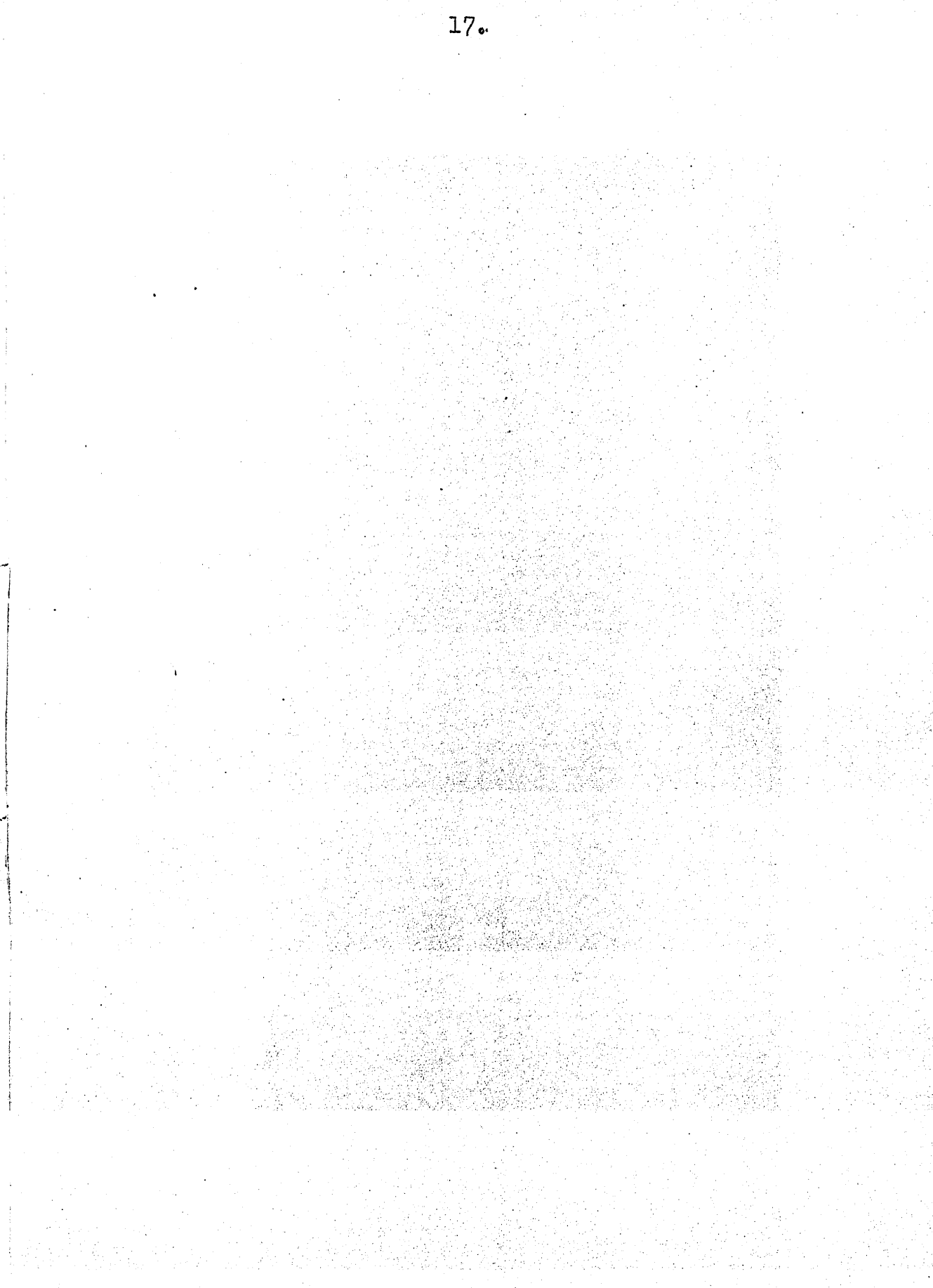
and tetraacetic acid lactones (56) in place of the normal tropolone metabolites[1] (57) and under normal conditions the mould was able to utilise these lactones for biosynthesising the tropolones[51].

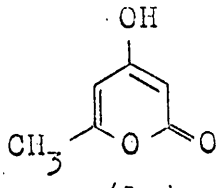
While the measurement of radioactive decay of incorporated atoms in natural products is undoubtedly the corner stone of experimental verification of the theories, other properties of certain isotopes have been exploited in biosynthetic studies. For example, the magnetic moment of the carbon-13 isotope has suggested n.m.r. studies of the fungal troplone, sepedonin (58), fed with ^{13}C acetate and formate precursors[52]; by this technique, equal labelling was observed throughout the molecule derived from acetate precursors, and ·sotope enrichment was obtained at C-8 with formate precursor, indicating that the biosynthesis of sepedonin involves a polyketide chain and a one carbon unit. A mass spectrometric study of biosynthesis[53] has elucidated the nature of the <u>in vivo</u> conversion of 6-methylsalicylic acid into patulin; thus, when (2,4,6-$^{2}H_3$)-3 methyl phenol (59) was administered to <u>P.patulum,</u> the derived patulin was shown by examination of the mass spectrometric (M+2) peak to have incorporated the precursor to an extent of 30%.

In addition, since deuterium from C-6 in (59) was retained at C-5 in patulin (61), ring fission of the presumed gentisaldehyde intermediate (60) must be followed by a stereospecific reduction (Fig. 10).

An important and interesting difference between the biosynthesis of fatty acids and aromatic polyketides is that, whereas, in formation of fatty acids, only a limited degree of assembly occurs without chain reduction, the cyclic unreduced structures of the aromatic polyketides imply that no reduction is involved in their assembly. Since the β-polyketone in the unreduced state must be highly reactive, and since aromatic systems of the type displayed in the secondary metabolites are not the result of spontaneous reactions of such β-polyketones, it is concluded that the assembly has some special means of stabilisation not involved in fatty acid synthesis. A plausible mechanism, yet one without much experimental verification, is that the poly-enol structure is stabilised by chelation with metals; more experimentally secure is the elegant demonstration [54-59] than β-polyketo esters, of general formula(62), may be stabilised as the corresponding polypyrones. Thus, dipyrones of type (63) may be regarded as the protected form of the triketo ester (62; n = 2) and trypyrones of type

(64) as the masked form of a tetraketo ester (62; n=3). Although the intermediacy of such entities has not yet been demonstrated in polyketide biogenesis, dipyrones of the type (63) may be converted either to phloroglucinols by magnesium methoxide induced Claisen condensation, or to orsellinates by aldol condensation with potassium hydroxide,[56,57,58] and the tripyrones (64) also react with base, though in a less specific manner, to give four structural types of aromatic compounds occurring in nature[54,55,59]. These correspond to the carbon skeletons of C-acetyl orsellinic acid (65), 6,8 di-hydroxyisocoumarin (66), curvulinic acid (67) and eugenin (68). An even closer model for aromatic polyketide biosynthesis utilises the previously discussed tetraacetic acid lactone in the 5,6 dihydro form, which could be converted to 6-methylsalicylic acid by methanolic potassium hydroxide and subsequent acidification. This represents a biogenetic analogy to the condensation of a polyketide chain in which one or more of the carbonyl groupings not involved in the cyclisation mechanism is reduced and the resultant hydroxyl function subsequently lost, an important feature of several biosynthetic pathways[60].





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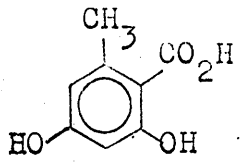

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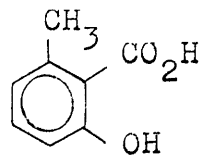

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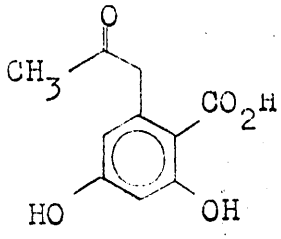

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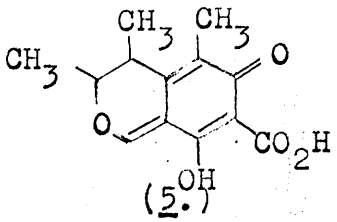

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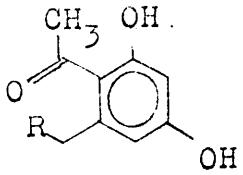

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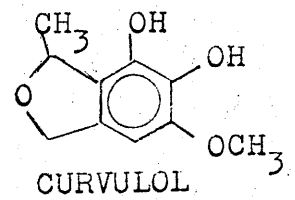
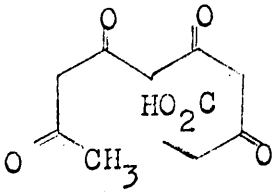


CURVULOL

R = $COOC_2H_5$; CURVULIN

R = COOH; CURVULINIC ACID



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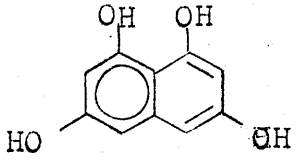

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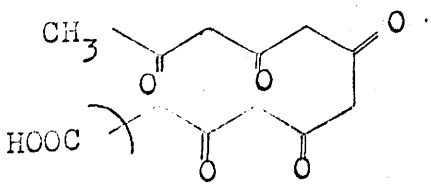

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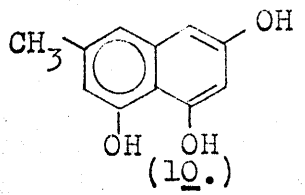

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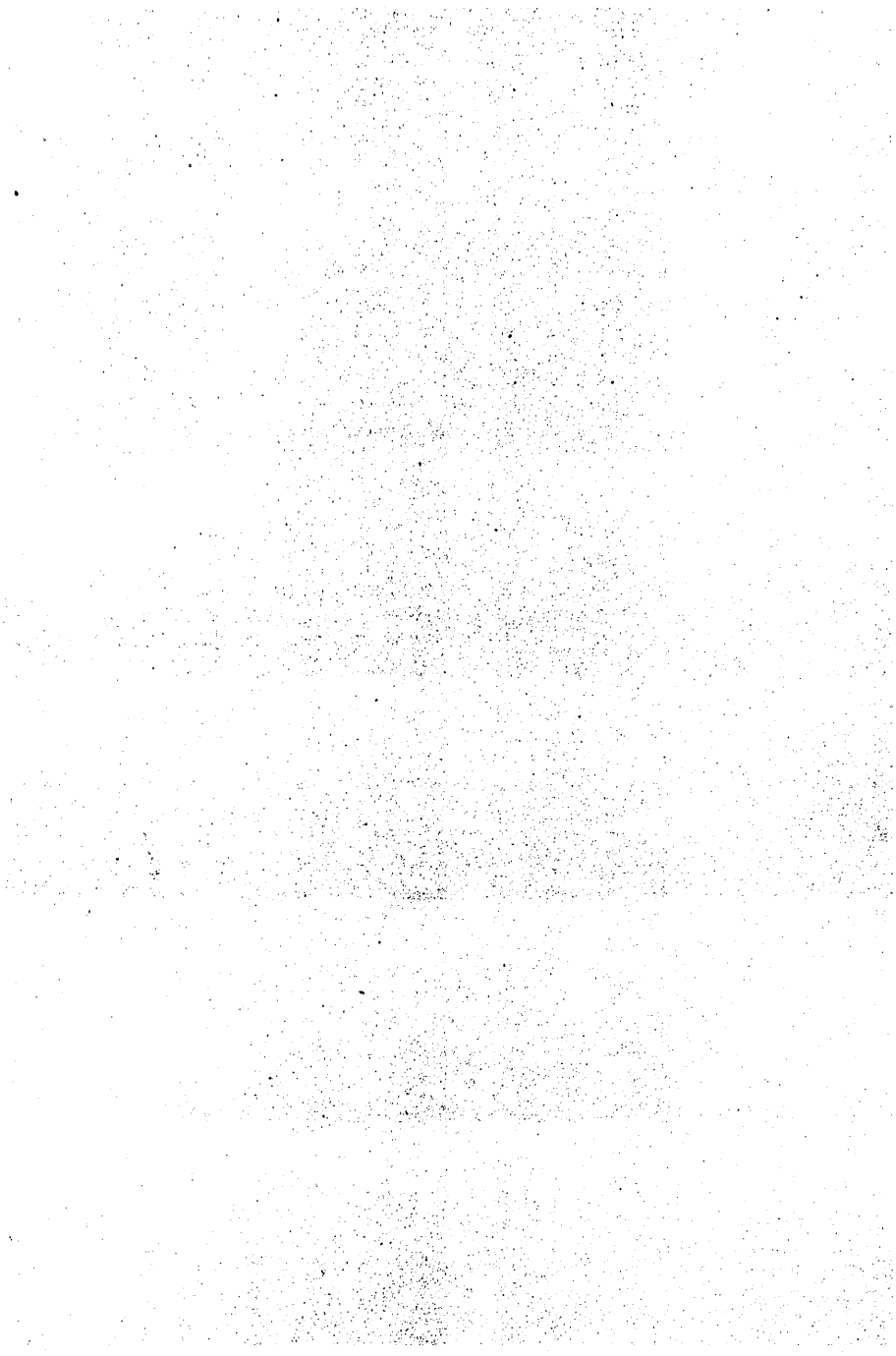

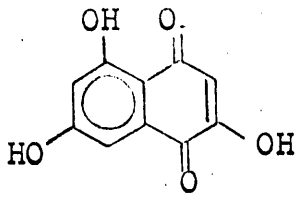

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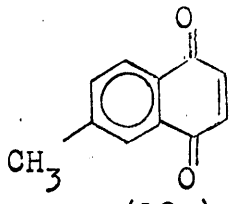

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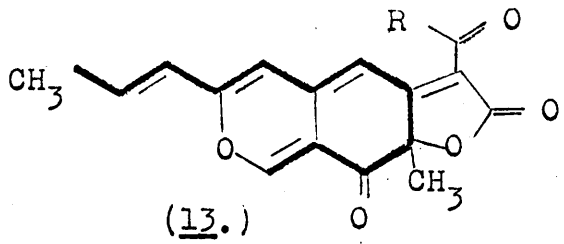
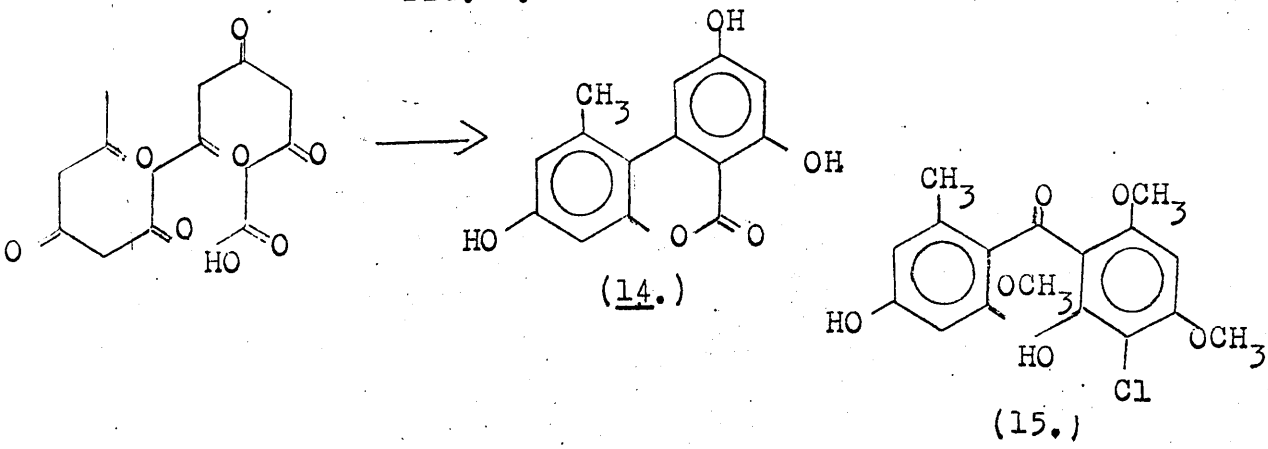


(13.)

R= n-C_7H_{15}; MONASCORUBIN, R= n- C_5H_{11}; RUBROPUNCTATIN.

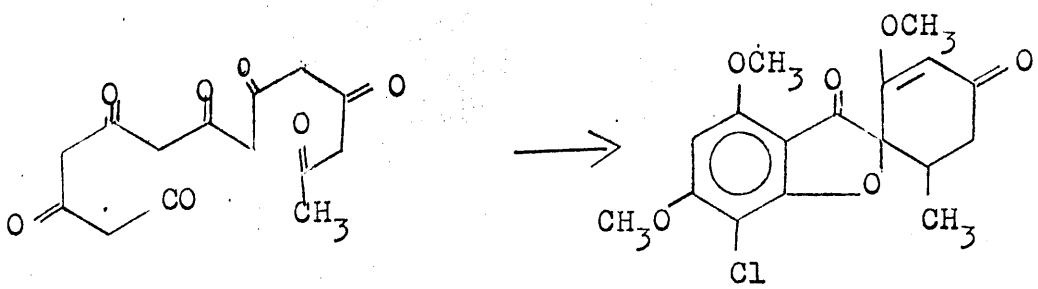
FIG. 1.



(14.)

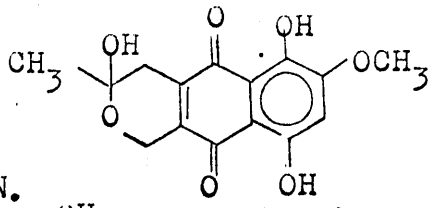
(15.)

FIG. 2.



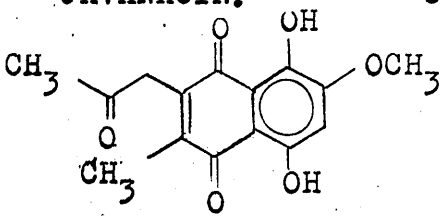
(16.)

FUSARUBIN.

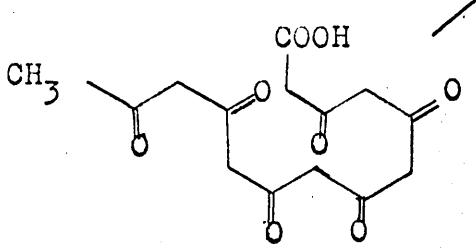


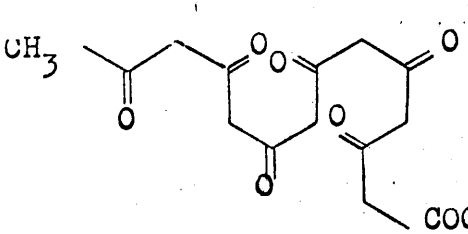
(18.)

JAVANACIN.

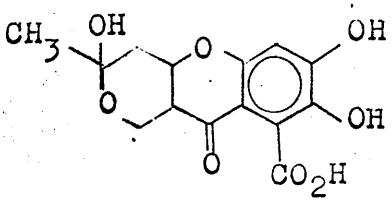


(19.)



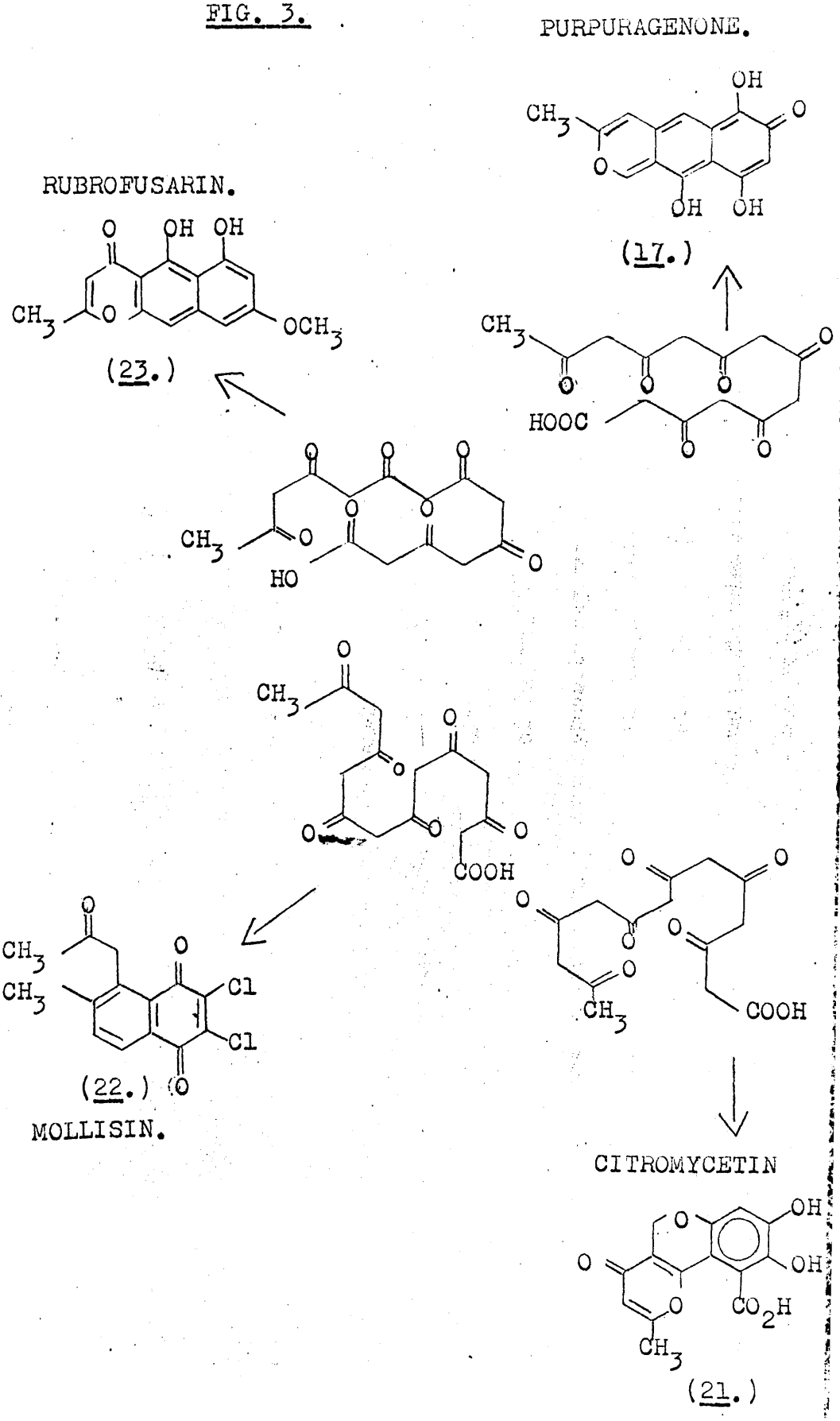


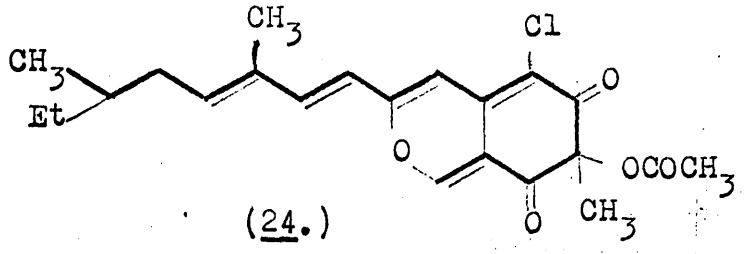
FULVIC ACID.



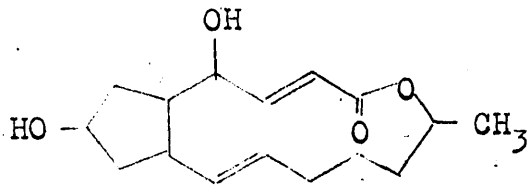
(20.)

FIG. 3.

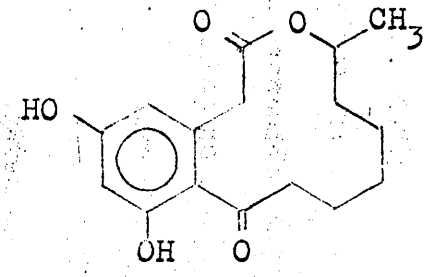

PURPURAGENONE.
(17.)
RUBROFUSARIN.
(23.)
HOOC
HO
COOH
COOH
(22.)
MOLLISIN.
CITROMYCETIN
(21.)



(24.)

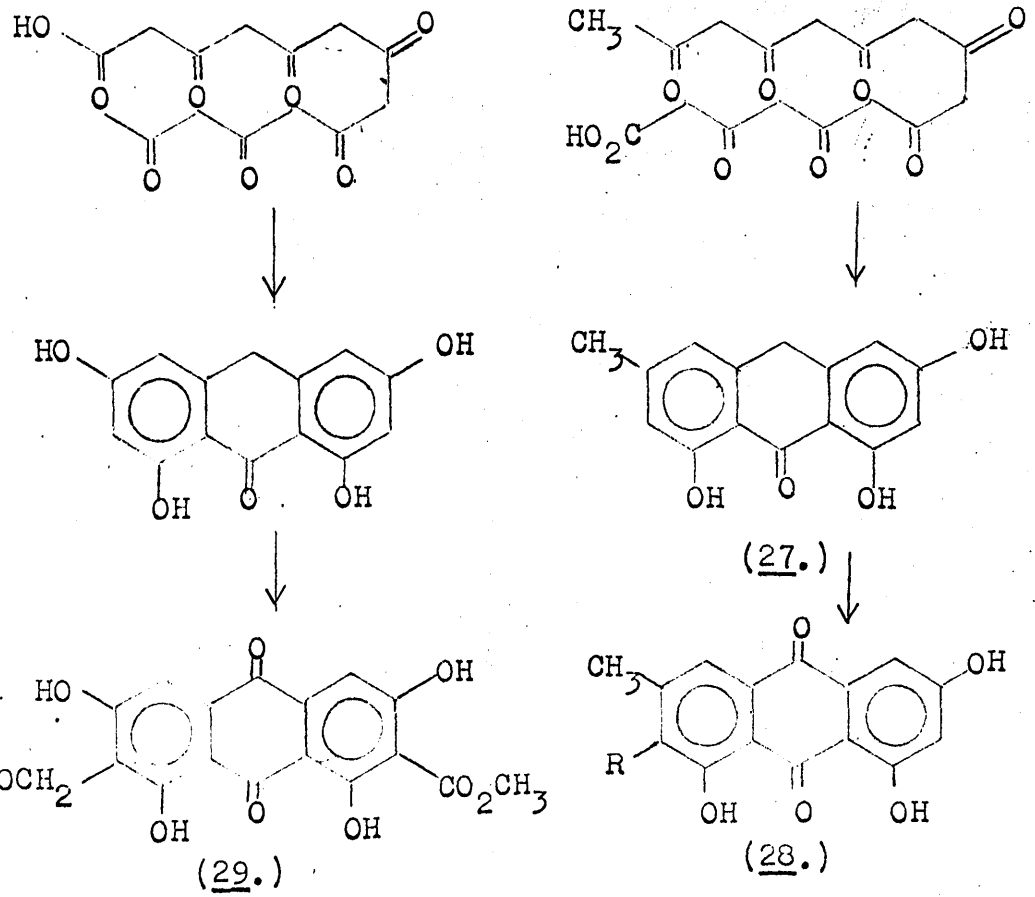


(25.)

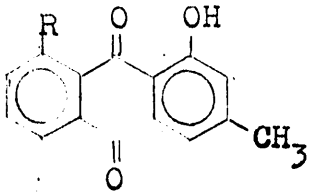


(26.)

FIG. 4.



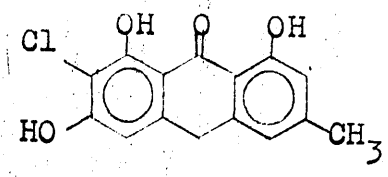
R = CO_2H; ENDOCROCIN, R = H; EMODIN.



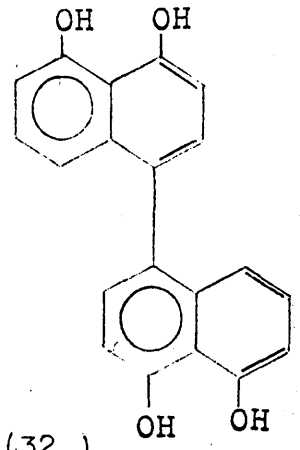
(30.)

R = H; PACHYBASIN.

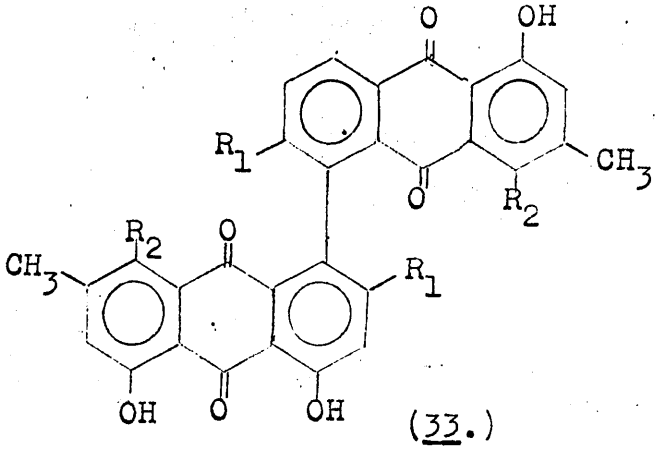
R = OH; CHRYSOPHANOL.



(31.)



(32.)

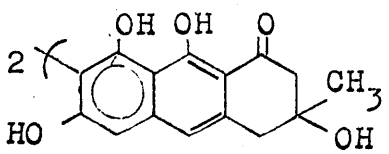


(33.)

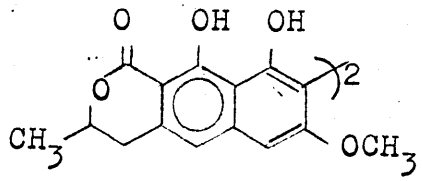
R_1 = OH, R_2 = H; SKYRIN.

R_1 = H , R_2 = OH; IRIDOSKYRIN.

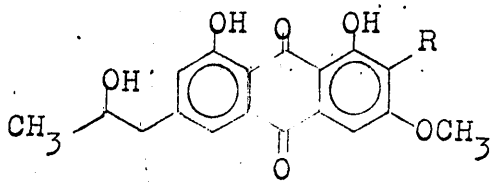
R_1 = OCH_3, R_2 = OH; FUSAROSKYRIN.



(34.)



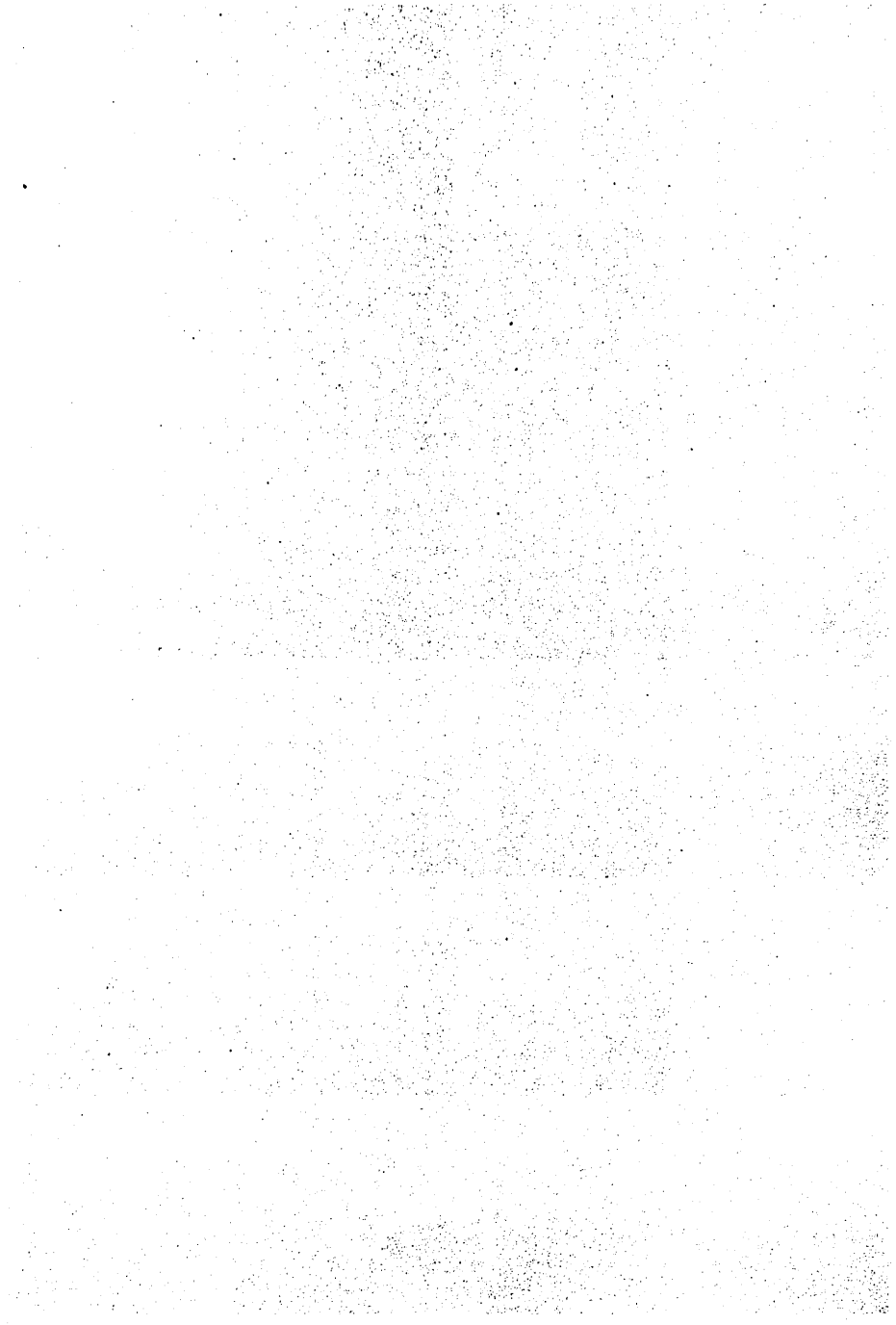
(35.)

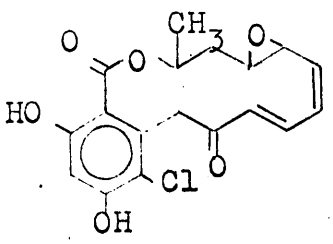


(36.)

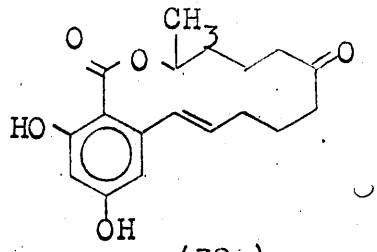
R = H; NALGIOVENSIN.

R = Cl; NALGIOLAXIN.



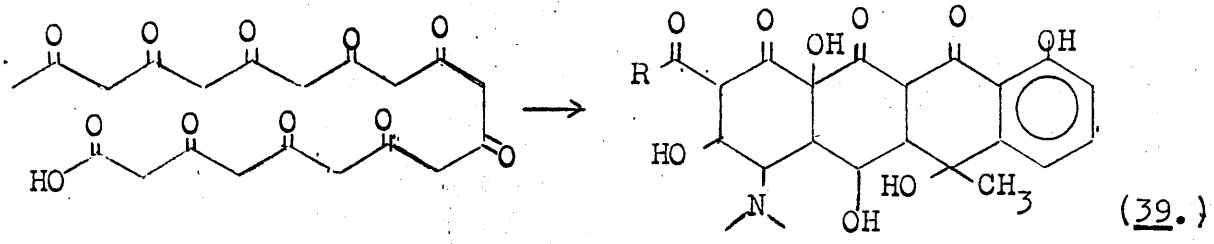


(37.)



(38.)

FIG. 5.

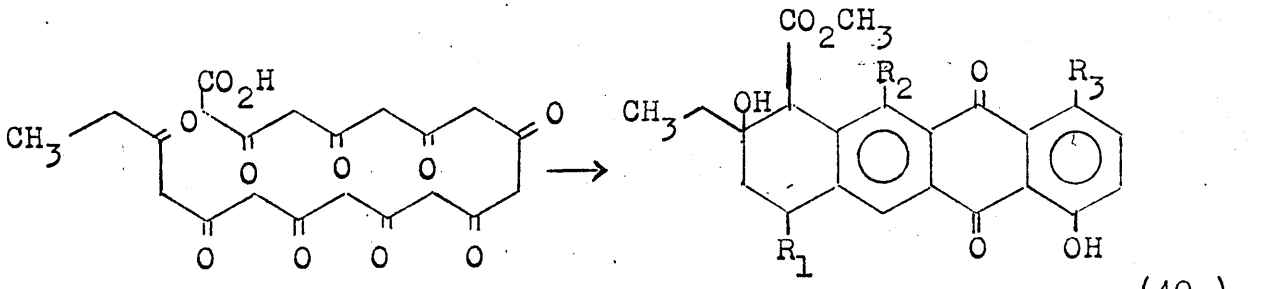


(39.)

R = CH_3; TERRAMYCIN X.

R = NH_2; TERRAMYCIN.

FIG. 6.

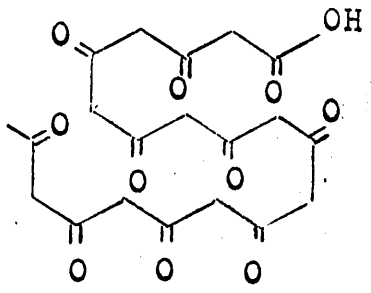


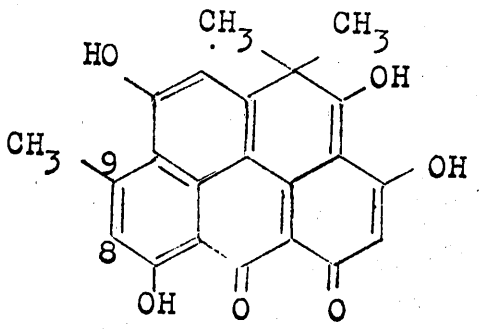
(40.)

R_1= H, R_2= OH, R_3= H; δ - RHODOMYCINONE.

R_1= R_2= OH, R_3= H; ε - RHODOMYCINONE.

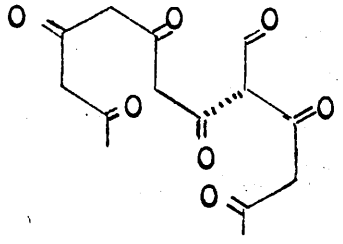
FIG. 7.

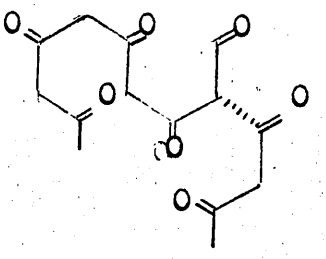


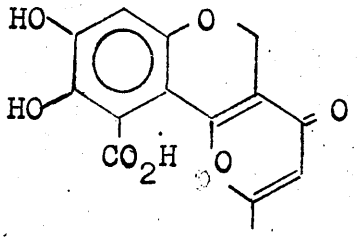


(41.)

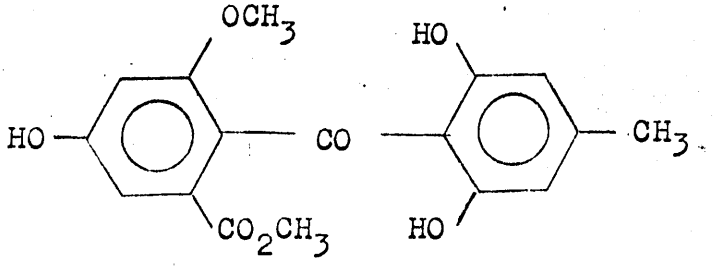
FIG. 8.



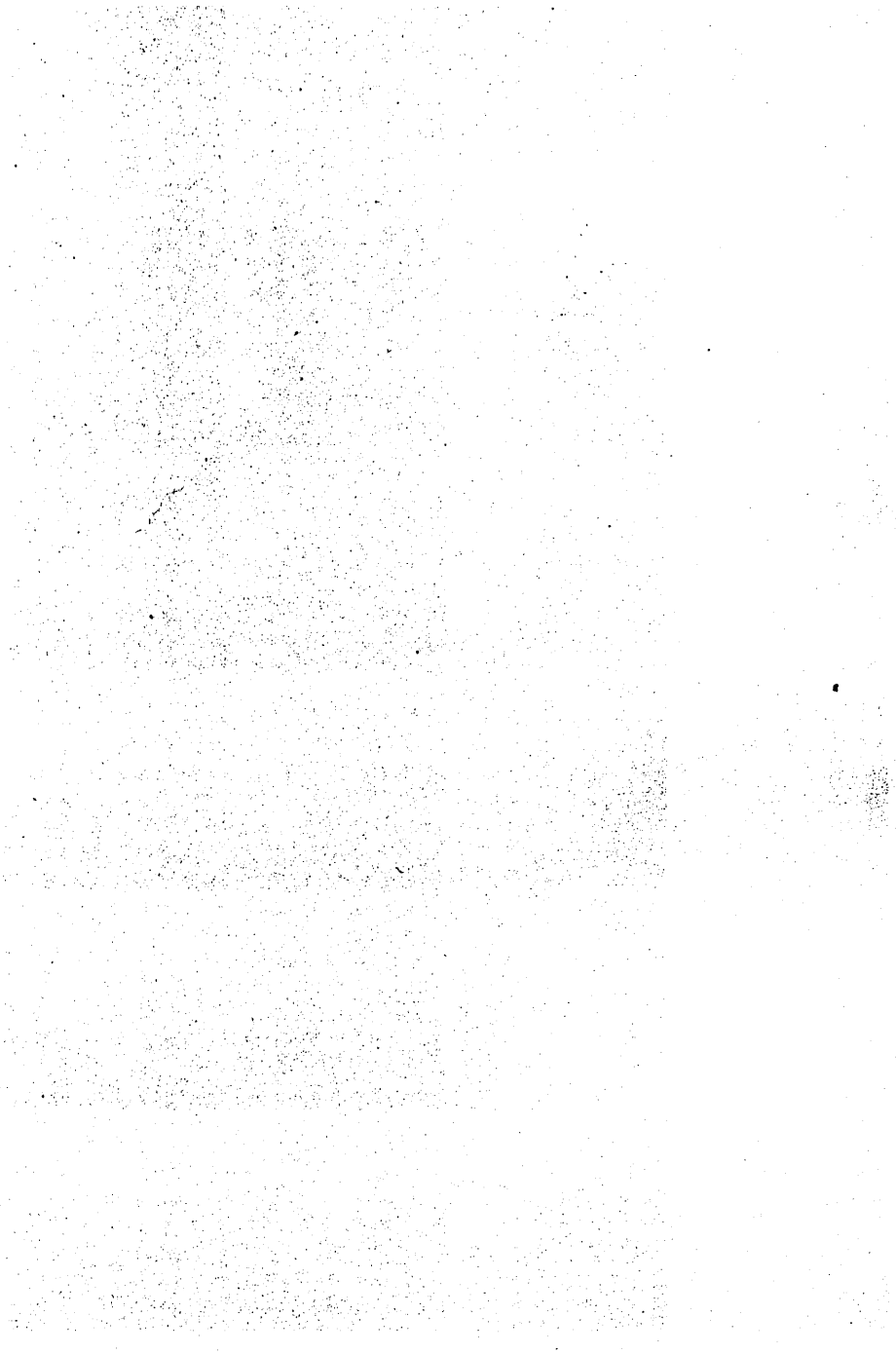


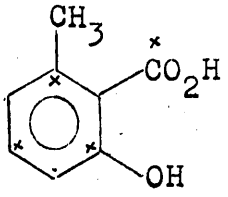


(42.)



(43.)

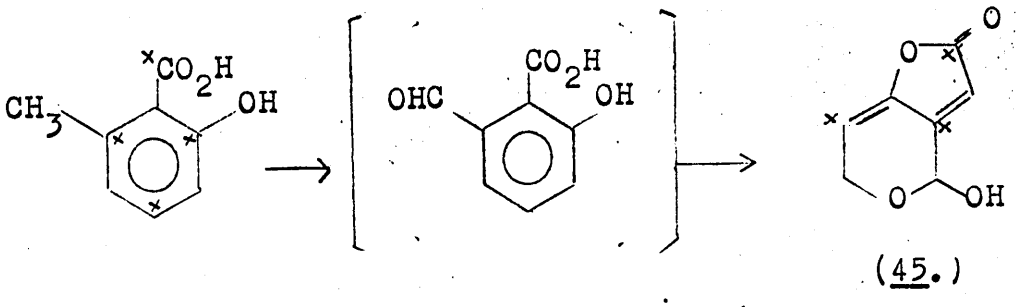




× = C^{14}

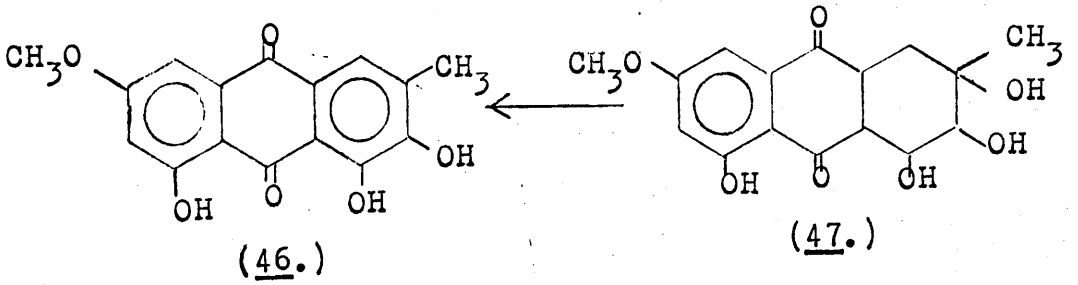
(44.)

FIG. 9.



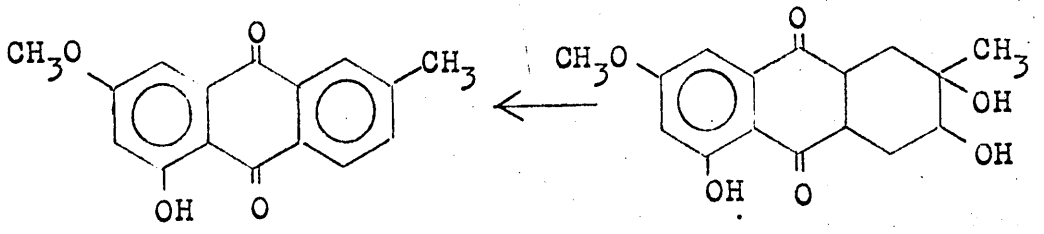
(45.)

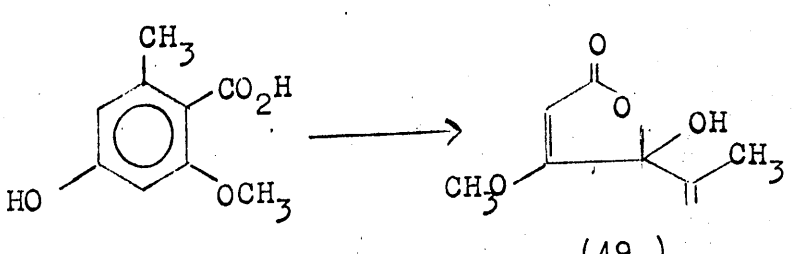
A.



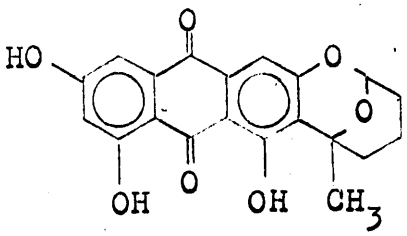
(46.) (47.)

B.

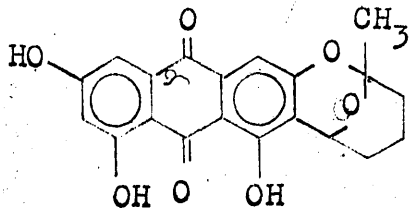




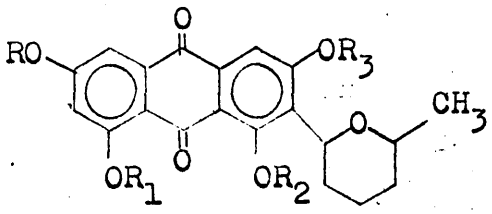
(48.) (49.)



(50.)

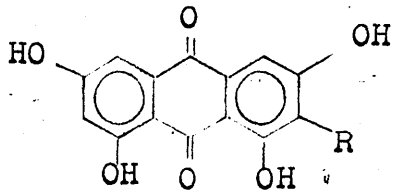


(51.)



(52.)

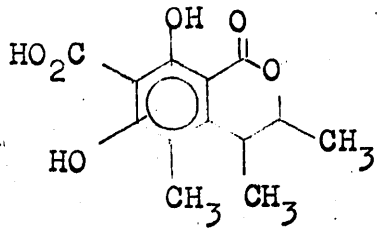
	R	R_1	R_2	R_3
(1)	H	H	H	H
(11)	H	CH_3	H	H
(111)	CH_3	CH_3	H	H



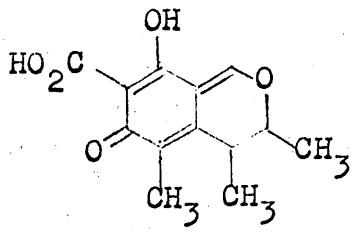
(53.)

R = $CH{:}CH\cdot(CH_2)_3\cdot CH_3$; AVERYTHRIN

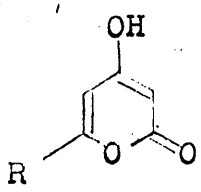
R = $CH(OH)\cdot(CH_2)_4\cdot CH_3$; AVERANTIN.



(54)



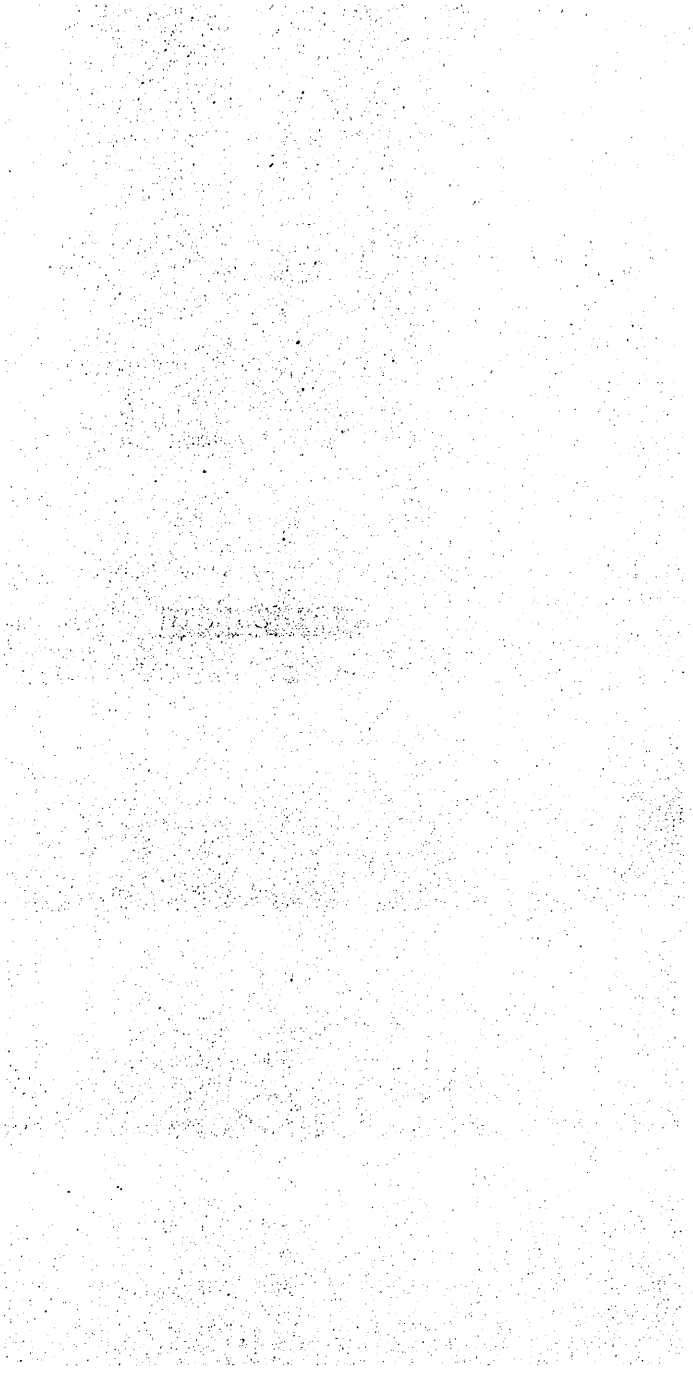
(55)

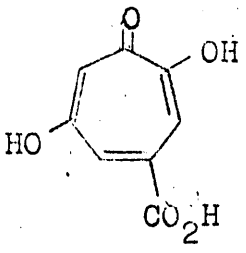


(56)

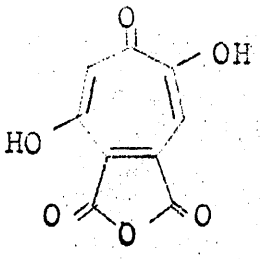
R = CH_3; TRIACETIC ACID LACTONE.

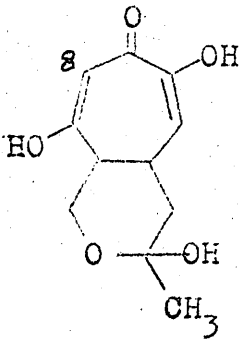
R = $CH_3\cdot CO\cdot CH_2$; TETRAACETIC ACID LACTONE.





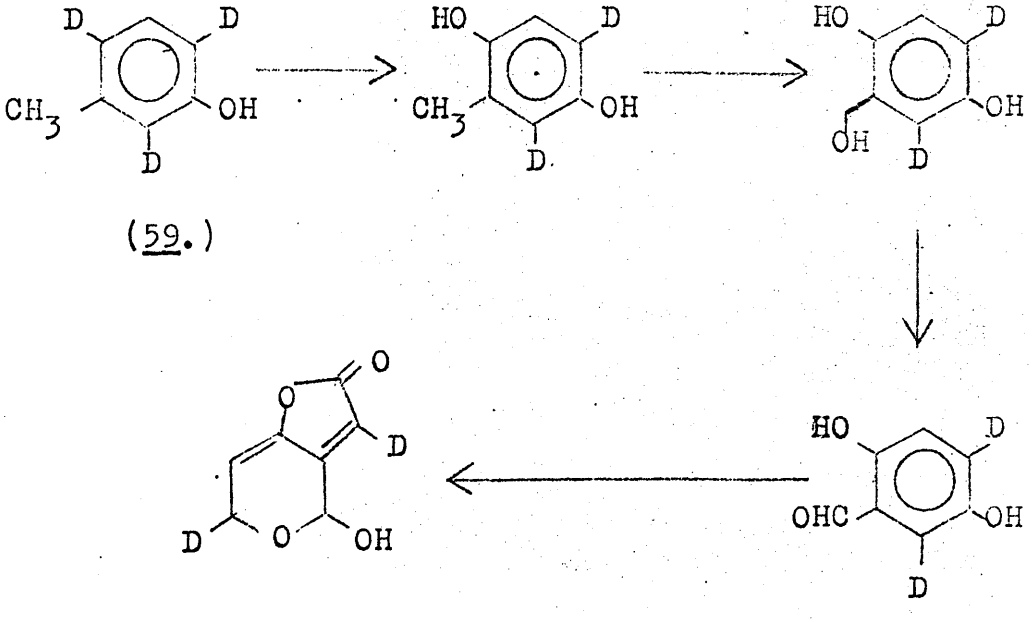
(57.)





(58.)

FIG. 10.



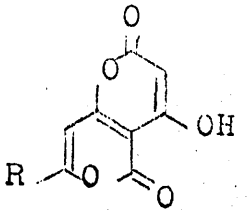
(59.)

(60.)

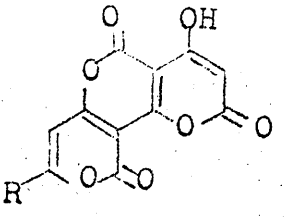
(61.)

$R.CO(CH_2CO)_n.CH_2.CO.S.Enzyme$

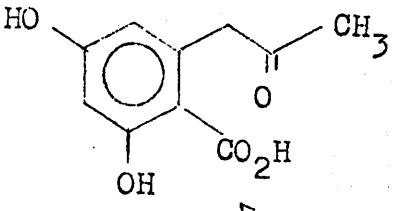
(62)



(63)



(64)



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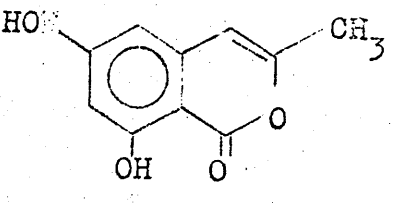

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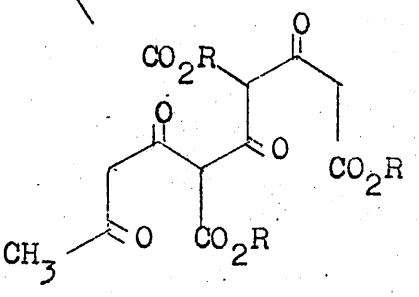

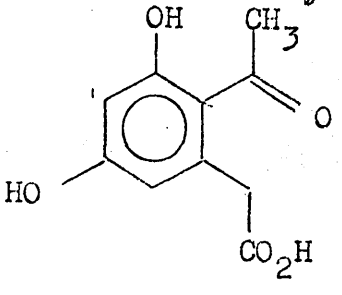

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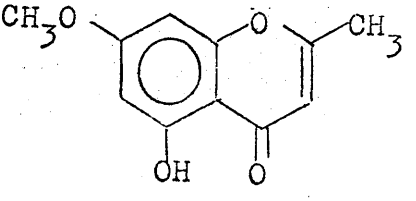


(68)

DISCUSSION.

DISCUSSION I.

The broth from cultures of the basidiomycete, Lenzites striata, which was found to have a final pH of ca.5 was subjected to liquid-liquid extraction using ethyl acetate, and the chemical constituents separated by column chromatography on silica gel (and silicic acid), using a light petroleum to ethyl acetate gradient. By this method, three related metabolites were isolated and purified; the code names LE/I, LE/0 and LE/0-I were assigned to these compounds, the t.l.c. properties of which are defined in Table I.

The most abundant and the most polar compound, LE/I, had molecular formula $C_{11}H_{10}O_5$ from elemental analysis and the parent molecular ion at m/e 222 in the mass spectrum. The structure (69) was assigned to LE/I on the basis of the following evidence: the presence of the hydroxylic side chain was apparent from the infra-red (i.r.) spectrum which showed broad absorption in the region 3,200-3,400 $cm.^{-1}$, and from the mass spectrum fragmentations, supported by the presence of appropriate metastable peaks (Table 2); these included losses of 18 mass units typical of primary and/or secondary hydroxyl functions and of 31 mass units, characteristic of the primary hydroxylic grouping. In the n.m.r. spectrum a two proton multiplet at 5.7τ and a one

proton multiplet at 4.5τ formed a typical ABX pattern, irradiation at 5.7 τ resulting in a collapse of the quartet at 4.5 τ to a singlet and irradiation at 4.5 τ simplifying the group of signals at 5.7τ. (An impurity in the CF_3CO_2H solvent obscured half of the resulting AB quartet). This is consistent with the presence of the grouping $HOCH_2.CH(OH).R$, where R is a deshielding substituent.

In accord with this, brief treatment of the natural metabolite with acetic anhydride/pyridine at room temperature yielded a crystalline compound, the mass spectrum of which showed the parent molecular ion at m/e 306, an increase of 84 mass units from that of LE/I. Losses of acetic acid (M^+-60), followed by ketene (M^+-60-42) (Table 3) confirmed that this was a diacetate, as did the n.m.r. spectrum, which showed two methyl singlets at 7.83 τ and 7.91τ (CH_3CO-) with appropriate downfield shifts for the C-9 proton and the C-10 methylene protons. The i.r. spectrum showed additional absorption in the carbonyl region, (υ max 1730, 1735 cm^{-1}). Confirmation of the presence of the grouping $CH_2OH.CHOH$ in LE/1 was obtained from treatment of the natural product with sodium metaperiodate. Steam distillation of the reaction mixture into an ethanolic solution of dimedone gave the crystalline condensation product of formaldehyde and dimedone, while extraction of the residue into chloroform yielded a crystalline compound

identified as the aldehyde (70), from the mass spectral parent ion at m/e 190, the n.m.r. absorption at 0.14 τ (one-proton singlet) and the superposition in the i.r. carbonyl region of a new carbonyl absorption on the original peak (ν max 1690 cm.$^{-1}$) resulting in broad absorption between νmax 1680 and ν max 1710 cm.$^{-1}$, due to an α,β-unsaturated aldehyde.

A purple ferric chloride reaction on t.l.c. and in solution, together with the appearance of a light yellow precipitate on addition of cupric acetate in acetone to an acetone solution of LE/1, indicated that the phenolic hydroxyl group was chelated as did the low i.r. carbonyl absorption (ν max 1670 cm.$^{-1}$). The ultraviolet (u.v.) spectrum showed two electron transfer bands at 259 and 336 nm, the latter undergoing a bathochromic shift to 358 nm at pH13, and a blue shift to 333 nm on etherification (see below), typical of o-hydroxy carbonyl compounds.[61]

A further indication of the ortho-hydroxy carbonyl system was provided by treatment of the natural metabolite with ethereal diazomethane, whereby a crystalline derivative was obtained in 63% yield, with molecular formula $C_{12}H_{12}O_5$ from elemental analysis. This was deduced to be a mono-methyl ether of LE/1 from the mass

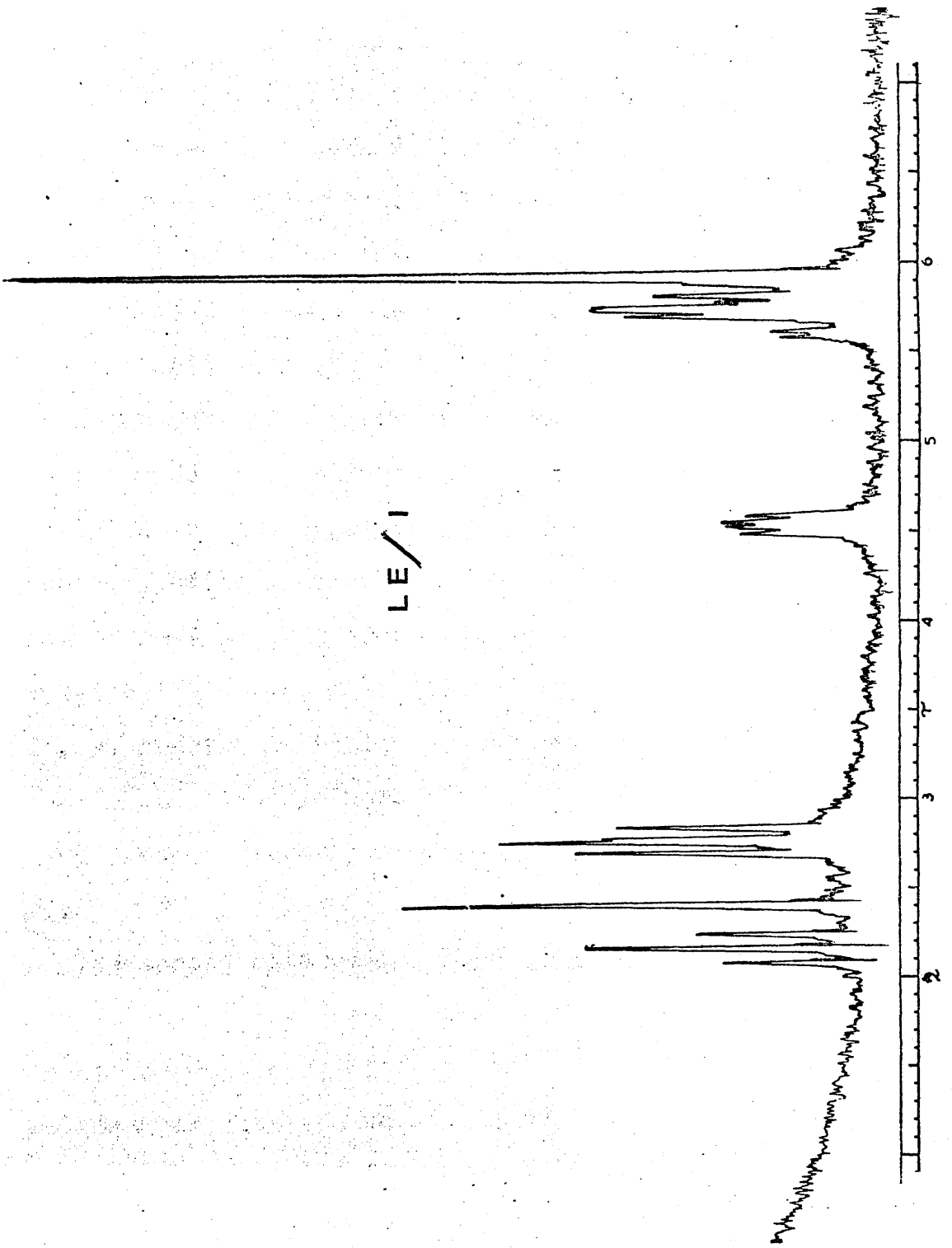
spectrum which showed a parent molecular ion at m/e 236 (LE/1 + 14 mass units) and had fragmentation pattern (Table 4) similar to that of LE/1. The appearance in the n.m.r. of a new methyl singlet (5.90 τ) was attributed to the CH_3O-Ar grouping, the low value being due to deshielding by the adjacent carbonyl function; the increased polarity of this methyl ether by t.l.c. comparison with LE/1, due to disruption of the chelated system, together with the absence of a base-shift in the u.v. spectrum also supported location of the methoxy grouping ortho to a carbonyl function.

The chelated nature of the phenolic grouping was again reflected in the slow rate of acetylation of LE/1 at room temperature. Under reflux, acetylation gave a clear gum, which, although t.l.c. pure, could not be induced to solidify, but sublimed easily. The n.m.r. spectrum of this derivative showed three methyl singlets, two of τ value, (7.84, 7.92), similar to those of the diacetate, and a third considerably lower (7.58 τ). This low-field methyl was assigned to the phenolic acetate, deshielded by adjacent carbonyl group; the absence of a base-shift in the u.v. spectrum and a negative ferric chloride reaction combined to confirm that the phenolic hydroxyl group was

now acetylated. Mass spectrometric analysis also indicated that a triacetate had been formed, both from the parent molecular ion at m/e 348 and from successive losses of 42, 60 and 42 mass units (Table 5).

The n.m.r. spectrum of LE/1 (p.33) also showed two doublets at 2.72 and 2.80 τ, assigned to protons H-5 and H-7, ortho and para to the chelated hydroxyl group, and in addition a double doublet at 2.15 τ corresponding to the meta H-6. Ortho coupling (H-6, H-7 and H-6, H-5) was present with J=8 c.p.s., and meta coupling (H-5, H-7) was negligible in this instance. The remaining signal in the n.m.r. spectrum was a one-proton singlet at 2.40 τ and this was assigned to the olefinic C-3 proton in structure (69).

The possible alternative structures (71) were eliminated by comparison of the downfield region of the n.m.r. spectra of LE/1 (69) and the aldehyde (70). In the aldehyde (70), the H-6 double doublet (2.10 τ) and the H-7 doublet(2.73 τ) possessed the same approximate chemical shift as in LE/1, while the remaining doublet (H-5) was now observed at 1.60 τ as opposed to 2.79 τ in LE/1, a deshielding explicable if the aldehyde carbonyl group is assigned the "homo-peri" position attached to C-4; the deshielding of the H-5 doublet alone tended to indicate a "through-space"

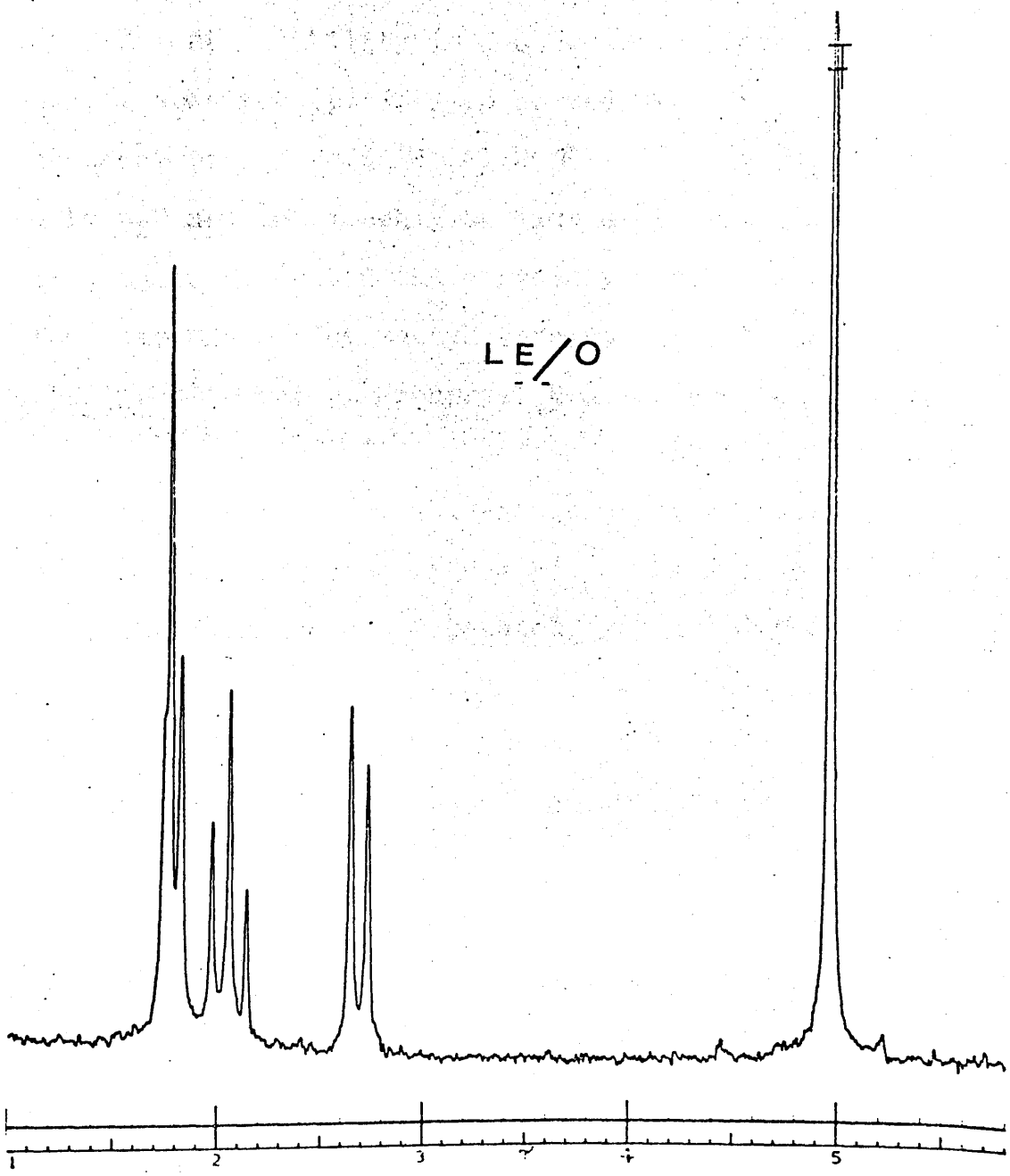

LE/I
2
3
4
5
6
τ

effect rather than an effect which was transmitted through the π-electron system of the molecule. Analagous deshielding effects have been observed in the polycyclic aromatic structures such as (72)[62] in which H_A resonates at much lower values (ca.0.4τ) than the remaining aryl protons (ca.3.4τ) and the n.m.r. spectrum of 1-naphthoic acid similarly shows a one-proton multiplet (ca.0.9τ) at lower field than the remaining aryl multiplet (1.5 - 2.6τ).

A compound of the same structure as LE/I has previously been isolated from cultures of an airborne microorganism classified as an Oospora species[63,64] . Comparison of the properties of this metabolite, named oospoglycol, with those of LE/I left no doubt that they are identical. Thus, oospoglycol had almost identical m.p., rotation, u.v. and i.r. spectra to LE/I; a diacetate and aldehyde prepared by periodate cleavage were also reported and these again showed close correspondence in m.p. to those obtained from LE/I.

The second metabolite of Lenzites striata, LE/0, C_{11},H_8O_5, had t.l.c. characteristics defined in Table 1. This was evidently related to LE/I from a comparison of the spectroscopic data and was assigned the structure (73). The molecular weight difference of two between LE/1 and LE/0 and the increased complexity of the i.r. carbonyl

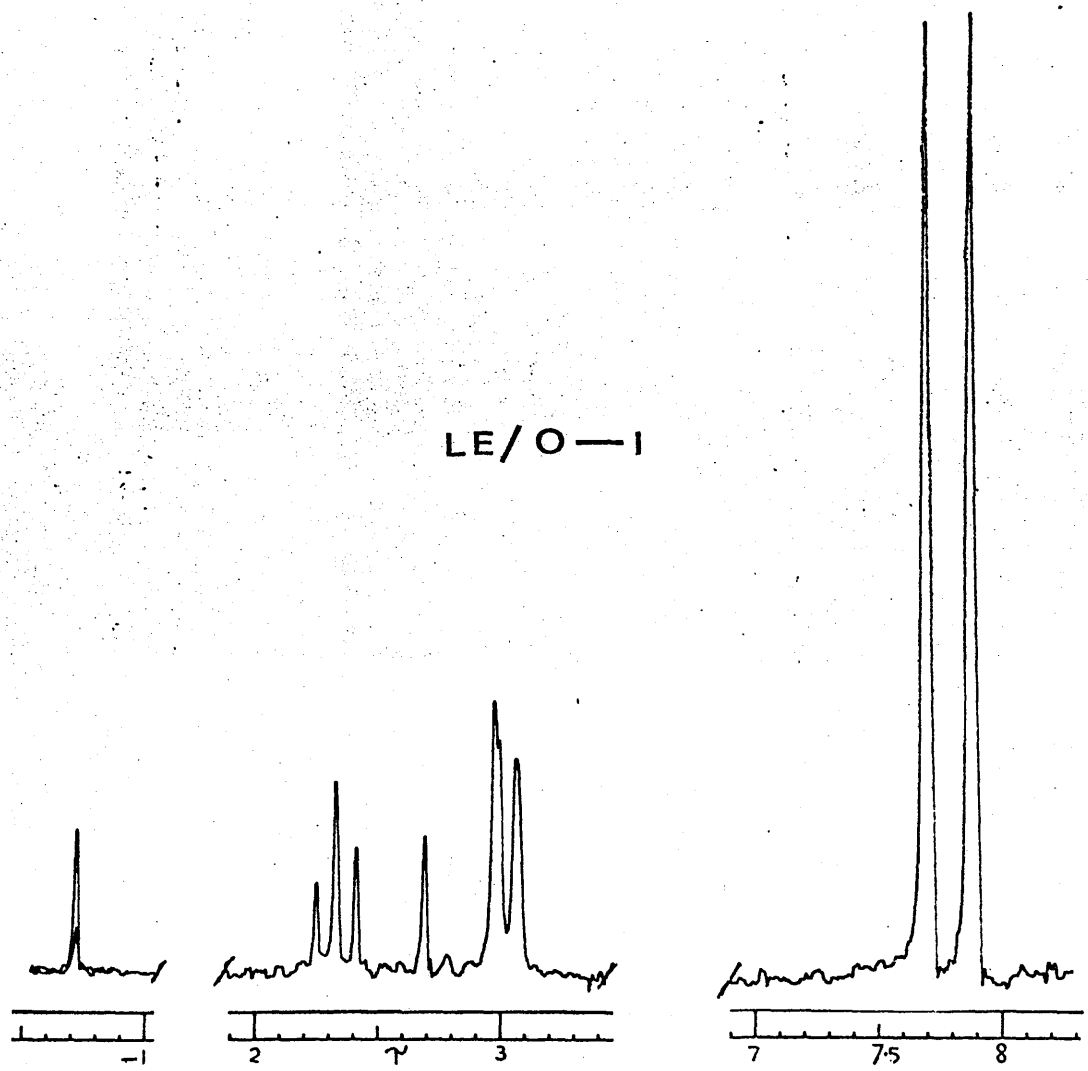
absorption suggested that one of the -CHOH- groups of the side chain had been replaced by a carbonyl group. From the loss of 31 units of mass from the parent ion (Table 6), the primary hydroxylic function evidently remained, indicating that the site of the new carbonyl function was C-9. This was further supported by the n.m.r. spectrum (p.36) which showed striking similarity in the region 1.6 τ to 2.8 τ to that of the aldehyde derivative of LE/1, above. Thus, the downfield shift of one of the pair of aryl proton doublets was again evident as was that of the C-3 proton. A two proton singlet at 4.98 τ bore evidence of the presence of the grouping $-CO.CH_2OH$. The similarity of the u.v. spectrum to that of LE/1 together with the formation of a precipate with cupric acetate in acetone again indicated the presence of a chelated phenolic grouping. Structure (73) could thus be assigned to LE/0 without resort to chemical degradation. From the same Oospora species which produced oospoglycol (LE/1), another metabolite, oosponol, assigned structure (73) was also reported[65,66]. The reported i.r. and u.v. spectra again compared well with those of LE/0 and the τ values for the hydroxymethylene and C-3 protons were also in close agreement; the structure of oosponol was confirmed by


LE/O
1
2
3
4
5

synthesis both of the degradation product oospoic acid (74)[67] and the diacetate (75)[68].

The third metabolite to be isolated, with molecular formula $C_{11}H_{10}O_3$, was assigned the code name LE/O-1 (Table 1) and structure (76). This was the first compound with sufficient solubility in deutero-chloroform to allow its n.m.r. spectrum (p. 38) to be measured in this solvent; the presence of a downfield (-1.29 τ) one-proton singlet exchanged by D_2O and the roughly similar aryl proton pattern to that of LE/1, again suggested the 8-hydroxy isocoumarin structure for the molecule. Two methyl singlets at 7.68 and 7.86 τ were assigned to methyl groups at C-3 and C-4 respectively With parent ion at m/e 190, the fragmentation pattern showed significant losses of 15, 43, and 28 units of mass, rationalised as indicated (Table 7). The i.r. spectrum revealed the presence of a chelated hydroxyl group (υ max 3,100 $cm.^{-1}$), the lactone carbonyl group (υ max 1688 $cm.^{-1}$; E = 843), and the double bond (υ max 1635 $cm.^{-1}$), while the typical ultraviolet spectrum previously noted was observed for this metabolite (reversible base shift, from λ max 342 at pH7 to λ max 370 at pH 13). A precipitate on addition of cupric acetate in acetone again indicated the presence of a chelated system in LE/O-I. This metabolite has also been isolated from the previously mentioned Oospora

LE/O—1



species[65,69], with comparable i.r. and u.v. spectra to LE/O-I and comparable m.p.; the structure (76) has been confirmed by synthesis[70,71].

The recently isolated Oospora metabolite, oospolide, with structure proposed initially as (77)[72] and corrected to (78)[73], was not detected in Lenzites striata, although dehydro-eburicoic acid is apparently a common metabolite of both fungal species.[63]

DISCUSSION II.

The isocoumarins comprise a group of aromatic natural products which has expanded considerably in size over recent years; developments in this field to 1964 have been adequately reviewed[74]. The variety of structures exhibited by this group of natural products requires that they must derive biogenetically from both the "shikimic acid" (see section III) and the "polyacetate" pathways. The isocoumarins arising from the former of these two routes such as hydrangenol (79)[75] and brevifolin carboxylic acid (80)[76] are mainly of plant origin and, as such, will not be considered here.

The isocoumarins of fungal origin can generally be derived by chain-folding of the polyacetyl chain as indicated (scheme 1); ramulosin (81)[77] appears to arise in this way, and a simple aromatic example, isolated from two strains of Marasmius ramealis[78,79], is 3-methyl-8-hydroxy-isocoumarin (82), (apparently derived from five "acetate" units), in which the oxygen function at C-6 has been reduced prior to cyclisation and subsequently eliminated. The structures (83), both metabolites of the fungus Ceratocystis fimbriata[80], show the fully oxygenated skeleton. A common structural variation is reduction of the 3,4-double bond, as is evident in mellein (or ochracin) (84)

from both Aspergillus melleus and Aspergillus ochraceus.[81] Related in structure, and again showing full oxygenation is 6-methoxy-mellein (85), originally isolated from infected carrots and thought to be produced as a form of resistance to the microorganism[82]; subsequent investigators demonstrated that this 'phytoalexin' theory was incorrect and that 6-methoxy-mellein was a 'true' fungal metabolite by isolating it from both Sporormia bipartis[83] and Sporormia affinis[84]. Also isolated from this latter source were the chloroisocoumarins (86).

Relatively few biosynthetic investigations have been carried out on the acetate-derived isocoumarins, the most extensive study[85] being that made on sclerin (87), a metabolite of a Sclerotinia fungus[86]. Of the possible modes of condensation of a pentaacetyl chain, schemes a and a' were considered less likely while scheme b, a pathway already demonstrated for citrinin (88)[87], and supported by the occurrence of Sclerotinin A and B (89)[88] as co-metabolites of sclerin, would require, in the case in point, C_1 alkylation at a ketonic site of the polyketide chain. This unusual process, and the biogenetically uneconomical loss of a C_1 unit necessary in all three routes, suggested that another mode of cyclisation was involved. Feeding experiments using sodium acetate, and sodium formate as a C_1 source, suggested a more likely biogenetic scheme c, in which two

distinct polyketide chains are condensed; condensation of the same two polyketide chains in the opposite direction (scheme d) would also give rise to sclerotinin A and B. This type of biogenetic pathway has already been established for a number of natural products, including rotiorin[89] and sulochrin[90].

Canescin (90), from Aspergillus malignus, represents another of the few isocoumarins for which a detailed biogenesis has been proposed[91] . Current interest in this metabolite has centred on proof of the incorporation of a C_1 unit (C^*) from (^{14}C-Me-)-methionine, while confirmation of the polyketide origin of the nucleus is forthcoming[92] . It was noted that the methionine derived atom is in the same position as the similarly derived carboxyl carbon of citrinin, indicating a close biogenetic relationship, which is supported by the fact that the canescin-producing strain of P.canescens is also a source of citrinin. This, together with the previously noted fact (p.13) that a mutant strain of the citrinin-producing Aspergillus terreus gave the isocoumarin (91) as a major metabolite, suggests that the polyacetate derived C-acetyl-orsellinic acid (92), itself a known constituent of P.brevicompactum, is a common biogenetic precursor of both citrinin and the isocoumarins.

INVESTIGATION OF THE BIOSYNTHESIS OF OOSPOGLYCOL.

A feasible biosynthetic route to the isocoumarins isolated from Lenzites striata is outlined in schemes e and f. Thus, oospolactone could be derived (scheme e) by insertion of a C_1 unit in the acid (93), the probable precursor of mellein, followed by cyclisation; such C_1-units are commonly found attached either to aromatic rings[93] or aliphatic chains[94]. The same intermediate could also be involved in a biosynthetic scheme f to oosponol and oospoglycol. To test the validity of the above hypothesis, a radio-tracer study of the constituents of the fungus, Lenzites striata, was inaugurated. Oospoglycol being the most abundant of the isocoumarin metabolites present, this was chosen as the molecule for biosynthetic investigation, since adequate supplies of unlabelled material were available for dilution purposes.

A necessary preamble to any biosynthesis experiment is, firstly, an examination of the rate of production, in vivo, of the metabolite under study, and, secondly, to devise an appropriate series of degradative reactions, whereby the activity of (ideally) individual atoms may be measured. The first requirement was satisfied for the case in point by

chromatographic analysis (thin layer and column) of the oospoglycol content of Lenzites striata cultures at increasing intervals of growth up to two months. These results (Table 8) allowed a plot of the type shown (Table 9), from which it was apparent that oospoglycol formation occurred very quickly between ten and fourteen days after setting up cultures. Thus, the optimum time for feeding labelled precursors was chosen as the beginning of this period of high metabolic activity with only a short time interval between inoculation of precursor and harvesting of metabolites to offset in vivo dilution of the active oospoglycol.

The radioactivity of the terminal CH_2OH group of oospoglycol could readily be measured by the periodate cleavage reaction previously discussed. In addition, examination of tritium labelling in the aromatic ring would be facilitated by exploiting the substitution reactions directed to ortho(C-7) and para (C-5) positions by the phenolic hydroxyl group; the substitution reaction chosen was bromination. Thus, treatment of a solution of oospoglycol in methanol at ice temperature resulted in formation of a crystalline derivative identified as 5,7-dibromo-oospoglycol from the following data: elemental

analysis and mass spectrum gave a molecular formula $C_{11}H_8O_5Br_2$; the parent ion was a triplet of peaks at m/e 382, 380, and 378, with relative intensities in the ratio 1:2:1, characteristic of a dibromo-compound. In addition, the fragmentation pattern showed typical losses of 18,28 and 31 units of mass already noted for oospoglycol (Table 2). The i.r. spectrum showed hydroxyl, lactone carbonyl and C-C double bond absorption, and while the u.v. spectrum was essentially unchanged in comparison with oospoglycol, the n.m.r. spectrum showed the expected simplification; thus, the ABX pattern of the hydroxylic side chain was still present (multiplets at 3.38 and 5.60τ), but the remainder of the spectrum consisted of two one-proton singlets at 2.21τ (C-3 proton) and 1.70τ (C-6 proton). This confirmed the structure (94) of the dibromo derivative.

Concentration of the mother liquors of the above reaction, or prolonged treatment of oospoglycol with bromine/water, afforded another bromo-derivative less polar than 5,7-dibromo-oospoglycol with molecular formula $C_{11}H_7O_5Br_3$. This was assigned the structure (95) on the following evidence: the parent molecular ion was a quartet of peaks at m/e 462, 460, 458 and 456, indicative of a tribromo compound, while the fragmentation pattern (Table 10)

showed an initial loss of 60 units of mass, as well as 81(Br atom), in place of the former elisions of H_2O and $-CH_2OH$; assuming substitution by bromine at C-5 and C-7 as before, the 3,4-double bond seemed the most likely site of further attack by bromine. This was confirmed by the disappearance in the i.r. spectrum of the C-C double bond absorption. The n.m.r. spectrum showed the following features, consistent with the structure (95); the one-proton singlet assigned to the remaining aryl proton of the dibromo derivative was also present here (1.79τ), but the C-3 proton now resonated as a singlet at 3.69τ. Also shifted upfield (4.31τ) was the side chain methine proton, while the adjacent methylene protons remained unchanged in chemical shift value. The bromination reaction proceeds presumably by scheme g, and also occurred using N-bromosuccinimide in place of bromine/water; two equivalents of this reagent gave 5,7-dibromo-oospoglycol, while three equivalents of N-bromosuccinimide gave the tribromo derivative. Other examples of bromination reactions involving the participation of a neighbouring hydroxyl group are known: one such is the reaction of terpene alcohols, e.g. linalool (96), with N-bromosuccinimide, resulting in formation of an α-bromotetrahydrofuran (97) as a major product[95], while in

dammarane-type triterpene series[96,97], betulafoliene-triol (98) also undergoes an anomalous side-chain bromination reaction, both with N-bromosuccinimide in acetone, and bromine/chloroform, to give the tetrahydrofuran (99).

The reaction of oospoaldehyde (70) with bromine/water resulted in formation of a compound, $C_{11}H_7O_5Br_3$, which like tribromo-oospoglycol with which it was isomeric showed a parent molecular ion as a quartet at m/e 462, 460, 458 and 456, as well as a loss of 60 units of mass. The i.r. spectrum again showed no C-C double bond absorption and the aldehyde carbonyl absorption frequency was now ν max 1731 cm^{-1} (cf. ν max 1710 cm^{-1} in oospoaldehyde) indicating saturation of the 3,4- double bond and the presence of Br α to the carbonyl group. By analogy with tribromo-oospoglycol, the compound was assigned structure (100) confirmed by the extremely simple n.m.r. spectrum; this consisted of four singlets assigned to an aldehyde proton (0.29τ), the aryl C-6 proton (1.82τ), the C-3 proton (4.31τ) and to a methoxyl group (6.26τ).

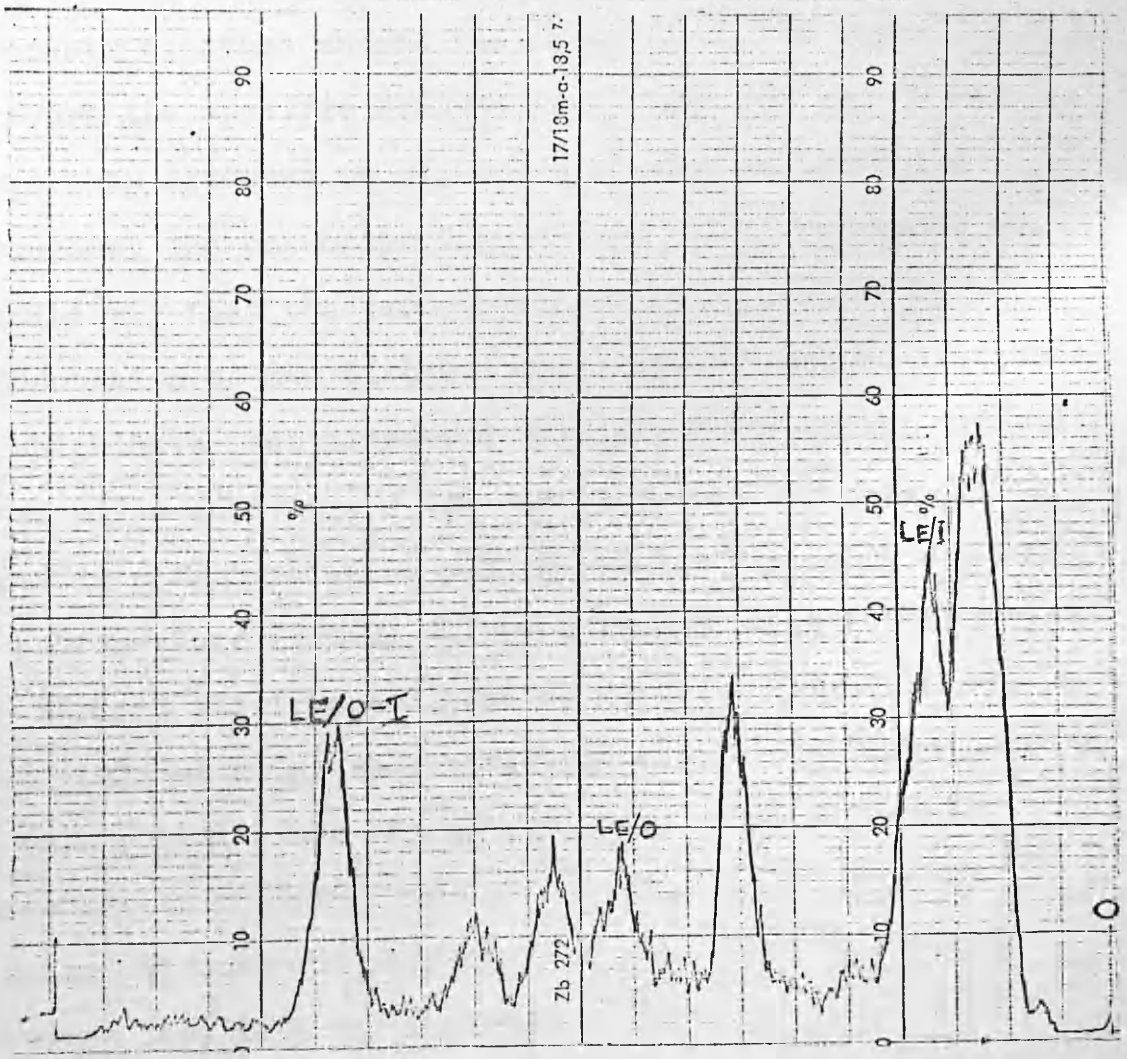
The degradation scheme chosen for examination of radioactive oospoglycol, outlined in scheme (h), involved preliminary cleavage of the side chain with sodium metaperiodate followed by bromination of the resultant aldehyde. Two separate labelling experiments were performed, one to investigate the incorporation of acetate into oospoglycol, and one employing methionine as a possible source of a C_1-unit.

In the first experiment, a mixture of (2 -^{14}C) sodium acetate and (2-^{3}H) sodium acetate was fed to cultures of L.striata on the tenth day of growth and the fungus harvested seventeen hours after inoculation. The ethyl acetate extract of the broth was examined on a radio t.l.c. scanner, from which (p. 49) oospolactone, oosponol and oospoglycol could be identified, together with three other minor constituents. The specific molar activities (^{14}C and ^{3}H) of the oospoglycol thus obtained and its degradation products are collected in table 11 . On the basis of incorporation into oospoglycol of five acetate molecules the relative molar activities (R.M.A.) were also calculated.

From these results it appeared that the oospoglycol molecule was labelled as indicated in (101). This was in agreement with the observed drop in specific activity of

BROTH METABOLITES

TLC SCAN : ACETATE FEED



^{14}C on cleavage to oospoaldehyde and also with the virtual elimination of remaining ^{3}H in the aldehyde on bromination. The ^{3}H activity in oospoaldehyde was lower than the theoretical value, which could possibly be due to partial equilibration of the aryl ^{3}H atoms under theconditions of the periodate reaction. The formaldehyde obtained by periodate cleavage showed the expected $^{3}H/^{14}C$ ratio (2 : 1) although the specific activities of both ^{14}C and ^{3}H were lower than expected on the basis of uniform labelling throughout the oospoglycol molecule. This suggested the possibility that the isocoumarin could be derived by condensation of two distinct poly-acetate chains, instances of which have been discussed earlier, these chains differing in specific activity. One way in which this might occur would be condensation of a penta-acetyl unit with a molecule of acetoacetate (scheme i) by a mechanism corresponding to the Michael reaction followed by a retro-Michael rearrangement yielding a suitable precursor (102) for the formation of both oospolactone and oospoglycol. Alternatively, condensation of (103) with a C_2-unit could give the required isocoumarin nucleus (scheme j), although this requires loss of a carbon atom.

The possibility of condensation of two poly-acetate

chains was further supported by the results of the second labelling experiment, in which a mixture of (^{14}C-)-methyl methionine and (^{3}H-)-methyl methionine was inoculated: t.l.c. scans of the crude broth extract showed in this case (p. 52) that while oospoglycol constituted the major proportion of the metabolites, it was by no means the most highly active component. After dilution with inactive material and recrystallisation in the usual manner the oospoglycol was still radiochemically impure to the extent of approximately 75% as determined by the t.l.c. scanning. On this basis the percentage incorporation was only 0.04 and the specific activities 0.058 x 10^6 ^{14}C d.p.m./m. mole and 0.093 x 10^7 ^{3}H d.p.m./m.mole. Since the "residual" ^{3}H activity corresponding to methoxy tribromo-oospoaldehyde (100) was 0.158 x 10^7 d.p.m. /m.mole, it was thus concluded that the incorporation of methionine into oospoglycol is neglibible. The direct precursor of a C_1 unit may sometimes be N-formyltetrahydrofolic acid rather than methionine, but the methyl group of methionine normally can be transferred to the C_1- pool. It therefore seems probable that a C_1- unit is not involved in the biosynthesis of oospoglycol.

BROTH METABOLITES

TLC SCAN: METHIONINE FEED

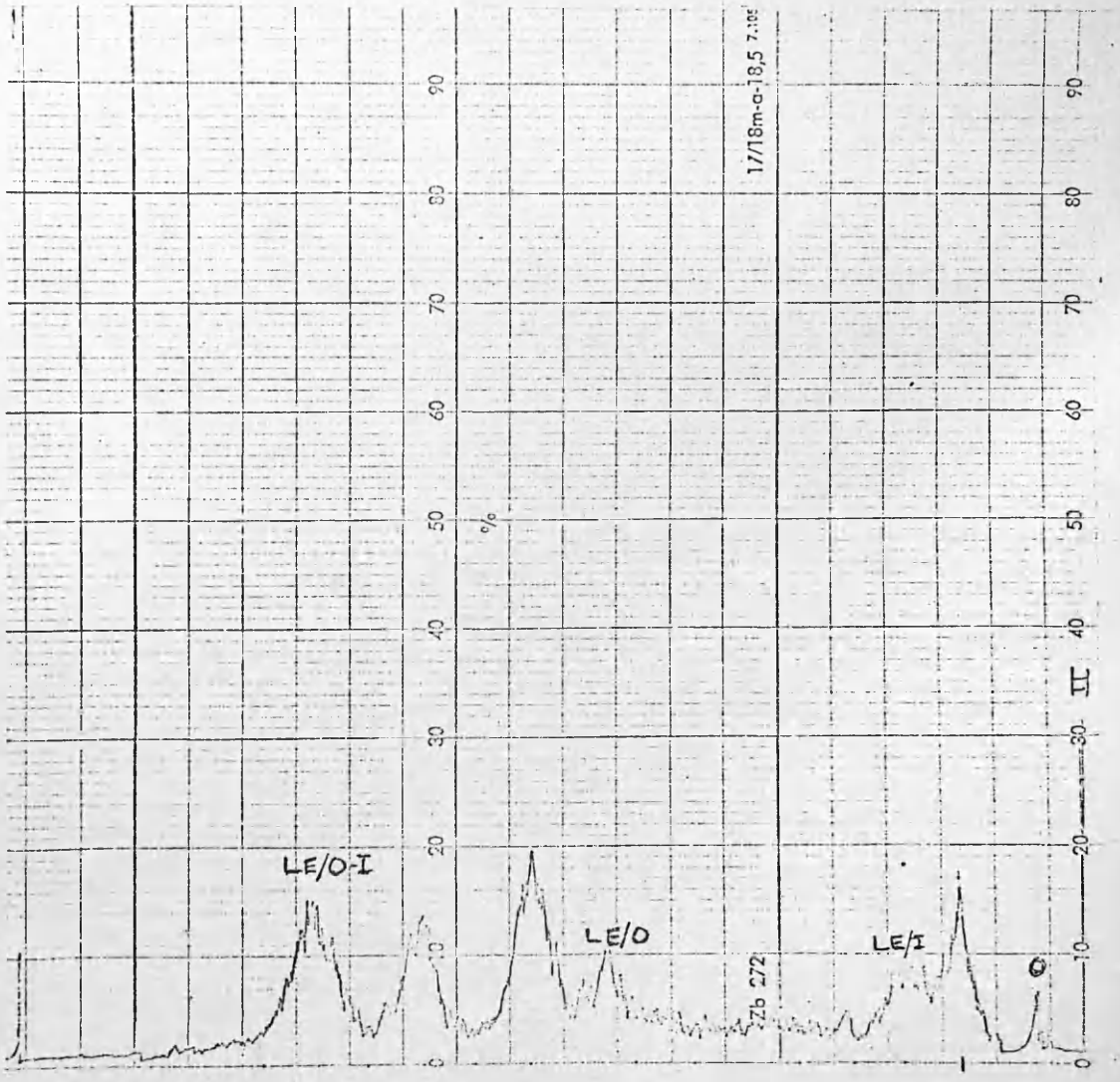


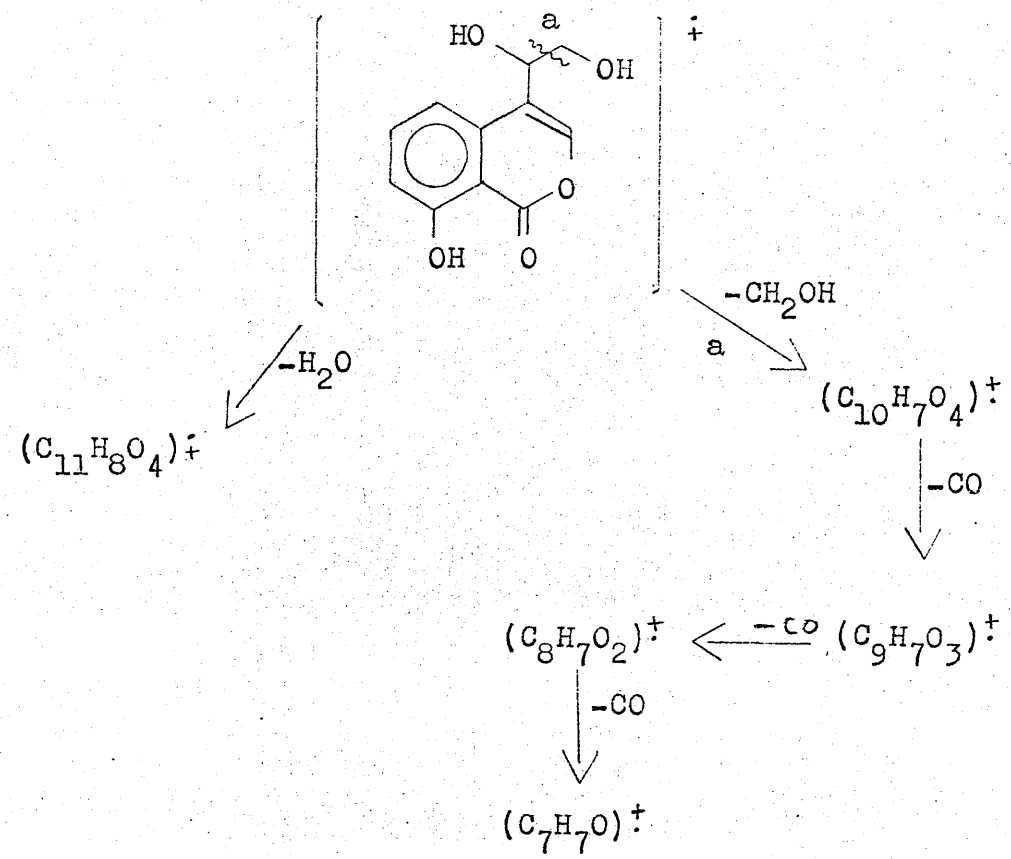
Table 1.

T.L.C. Properties of Broth Metabolites.

Code	Rf	Ceric Amm. Sulphate /H_2SO_4	$FeCl_3/CH_3OH$
LE/1	0.3 : (3%CH_3OH/97%$CHCl_3$)	Blue	Purple
LE/0	0.7 : (3%CH_3OH/97%$CHCl_3$)	Yellow	Blue
LE/0-1	0.9 : ($CHCl_3$) .	Brown	Light Blue

Table 2.

Proposed Fragmentation of LE/1.



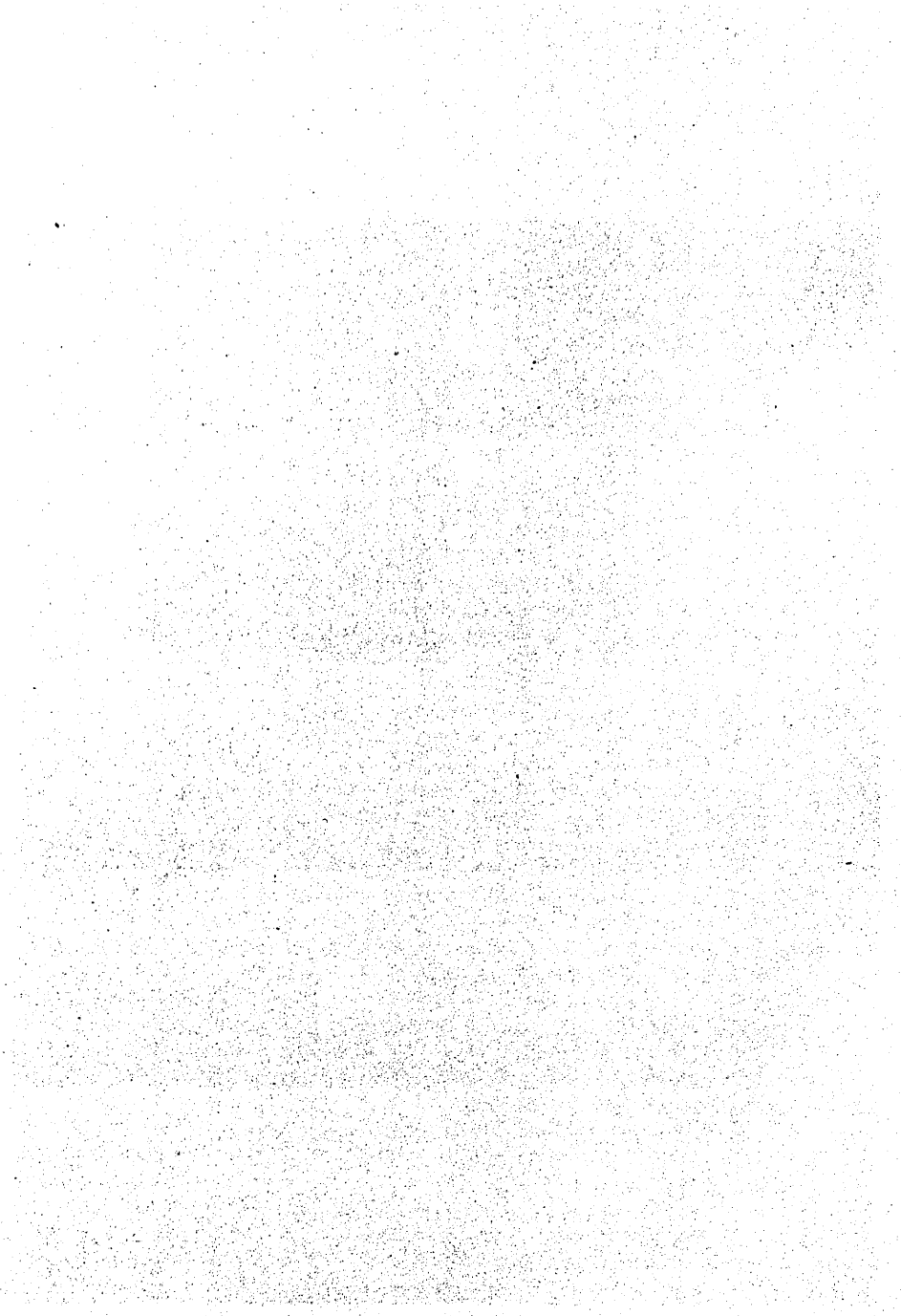


Table 3.

Proposed Cracking Pattern of LE/1 diacetate.

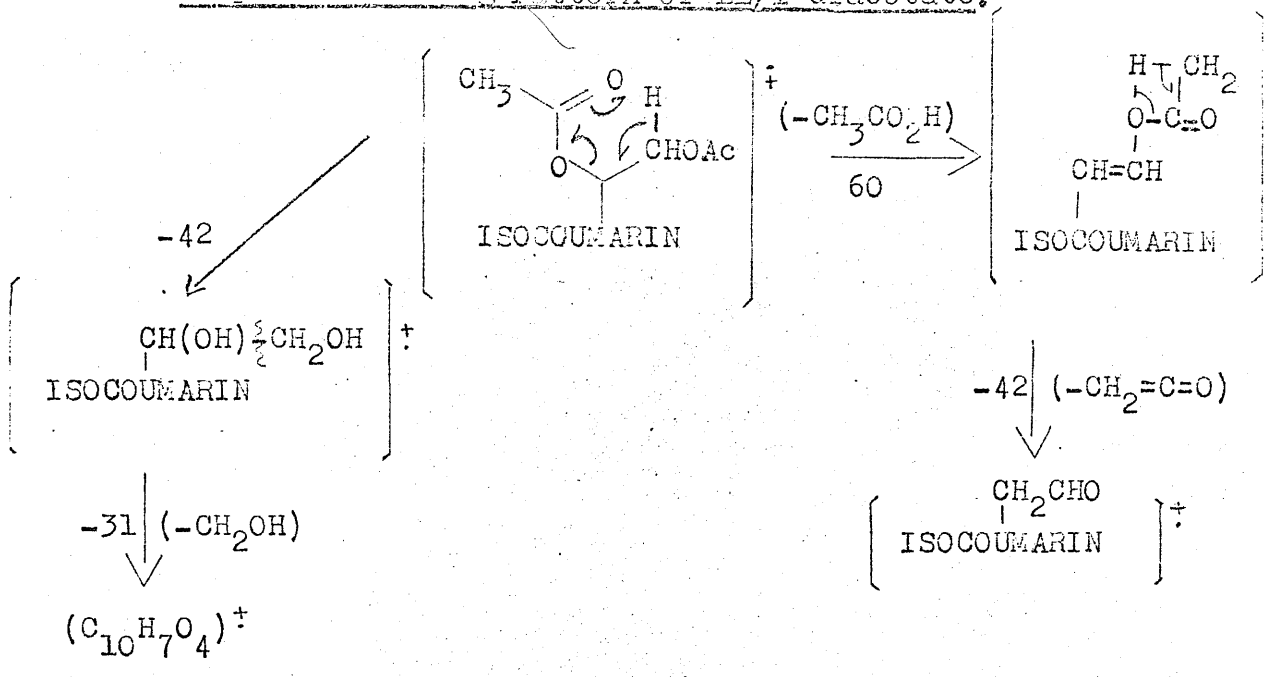


Table 4.

Proposed Cracking Pattern of LE/1 Monomethyl Ether.

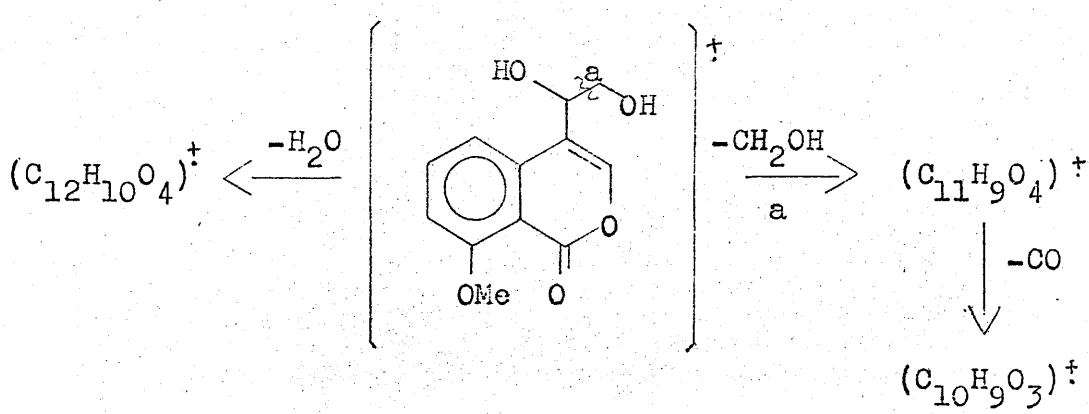
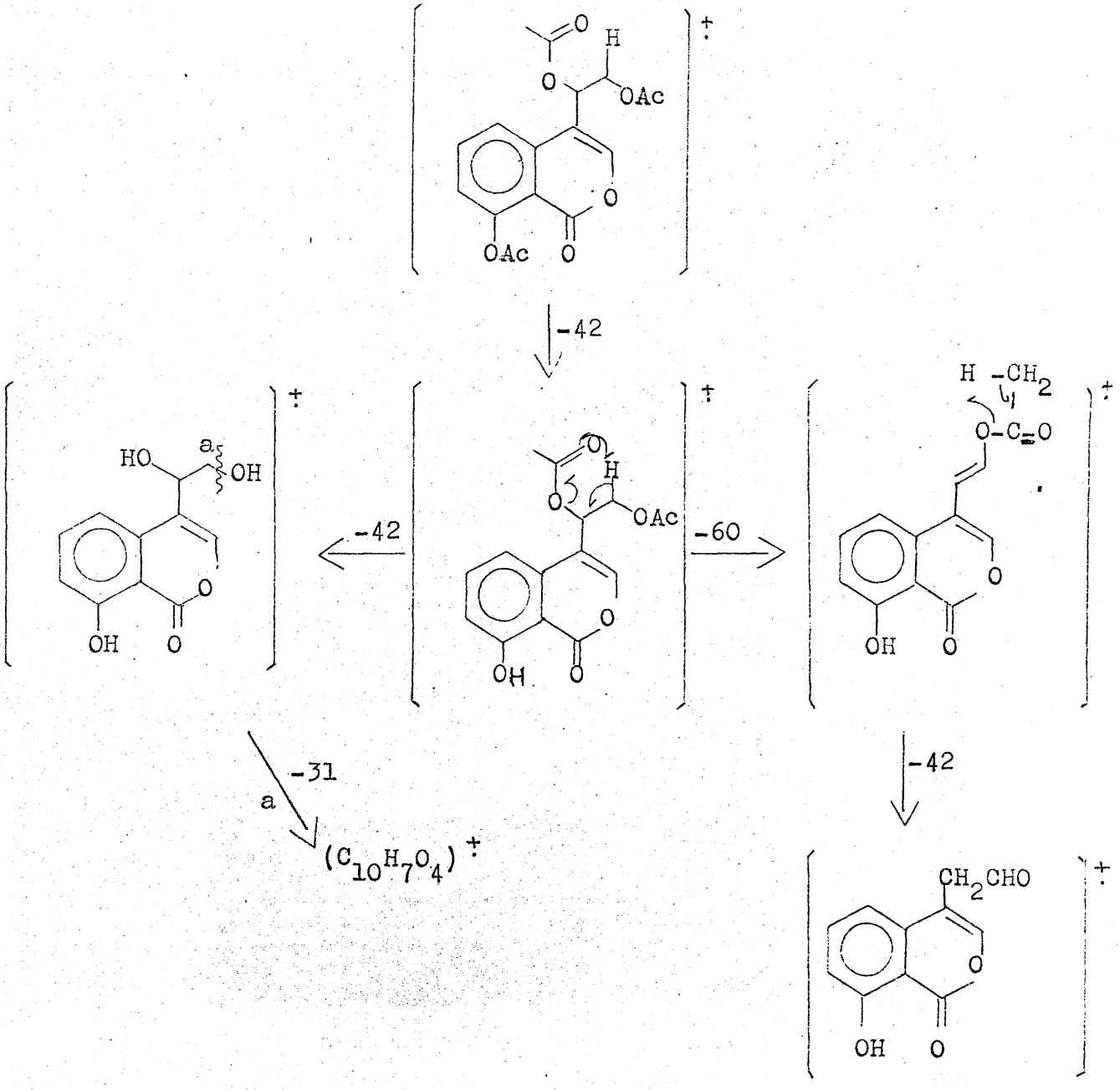


Table 5.

Proposed Cracking Pattern of LE/1 Triacetate.



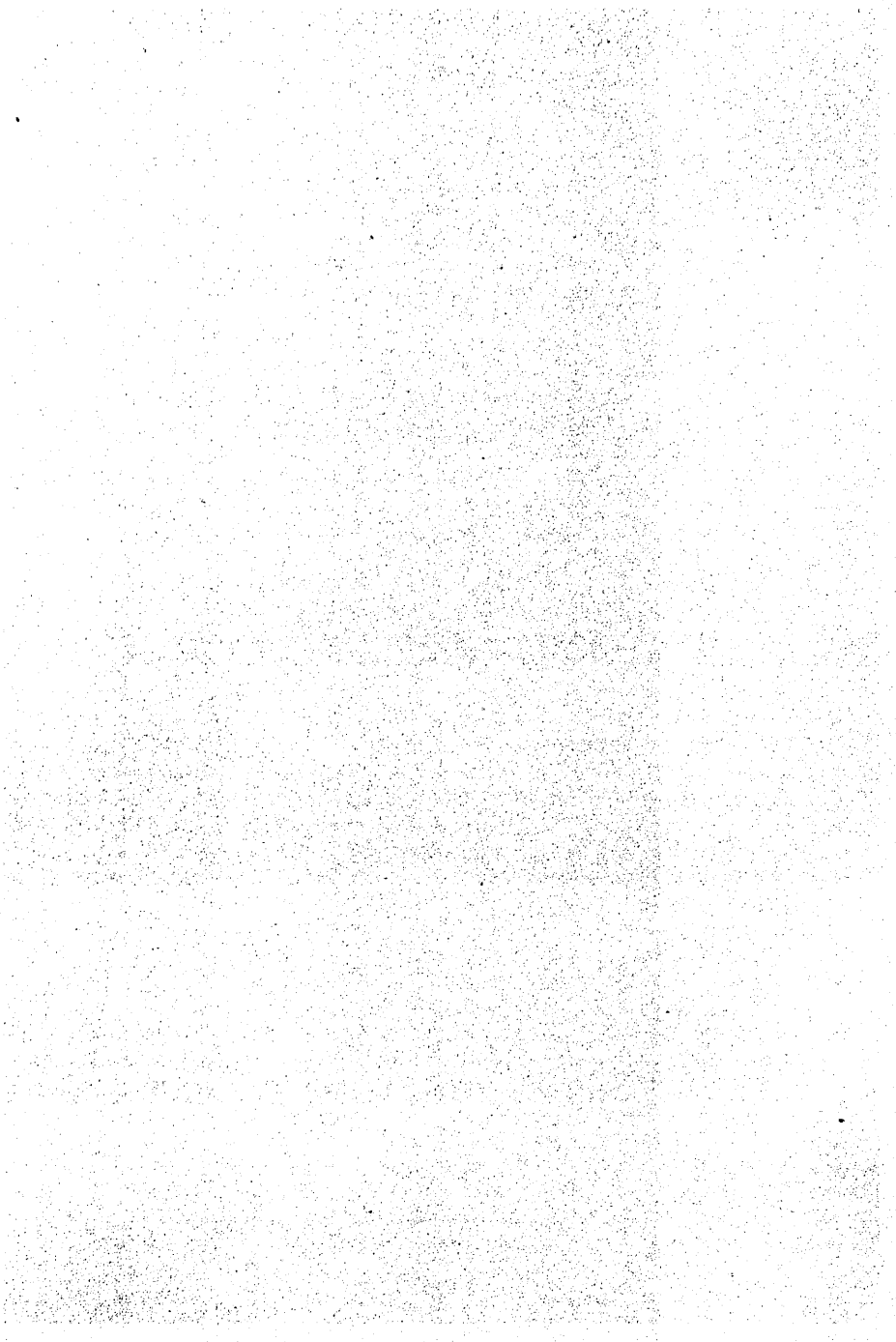


Table 6.

Proposed Cracking Pattern of LE/O.

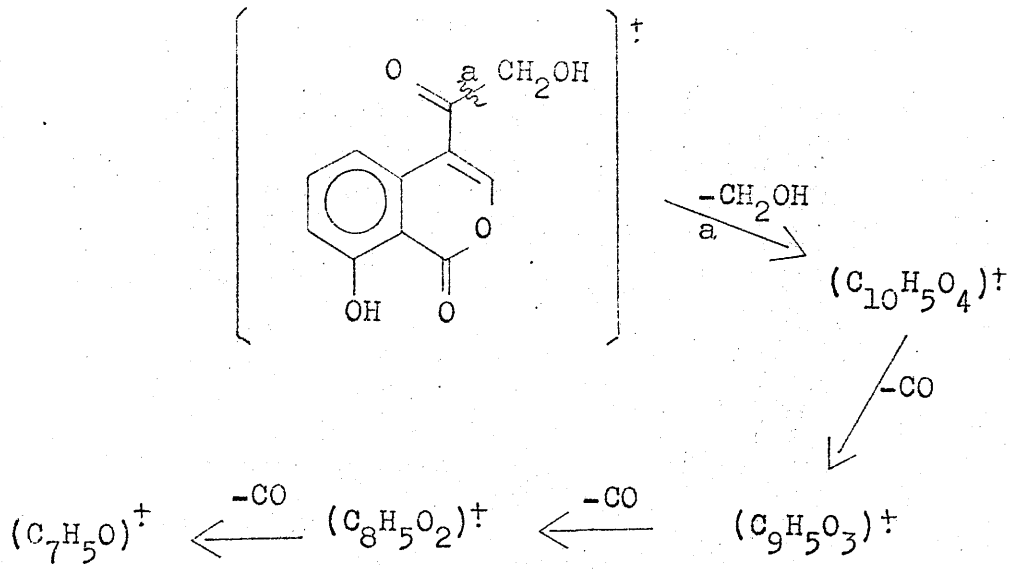


Table 7.

Proposed Cracking Pattern of LE/O-1.

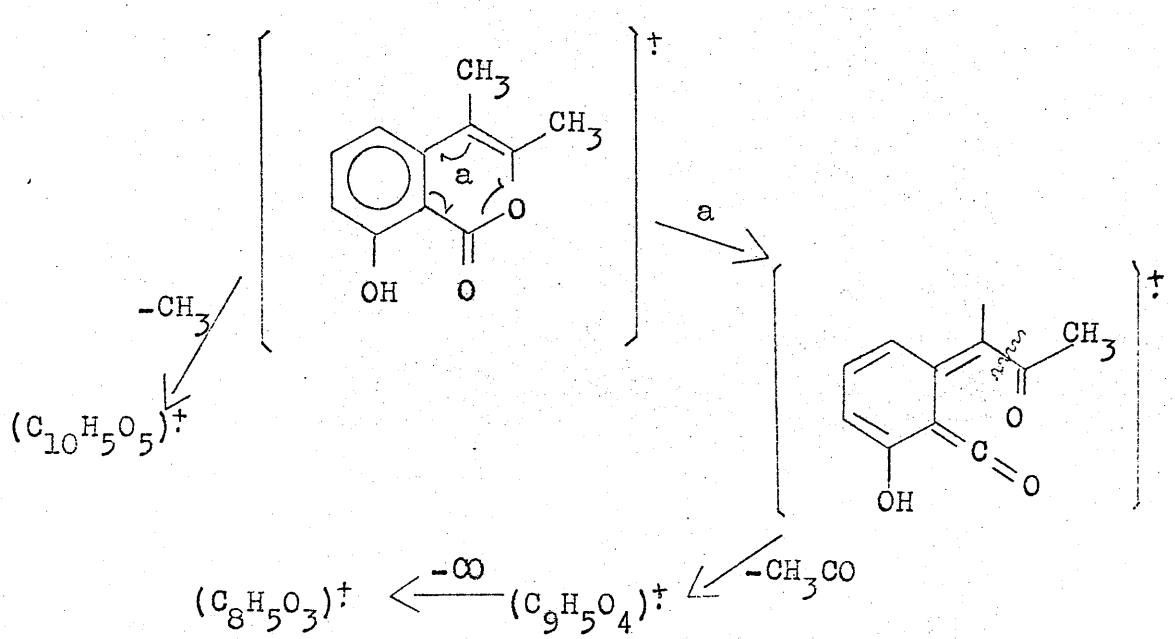


Table 8.

Oospoglycol Content of Lenzites striata.

Time of Culture* Growth	Mycelium wt.(g.)	LE/l wt (mg.)
Days		
10	0.22	10
14	0.35	65
28	0.97	76
56	0.85	80

*Ten bottle batches.

Table 9.

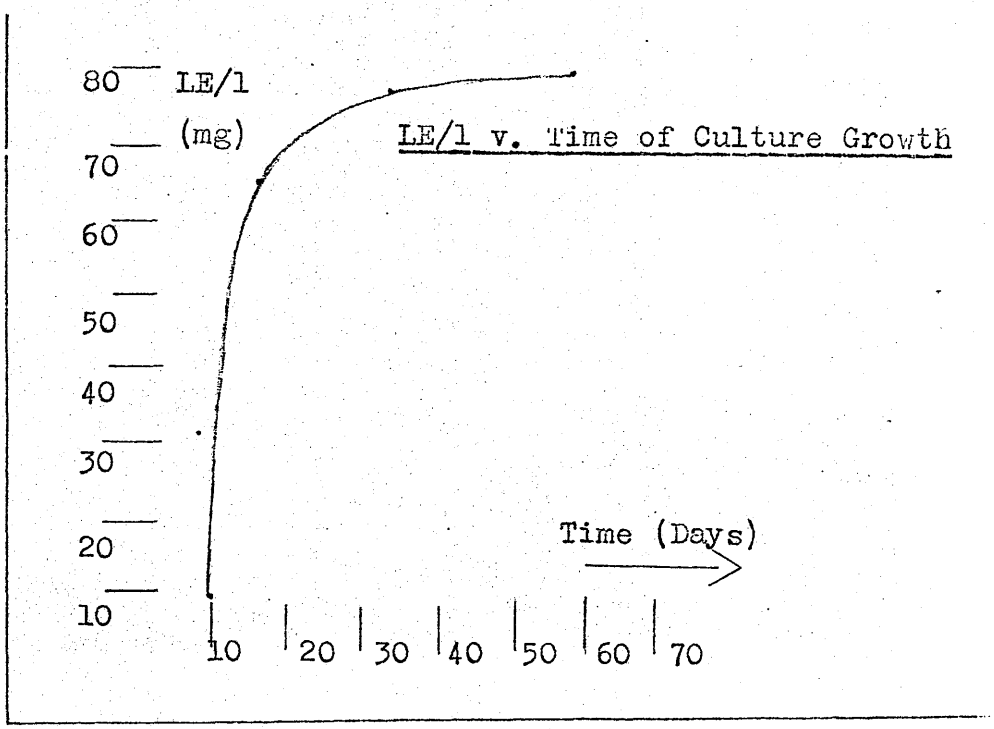


Table 10.

Initial Mass Spectrum Fragmentation of Tribromo Oospoglycol.

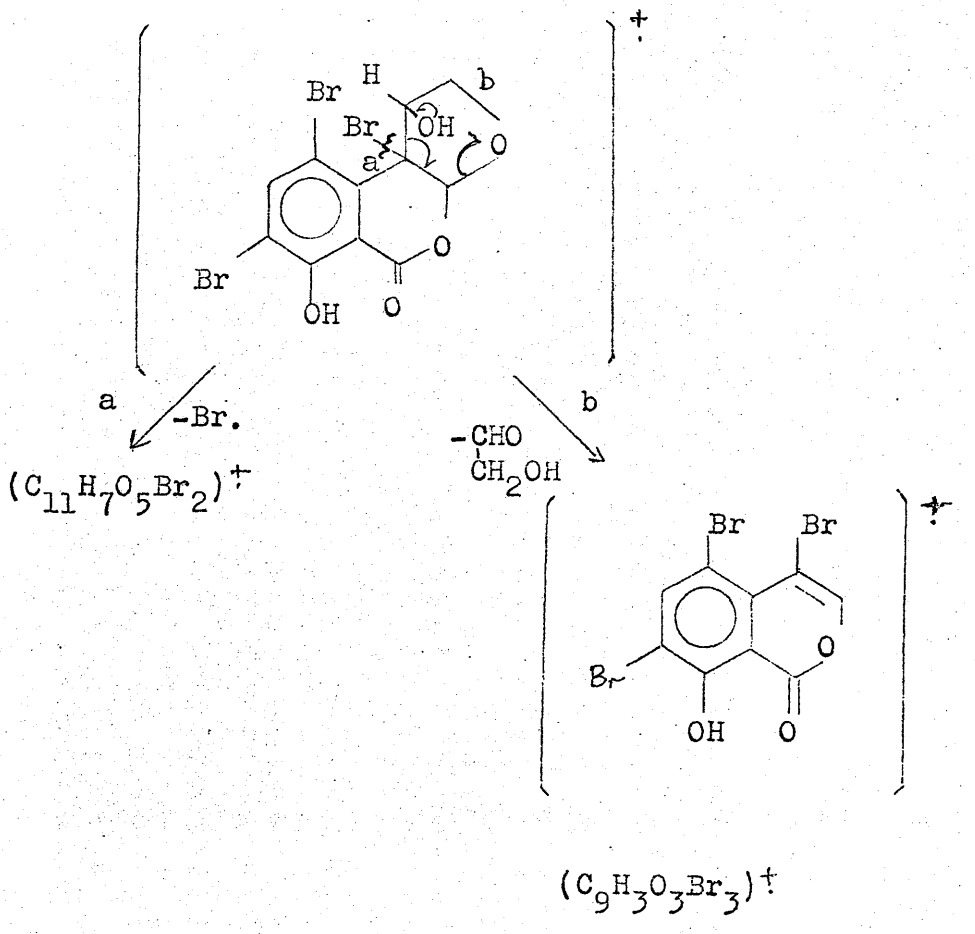
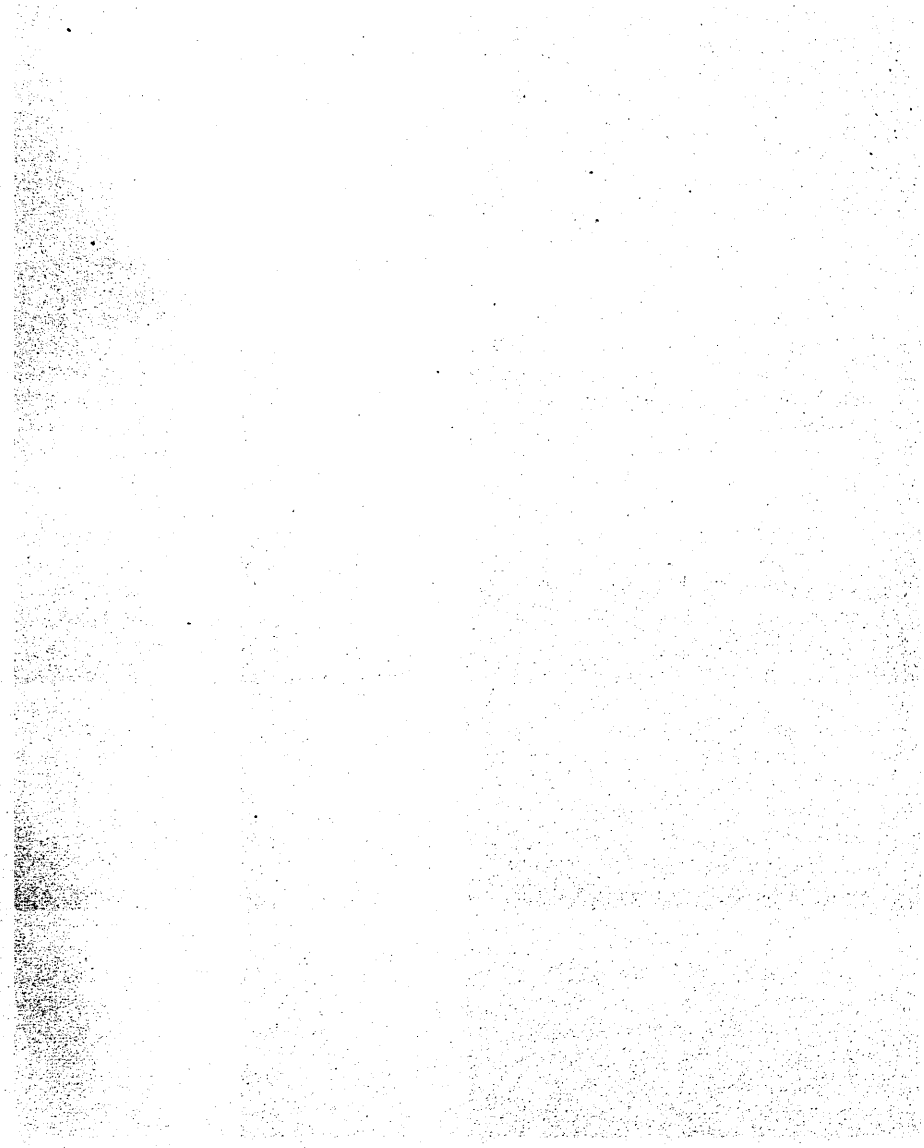
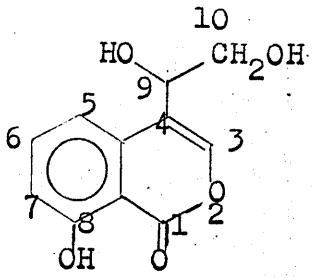


Table II.

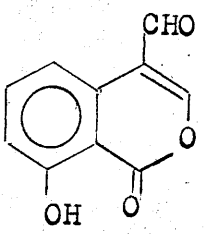
Specific Molar Activities of Oospoglycol and Degradation Products.

	^{14}C d.p.m./ m.mole x10^6	R.M.A.	^{3}H d.p.m./ m.mole x10^7	R.M.A.
Oospoglycol	8.569	5.0	7.787	4.0
Oospoaldehyde	7.190	4.20	3.032	1.56
Formaldehyde	1.147	0.669	2.250	1.16
Methoxy Tri-Bromo-Oospoaldehyde	7.429	4.33	0.158	0.082

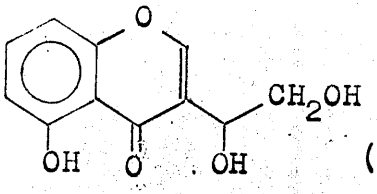




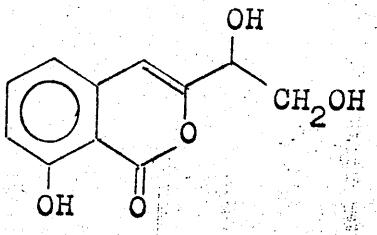
(69.)



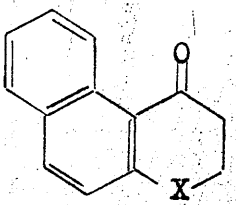
(70.)



(71.)



X = S or O



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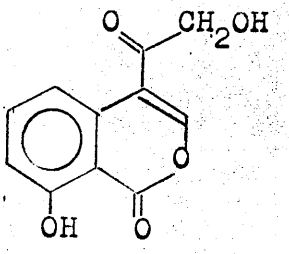
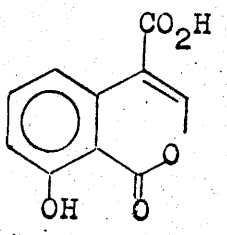
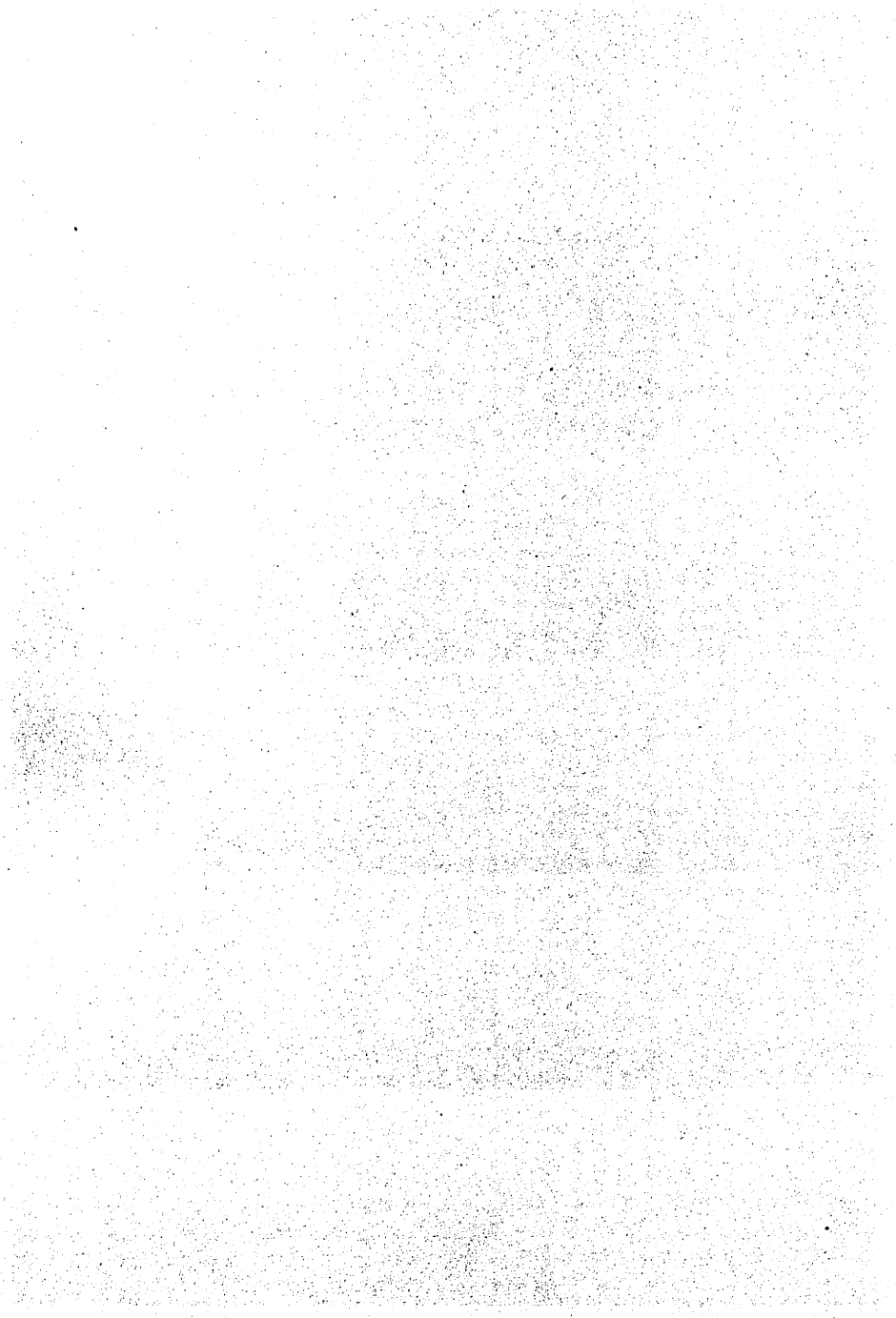
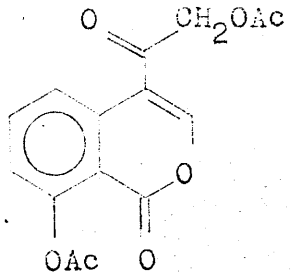


(73.)



(74.)





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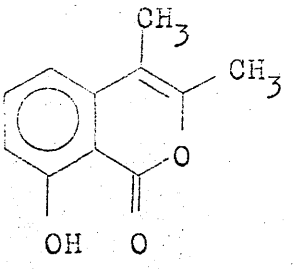
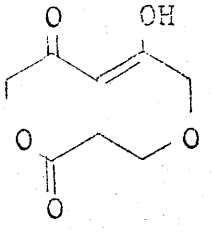
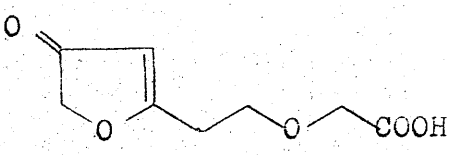


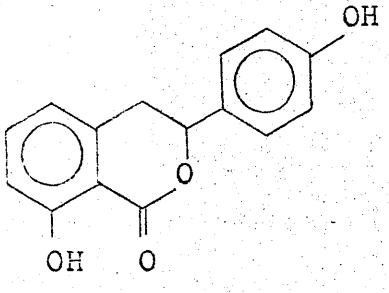
(76.)



(77.)



(78.)



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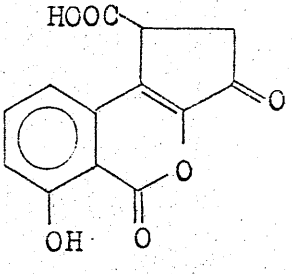

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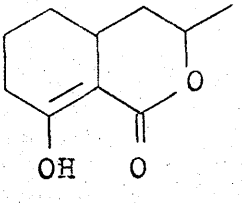
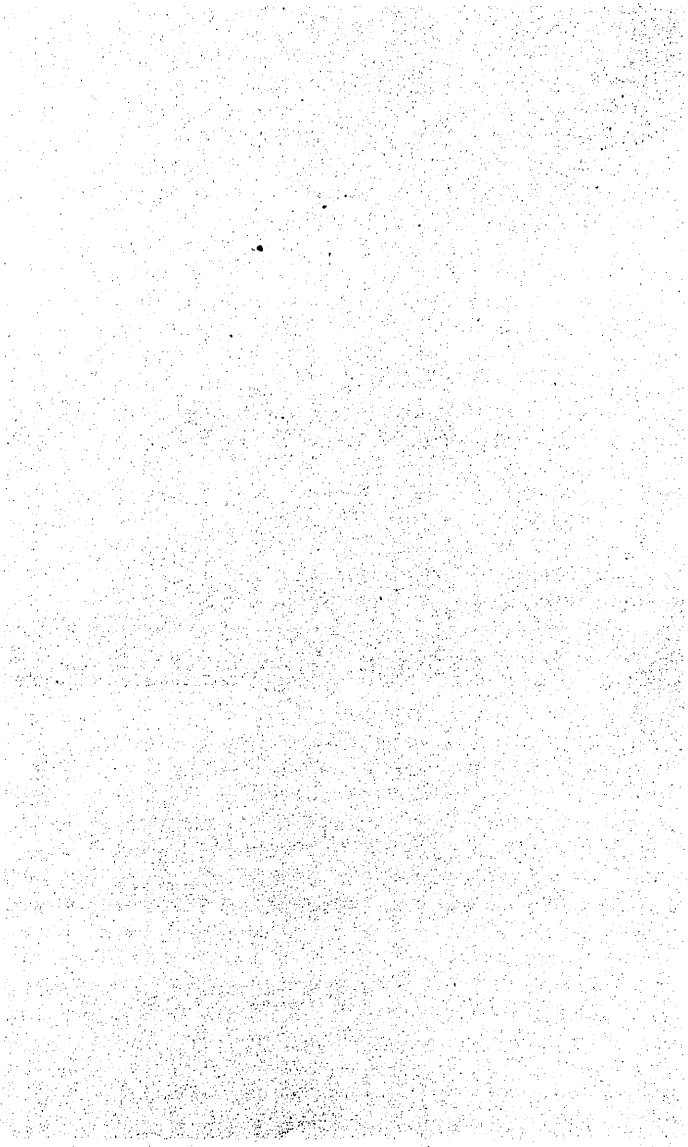
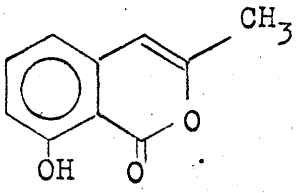


(81.)





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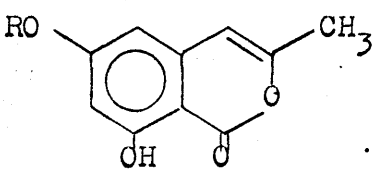
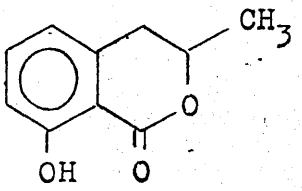


(83.)

(1) R = H; (11) R = CH_3



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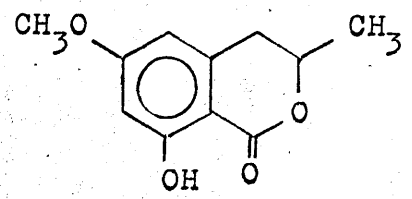
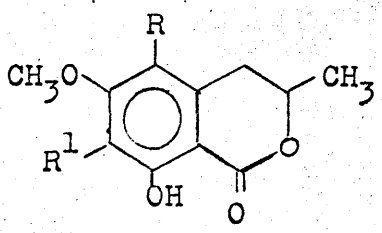


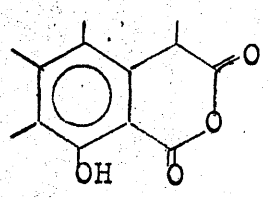
(85.)



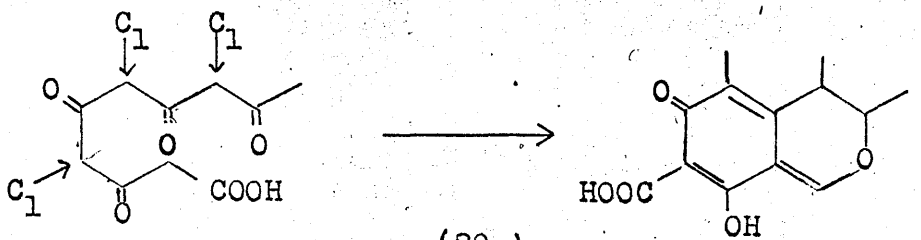
(86.)

(1) R = R^1 = Cl

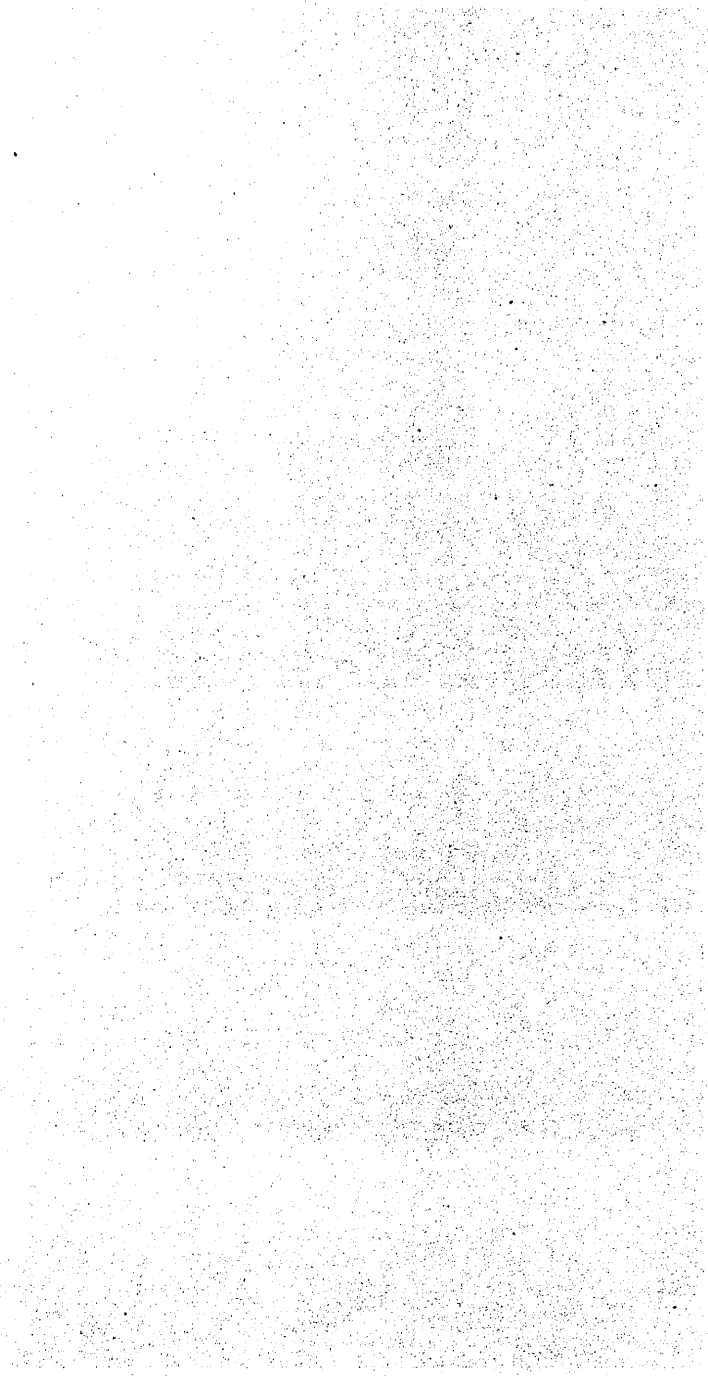
(11) R = H; R^1 = Cl

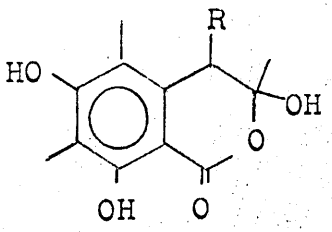


(87.)



(88.)

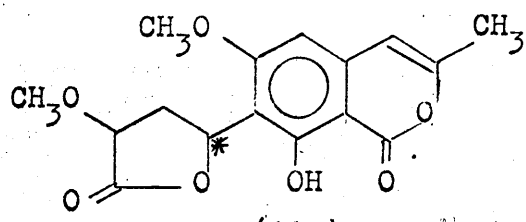




(89.)

R = CH_3; SCLEROTININ A.

R = H; SCLEROTININ B.



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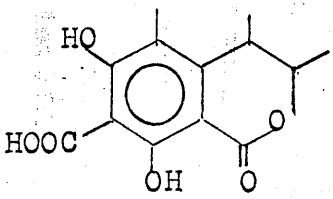
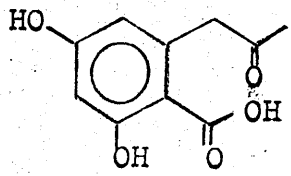


(91.)



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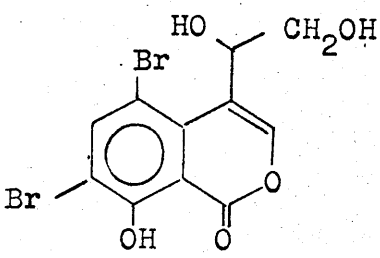

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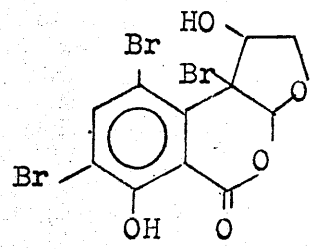
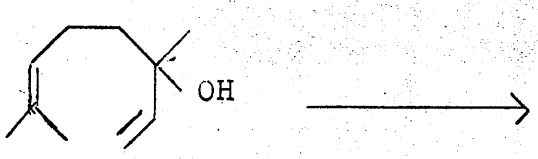
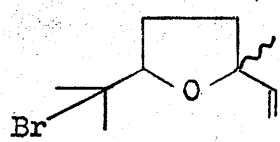


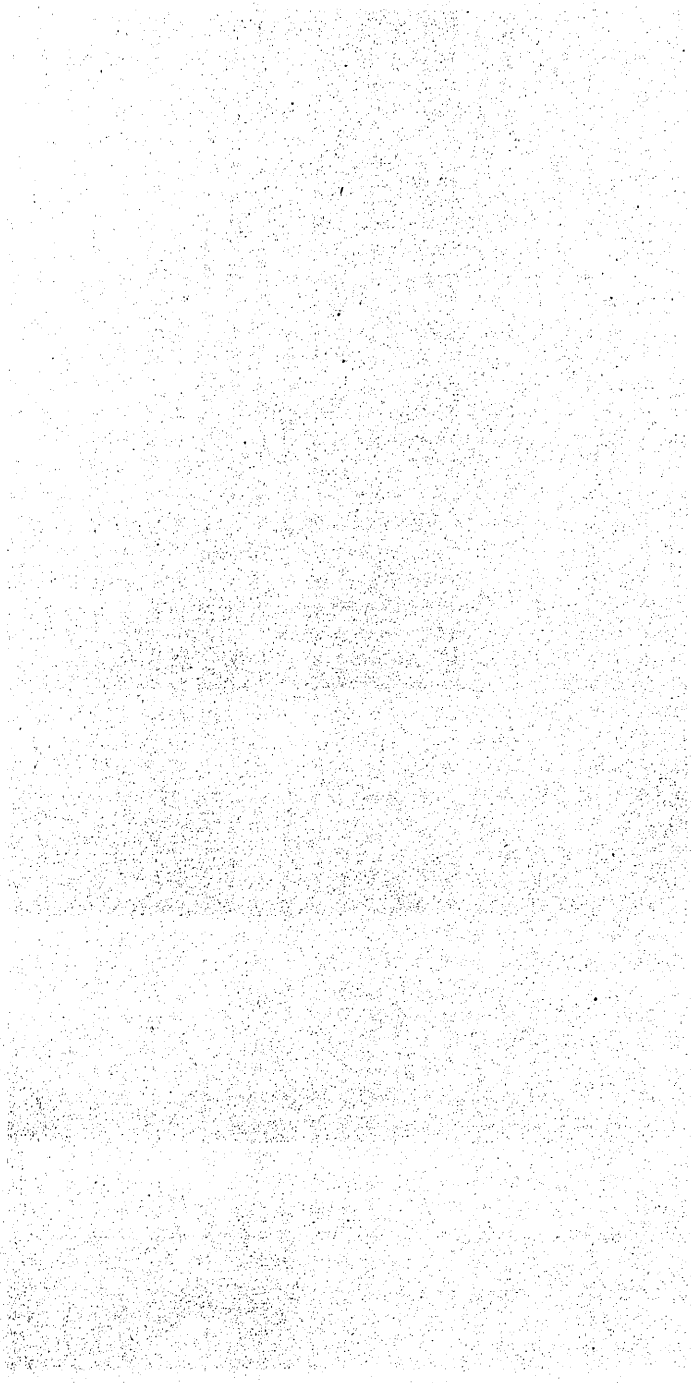
(95.)



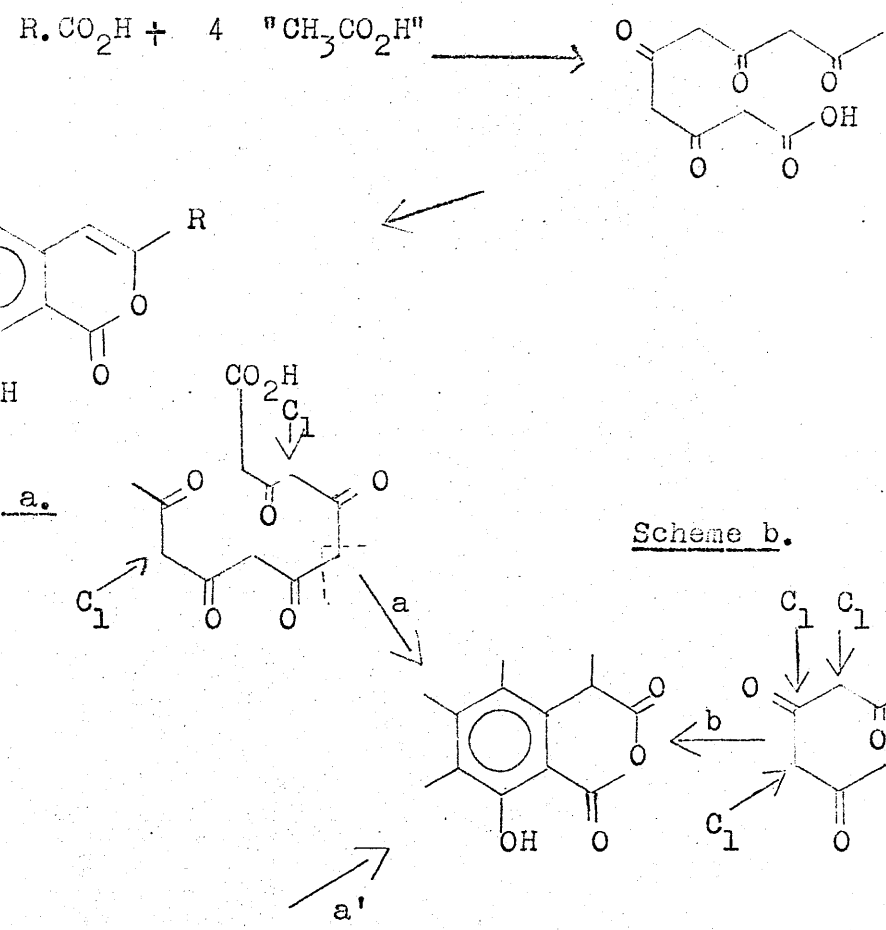
(96.)



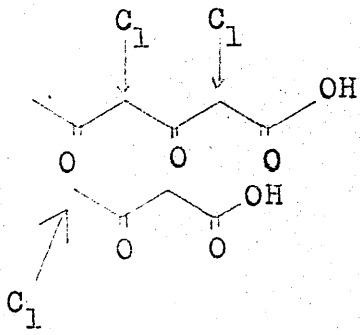
(97.)



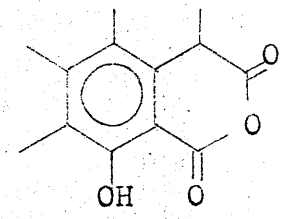
Scheme 1. Formation of Isocoumarin nucleus.

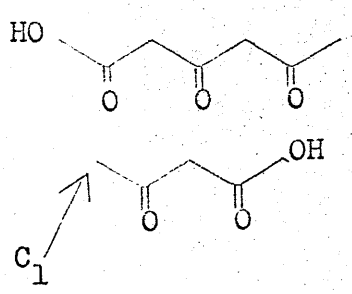


Scheme a'

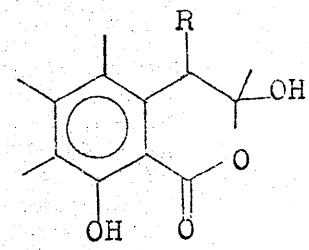


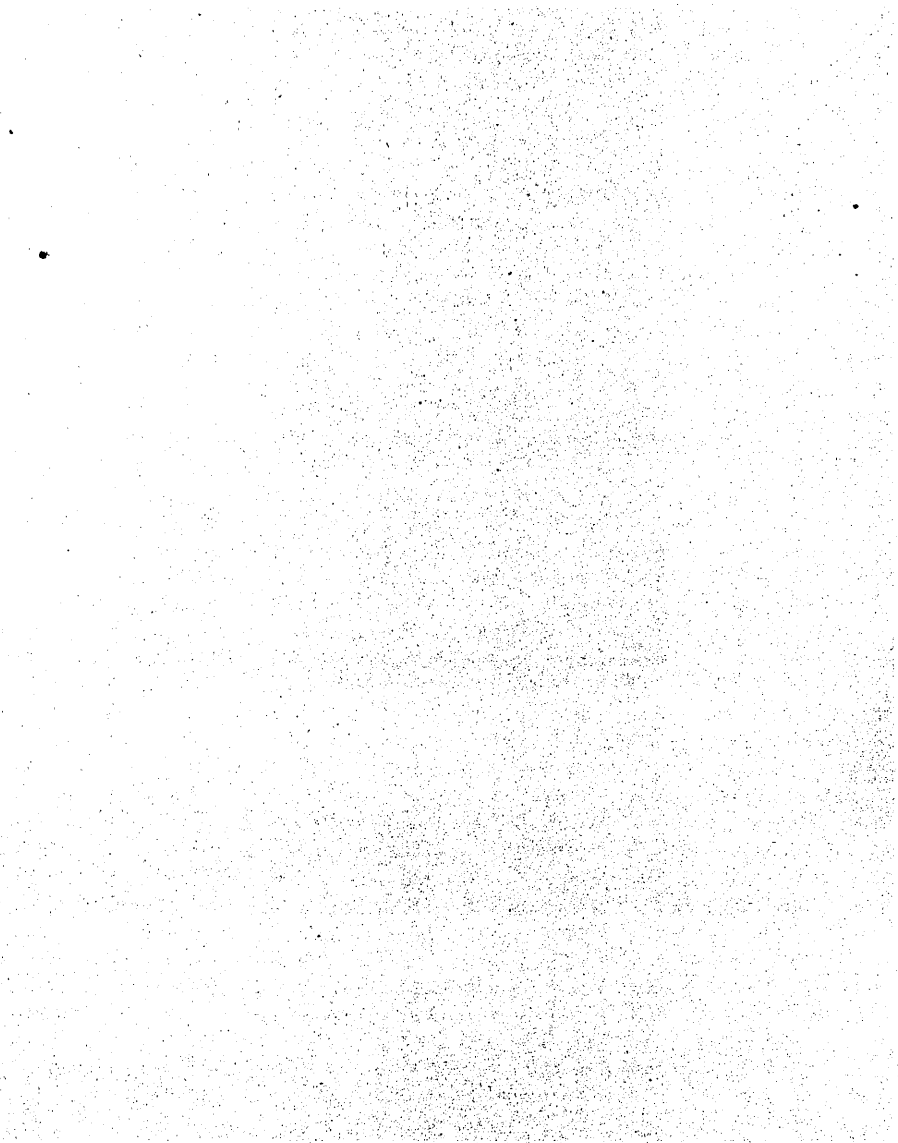
Scheme c

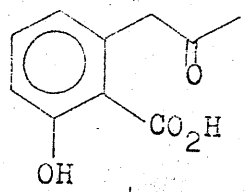




Scheme d

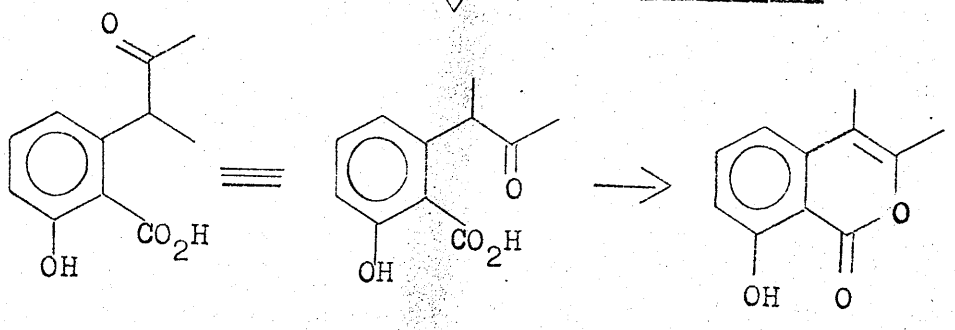




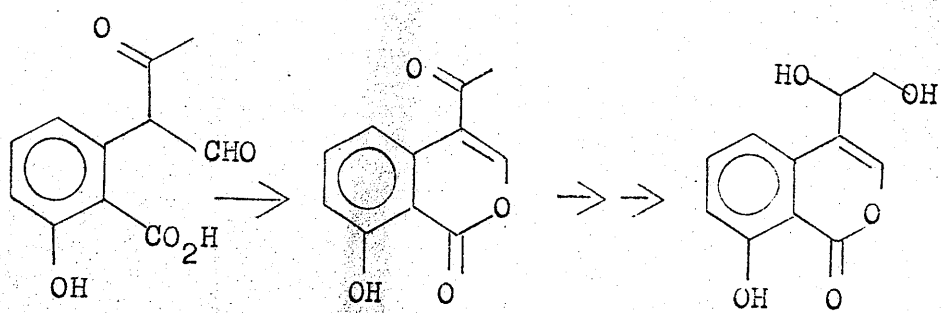


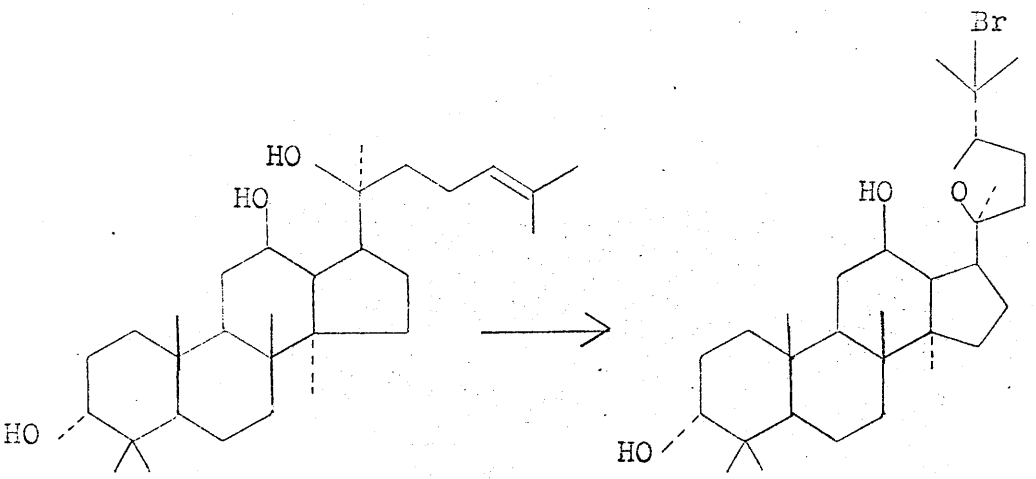
Scheme (f)

Scheme (e)

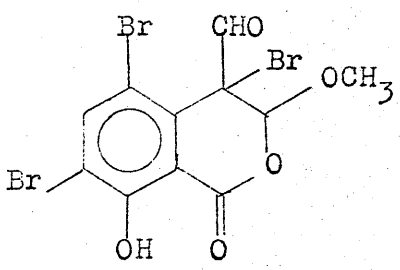


(O)

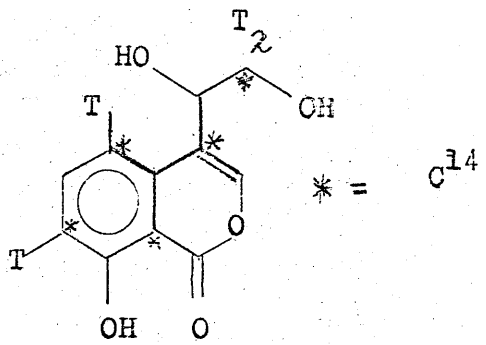




(98.) (99.)

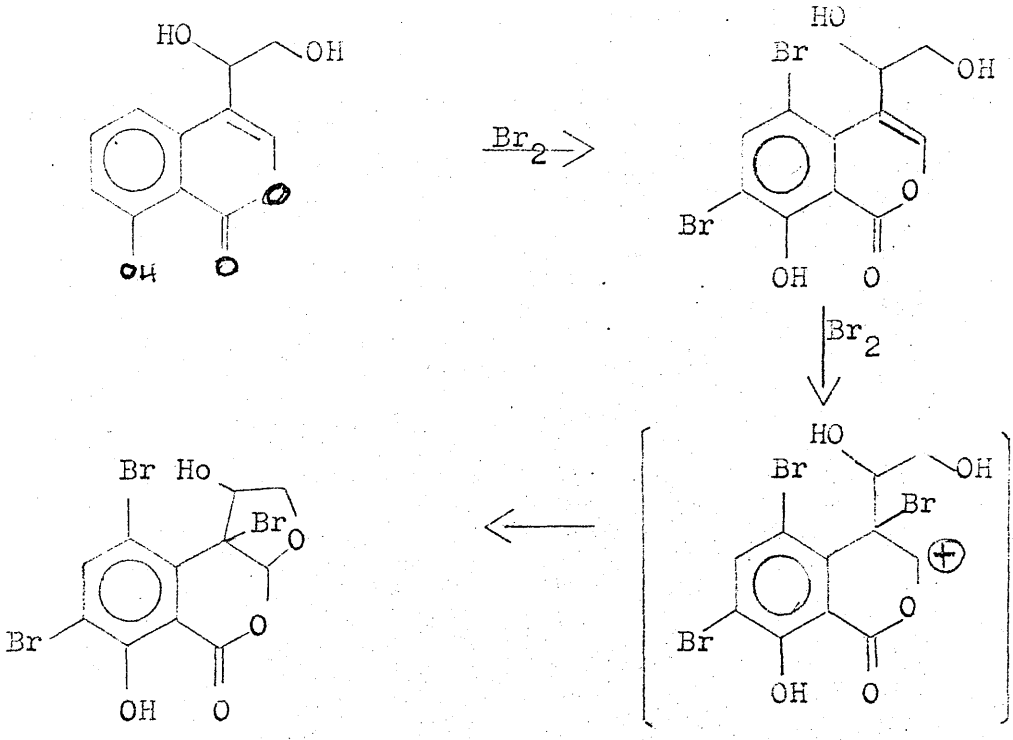


(100.)

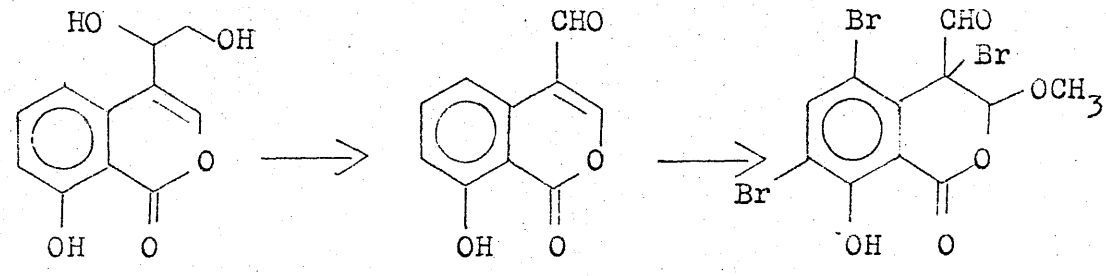


(101.)

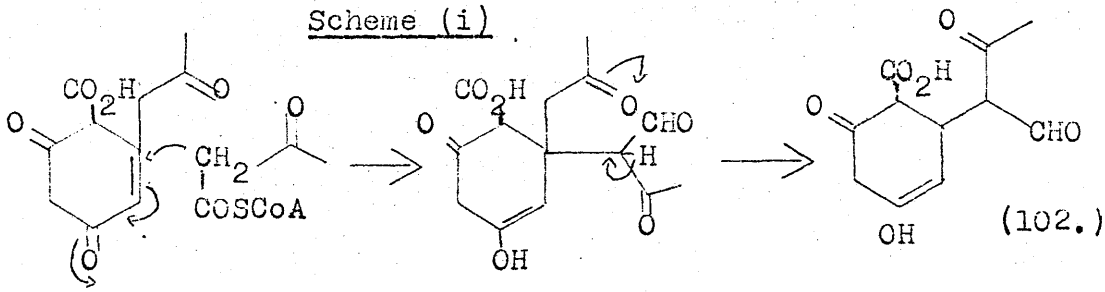
Scheme G. : Possible mechanism of formation of Di and Tribromo Cosposglycol.



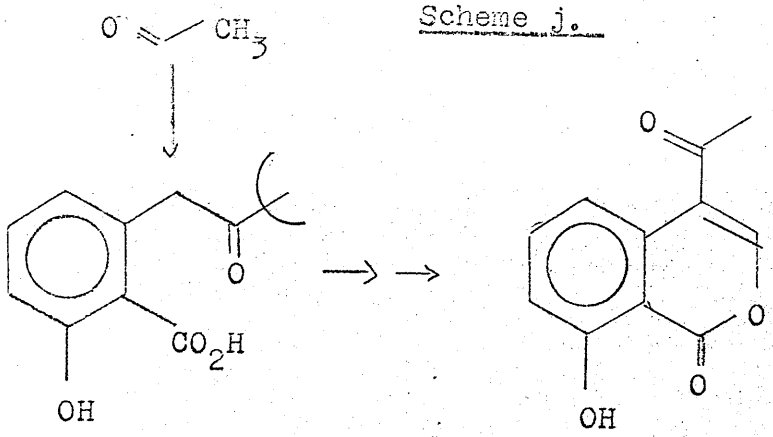
Scheme (h)



Scheme (i)



Scheme j.



(103.)

EXPERIMENTAL.

(1) Instrumentation

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected; boiling points are uncorrected. Ultraviolet spectra were obtained on a Unicam S.P.800 recording Spectrophotometer. Infra red spectra were measured using a Unicam S.P. 200 instrument and, for high resolution (KBr disc and solution spectra) with a Unicam S.P. 100 double beam infra red spectrometer equipped with an S.P. 130 sodium chloride prism grating double monochromater, operated under vacuum. Nuclear magnetic resonance spectra were recorded with Perkin-Elmer R.10 60 Mc./s. and Varian T-60 60 Mc./s. spectrometers as well as a Varian HA-100 100 Mc./s spectrometer, using tetramethylsilane as internal standard. Mass spectra were obtained with an A.E.I. MS-12 single focussing low resolution high sensitivity mass spectrometer. Optical rotations were measured on a Hilger-Watts microptic photoelectric polarimeter.

GENERAL

Diazomethane was prepared by the method of Moore/Reed[98] from bis(N-methyl-N-nitroso)- terephthalamide. "Light-petroleum", unless otherwise stated, refers to light petroleum ether, b.p. 40 - 60°C.

The following abbreviations are used in reporting spectral date : m^*, metastable ion: S, singlet: d, doublet: t, triplet: q, quartet: m, multiplet: infl., point of inflexion.

(2) Culture and extraction of Lenzites striata.

5% Malt Agar slants of L.striata, (strain 204^A, Forest Products Research Lab.), were inoculated into 10 Roux bottles containing 5% Malt extract and maintained at 24°C., 77% relative humidity for one month. After this time the contents of the bottles were blended and dispersed over 20 l. (100 Roux bottles) of 5% Malt extract.

Growth was continued under the above conditions for the required time. The mycelium was separated from the broth, blended and extracted with cold methanol for 24 hr.; the broth was extracted at the final pH(ca.5) with ethyl acetate for 24 hr.

(3a) Column chromatography of fifteen-day cultures of Lenzites striata.

The ethyl acetate extract of the broth, obtained as described in section (2), was evaporated to dryness yielding a yellow oil (3.7g) which was repeatedly extracted with light petroleum. Concentration of the light petroleum extracts gave a crystalline material (ca.30 mg); t.l.c. showed that a similar quantity of this substance remained in the residual oil. This oil was adsorbed onto silicic acid (5g.) and placed on top of a column of the same (100g.), packed in 10% ethyl acetate/90% petroleum ether. Fractions from the column were collected at the rate of six per hour, while the proportion of ethyl acetate in the eluting solvent was gradually increased. T.l.c. analysis of the fractions allowed the groupings indicated in the table below:

Fractions	Species Code Number	Weight (mg)
5- 10	Oospolactone (LE/O-I)	50
29- 61	Oosponol (LE/O)	196
73-100	Oospoglycol (LE/1)	215*

*Obtained after further purification by p.l.c. (three 20x20x 0.5 m.m. Kieselgel G nach Stahl), using 15% methanol/85% chloroform as developing solvent.

(3b) Column chromatography of two-month cultures of Lenzites striata.

Following an identical proceedure to that described in Section (3a), 6.2g. of broth extract yielded LE/0 (0.2g) and LE/1 (2.5g). None of the species, code name LE/0-I was isolated.

(4) The substance LE/0-I: oospolactone.

Isolated as described above, this was recrystallised from diethyl ether as white needles, m.p. 127-129°C. $R_f(CHCl_3)$ 0.9. (Found: C, 69.76%; H, 5.35%; M^+ at m/e 190; calculated for $C_{11}H_{10}O_3$: C, 69.46%; H, 5.30%; m.w. 190.2) υ max (KBr): 3100 (broad; chelated hydroxyl), 2990, 2930, 1673 (conjungated carbonyl), 1635 (C=C), 1605, 1566, 1495, 1455, 1387, 1346, 1329, 1315, 1278, 1213, 1207, 1170, 1138, 1120, 1066, 1056, 992, 975, 854, 811, 793 $cm.^{-1}$.

υ max ($CHCl_3$): 1688 $cm.^{-1}$ (E = 843).

λ max (EtoH): 210 (E=22,500), 230 (E=24,830), 237 (E=8,766), 260 (E=15,220), 275 (infl.), 342 (E = 8,766) nm.

τ (100 Mc./s. $CDCl_3$): -1.29(1H,S, exchanged by D_2O - O<u>H</u>); 2.35 (1H, d.d., J=8 c.p.s., H-6), 3.00 (1H, S, H-5), 3.05 (1H, d, J=7c.p.s., H-7), 7.68 (3H, S, <u>CH_3</u>.C(O):C), 7.86 (3H, S, <u>CH_3</u>.C:C.O) .

m/e 190 (74%), 175 (11%), 162 (12%), 161 (26%),
148 (71%), 147 (100%), 133 (11%), 130 (18%),
120 (15%), 119 (45%), 115 (12%), 105 (12%),
102 (21%), 91 (54%), 89 (30%), 65 (47%),
63 (40%),

m* corresponding to m/e 190 ⟶ 175,

m/e 190 ⟶ 147 , m/e 147 ⟶ 119.

Literature data for oospolactone : m.p. 129°C.

ν max (nujol): 1675, 1630, 1600, 1560, 1210, 1170, 1140, 1060, 830, $cm.^{-1}$

λ max (E_tOH) : 230, 260, 275 (infl.), 342 nm.

(5) <u>The substance LE/0: oosponol.</u>

Isolated as described in Section (3a), this was recrystallised from ethyl acetate as pale yellow needles, m.p. 163 - 165°C. R_f (3% CH_3OH/97% $CHCl_3$) 0.7. (Found: C, 59.98%; H, 3.77%; M^+ at m/e 220: Calculated for $C_{11}H_8O_5$: C, 60.01%; H, 3.66%, m.w. 220.2).

ν max (KBr) 3448, 3246 (hydroxyls), 3134, 3082, 2924, 2872, 1720 (C-9 carbonyl), 1698, 1677, (conjugated carbonyl), 1632 (C = C) 1599, 1575, 1491, 1448, 1405, 1312, 1278, 1187, 1176, 1158, 1102, 1081, 1026, 1015, 1000, 921, 876, 826, 802, 771 cm^{-1}.

λ max (EtOH): 217 (E = 18,600), 234 (infl.), 256 (E = 6,875), 335 (E = 6,270) nm.

τ (100 Mc/s: CF_3CO_2H): 1.78 (1H, S, C:C(O) - H); 1.79 (1H, d, J = 8 cps., H-5); 2.07 (1H, d.d., J = 8 cps. H - 6); 2.70 (1H, d, J=8cps, H-7); 4.98 (2H, S, $CO\text{-}\underline{CH_2}\text{-}OH$).

m/e 220 (99%), 202 (17%), 192 (17%), 191 (72%), 190 (78%), 189 (100%), 176 (7%), 161 (72%), 145 (17%), 133 (14%), 105 (99%), 77 (74%), 51 (91%).

m* corresponding to m/e 220 —— 189, m/e 189 —— 161, m/e 161 —— 133, m/e 133 —— 105, m/e 105 —— 77.

Literature data for oosponol:

m.p. 176°C.

ν max (nujol): 3400, 1720, 1700, 1670, 1630, 1300, 1280, 1210, 1185, 1160, 1080, 1030, 1000, 920, 870, 825 $cm.^{-1}$

λ max (EtOH): 234 (infl.), 256, 335 nm.

τ (HCOOH) : 5.06 ($CO.\underline{CH_2}OH$), 2.07 (C:C(O).H).

(6) The substance LE/1: oospoglycol.

This was obtained as described in Section (3a) and recrystallised from ethyl acetate/light petroleum as a microcrystalline solid; m.p. 110-112°C.

R_f (3% CH_3OH/97% $CHCl_3$), 0.3. $(\alpha)_D$ (C, 0.82) - 73°. (Found : C, 59.27%; H, 4.34%; M^+ at m/e 222. Calculated for $C_{11}H_{10}O_5$: C, 59.46%; H, 4.54%; m.w. 222.2).

υ max (KBr) : 3346, 3214 (broad; hydroxyls), 3042 2970, 2942, 2890, 1670 (conjugated carbonyl) 1639 (C = C), 1611, 1566, 1502, 1455, 1349, 1323, 1306, 1287, 1259, 1242, 1183, 1142, 1098, 1083, 1062, 1042, 978, 927, 871, 820, 736, 700 $cm.^{-1}$

λ max (EtOH) : 229 (E = 17,110); 236 (E = 17,030); 259 (E = 8,172), 273 (infl.)., 336 (E = 6,386) nm.

τ (100 Mc./s. CF_3CO_2H) : 2.15 (1H, d.d., J. = 8 c.p.s, H-6); 2.40 (1H, S, C:C(O)-H): 2.72 (1H, d, J = 6 c.p.s., H-5); 2.80 (1H, d, J = 6 cps., H-7); 4.55 (1H, m, X part of ABX system, - C<u>H</u>(OH).CH_2OH); 5.74 (2H, m, AB part of ABX system, -CH(OH)-C<u>H</u>$_2$OH).

m/e : 222 (40%), 204 (3%), 191 (100%), 186 (5%) 176 (4%), 163 (61%), 147 (13%), 145 (10%), 135 (35%), 119 (5%), 107 (16%), 89 (13%), 79 (14%), 77 (27%).

m* corresponding to m/e 222 —— 204, m/e 222 —— 191, m/e 191 —— 163, m/e 163 —— 135

Literature date for oospoglycol

m.p. 116° C. $(\alpha)_D$ - 71°

ν max (nujol): 3400, 3200, 1675, 1635, 1610, 1260, 1180, 1150, 1100, 1080, 1060, 1040, 980, 930, 880, 825, 730 $cm.^{-1}$.

λ max (EtOH) ; 229, 243, 259, 275 (infl.), 336 n.m.

(7) Treatment of oospoglycol with diazomethane.

To a solution of oospoglycol (30mg.) in methanol (3ml.) was added a large excess of ethereal diazomethane, prepared as described (p. 70), and the reaction mixture allowed to stand at room temperature for fifteen hours. Concentration of the solution, followed by p.l.c. (one 20 x 20 x 0.5 mm. Kieselgel HF_{254} nach Stahl) in 10% methanol/90% chloroform gave oospoglycol monomethyl ether (20 mg. 63%), recrystallised from ethyl acetate as colourless plates, m.p. 155 - 158°C.

R_f(3% CH_3OH/97%$CHCl_3$) 0.1.

(Found: C, 60.82%; H, 5.22%; M^+ at m/e 236.

$C_{12}H_{12}O_5$ requires C, 61.02%; H, 5.12%; m.w. 236.2).

ν max (KBr): 3458, 3242 (hydroxyls), 3098, 3048, 2946, 2913, 2834, 1713 (carbonyl), 1645 (C=C), 1594, 1568, 1481, 1459, 1443, 1430, 1388, 1360, 1327, 1261, 1231, 1183, 1162, 1144, 1095, 1066, 1032, 1022, 1002, 893, 872, 850, 812, 767, 700 $cm.^{-1}$.

λ max (EtOH):210 (E = 15,390), 232 (E = 18,310),

238 (E = 20,660), 251 (E=10,230), 258 (E=10,610) 270 (infl), 333 (E = 6,298) nm.

τ (100 Mc./s. CH_3CO_2H) : 2.05 (1H, d.d., J = 8 cps H-6), 235 (1H, S, C:C(o)-H), 2.62 (1H, d, J = 8 cps, H-5), 2.73 (1H, d, J = 8 cps, H-7), 4.48 (1H, m, -C<u>H</u>(OH)CH_2OH); 5.77 (2H, m, -CH(OH).C<u>H</u>$_2$OH), 5.90 (3H, S, C<u>H</u>$_3$OAr.).

m/e : 236 (39%), 218 (22%), 205 (100%), 189 (17%), 177 (67%), 161 (22%), 149 (44%), 134 (17%), 105 (17%), 91 (33%), 77 (22%).

m* corresponding to m/e 236 ___205, m/e 205 __ 177.

(8) <u>Treatment of Oospoglycol with sodium metaperiodate.</u>

A solution of sodium metaperiodate (80 mg.) in water (1.0 ml.) was added to oospoglycol (40 mg.) in methanol (3 ml.); after an initial rise in temperature, a white precipitate was formed. The solution was stirred overnight at room temperature, filtered, then steam distilled into a precalculated quantity of dimedone in ethanol (10 ml.): without concentration, white needles (m.p. 190-194°C.) were formed of a substance identified, by analytical t.l.c. and mixed-melting point, as the condensation product of dimedone with formaldehyde.

Ethyl acetate extraction of the steam distillation residue and white precipitate gave oospoaldehyde (26.3 mg. 76%), which crystallised from methanol to m.p. 170 - 173°C.

R_f (3% CH_3OH/ 97% $CHCl_3$) 0.7.

(Found : C, 63.06%; H, 3.38%; M^+ at m/e 190.
Calculated for $C_{10}H_6O_4$: C, 63.16%; H, 3.18%, m.w. 190.2).

υmax (KBr) :3210 (chelated hydroxyl), 3120, 3090, 3058, 2890, 1680-1710 (broad; aldehyde + lactone carbonyl), 1637 (C =C), 1602, 1573, 1500, 1456, 1401, 1368, 1330, 1264, 1255, 1229, 1190, 1170, 1153, 1079, 1020, 944, 913, 900, 826, 802, 781, 700 $cm.^{-1}$

λ max (EtOH) : 216 (E = 27,390), 235 (E = 15,030), 257 (E = 12,350), 335 (E = 6,468) nm.

τ (100 Mc./s. CF_3CO_2H) : 0.14 (1H, S, - C<u>H</u>O), 1.60 (1H, d, J = 8 c.p.s., H-5), 1.80 (1H, S, C:C(O)<u>H</u>), 2.10 (1H, d.d. J = 8 c.p.s H-6); 2.73 (1H, d, J = 8 c.p.s. H-7).

m/e : 190 (100%), 162 (36%), 134 (61%), 106 (39%), 105 (67%), 78 (58%).

m* corresponding to m/e 190 —— 162; m/e 162——134 m/e 134 —— 106; m/e 106 — 78

(9) Acetylation of oospoglycol.

(a) Oospoglycol (50 mg.) was treated with pyridine (0.2 ml.) and acetic anhydride (0.2ml.) at room temperature for fifteen hours. The reaction mixture was poured onto crushed ice (ca.1g.) and extracted with chloroform. After successive washing with dilute hydrochloric acid (2 x 2ml.) and water (2 x 2ml.), the chloroform solution was dried over magnesium sulphate. Evaporation to dryness gave the diacetate as a solid (45 mg., 65%), which could be recrystallised as plates from ethyl acetate/light petroleum to m.p. 120-121°C.

R_f ($CHCl_3$) 0.6.

($C_{15}H_{14}O_7$ requires C, 58.83%; H, 4.61%; m.w. 306.3
Best analysis gave C, 56.54%; H, 4.70%; M^+ at m/e 306.).

ν max (nujol) : 3500 (broad), 1735 (infl.), 1730 (acetate), 1680 (lactone carbonyl), 1640 (C = C), 1605, 1500, 1260, 1230, 1180, 1160, 1155, 1085, 1040, 960, 935, 890, 825 $cm.^{-1}$

λ max (EtOH): 229, 236, 255, 273, (infl), 333n.m.

τ (100 Mc./S. : $CDCl_3$) : 2.33 (1H, d.d., J = 8 c.p.s., H - 6), 2.78 (1H, S, C: C(H) - O), 2.89 (1H, d, J = 8 c.p.s., H - 5), 2.97 (1H, d, J = 8 c.p.s., H - 7), 3.80 (1H, m, X part of ABX system, J_{AX} = 8 c.p.s., J_{AB} = 4 c.p.s.), 5.60 (2H, m, AB part of ABX system, J_{AB} = 12 c.p.s.), 7.83, 7.91 (3H each, S, CH_3COO).

m/e 306 (7%), 264 (2%), 246 (3%), 222 (2%), 204 (33%), 191 (24%), 186 (8%), 176 (8%), 175 (5%), 163 (6%), 160 (11%), 147 (6%), 145 (3%), 134 (4%), 130 (10%) 102 (7%), 91 (5%), 89 (5%), 85 (6%), 83 (9%), 77 (10%), 60 (7%), 43 (100%).

(b) Prolonged treatment of oospoglycol (50 mg.) with acetic anhydride/pyridine followed by the above extraction gave oospoglycol triacetate as a clear gum refusing to crystallise. b.p. 160 - 165°C./2 m.m. (50 mg., 64%).

R_f ($CHCl_3$) 0.7.

($C_{17}H_{16}O_8$ requires C, 58.62%; H, 4.63%; m.w. 348.3.
Found: C, 58.18%; H, 4.54%; M^+ at m/e 348).

ν max : (thin film) : 3020, 2950, 1740, (broad ; ester carbonyl), 1645 (C = C), 1605, 1570, 1485, 1440, 1370, 1220 (broad), 1145, 1050, 1010, 940, 880, 830. $cm.^{-1}$

λ max (EtOH) : 230, 245, 270 (infl.), 325 n.m.

τ (60 Mc./S. ; $CDCl_3$) : 2.20 - 2.90 (4H, m, aryl and H - 3) 3.77 (1H, m, X part of ABX system, J_{AX} = 8 c.p.s., J_{BX} = 4 c.p.s.), 5.60 (2H, m, AB part of ABX system, J_{AB} =12 c.p.s.), 7.58 (3H, S, CH_3COOAr), 7.84, 7.92 (3H each, S, CH_3COO-).

m/e 348 (26%), 306 (43%), 264 (16%), 246 (39%), 233 (10%), 222 (13%), 204 (45%), 191 (55%), 186 (50%), 176 (37%), 175 (25%), 163 (18%), 160 (13%), 147 (16%), 134 (13%), 131 (10%), 105 (8%), 91 (7%), 89 (7%), 77 (12%), 43 (100%).

m* for m/e 348 ⟶ 306, m/e 306 ⟶ 264, m/e 306 ⟶ 246, m/e 246 ⟶ 204.

(10) Bromination of oospoglycol.

(a) 5, 7-dibromo-oospoglycol.

Oospoglycol (50 mg.) was dissolved in methanol (2 ml.) at ice temperature and bromine-water added dropwise until precipitation just began. Addition of a further drop of bromine-water gave a colourless crystalline deposit which crystallised from aqueous methanol to m.p. 187 - 190°C. (67 mg., 82%).

R_f (10% CH_3OH/ 90% $CHCl_3$) 0.5.

(Found : C, 34.87%; H, 2.21%; M^+ at m/e 380.

$C_{11}H_8O_5Br_2$ requires C, 34.76%; H, 2.12%; m.w. 379.9),

νmax (KBr) : 3440, (hydroxyl), 3120, 3050, 2920, 1665 (lactone carbonyl), 1623 (C = C), 1576, 1432, 1410, 1348, 1316, 1295, 1242, 1207, 1190, 1155, 1075, 1016, 909, 882, 862, 800, 786, 723 $cm.^{-1}$

λmax (EtOH) : 239 (E = 20,670); 275 (E = 9,881); 288 (infl.); 362 (E = 6,233).

τ(100 Mc./s. CF_3CO_2H): 1.70 (1H, S, H - 6)
2.21.(1H, S, C:CH(O)); 3.38 (1H, m, - CH(CH) -)
5.55, 5.65, (2H, m, - CH_2 - OH).

m/e 382 (17%), 380 (31%), 378 (18%), 364 (4%),
362 (8%), 360 (4%), 351 (50%), 349 (100%),
347 (51%), 323 (31%), 321 (59%), 319 (31%),
307 (11%), 305 (24%), 303 (17%), 295 (22%),
293 (44%), 291 (22%), 283 (12%), 281 (12%),
271 (17%), 270 (26%), 269 (22%), 268 (26%),
243 (13%), 242 (15%), 241 (15%), 240 (15%),
239 (13%), 237 (13%), 227 (13%), 225 (15%),
214 (21%), 212 (22%), 198 (11%), 196 (12%),
185 (24%), 183 (24%), 158 (21%), 156 (22%),
134 (11%), 118 (34%), 105 (37%), 75 (61%).

m* corresponding to m/e 380 ⟶ 362, m/e 380 ⟶ 349, m/e 349 ⟶ 321, m/e 349 ⟶ 305.

(b) 4,5,7,-tribromo-oospoglycol

Concentration of the mother liquors of this reaction yielded a further crop of crystals, recrystallised from aqueous methanol as a microcrystalline powder, m.p. 148 - 150°C. (13 mg., 13%).

R_f (10% CH_3OH/90% $CHCl_3$) 0.8

(Found : C, 28.63%; H, 1.63%; M^+ at m/e 459.
$C_{11}H_7O_5Br_3$ requires C, 28.78%; H, 1.54%; m.w. 458.9).

νmax (KBr) : 3375 (hydroxyl), 3060, 2960, 1691 (lactone carbonyl), 1577, 1411, 1305, 1212, 1184, 1140, 1055, 1030 (infl.), 987, 940, 906, 875, 830, 790, 735 cm.$^{-1}$

λmax (EtOH) : 229 (E = 24,400), 265 (infl.), 341 (E = 5,555) n.m.

τ(100 Mc./s.; CF_3CO_2H) : 1.79 (1H, S, H - 6); 3.69 (1H, S, (O)CH(O)); 4.31 (1H, broad S. - CH(OH)) 5.86, 5.90 (2H, m, $-CH_2-$ O).

m/e : 462 (8%), 460 (16%), 458 (16%), 456 (8%), 402 (24%), 400 (59%), 398 (62%), 396 (24%), 381 (13%), 379 (20%), 377 (13%), 374 (10%), 372 (20%), 370 (20%), 368 (10%), 364 (5%), 362 (10%), 360 (5%), 353 (5%), 351 (10%), 349 (5%), 346 (16%), 344 (27%), 342 (16%), 337 (10%), 335 (27%), 333 (27%), 331 (10%), 323 (10%), 321 (24%), 320 (24%), 319 (20%), 318 (34%), 317 (10%), 316 (20%), 309 (19%), 307 (30%), 305 (27%), 303 (19%), 265 (15%), 263 (24%), 261 (15%), 211 (19%), 209 (27%), 207 (16%), 198 (20%), 196 (19%), 167 (13%), 129 (24%), 105 (19%), 101 (35%), 89 (30%), 87 (51%), 75(100%), 74 (92%).

M* for m/e 462 ——— 402, m/e 402 ——— 374.

(11) Treatment of oospoglycol with N-bromosuccinimide.

Freshly crystallised N-bromosuccinimide (36 mg. 0.2 m. mole) was dissolved in a stirred solution of oospoglycol (22 mg. 0.1 m.mole) in methanol (0.1 ml.) and carbon tetrachloride (2ml.): the solution quickly became brown in colour. After one hour stirring at room temperature, the solvent was evaporated under reduced pressure and the residue taken up in ethyl acetate, which was washed with water (3 x 3 ml.) and dried over magnesium sulphate. The residue after evaporation crystallised as prisms from methanol m.p. 187 - 190°C., (21 mg., 55%), with R_f and spectroscopic properties identical to the dibromo compound described in section (10a).

With a 3:1 molar excess of N-bromosuccinimide, the formation of the tribromo compound described in section (10b) was observed by t.l.c.

(12) Treatment of oospoaldehyde with bromine-water:3-methoxy-4,5,7-tribromo-oospoaldehyde.

Bromine-water was added dropwise, with occasional warming on the steam bath, to a solution of oospoaldehyde (20 mg.) in methanol (3 ml.). After the appearance of a faint precipitate, the solution was evaporated: the residue crystallised from methanol as prisms, m.p. 163 - 165°C, (40 mg., 83%).

R_f (3% CH_3OH/97% $CHCl_3$) 0.8.

(Found : C, 28.89%; H, 1.59%; M^+ at m/e 459.

$C_{11}H_7O_5Br_3$ requires C, 28.78%; H, 1.54%; m.w. 458.9).

νmax (KBr) : 3420, 3380, 3070, 2940, 2850, 1731 (α-bromo aldehyde carbonyl), 1693 (lactone carbonyl), 1580, 1407, 1385, 1350, 1292, 1171, 1126, 1098, 1072, 1057, 1013, 842, 785, 756, 643 $cm.^{-1}$

λmax (EtOH) : 228 (E = 28,180), 265 (infl), 343 (E = 7,573)nm.

τ(100 Mc./s., CH_3CO_2H): 0.29 (1H, S, -CHO); 1.82 (1H, S, H - 6); 4.31 (1H, S, (O)-CH-(O)); 6.26 (3H, S, CH_3O -).

m/e : 462 (4%), 460 (10%), 458 (10%), 456 (4%), 402 (15%), 400 (42%), 398 (42%), 396 (15%), 382 (2%), 380 (2%), 378 (2%), 374 (6%), 372 (14%), 370 (14%), 368 (6%) , 353 (4%), 352 (3%), 351 (8%), 350 (8%), 349 (6%), 348 (9%), 347 (2%), 346 (4%), 338 (6%), 336 (7%), 334 (6%), 323 (4%), 322 (7%), 321 (8%),320 (16%), 305 (11%), 294 (7%), 292 (15%), 290 (9%), 269 (35%), 267 (37%), 265 (16%), 263 (31%), 261 (16%), 241 (12%), 239 (11%), 227 (11%), 185 (26%), 183 (24%), 155 (23%), 153 (15%), 143 (10%), 105 (11%), 103 (31%), 101 (19%), 86 (27%), 75 (100%).

BIOSYNTHESIS OF OOSPOGLYCOL

EXPERIMENTAL.

(1) Instrumental :

Assays of radioactivity of material on t.l.c. were carried out using a Panax Thin Layer Scanner, RTLS-1A, and in solution on a Nuclear Chicago Scintillation Spectrometer using channels A(carbon -14) and B(tritium). Under the conditions employed for scintillation counting, the efficiency of tritium counting was 53.2% and of carbon - 14 counting 32.1%. To compensate for channels overlap, the reading in the channel A , divided by the factor 1.92 (calculated from the measurement of a standard carbon-14 sample) was subtracted from each reading in channel B. From measurement of a standard tritium sample, the overlap into channel A was found to be negligible.

(2) Introduction of radioactive acetate into cultures of Lenzites striata.

A mixture of (2 - ^{14}C) sodium acetate, (total activity 0.15 mc.) and (2 - ^{3}H) sodium acetate (total activity 7.5 mc) in sterilised water (20 ml.) was spread evenly over 10 Roux bottles containing ten-day old cultures of Lenzites striata, which were harvested 17 hr. later in the usual manner (p. 71). The ethyl acetate extract of the broth was concentrated to 10 ml. and, after preliminary thin layer scanning of the radioactivity, a total weight of 159.6 mg. of inactive

oospoglycol added. The oospoglycol was reisolated by p.l.c. on Kieselgel HF_{254} (four plates, 20x20x0.5 mm.) using 10% ethyl acetate/ 90% light petroleum as eluting solvent. The total weight of oospoglycol thus recovered was 153.4 mg. A sample (20 mg.) of this material was further purified by p.l.c. and then crystallised several times from ethyl acetate/ light petroleum, the radioactivity being measured after each recrystallisation, until constant. After four such recrystallisations, oospoglycol was obtained of constant activity, and radiochemical purity was confirmed by a t.l.c. scan after the final crystallisation. The percentage incorporation of radioactivity was 1.8. Scintillation counting results are summarised in table 12.

(3) Periodate cleavage of radioactive oospoglycol

Oospoglycol (75 mg.) was treated in aqueous methanol (5 ml.) with sodium metaperiodate (300 mg.) under the conditions previously described. The oospoaldehyde thus obtained (59 mg.) was recrystallised from methanol to constant activity and radiochemical purity confirmed by t.l.c. scanning.

In a similar reaction oospoglycol (20 mg.) was treated with sodium metaperiodate (100 mg.) in aqueous methanol (2 ml.) and the solution distilled into an ethanolic

dimedone solution (30 mg. in 0.5 ml. ethanol). The product (35 mg.) was recrystallised to constant activity, the radiochemical purity again being confirmed by a t.l.c. scan.

Scintillation counting results are summarised in Tables 13 and 14.

(4) Bromination of radioactive oospoaldehyde

Oospoaldehyde (20 mg.) obtained as described in section (3) in methanol (1 ml.) was treated with bromine/water until a precipitate appeared. The bromoaldehyde (35 mg.) was recrystallised to constant activity and radiochemical purity. Scintillation counting results are summarised in table 15 .

(5) Introduction of radioactive methionine into cultures of Lenzites striata

A mixture of (^{14}C -)-methyl methionine (total activity 0.04 mc.) and (^{3}H-) methyl methionine (total activity 2.00 mc.) was fed to L.striata cultures as in the above experiment using acetate and harvesting carried out after 17 hr. After a preliminary t.l.c. scan of the broth extract, inactive oospoglycol (152.2 mg.) was added and reisolated by p.l.c. in the usual manner. The total weight of oospoglycol

isolated by this means was 133.8 mg. after crystallisation from ethyl acetate/light petroleum.

A sample (20 mg.) was resubjected to p.l.c. then crystallised five times and examined by radio t.l.c. scanning and scintillation counting (Table 16)

ACETATE FEED

TABLE (12) : OOSPOGLYCOL

Cryst.	^{3}H cpm/mg	^{14}C cpm/mg	^{3}H dpm/mg	^{14}Cdpm/mg
(1)	179,039.7	11,896.9	336,794.0	37,096.7
(2)	192,556.6	12,797.0	381,032.0	39,903.3
(3)	188,235.3.	12,414.8	354,092.0	38,711.6
(4)	191, 498.0	12,774.2	360,229.5	39,832.2.

TABLE (13) : OOSPOALDEHYDE

(1)	81,615.9	11,724.7.	153,601.3.	36,525.5.
(2)	80,764.2.	11,529.6	151,812.4	35,918.1
(3)	92,457.7	13,147.0	173,792.7	40,956.4.

TABLE (14) : FORMALDEHYDE

(1)	41,759.0	1145.3	78,494.4	3,895.6
(2)	40,731.2	1256.8	76,562.4	3,915.3
(3)	40,863.9	1250.5.	76,811.8	3,895.6
(4)	40,597.3.	1253.1	76,310.7	3,903.7

TABLE (15) : METHOXY TRIBROMO-OOSPOALDEHYDE

(1)	2,092.9	5,444.3	3934.0	16,960.4.
(2)	1,761.70	5,249.9	3311.5	16,354.8
(3)	1,801.0	5,163.8	3385.3	16,086.6

METHIONINE FEED

TABLE (16) : OOSPOGLYCOL

Cryst.	^{3}H cpm/mg	^{14}C cpm/mg	^{3}H dpm/mg	^{14}C dpm/mg
(5)	8,900	333.8	16,730	1055

SECTION II.

FUNGAL TERPENES.

INTRODUCTION

Wallach's original coining of the word "terpene" was to describe a group of volatile C_{10} hydrocarbons isolated from plant essential oils;[99] the description was soon extended to include the oxygenated analogues of these C_{10} compounds, and then to embrace products of a similar origin which contained fifteen or twenty carbon atoms until today it is applied collectively to a large number of widely distributed natural products of the plant and animal kingdom. From a comparison of the structures of various compounds, there arose the so-called empirical isoprene rule, by which terpenes were designated as having carbon skeletons composed of C_5H_8 (isoprene) units, linked in regular or irregular arrangement.[100] This definition, was ultimately replaced by a "biogenetic isoprene rule", which allowed deduction of the structures of terpenoids by "accepted reaction mechanisms from the hypothesized simple precursors, squalene, geranyl-geraniol, farnesol, geranol."[101] The value of this rule is two-fold: while it restricts the number of carbon skeletons which can be proposed on the basis of the empirical isoprene rule, it also allows that the carbon skeleton of the end product need not be identical to that of the precursor, thus rationalising

a large number of structures which have undergone backbone rearrangements of the type corresponding to 1,2-alkyl or hydride shifts.

It is known that mevalonic acid (104) is the precursor of the terpene building unit, itself isopentenyl pyrophosphate (105), which in its biologically active form is dimethyl allyl pyrophosphate (106); a strict definition of a terpene should take into account this mevalonic origin, which would also include the so-called molecules of "mixed biogenesis", owing their origin to separate biosynthetic pathways. However, since rigorous proof of the mevalonate derivation of the large number of natural products isolated would be prohibitive, it is still of value that mere recognition of the simple isoprene unit as a part of a structure suggests terpenoid origin. The prefixes hemi, mono, sesqui, di, sester, tri, tetra are commonly applied to structures derived from the isopentenyl monomer, dimer, trimer, tetramer, pentamer, hexamer, and octamer, respectively.

From the almost overwhelming number of fungal metabolites of varied type and usefulness, the terpenes have by no means been omitted. One of the simplest structures is 2-methylbut-2-ene (107) a hemiterpene[102]

from Puccinia graminis , while the monomeric isopentenyl unit is evident in flavoglaucin (108)[103], from Aspergillus glaucus, and also in fuscin(109)[104], agroclavine (110)[105], and echinulin (111)[106], all examples of "mixed biogenesis". Mycelianamide (112)[107] is another such example, with the isopentenyl dimer forming the terpene portion, and this geranyl unit is also apparent in degraded form as the side-chain of mycophenolic acid (113)[108].

The fungal sesquiterpenes are derived enzymically from farnesol (114), or a suitable form thereof: the structural varieties range from the azulenes, such as lactaroviolin (115)[109], from Lactarius delicosus to the more complicated skeletons of helminthosporal (116)[110], culmorin (117)[111] and hirsutic acid (118)[112]. Other examples are illudalic acid (119)[113], from the basidiomycete, Clitocybe illudens, and marasmic acid[114] (120), both derivable from the same basic "protoilludane" skeleton (121). From an unidentified Fusarium species have been isolated examples of terpenes of mixed biogenesis, showing both the simple farnesyl side-chain (122), and a cyclised form of it (123)[115]. The siccanochromenes A and B, (124), (125)[116] are other

examples of prenylphenols, in which the sesquiterpene portion has the drimane skeleton.

The isopentenyl tetramer, geranyl geraniol, cyclises in a number of ways to give the diterpenes. From fungal sources, structures like pleuromutilin (126)[117], rosenonolactone (127)[118], and the Giberella metabolites,[119] e.g. giberellic acid (128), are a few examples, while the kaurene derivatives, such as 16 α -hydroxy-(-)-kauran-19-al (129)[120], from Fusarium monoliforme, show another structural diversification.

The most recently discovered class of terpenes are the sesterterpenes, the first isolated being ophiobolin (130), from the plant pathogenic fungus, Ophiobolus miyabeanus.[121] These metabolites are presumably derived[122] from the C_{25} geranyl-farnesol, the only source of which as yet is an insect wax[123]. The carotenoids, such as β - carotene (131), and phytoene (132), are examples of fungal tetraterpenes.

The triterpenes, the class of interest in this work, are taken as being derived from the isopentenyl hexamer squalene (133), the occurrence of which has been reported in one fungus, Amanita phalloides[124], but which may well occur widely. Much effort has been devoted to the

problem of formation of the interfarnesyl bond of squalene, a process which proceeds in a highly stereospecific manner; the intermediate or "presqualene" molecule has recently been identified as (134)[125].

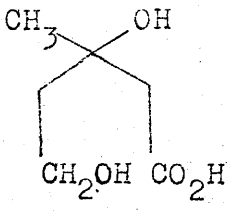
The vast majority of the fungal triterpenes possess the (tetracyclic) lanostane skeleton, the formation of which requires cyclisation of squalene in the enzyme-bound chair-boat-chair- configuration[126]. The intermediate carbonium ion (135) is converted to lanosterol (136) by a series of hydride and methyl shifts, while elimination of a proton from (135) without these shifts leads to the fusidane skeleton, exhibited by fusidic acid (137)[127]. 23-hydroxylanosterol (138) is a metabolite of the Common Earth-ball fungus[128,129], while pinicolic acid (139)[130], and trametenolic acid (140)[131] represent two other C_{30} tetracyclic structures: dustanin (141)[132] is a rare example of a pentacyclic fungal triterpene.

These C_{30} compounds may undergo loss of carbon atoms in a manner considered similar to the oxidative decarboxylation of cholesterol[133]. In this category occur ergosterol (142)[134] and its derivatives, such as 14-dehydroergosterol (143), a metabolite of

Aspergillus niger[135]; others are the antibiotic cephalosporin P_1 (144)[136], viridin[137], and its recently isolated dihydro analogue, viridiol (145)[138].

The ubiquitous process of C_1-unit insertion is also operative for the triterpenes and C_{31} compounds are common in this class. The known examples, (146) to (149), all again possess the lanostane stereochemistry and have a C-20 carboxyl function, the exception being polyporenic acid A (150)[143] which has this grouping at C-25.

The fungal terpenes are known to exist in various "conjugated" forms: on a simple level, the conjugated species may be acetic acid, such as occurs in eburicoic acid isolated from Polyporus anthrocophilus[144]. Polyporenic acid A (150) has been obtained from P.betulinus conjugated with four different acids[145], acetic, caproic, malonic and hydroxymethylglutaric and from Daedalea quercina the malonate ester has been found as part of structures such as (151)[146]. The first triterpene glucosides e.g. (152)[147] have been isolated from Leptoporus stipticus. As an extension to this work carried out in this laboratory, an examination of the triterpene constituents of the basidiomycete Lenzites striata was undertaken.



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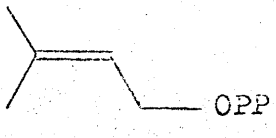

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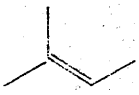

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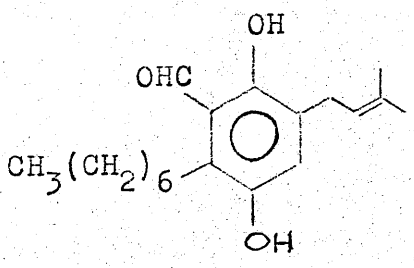

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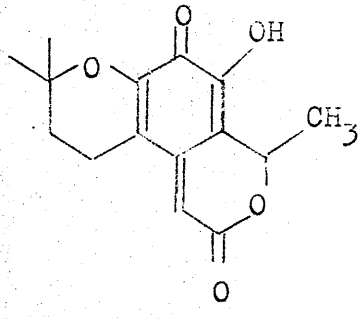

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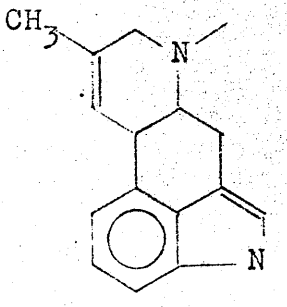

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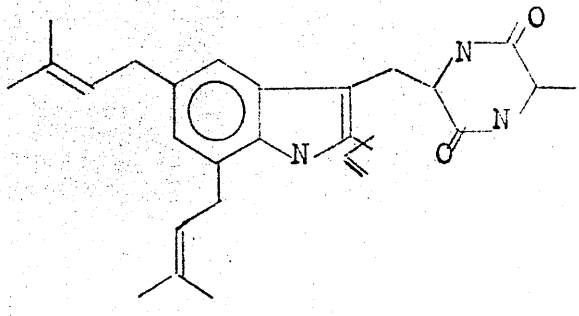

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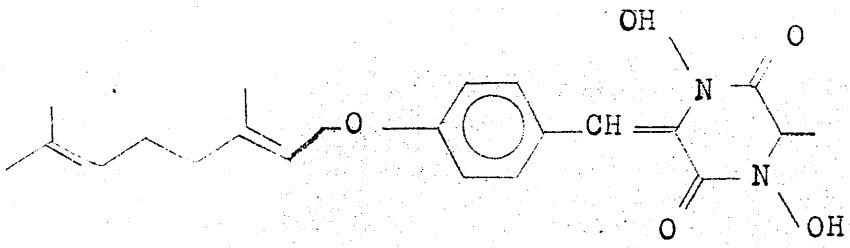

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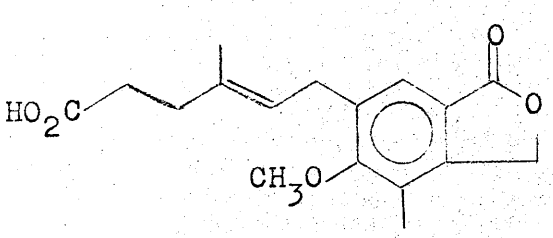

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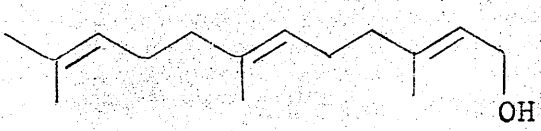

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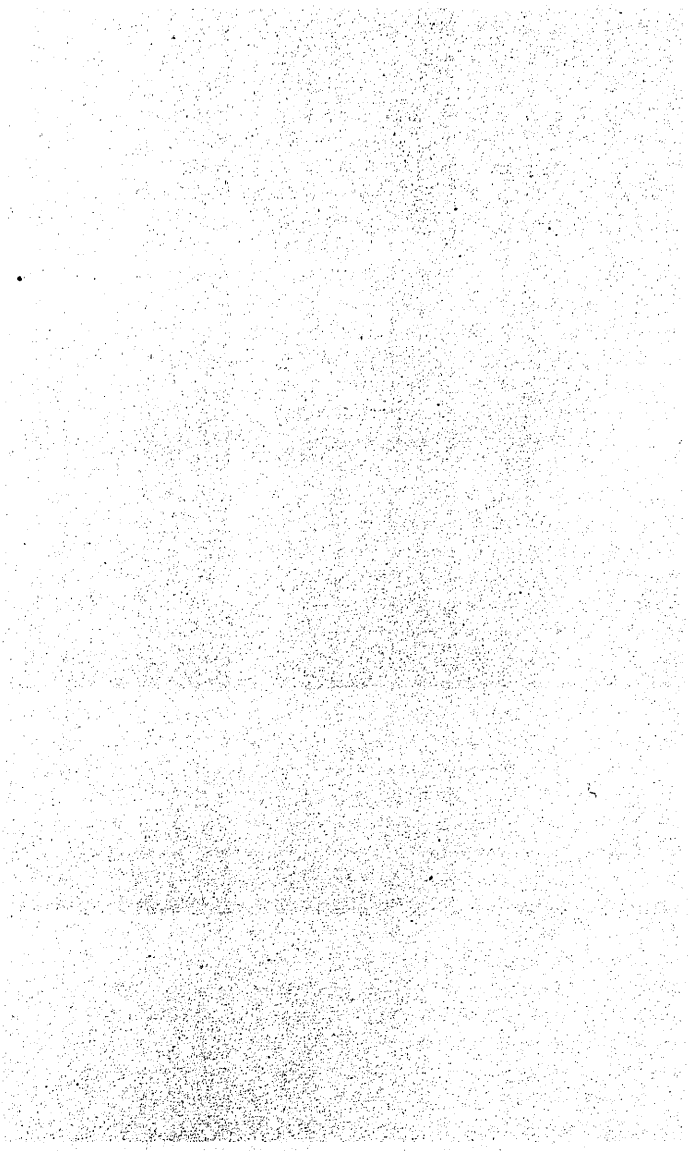

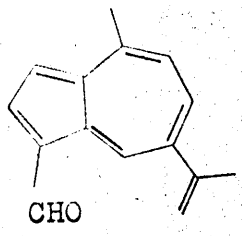

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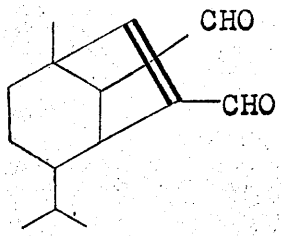

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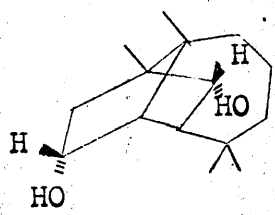

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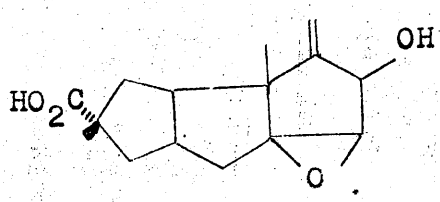

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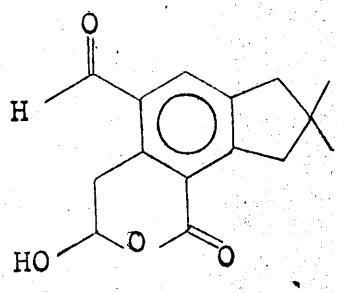

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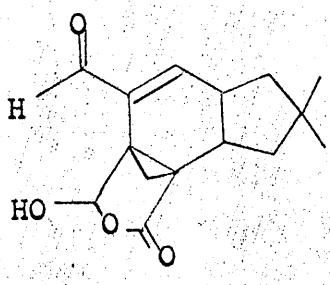

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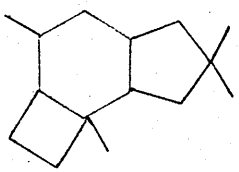

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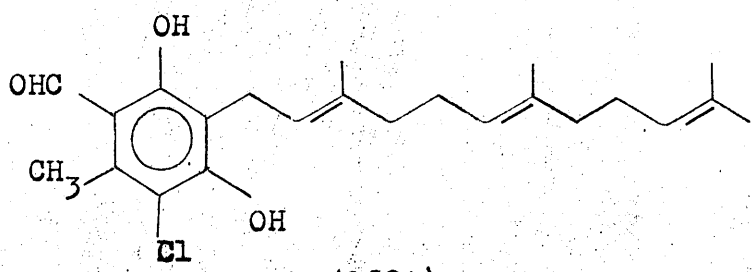

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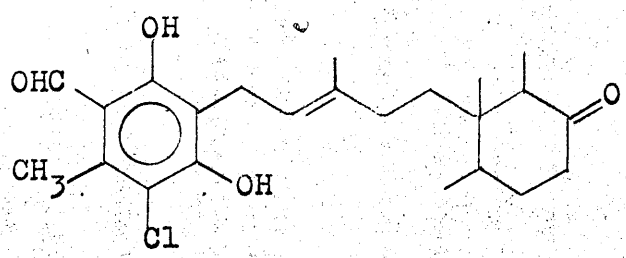

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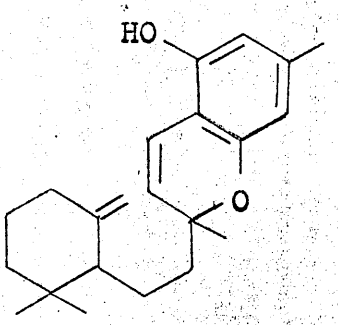

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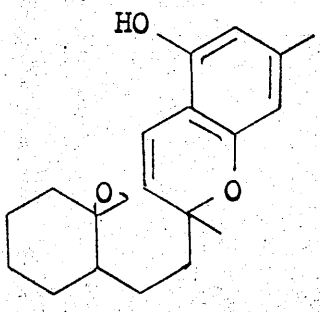

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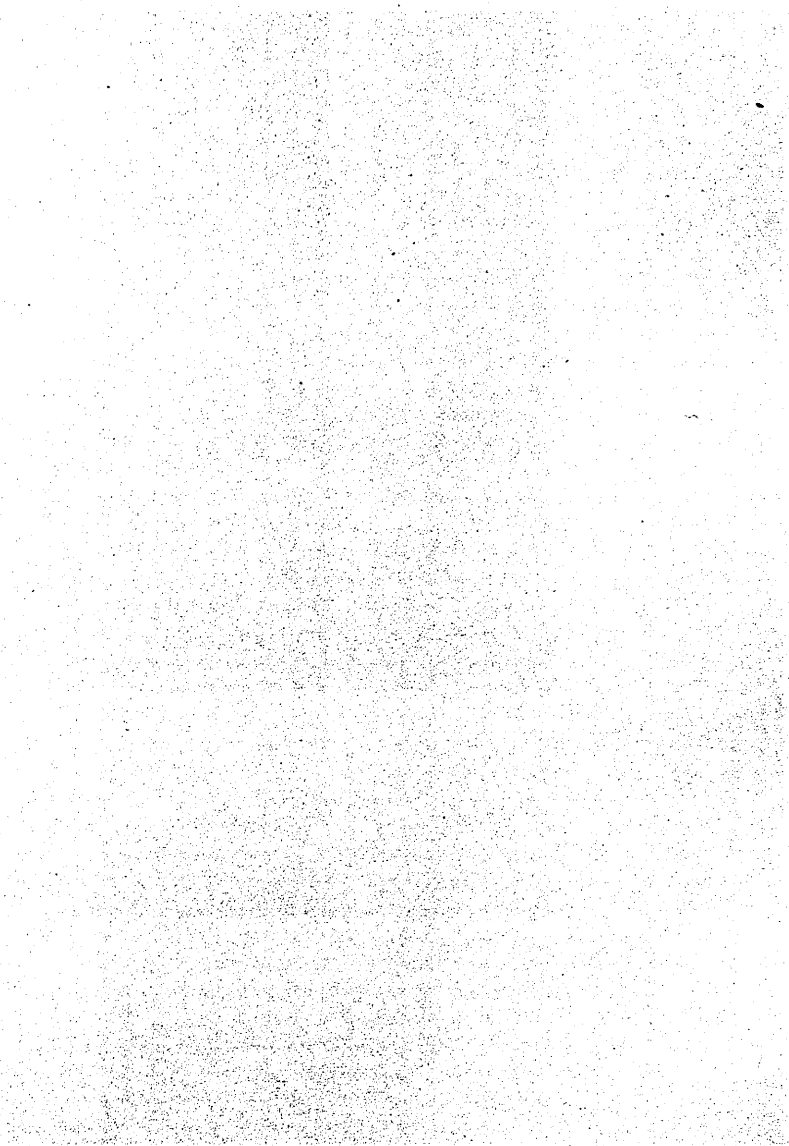

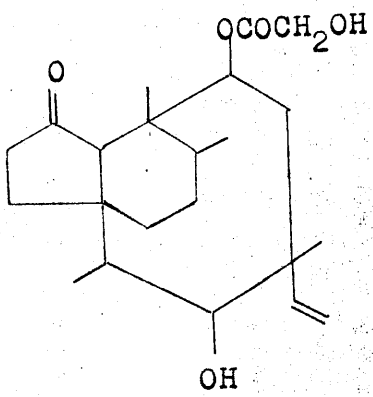

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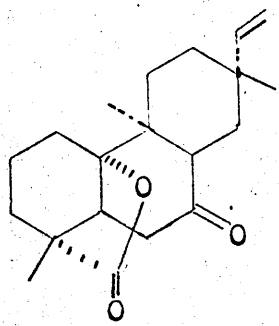
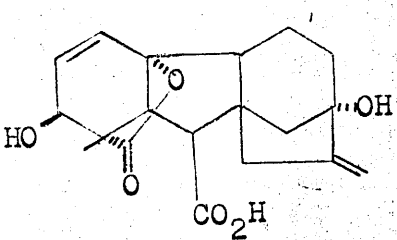


(127.)



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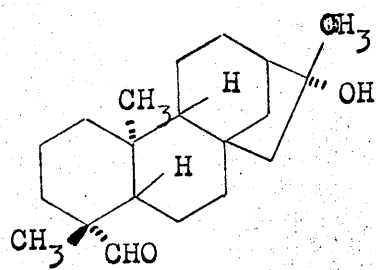
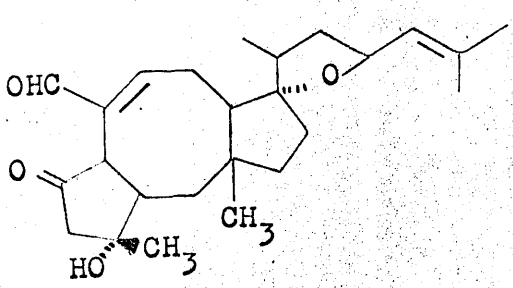


(129.)



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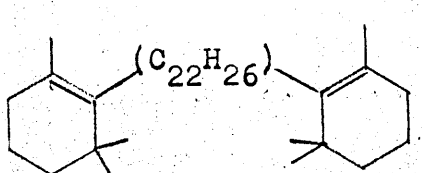

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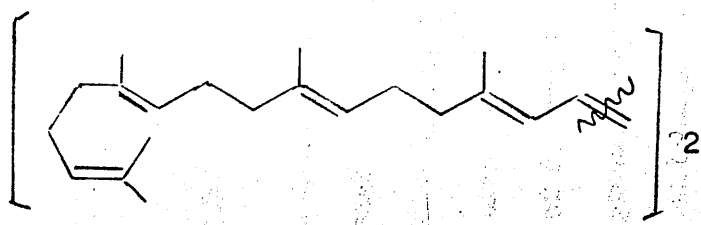

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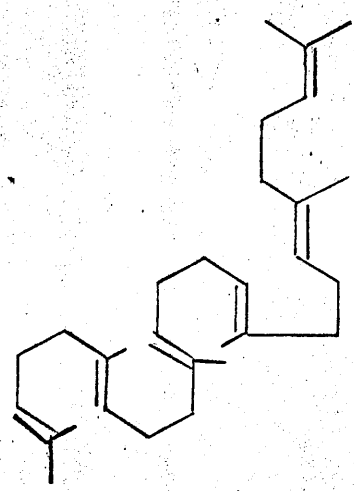

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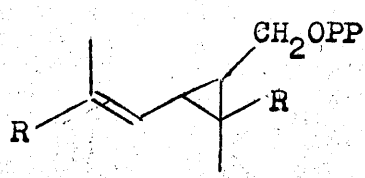
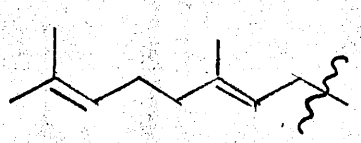
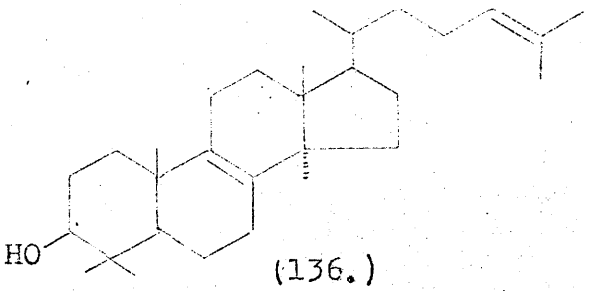


(134.)

R =





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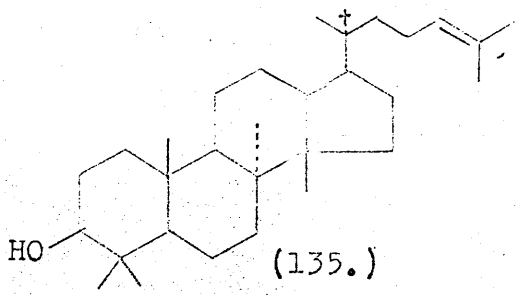

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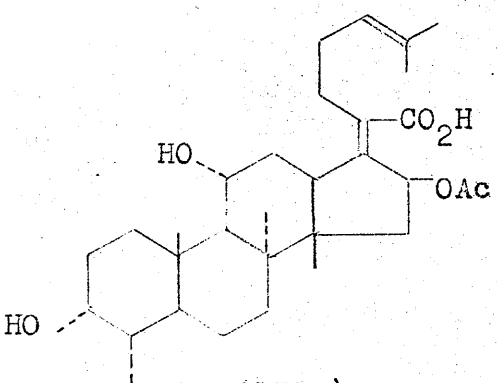

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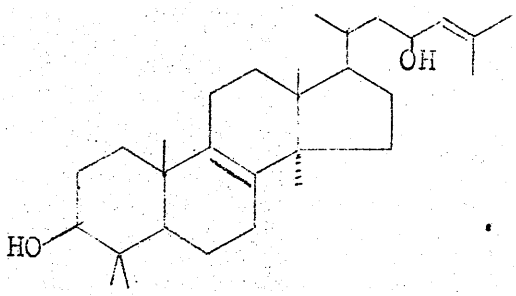

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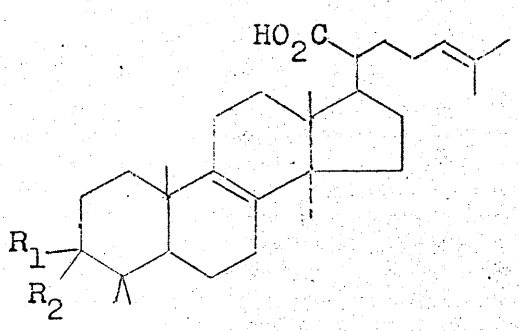
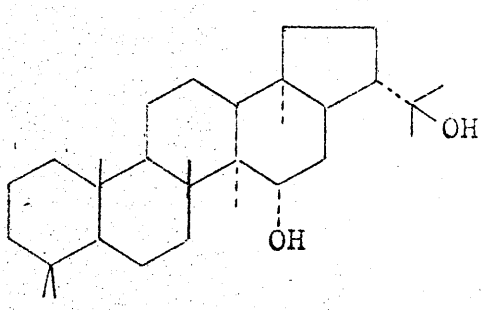
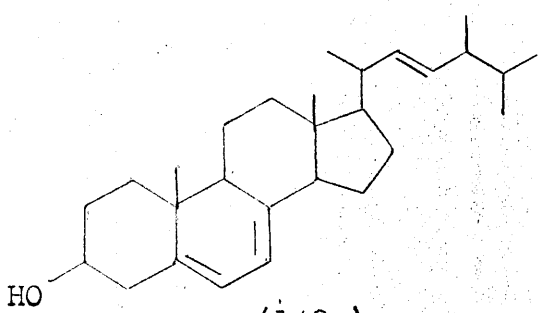


(139.) $R_1 = R_2 = O$.

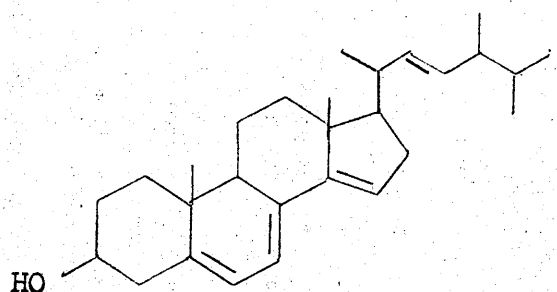
(140.) $R_1 = OH$, $R_2 = H$.



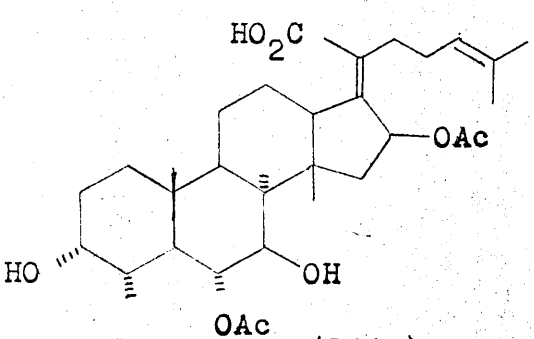
(141.)



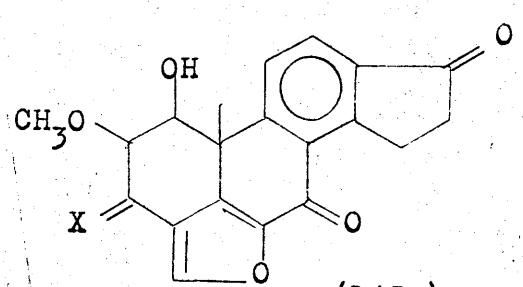
(142.)



(143.)



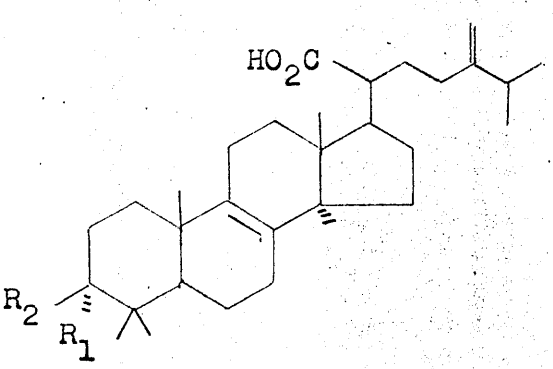
(144.)



(145.)

X = O; VIRIDIN.

X = H,OH; VIRIDIOL.



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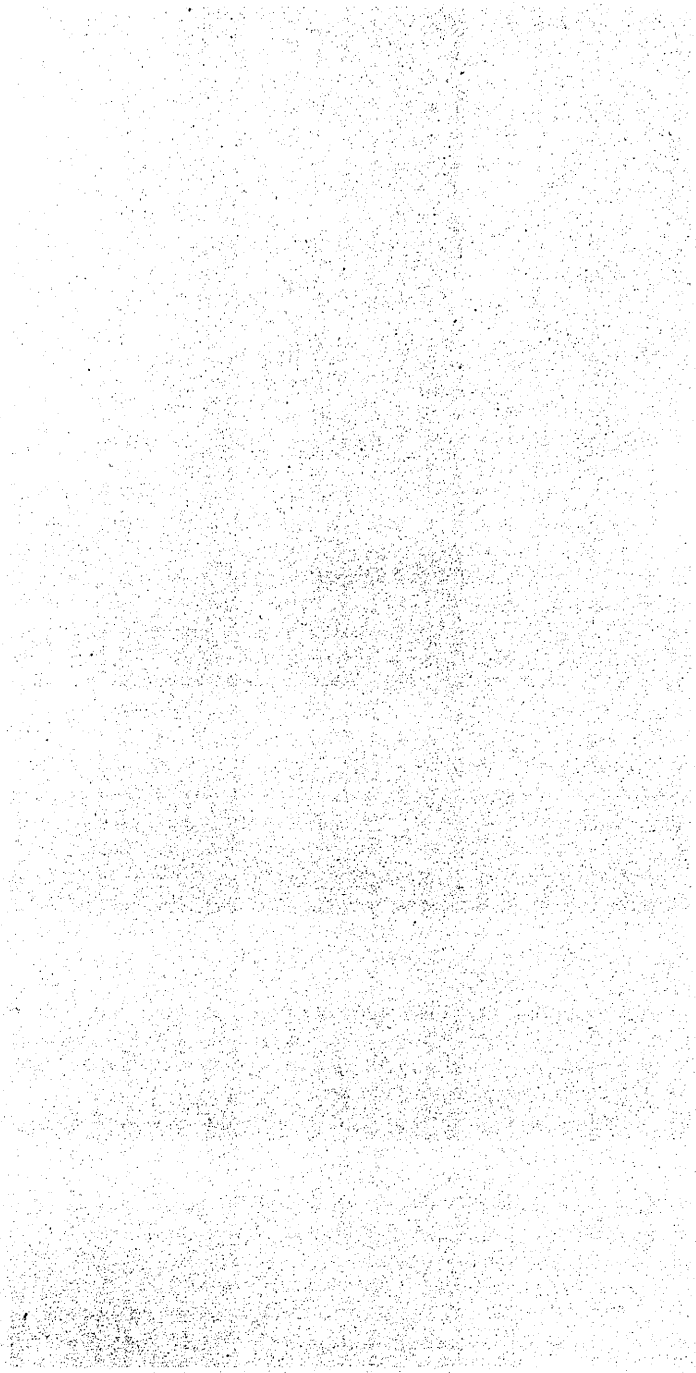
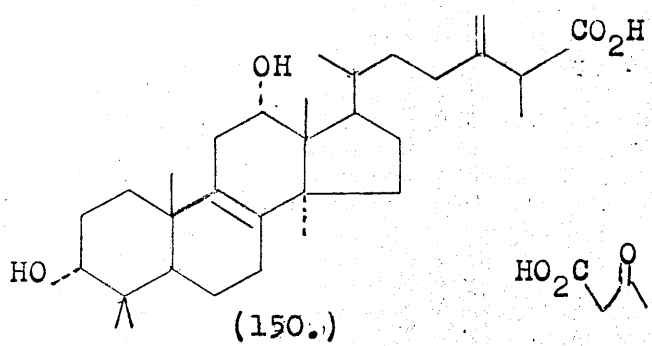
146 R_1 = H, R_2 = OH. [139]

147 R_1 = R_2 = O, 16α-OH [140]

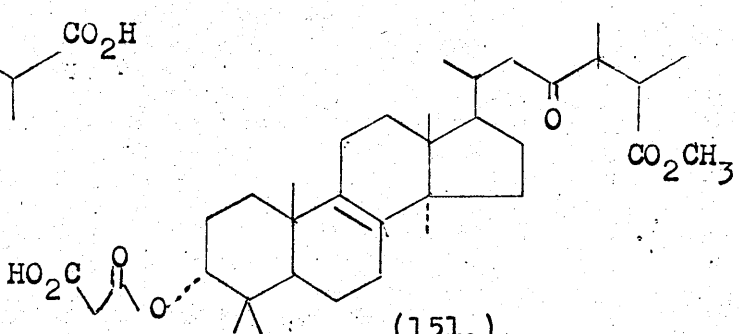
148 R_1 = H, R_2 = OH, 16α-OH [141]

149 R_1 = H, R_2 = OH, 15α-OH [142]

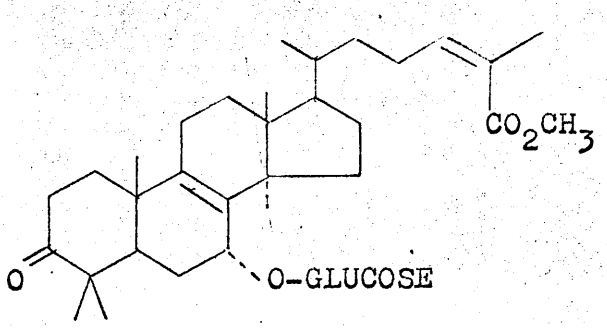




(150.)



(151.)



(152.)

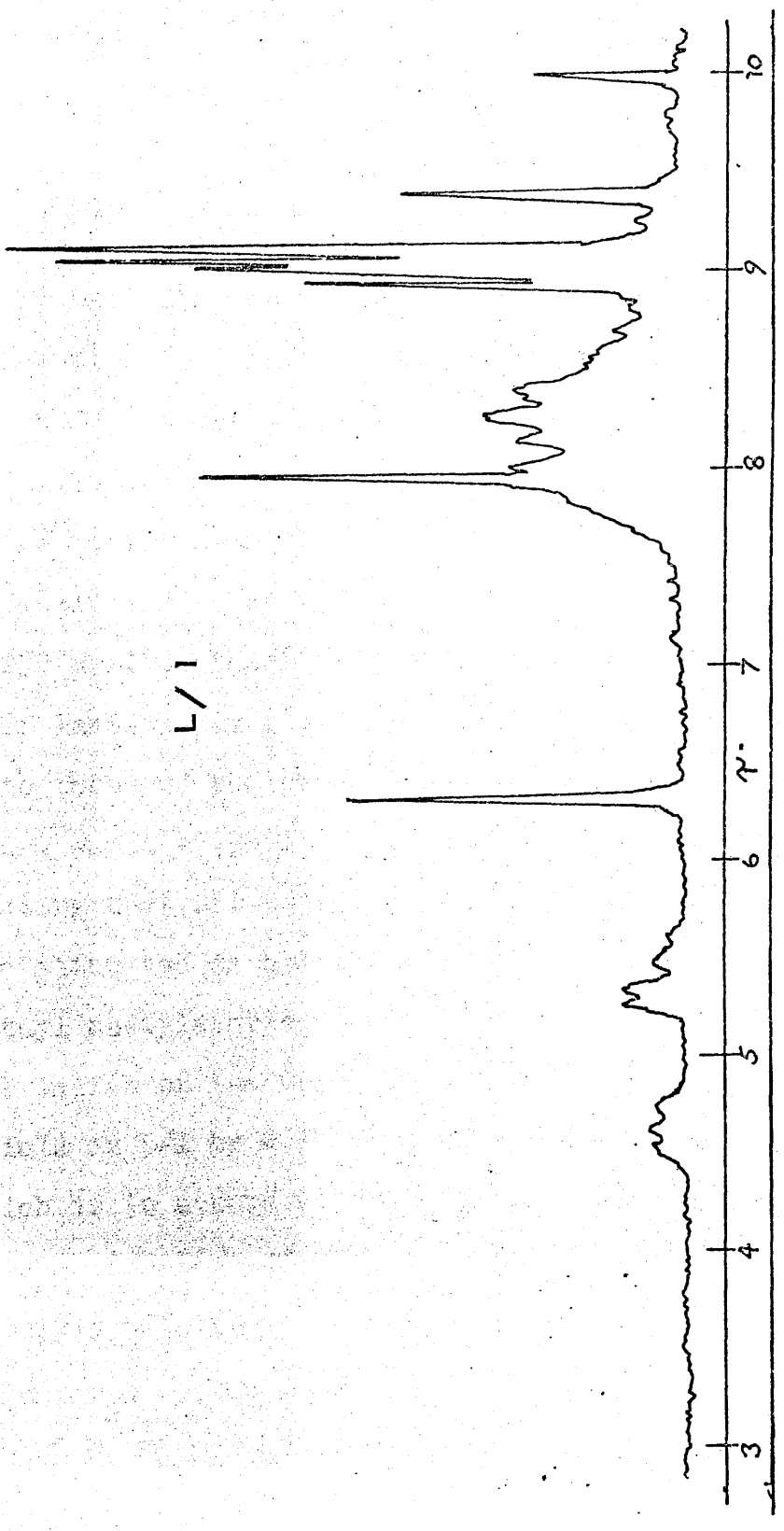
DISCUSSION.

FUNGAL TERPENES

DISCUSSION I

As a preliminary to the separation of the triterpene constituents of *Lenzites striata*, the mixture of acids obtained by cold methanol extraction of the mycelium was treated with an excess of ethereal diazomethane, at room temperature overnight; the triterpenes could then be separated as their methyl esters by column chromatography on silica gel, using a light petroleum to ethyl acetate elution gradient. Four triterpenes were isolated in this manner, to which the code names L/1, L/2, L/3 and L/4 were assigned in order of increasing polarity; the t.l.c. properties of these compounds are defined in Table 17.

The compound L/1 had molecular formula $C_{34}H_{52}O_4$ as determined by elemental analysis and parent molecular ion at m/e 524. L/1 was deduced to be methyl acetyl -7,11- dehydroeburicoate (153) from the following spectroscopic evidence: the presence of an acetoxy grouping was apparent from the loss in the mass spectrum fragmentation (Table[18]) of 60 mass units, together with the 3H singlet in the n.m.r. (p. 109) at 7.98 τ (CH_3CO-) and carbonyl absorption at 1734 cm^{-1} in the i.r. The absorption coefficient (1052) indicated the presence of two superimposed carbonyl groups


L/1
τ.
3
4
5
6
7
8
9
10

and the second carbonyl function was assigned to that of a carbomethoxy grouping from the loss in the mass spectrum of 59 mass units and a 3H singlet at 6.33 τ in the n.m.r.

Double bond absorption was evident in the i.r. spectrum at 1634 $cm.^{-1}$. This was of two distinct kinds: firstly, a terminal methylene grouping was present from the out-of-plane deformation frequency at 882 $cm.^{-1}$ and the two-proton doublet at 5.3 τ in the n.m.r. spectrum. Secondly, the presence of a 7,9 (11)-diene system was inferred from the presence in the n.m.r. of a two-proton multiplet at 4.6 τ and the typical triplet of peaks in the u.v. spectrum at 236, 243 and 252 n.m. ; these absorption maxima were nearer to those of lanosta-7,9 (11)-diene (237, 243, 252 n.m.) than to those of the other most common tetracyclic triterpene nucleus, eupha -7,9 (11)-diene (232, 240, 247 n.m.)[148] , indicating that L/1 possessed the former skeleton. Comparison of the saturated methyl signals in the n.m.r. of L/1 with those of methyl acetyleburicoate (154) showed (Table 19) that the chemical shifts of the C-18 and C-19 methyl singlets were shifted upfield in L/1 by 0.14 p.p.m. and 0.08 p.p.m. respectively, which is in accord with the presence of a 7,9 (11)-diene system in place of the 8,9-monoene; the C-18 methyl group is mainly affected, since it lies directly over

the double bond system.

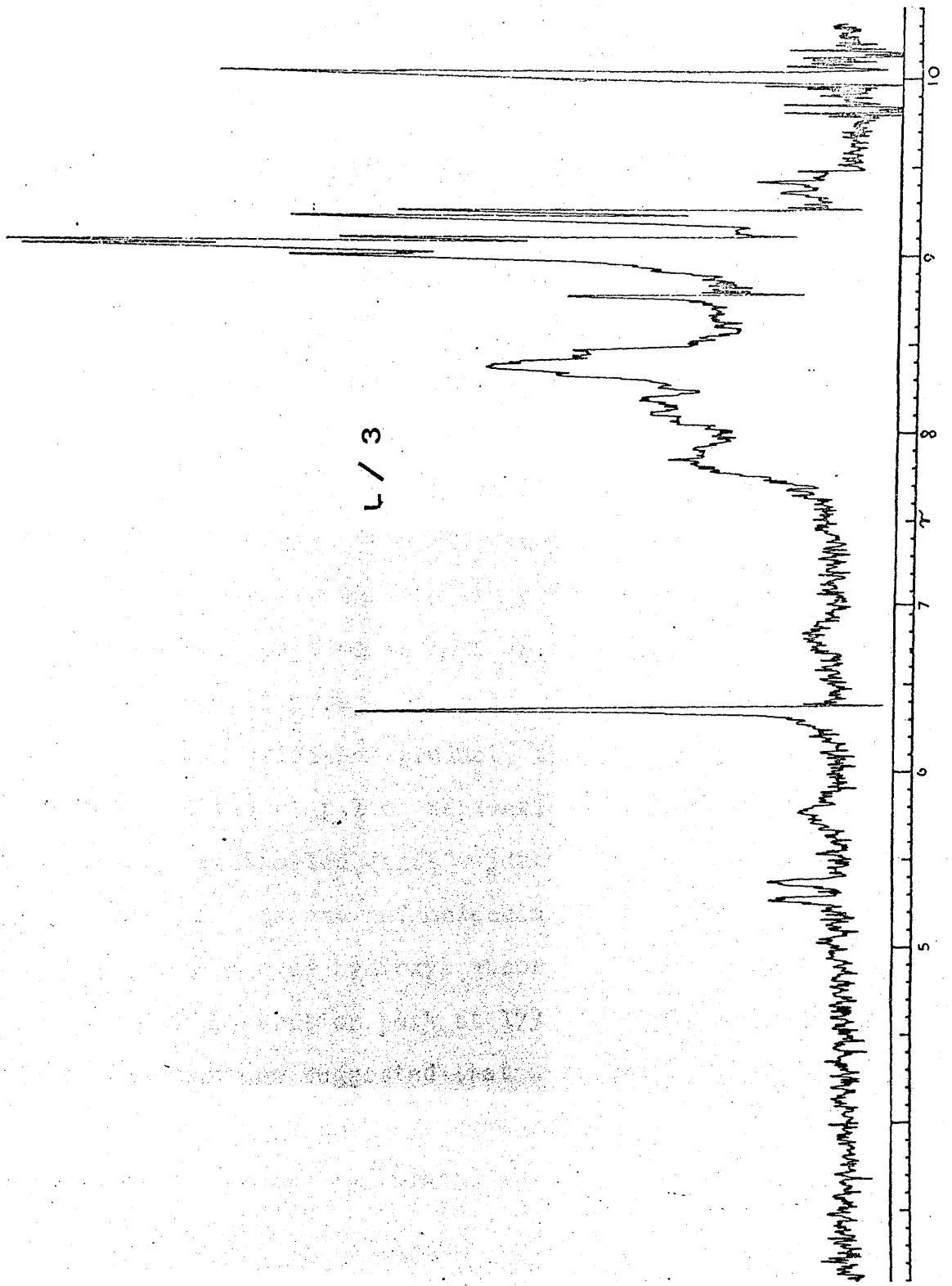
The loss of 169 mass units in the mass spectrum suggested a formula $C_{10}H_{17}O_2$ for the C-17 side chain incorporating both the terminal methylene and carbomethoxy groupings; the carbomethoxy group was assigned to C-20 from the appearance in the n.m.r. of the characteristic doublet (8.95, 9.03 τ) for the C-26 and C-27 methyl groups.

The second metabolite isolated, code name L/2, was identified as methyl eburicoate from comparison with authentic material. From the n.m.r. spectrum, the presence of a mixture of 8,9-monoene and 7,9 (11)-diene was evident (m. at 4.60 τ ,<1H); this co-occurrence of monoene and diene is common for the triterpenes, separation often being tedious and difficult. While eburicoic acid is a metabolite common to several fungi, the 3 - acetate has previously only been isolated from sources where growing conditions closely resemble those of the natural environment; for example, from Polyporus anthrocophilus grown on decaying Eucalyptus regnans the acetate could be isolated, whereas, the same organism, cultured on synthetic medium, yielded the free hydroxy acid [144].

The third triterpene metabolite of Lenzites striata, L/3, had molecular formula $C_{32}H_{52}O_4$ from elemental analysis and parent molecular ion at m/e 500. A partial structure (155)

was assigned to L/3, from a consideration of the spectroscopic properties of the compound. Thus, a two proton doublet in the n.m.r. at 5.30 τ , in conjunction with the i.r. absorption at 885 cm.$^{-1}$ suggested the presence of a terminal methylene grouping, while carbonyl absorption at 1732 cm.$^{-1}$ (E=393) and a 3H singlet at 6.34 τ in the n.m.r. spectrum(p.113) confirmed the presence of the carbomethoxy grouping. No evidence of 7,9-(11)-diene character was evident in the n.m.r. or u.v. spectra. Two 1H multiplets at 5.73 τ and 6.75 τ in the n.m.r. were attributed to protons geminal to hydroxyl groups and this was confirmed by the disappearance of these signals upon oxidation (cf. below). Hydroxyl absorption was evident in the i.r. (ν max 3555, 3370 cm.$^{-1}$) while successive losses of 18 mass units in the mass spectrum were also in accord with the presence of secondary hydroxyl groups.

Since there are no known tetracyclic triterpenes which are not oxygenated at C-3, (cf. ref. (148) p.28), one hydroxyl group was assigned to this position. The configuration of this C-3 hydroxyl group was suggested as β from a comparison of the position (6.75τ) and half-band width (ca. 8cps.) of the C-3 proton in the n.m.r. spectrum with other 3 α -and 3β-hydroxylated lanostane derivatives[146], (Table20). The remaining hydroxylic function was assigned


L / 3
10
9
8
7
6
5

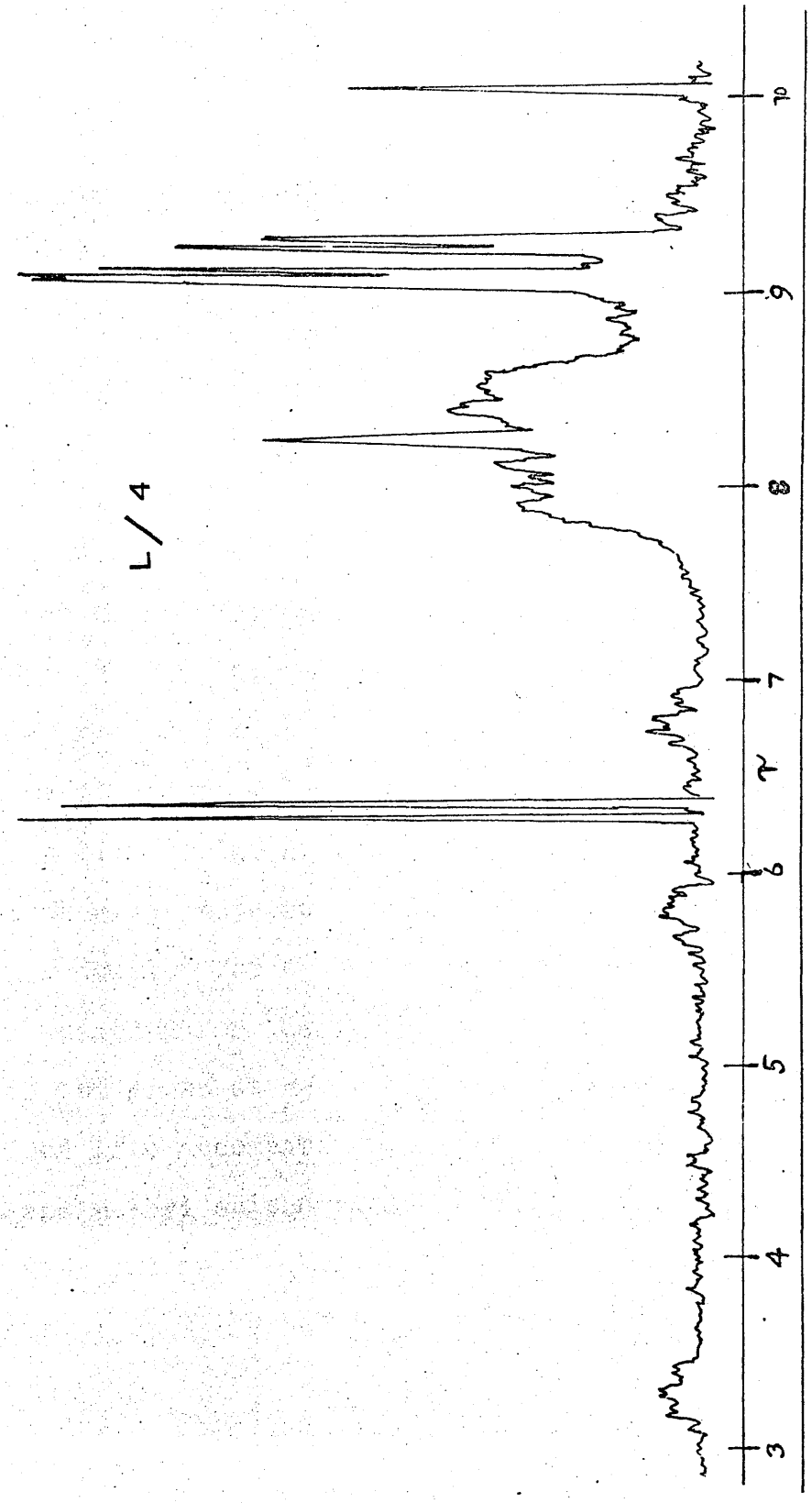
to ring D (C-15 or C-16) by Jones oxidation studies on L/3. T.l.c. monitoring showed the formation of two less polar compounds of R_f ($CHCl_3$) 0.2 and 0.6; at complete oxidation only one spot of R_f 0.6 remained and this derivative was examined first.

This crystalline compound of molecular formula $C_{32}H_{48}O_4$ was a diketone since the n.m.r. spectrum showed no aldehydic proton signals, indicating that both hydroxyl groups of L/3 were secondary. Examination of the i.r. spectrum showed two peaks in the carbonyl region at 1736 $cm.^{-1}$ (E=964) and 1706 $cm.^{-1}$ (E=502); the latter absorption was assigned to a 3-keto carbonyl function and the former more intense absorption to both the carbomethoxy group and a ring D carbonyl group.

The "half-oxidised" product, that is the compound of R_f 0.2, obtained by p.l.c. separation of the product obtained by controlled Jones oxidation of L/3 was also highly crystalline and had molecular formula $C_{32}H_{50}O_4$. The i.r. spectrum showed hydroxyl absorption at 3474 cm^{-1} and only one carbonyl absorption peak at 1735 $cm.^{-1}$ (E=1032); both these observations suggested that this compound had a 3-hydroxyl and ring D carbonyl grouping. The optical rotatory dispersion curve of a saturated 16-oxosteroid is characterised

by a strongly negative Cotton effect in contrast to that of a 15-oxosteroid which exhibits a moderately strong positive Cotton effect[149]. Thus, since this keto-alcohol of L/3 showed a positive Cotton curve, it was concluded that the carbonyl grouping was located at C-15, which in turn requires a structure (156) for the natural metabolite. This structure has been assigned to the fungal triterpene, sulphurenic acid, from Polyporus sulphureus[142]; the methyl ester prepared from a sample of this acid[150] was compared with L/3 and shown to be identical from comparison of physical properties and mixed melting point.

The fourth metabolite of the Lenzites striata mycelium extract, L/4, had a parent ion at m/e 530 and was assigned the partial structure (157) on the basis of the evidence discussed below. The presence of an α,β-unsaturated ester in the side-chain was deduced from the n.m.r. (p.116) singlet (6.39 τ), the u.v. absorption at λ max 215 n.m. (E=20,900), and also by comparison with methyl tyromycate isolated from Leptoporus stipticus (see later), and assigned the structure (158). Thus the i.r. absorption for L/4 at ν max 1707 $cm.^{-1}$ (E=864) compared well with that of the carbomethoxy grouping in methyl tyromycate (ν max 1706 $cm.^{-1}$, E=746). The presence of the vinyl methyl group in L/4, observed as a singlet at 8.22 τ (3H) located the double bond


L/4
3
4
5
6
7
8
9
10
τ

as $\Delta^{24(25)}$. The stereochemistry of this double bond was deduced to be the opposite of that in methyl tyromycate by comparison of the τ values of the vinyl protons; these were 4.00 τ in methyl tyromycate and 3.30 τ in L/4 suggesting that in the latter the vinyl proton and carbomethoxy grouping were cis to each other. A similar comparison has been made in a variety of systems including the simple tiglate and angelate esters (159) and the triterpene esters, methyl mangiferolate (160) (3.37 τ) and its trans isomer, methyl masticadienote (4.19 τ)[151].

The presence of an additional carbomethoxy grouping in L/4 was inferred from the second n.m.r. 3H singlet at 6.46 τ and the i.r. absorption at ν max 1723 cm.$^{-1}$ (E=889). This group was assigned to C-2 0 from consideration of the methyl contour region of the n.m.r. spectrum, which consisted of five methyl singlets integrating for fifteen protons; the absence of a doublet for a C-2.0 methyl substituent thus indicated this position for the carbomethoxy grouping. Confirmation of the gross structure of the side chain in L/4 was also obtained from a comparison of the mass spectra of this compound and methyl sulphurenate (L/3). The latter showed an ion fragment at m/e 311 attributed to a loss of the total side chain $C_{10}H_{17}O_2$ by the process indicated (161);

the corresponding ion for L/4 at m/e 311 was in agreement with loss of a side chain, $C_{10}H_{15}O_4$, by an analagous process.

The mass spectra of L/3 and L/4 were also very similar in the groupings below m/e 200; previously observed for other triterpenes from L. Stipticus and D.quercina, these appear to be characteristic of the lanostane skeleton. Both L/3 and L/4 also showed successive initial losses of 15 and 18 (2x) mass units, suggesting that the latter also possessed two hydroxylic groupings; these were also inferred from the i.r. absorption at νmax 3420 $cm.^{-1}$ and the n.m.r. 1H multiplets at 6.77 and 5.80 τ . The former, with half-width ca. 8 c.p.s., was taken to indicate the presence of a C-3 β-hydroxyl group.

Jones oxidation of L/4 was carried out under the same conditions described for methyl sulphurenate; again t.l.c. showed the presence of two less-polar compounds during the oxidation, and of only one compound when the reaction had gone to completion. This compound had molecular formula $C_{32}H_{46}O_6$ and parent ion at m/e 526 i.e. 4 mass units below that of L/4. Since no aldehydic protons were evident in the n.m.r. spectrum, the hydroxyl groupings of L/4 were confirmed as secondary. The carbonyl region of the i.r. spectrum of L/4 diketone showed two bands of approximately equal intensity:

one of these, at νmax 1711 cm.$^{-1}$ (E=951) was assigned to the C-27 α,β-unsaturated carbonyl group plus a ring-A carbonyl group, and the other, at νmax 1736 cm.$^{-1}$ (E=1082), to the C-20 ester carbonyl group plus a ring-D carbonyl group. The shift of the C-20 ester carbonyl frequency from νmax 1723 cm.$^{-1}$ in L/4 to ν max 1736 cm.$^{-1}$ in the diketone was in keeping with the removal of intra-molecular hydrogen-bonding on oxidation of L/4 to its diketone. Such hydrogen-bonding, between C-20 carbonyl and ring-D hydroxyl functions, could only occur if L/4 were a 16 β-hydroxy triterpene (162); in 16 α -hydroxy analogues such as methyl polyporenate C (163), where no such bonding is possible, the carbomethoxy ester carbonyl group appeared at νmax 1736 cm.$^{-1}$.

Further evidence for a C-16 rather than a C-15 hydroxyl grouping was obtained from a comparison of the values of saturated methyl groups in methyl eburicoate, methyl sulphurenate, and L/4 (Table 21). This showed that the C-18 and C-32 methyl chemical shifts in methyl eburicoate and L/4 were virtually identical and considerably different from those for methyl sulphurenate, which were deshielded. This deshielding of C-18 methyl groups is a common feature of 15-hydroxy steroids[152] and extends to C-32 methyl groups in the triterpenes[153]; as expected the deshielding is greater

for 15 β-hydroxy than for 15 α-hydroxy groups, although it is still significant for the latter. The lack of deshielding at C-18 and C-32 in L/4 thus suggested that the ring D-hydroxyl grouping was located at C-16 rather than at C-15.

DISCUSSION II

It has long been realised in the field of natural product chemistry that the metabolites produced by any particular source are subject to changes in their chemical nature as soon as that source is removed from its environment, be it natural or artificially controlled. A good example, with respect to fungi, of the wisdom of examining freshly harvested cultures is ergosterol peroxide (164): although a reported metabolite of several different fungi[154-157], this compound could only be detected in extracts of sporophores of Piptoporus betulinus or of Daedalea quercina which had been exposed to daylight for several days.[158] Subsequently, it was demonstrated that ergosterol could be converted to its peroxide in presence of oxygen, light and photosensitizing substances commonly present in fungal extracts.

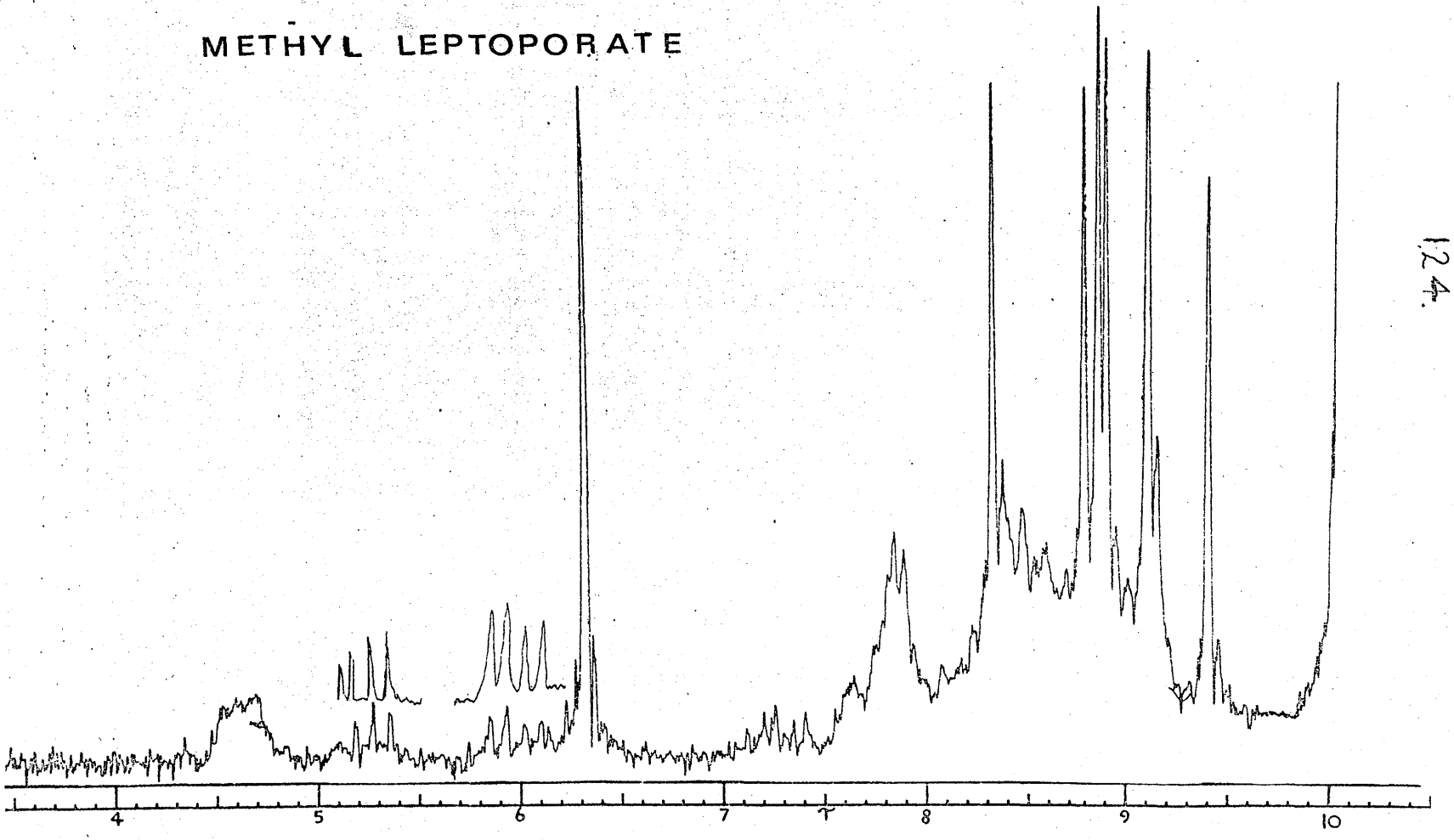
Methods used in extraction may also be a cause of artefact formation; Ourisson et. al.[159] have noted that, in a large number of studies on both plant and fungal triterpenes, the natural extract was subjected to initial saponification by strong alkali, such treatment producing marked changes in the substituents present; ester groups, in particular could have been hydrolysed. Recent re-examination of Piptoporus betulinus showed that polyporenic

acid A occurs as various esters other than as the free acid as previously reported using base during the extraction (cf. p. 98). A common procedure in this laboratory is the treatment of the crude triterpenoid acid mixture with ethereal diazomethane prior to separation of the constituents; the mixture of methyl esters thus formed is more readily separated into individual metabolites by column chromatography. We have now found two cases where this process results in the formation of fungal artefacts, products of the well-known addition of diazomethane to an α,β — unsaturated carbonyl system.[160]

The first of these adducts was isolated from Leptoporus stipticus[147] ; this fungus, the source of some interesting triterpene glycosides, also furnishes two less polar metabolites, methyl tyromycate and methyl leptoporate. Methyl tyromycate was assigned a structure (165), which was in perfect agreement with analytical and spectroscopic data for both the metabolite and its derivatives. A structure (166), assigned to methyl leptoporate proved less satisfactory however, in view of recurrent analytical difficulties. Re-examination of this metabolite showed that it in fact contained nitrogen and it was re-assigned

a molecular formula $C_{32}H_{48}O_3N_2$, with parent ion at m/e 508. On the basis of the following evidence, structure (167) was assigned to methyl leptoporate. The u.v. spectrum (227, 235, 243, 252 n.m.), and n.m.r. 2H multiplet at 4.60 τ (p.124) indicated the presence of a simple lanosta -7,9 (11) -diene chromophore, while the i.r. spectrum confirmed that the molecule possessed a saturated ester (υ max 1737 $cm.^{-1}$, E = 444) and a 6-membered ring ketone (υ max 1704 $cm.^{-1}$, E = 479). The loss in the mass spectrum of 59 mass units suggested that the ester function was present as a carbomethoxyl group, as did the singlet at 6.25 τ in the n.m.r. spectrum.

The presence of a pyrazoline system in methyl leptoporate was inferred both from the loss of 28 mass units from the parent ion and the appearance in the i.r. of weak absorption at 1556 $cm.^{-1}$ typical of the -N = N- system and observed for the pyrazolines of tiglic and angelic acids[161]; the presence in the u.v. spectrum of weak absorption at λ max 324 nm(E=310) was also attributed to the -N = N- chromophore. Additional evidence for the pyrazoline structure was obtained from the n.m.r. spectrum, which showed an ABX system. τ_A 5.2, τ_B 5.96, τ_X 8.09, J_{AB} = 17, $J_{AX} = J_{BX}$ = 8 c.p.s.: irradiation at 8.09 τ reduced the


METHYL LEPTOPORATE
4
5
6
7
τ
8
9
10

low-field signals essentially to a quartet. The deshielded methyl group which appeared as a sharp singlet at 8.34 τ was assigned to the carbon geminal to the -N=N-group and bearing the carbomethoxy grouping; this is in close agreement with the reported chemical shift value(8.38 τ) of the similarly located C-3 methyl group of cis -3,5-dimethyl-3-carbomethoxy- Δ'-pyrazoline[152] (168).

It may be deduced that methyl leptoporate arose by addition of diazomethane to the αβ - unsaturated ester, methyl tyromycate, or its geometrical isomer. In order to test this, methyl tyromycate was treated with diazomethane under the same conditions employed for the methylation of the crude terpenoid extract. By this method, methyl leptoporate was obtained in 58% yield, identical in every respect with the material obtained originally by column chromatography of the crude L. Stipticus extract.

This result prompted a re-examination of the structure of another metabolite, which had previously been isolated from Daedalea quercina by a mild non-hydrolytic isolation procedure. This mould produces a variety of triterpenes first isolated by Campbell [163] and examined in some detail by Adam [147] ; a number of these, including the previously mentioned carboxyacetylquercinic acid (169), possess

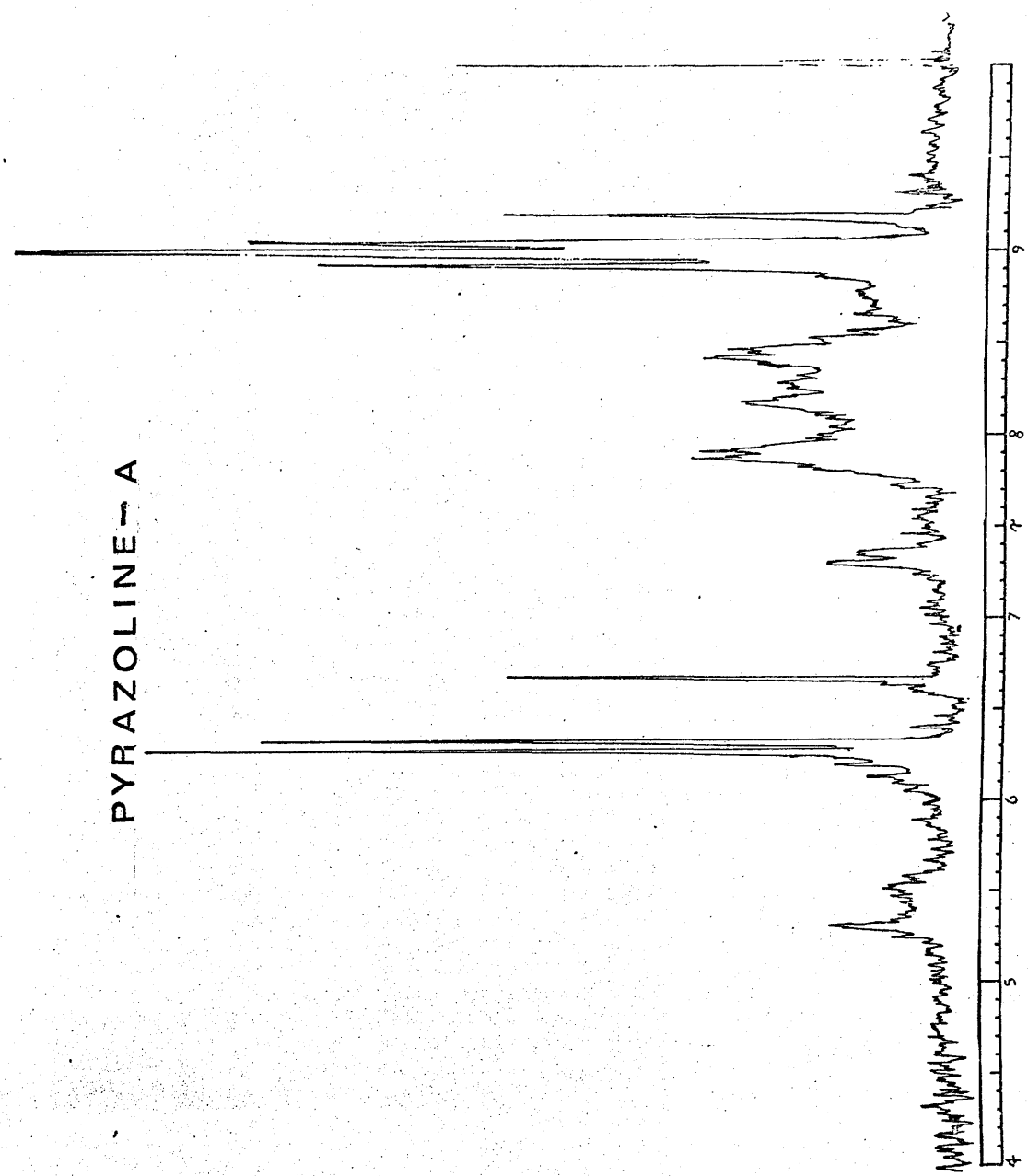
the formerly unknown structural features of a malonate ester and 23-keto function together with carboxyl group at C_{25}. The 3α malonate grouping was also recognised as a structural feature of a metabolite, code name DQ7, assigned a formula $C_{37}H_{56}O_7$ from high resolution mass spectrometry, although no satisfactory elemental analysis in agreement with this could be obtained; a tentative structure (170) was also proposed on the basis of available physical evidence, the molecular formula for which, $C_{37}H_{56}O_9$ was in better agreement with analytical findings, although still not precisely so.

In an attempt to obtain a sufficient supply of this metabolite for re-examination, fresh sporophores of Daedalea quercina were extracted in the usual manner, treated with excess ethereal diazomethane and the triterpenes separated by column chromatography. The fraction which corresponded in R_f to D.Q.7 could not be crystallised as previously reported, despite repeated purification by p.l.c.; when the compound was examined on a t.l.c. plate impregnated with silver nitrate, it appeared as two distinct spots of close R_f. These two compounds were separated by further column chromatography on silicic acid/silver nitrate, and were obtained

as highly crystalline compounds, for which elemental analysis gave a molecular formula, $C_{37}H_{56}O_7N_2$, in both cases. The mass spectra of these compounds were identical and showed the highest molecular ion at m/e 612, i.e. 28 mass units below the expected parent ion. This, and the i.r. absorption at 1550 $cm.^{-1}$, again suggested the presence in both compounds of a pyrazoline structure from which elemental nitrogen was readily lost. Weak absorption in the u.v. at λ_{max} 330 nm also supported this conclusion. Code name pyrazoline-A was given to the compound of R_f 0.44 and pyrazoline-B to the compound of R_f 0.42.

The presence in both compounds of a carbomethoxyacetate grouping was evident from the n.m.r. signals (p.128) at 5.28τ (1H, multiplet), 6.25τ, (3H, singlet) and 6.64τ (2H, singlet) as well as the i.r. peaks at ν max 1755 $cm.^{-1}$ and 1737 $cm.^{-1}$; this was confirmed by the loss of 118 mass units in the mass spectrum. Additional i.r. absorption at νmax 1737 and in the region ν1716 $cm.^{-1}$ was ascribed to a methyl ester and ketone respectively, the methoxyl group of the former resonating as a 3H singlet at 6.3τ in the n.m.r.

All these features were also present in the methyl ester of carboxyacetylquercinic acid, for which the structure (169) had been rigorously proven. It thus seemed reasonable to


PYRAZOLINE- A
4
5
6
7
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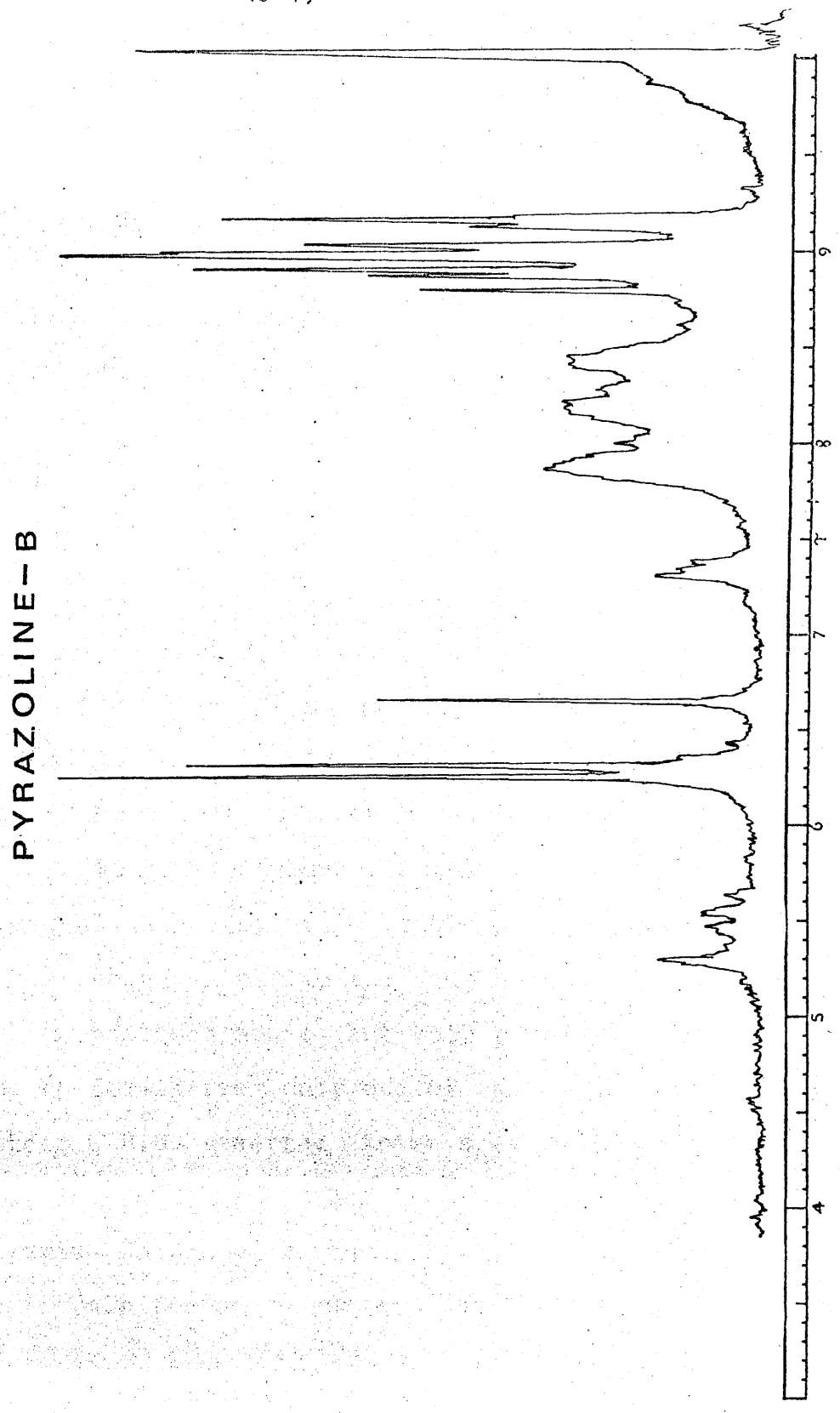
9


PYRAZOLINE-B
4
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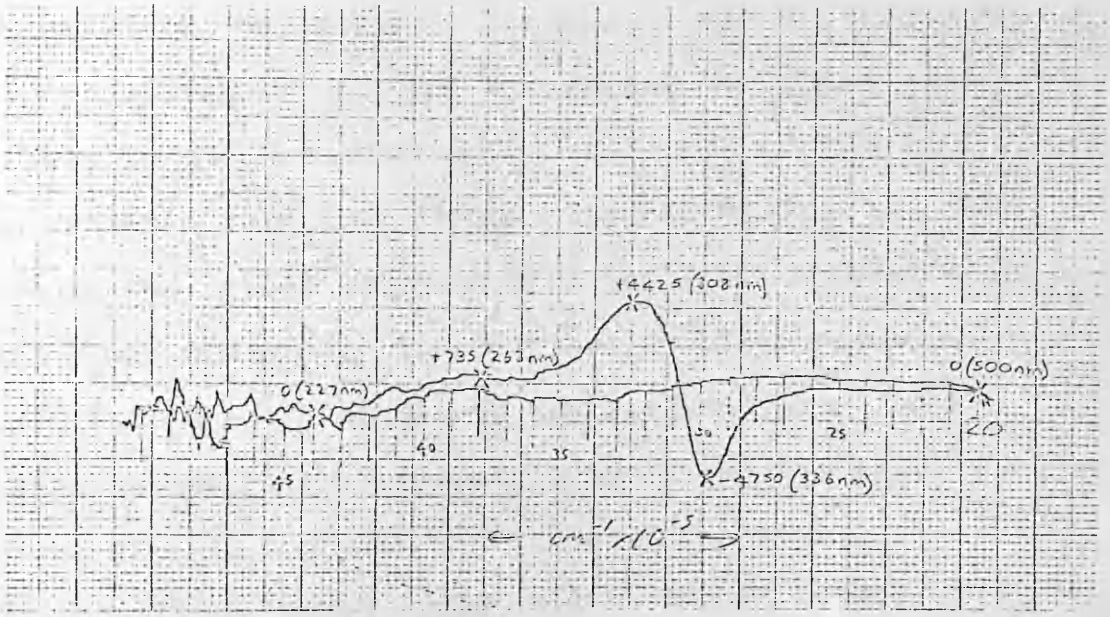
τ

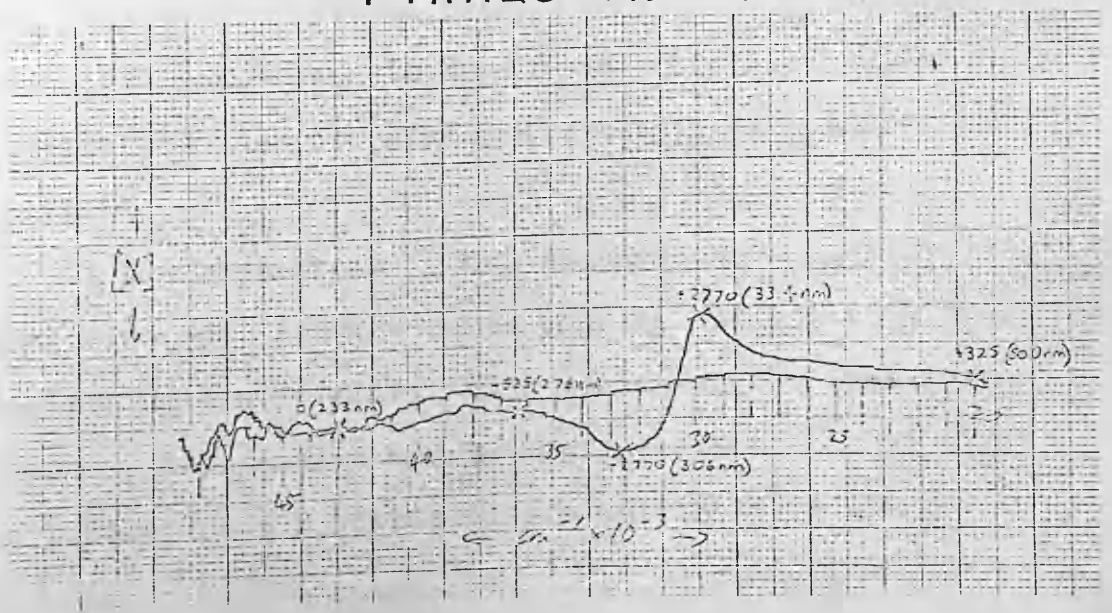
assume that the pyrazolines-A and B had been formed by diazomethane addition to a dehydro-analogue of carboxyacetylquercinic acid. That this addition had occurred in the side-chain of the triterpene was evident from a comparison of the mass spectral fragmentations of methyl carboxyacetylquercinate and the pyrazolines: thus, in methyl carboxyacetylquercinate, after preliminary elision from the parent ion of an angular methyl group ($M^+ - 15$), the resulting ion thenlost 158 mass units, equivalent to $C_8H_{14}O_3$ and rationalised as indicated (171). In the pyrazolines, however, the highest ion of the mass spectrum at m/e 612 ($M^+ - 28$) underwent loss of 15 followed by 170 mass units, this latter corresponding to $C_9H_{14}O_3$ i.e. 12 mass units more than the corresponding loss from methyl carboxyacetylquercinate.

Three possible α,β unsaturated carbonyl systems 172, 173, 174 could be envisaged in the side chain of methyl carboxyacetyl-dehydroquercinate, but both pyrazolines were considered to be formed from only one of these structures because of their O.R.D. spectra: these spectra were in fact mirror images of each other (p.131), pyrazoline-A showing a negative Cotton curve and pyrazoline-B a positive Cotton curve. This tended to suggest that the pyrazolines

PYRAZOLINE – A



PYRAZOLINE – B



were stereoisomers Of the the three possible unsaturated carbonyl systems 172 could be immediately eliminated by comparison of the n.m.r. spectra of methyl carboxyacetylquercinate and pyrazoline - B: two methyl doublets in the former at 8.76, 8.82 τ and 8.82, 8.91 τ were assigned to methyl groups attached to C-24 and C-25, while in the latter only one such low field doublet (8.78, 8.86 τ) was observed; in addition, double irradiation showed that the 2H signal in the region 5.50 τ, (not present in methyl carboxyacetylquercinate) was coupled to protons at ~7.30 and ~8.20 τ, indicating a grouping $-CH_2.CH_2.N=N-$, which could only be formed by addition of diazomethane to the α,β unsaturated systems (173) or (174) Although the adducts (175) and (176) would not be distinguishable from their physical properties, the α,β unsaturated ester (173) would seem to be the more "biogenetically satisfactory" dehydro analogue of methyl carboxyacetylquercinate.

It has been shown[164] that camphor- 3S - spiro - 3'-Δ^1-pyrazoline (177) and the corresponding alcohol both show negative Cotton effects; this would suggest that pyrazoline — A possesses the S configuration at C-25 (178) and pyrazoline - B with positive Cotton curve the R.

configuration (179). This result could also be deduced by application of an "inverse octant rule" to the -N=N- chromophore, as has been demonstrated for cyclopentenones compared to cyclohexenones.[165]

Table 20.

τ values of H at C_3

3β-alcohols		3α-alcohols	
Methyl eburicoate	6.78	methyl polyporenate A	6.63
Methyl dehydrotumulosate	6.75	methyl dihydropolyporenate A	6.65
24-dihydrolanosterol	6.78	methyl 3-epidehydrotumulosate	6.60
-lanosterol	6.75	methyl isopolyporenate A	6.63

Table 21.

	C_{19}	C_{31}	C_{32}	C_{30}	C_{18}
Methyl eburicoate	9.01	9.04	9.12	9.20	9.27
L/4	9.04	9.05	9.10	9.20	9.26
Methyl sulphurenate	9.00	9.04	9.01	9.20	9.22

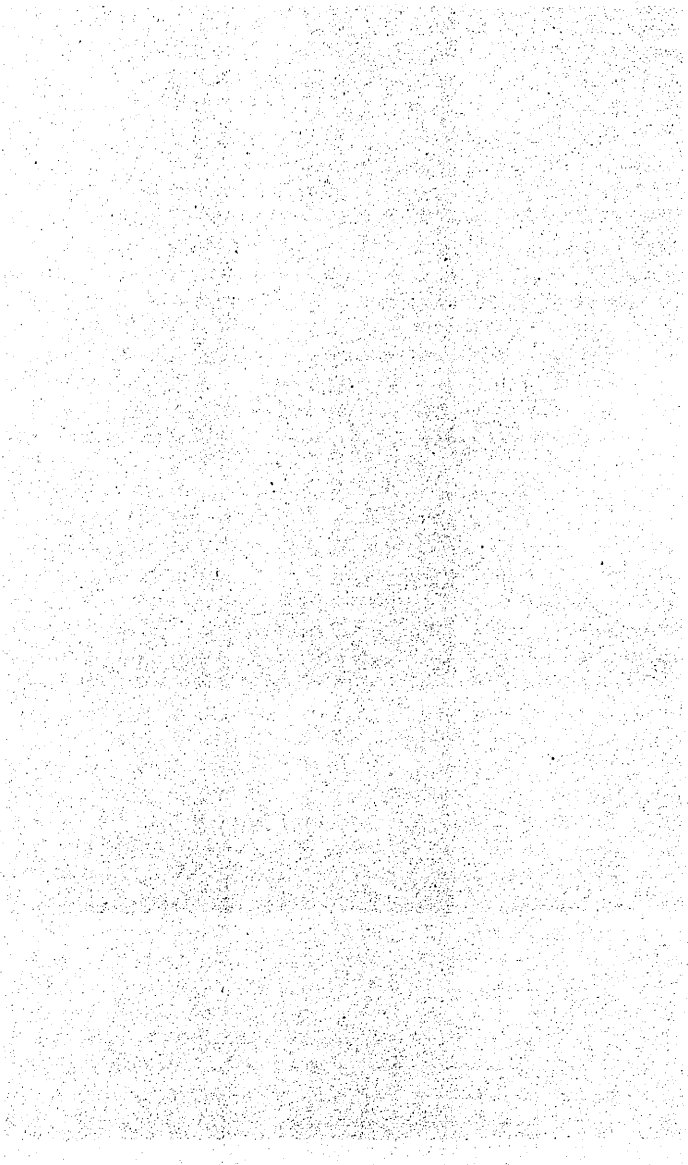


Table 17.

CODE	R_f	CERIC AMM. SULPH./ H_2SO_4
L/1	0.8 ($CHCl_3$)	BROWN.
L/2	0.3 (")	BROWN.
L/3	0.1 (")	BROWN.
L/4	0.4 (97%$CHCl_3$/ 3%CH_3OH)	BROWN.

Table 18.

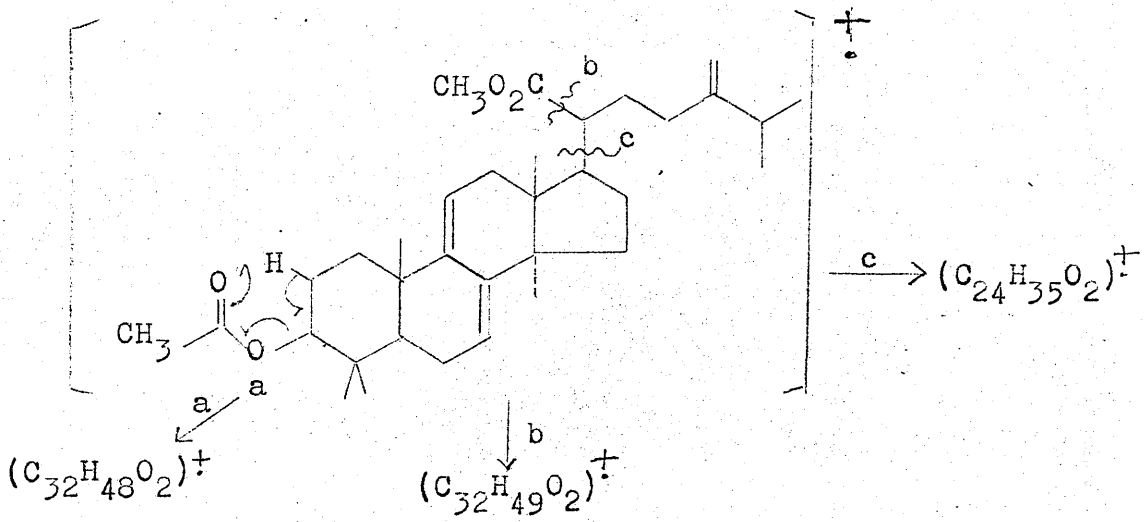
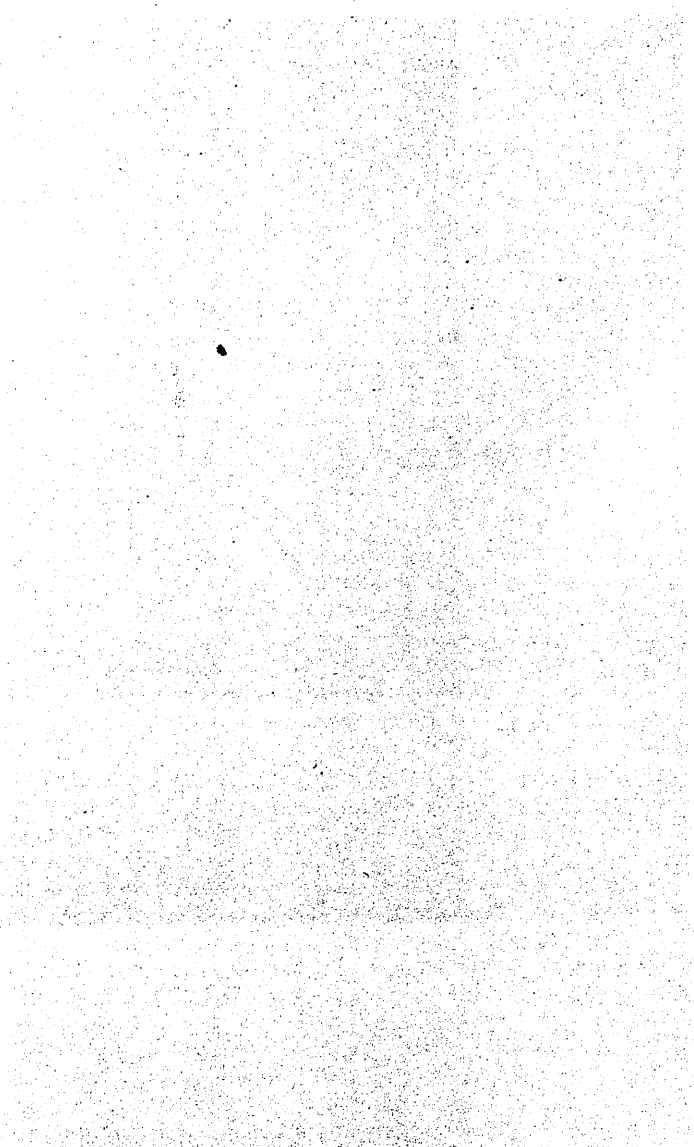


Table 19.

	C_{26}, C_{27}	C_{19}	C_{30}, C_{31}, C_{32}	C_{18}
3 -acetoxymethyleburicioate	9.00	9.00	9.12	9.27
L/1.	8.95, 9.03 (d)	9.08	9.13	9.41



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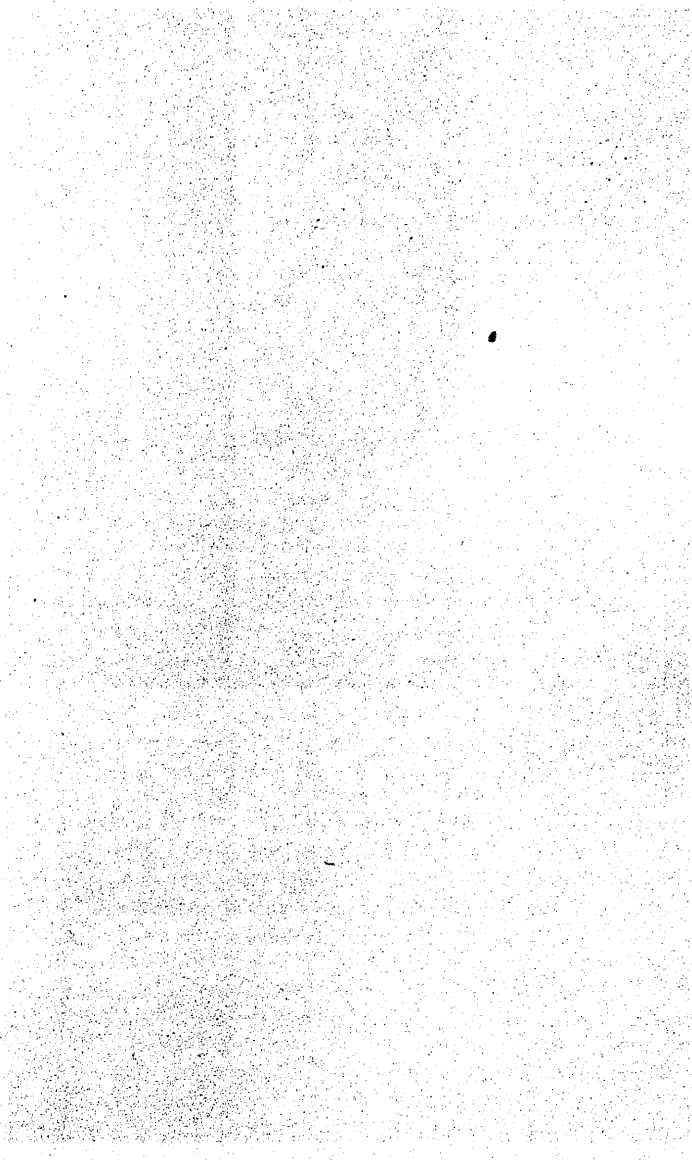

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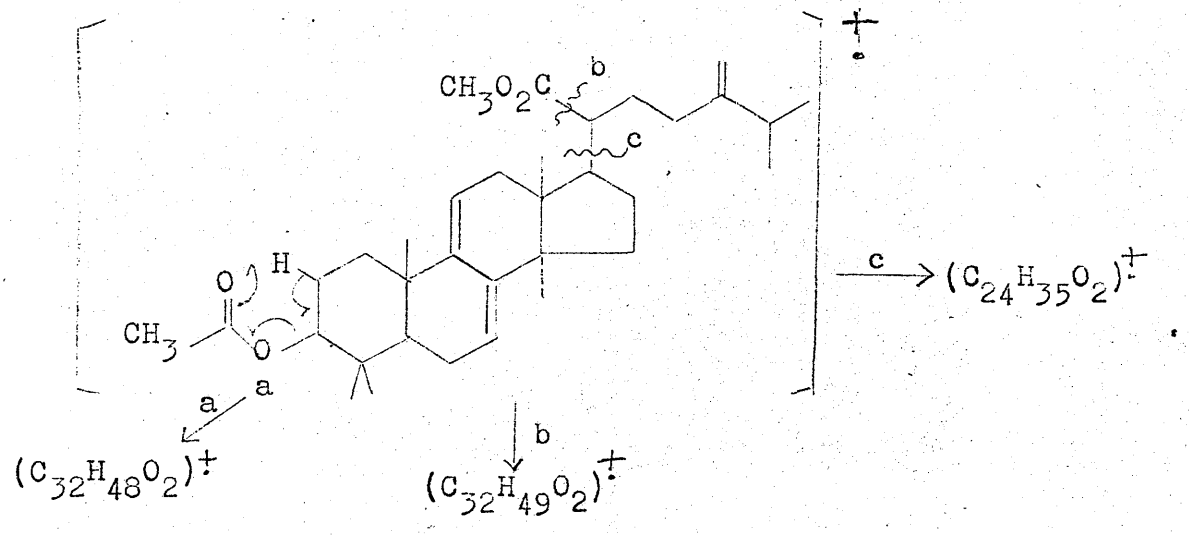

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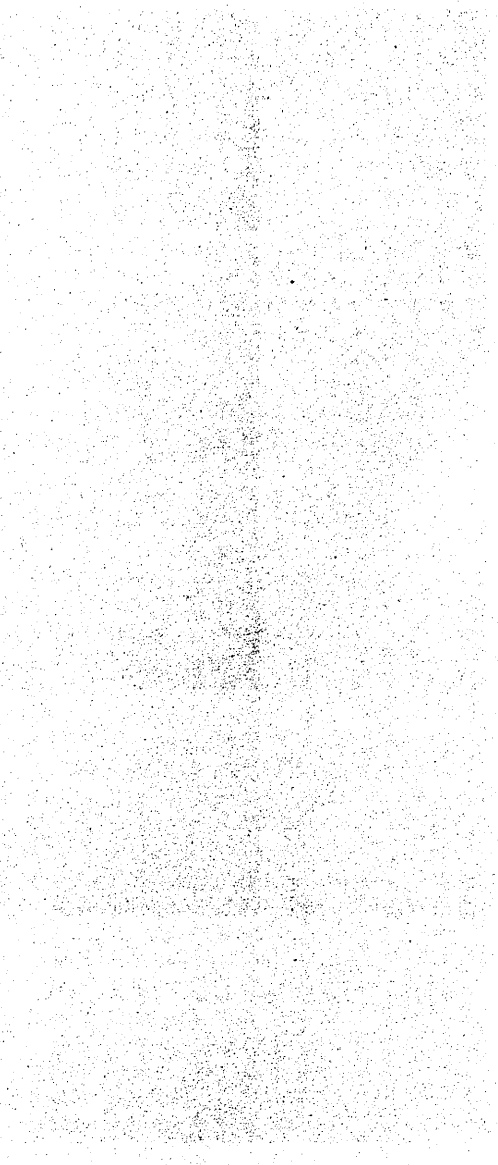
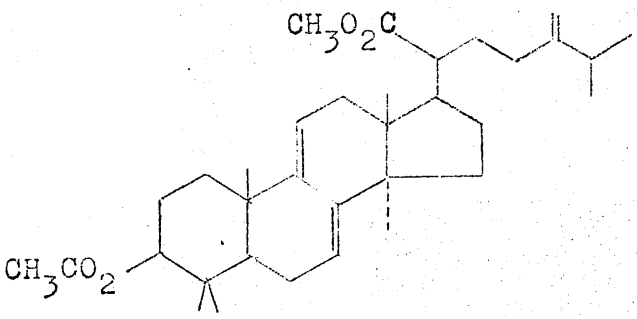
L/1.	8.95, 9.03 (d)	9.08	9.13	9.41





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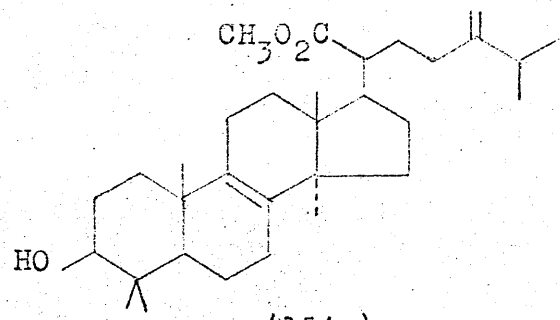

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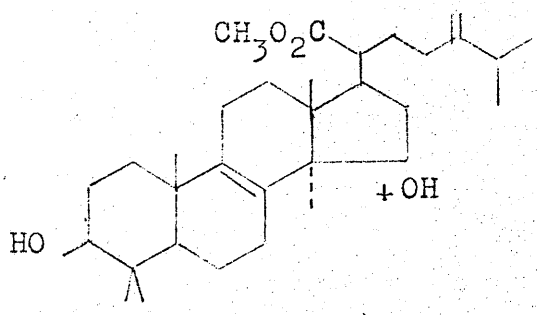

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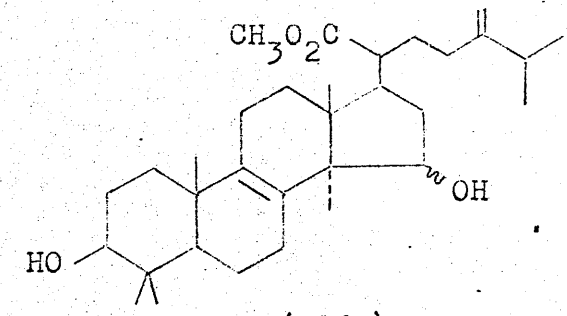

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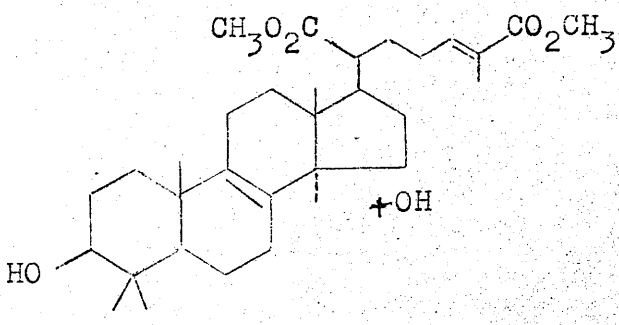

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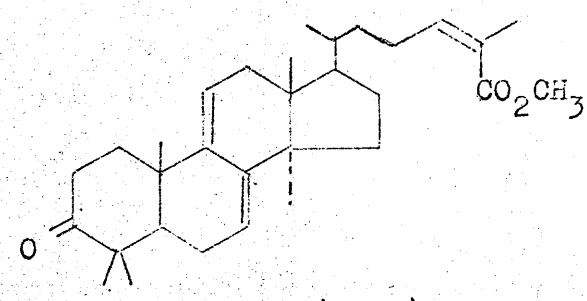
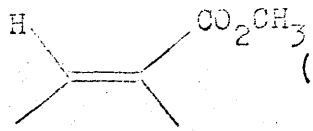


(158.)

3.27 τ



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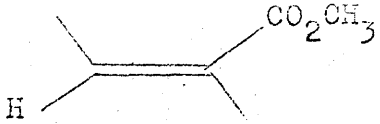
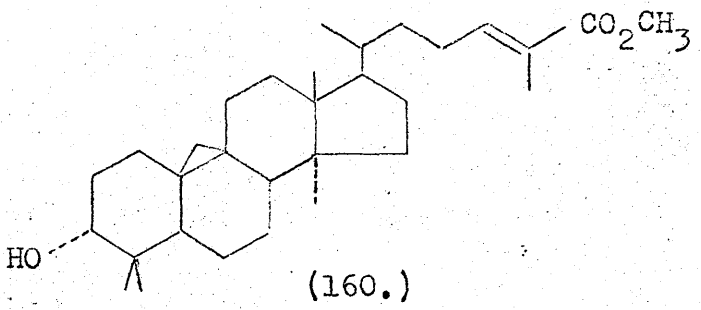


4.12 τ



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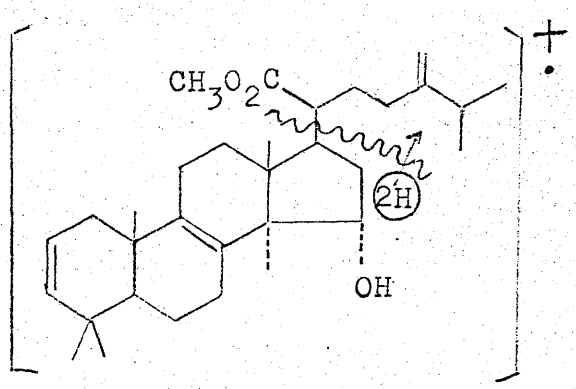

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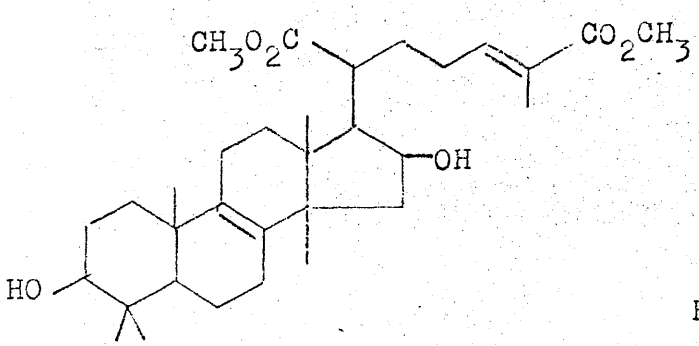
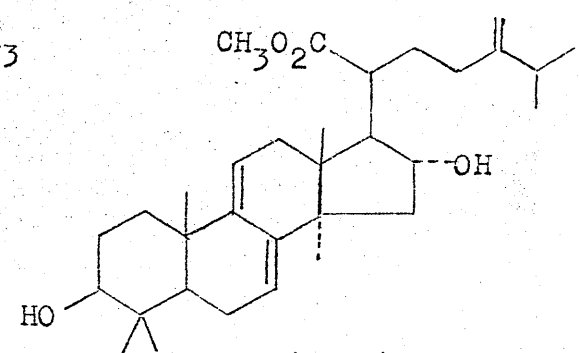
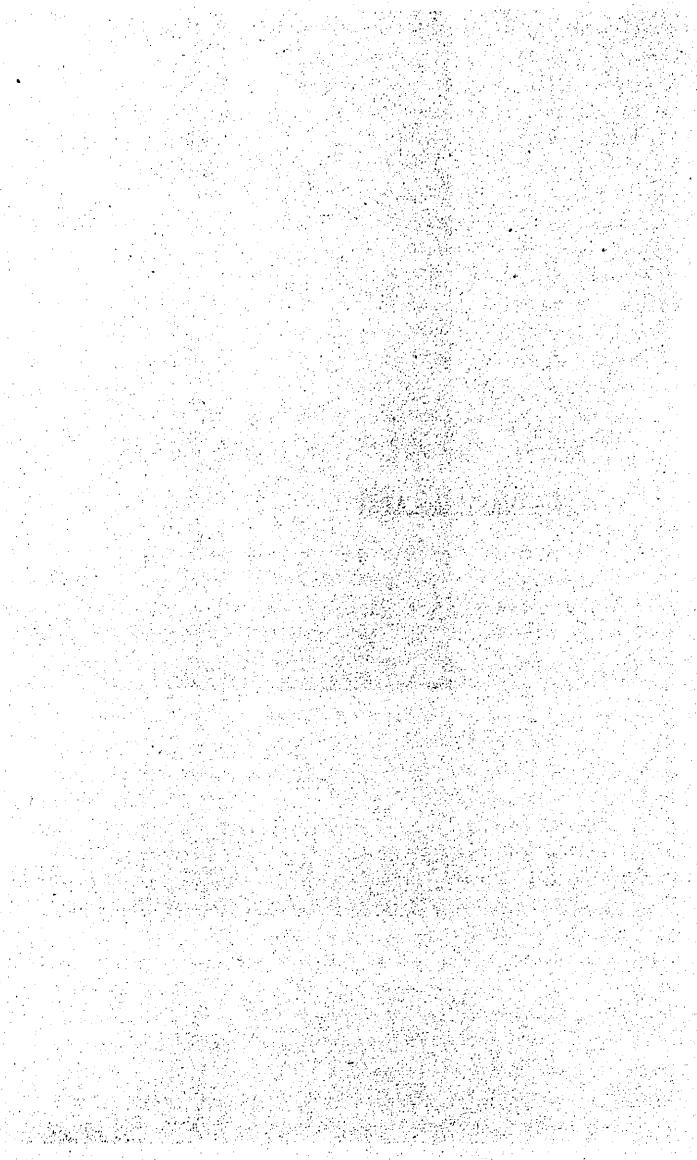
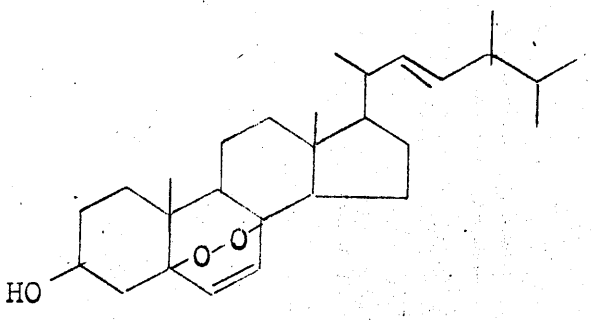


(162.)

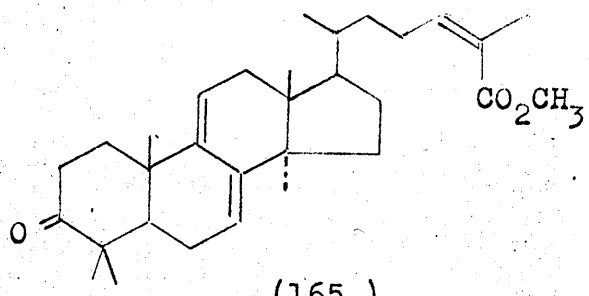


(163.)

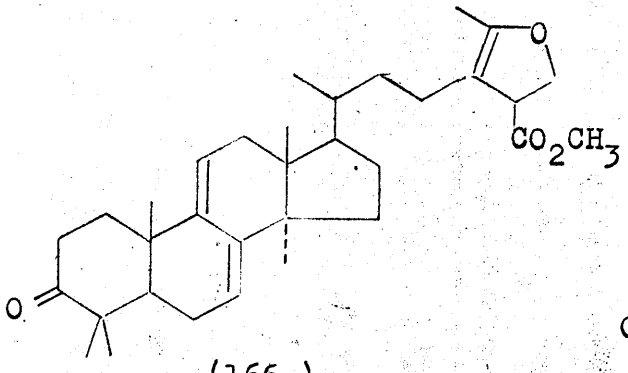




(164.)



(165.)



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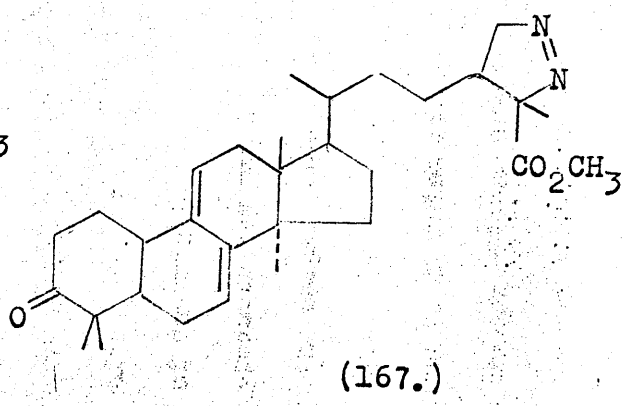
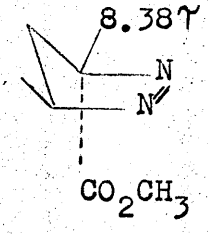
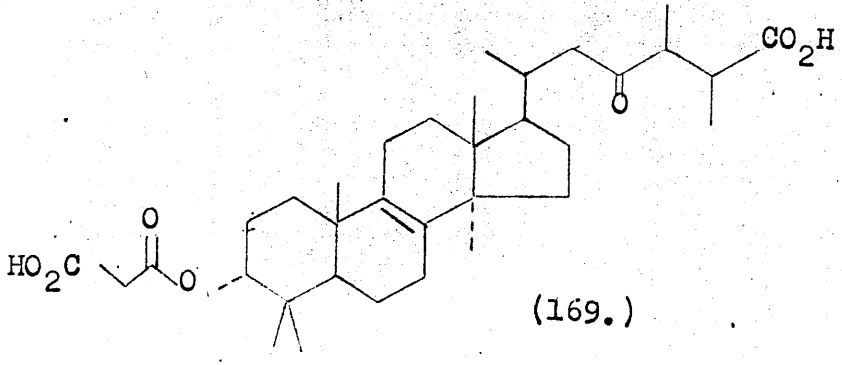


(167.)



(168.)



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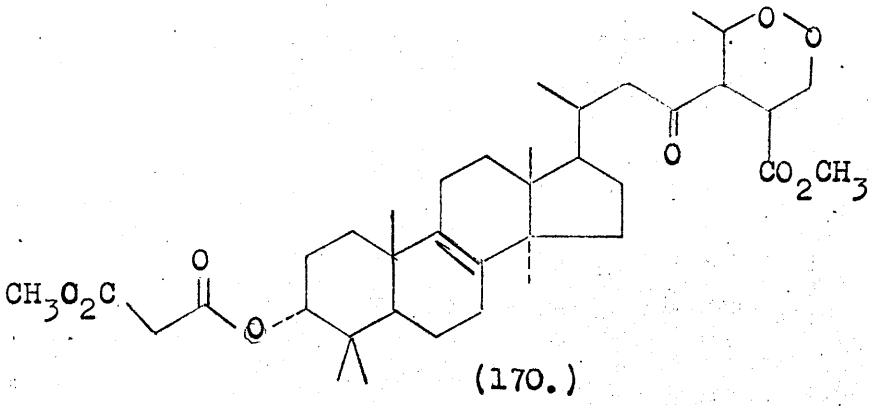
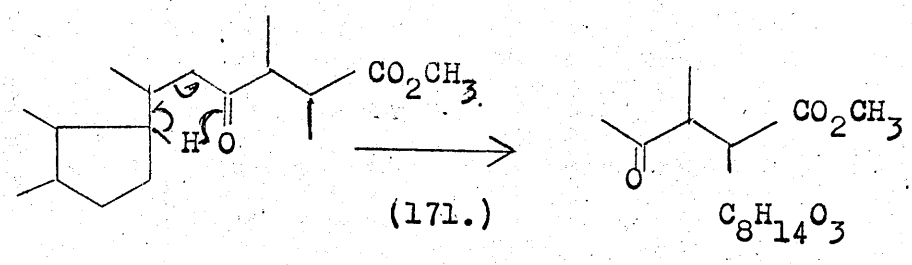
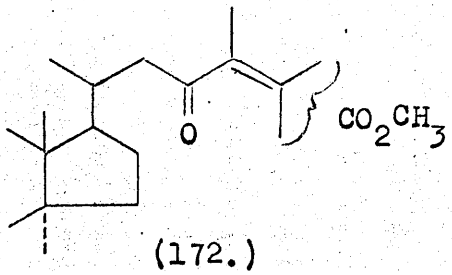


(170.)

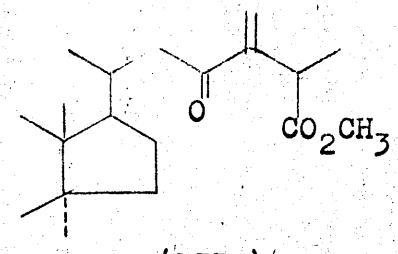


(171.)

$C_8H_{14}O_3$



(172.)



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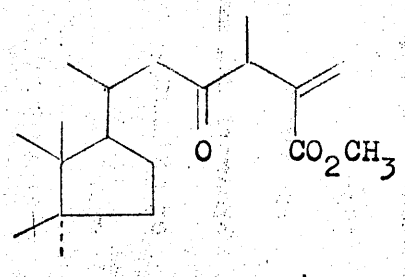
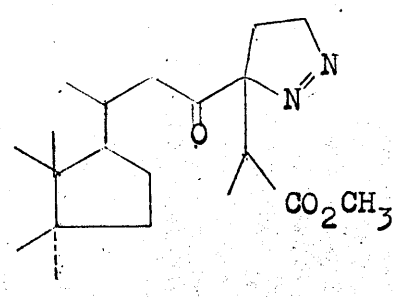
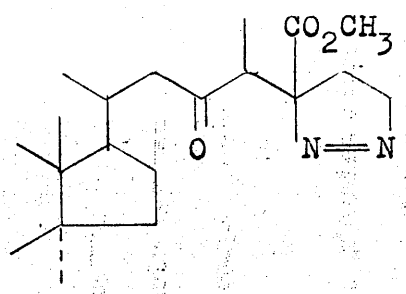


(174.)



(175.)



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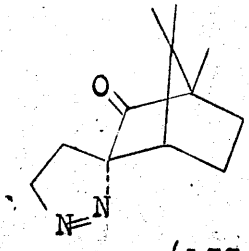
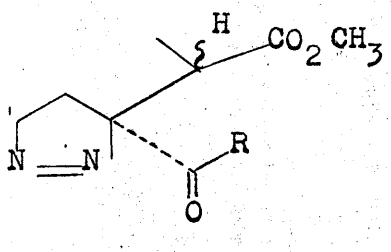
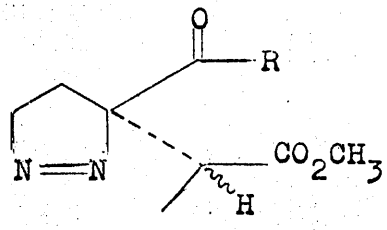


(177.)



(178.)



(179.)

R = Triterpene Skeleton.

EXPERIMENTAL.

(1) Separation of the mycelial metabolites of Lenzites striata.

The crude cold methanol extract (26.8 g.) of the mycelium (p.71), obtained after removal of the light petroleum soluble material was treated overnight with an excess of ethereal diazomethane. The oil thus obtained (26.1g.) was adsorbed onto silica gel (30 g.) and placed on top of a column of silica gel (900 g.) packed in 5% ethyl acetate/ 95% light petroleum. Fractions (200 ml.) from the column were collected at the rate of three per hour while the polarity of the eluting solvent was increased to 100% ethyl acetate. T.l.c. analysis of the fractions thus obtained allowed the groupings indicated in the table below:

Code	Fractions	WT.(g.)
L/1	12 -16	0.065
L/2	23 -46	1.614
L/3	55 -80	1.618
L/4	85 -93	0.132

(2) The compound L/1: methyl acetyldehydroeburicoate.

Isolated as described above, this compound crystallised from methanol as colourless needles, m.p. 159 - 161°C.

R_f ($CHCl_3$) 0.8. $(\alpha)_D$ ($CHCl_3$; C, 1.35) + 104°

(Found : C, 77.69%; H, 10.21%; M^+ at m/e 524:
calculated for $C_{34}H_{52}O_4$: C, 77.82%; H, 9.99%; m.w. 524.8).

υ max (KCl) : 3070, 3026, 2952, 2870, 2836, 1734, (ester carbonyl), 1634 (C = C), 1452, 1430, 1387, 1374, 1362, 1271, 1249 (acetate), 1181, 1083, 1068, 1027, 978, 882 (C = CH_2), and 800 $cm.^{-1}$

$\tilde{\nu}$ max ($CHCl_3$) : 1734 (E = 1052) $cm.^{-1}$

λ max (EtOH) : 236 (E = 8,917), 243 (E = 10,000), 252 (E = 7,214) nm.

τ (60 Mc./s.; $CDCl_3$) : 4.60 (2H, m, - C<u>H</u> = C - C = C<u>H</u> -); 5.32 (2H, d, J = 6 c.p.s. C = C<u>H</u>$_2$); 5.55 (1H, m, - C<u>H</u> - OAc); 6.33 (3H, S, - $CO_2C\underline{H}_3$); 7.98 (3H, S, $C\underline{H}_3.CO_2$ -); methyl contour 8.95, 9.03, 9.08, 9.13, 9.41.

m/e : 524 (100%), 511 (17%), 509 (17%), 465 (17%), 464 (25%), 448 (34%), 355 (25%), 353 (75%), 339 (19%), 313 (34%), 314 (34%), 295 (29%), 279 (25%), 252 (79%), 240 (46%), 225 (42%), 211 (29%), 185 (46%), 171 (54%), 159 (58%), 157 (54%), 145 (63%), 135 (68%), 133 (63%), 123 (79%), 121 (83%), 119 (83%), 111 (68%), 109 (83%), 107 (83%), 105 (79%), 93 (79%), 83 (75%).

(3) The compound L/2 : methyl eburicoate - methyl dehydroeburicoate.

Isolated as described (section 1) this compound crystallised from methanol as colourless needles, m.p. 133 - 135°C. This was identified as methyl eburicoate by comparison of the following data with that of authentic material.

R_f ($CHCl_3$) 0.3.

ν max (KBr) : 3470 — 3340 (hydrogen-bonded hydroxyl), 2960, 1733 (ester carbonyl), 1640 (C = C), 1450, 1430, 1375, 1262, 1160, 1062, 1030, 885 ($>C = CH_2$) $cm.^{-1}$

τ (100 Mc./s.; $CDCl_3$) : 4.60 (< 1H, m, -CH = C - C = CH -); 5.31 (2H, d, J = 8 c.p.s., $>C= CH_2$), 6.35, 6.37 (3H,S,- CO_2CH_3) 6.80 (> 1H, m, - CH - OH); methyl contour 8.76, 8.98, 9.01, 9.04, 9.12, 9.20, 9.27, 9.42.

(4) The compound L/3 : methyl sulphurenate.

Isolated as above (section 1), this compound crystallised from methanol as colourless needles, m.p. 167.5 - 168°C.

R_f ($CHCl_3$) 0.1; $(\alpha)_D$ ($CHCl_3$; C, 0.83) + 94°

(Found : C, 76.46%; H, 10.35%, after 4 hours at 120°C. in vacuo; M^+ at m/e 500: calculated for $C_{32}H_{52}O_4$; C, 76.75%; H, 10.47%; m.w. 500.8)

ν_{max} (KBr) : 3,555 (sharp); 3370 (broad; hydroxyl) 2960, 2935, 1733 (ester carbonyl), 1640 (C = C), 1450, 1375, 1356, 1285, 1273, 1200, 1146, 1093, 1045, 1023, 995, 885 ($>C = C\underline{H}_2$) $cm.^{-1}$

ν_{max} ($CHCl_3$) : 1732 (E = 393) $cm.^{-1}$ (ester carbonyl).

τ(100 Mc./s.; $CDCl_3$) : 5.30 (2H, d, J = 10 c.p.s., $>C=C\underline{H}_2$) 5.73 (1H, m, C<u>H</u>OH, ring D), 6.75 (1H, m, C<u>H</u>OH; ring A), 6.34 (3H, S, $-CO_2C\underline{H}_3$), methyl contour 8.94, 8.97 (d), 9.00, 9.01, 9.04, 9.20, 9.22.

m/e : 500 (54%), 498 (13%), 486 (49%), 485 (30%), 482 (22%), 467 (65%), 453 (49%), 449 (41%), 435 (38%), 311 (19%), 298 (22%), 289 (27%), 279 (35%), 271 (16%), 259 (24%), 241 (24%), 229 (22%), 215 (24.), 211 (19%), 201 (24%), 199 (24%), 187 (18%), 185 (35%), 173 (44%), 171 (35%), 161 (30%), 159 (46%), 157 (38%), 147 (38%), 145 (48%), 143 (32%), 135 (54%), 133 (49%), 131 (38%), 123 (32%), 121 (62%), 119 (73%), 109 (54%), 107 (66%), 105 (62%), 95 (70%), 93 (60%), 91 (46%), 81 (57%), 55 (100%).

M* for m/e 500 —— 485; m/e 485 —— 467; m/e 467 —— 449.

(5) Methyl ester of sulphurenic acid.

A solution of sulphurenic acid (19 mg.) in methanol (20 ml.) was treated with ethereal diazomethane, until the solution remained yellow after twenty minutes; evaporation of solvent gave a solid which crystallised from methanol as colourless needles (20 mg.) m.p. 165 - 166.5°C.

R_f ($CHCl_3$) 0.1.

This compound had identical t.l.c. properties, infra-red, n.m.r. absorption, and mass spectrum fragmentation to the compound L/3; the melting point of a mixture of the two was undepressed.

(6) Jones oxidation of methyl sulphurenate; bis-dehydro methyl sulphurenate.

(a) Jones reagent was added dropwise to an ice-cooled solution of L/3, (50 mg.), in acetone (4.0 ml.), and the progress of the reaction monitored by analytical t.l.c. At an incompletely oxidised stage of the reaction, two less polar compounds were observed by t.l.c. of R_f 0.2 and R_f 0.6; when oxidation was complete i.e. the original colour of the Jones reagent was not discharged, only the compound of R_f 0.6 remained. Thesolution was diluted with water (2 ml.) extracted into chloroform (15 ml.), washed with brine (2 x 3 ml.), water (2 x 3 ml.) and dried.

Evaporation of solvent gave the diketone as a white solid (32 mg., 64%), which crystallised from methanol as prisms, m.p. 139.5 - 140.5°C.

R_f ($CHCl_3$) 0.6.

(Found : C, 76.98%; H, 9.70%; M^+ at m/e 496:
calculated for $C_{32}H_{48}O_4$: C, 77.38%; H, 9.74%; m.w. 496.7)

νmax (KBr) : 3084, 2966, 2944, 2888, 1741, 1710, 1644 (C = C), 1461, 1439, 1388, 1347, 1306, 1222, 1192, 1155, 1120, 1077, 1038, 1008, 962, 933, 908, 892 (>C = CH_2) $cm.^{-1}$

νmax ($CHCl_3$) : 1736 (E = 964, ring D + ester carbonyl); 1706 (E = 502, ring A carbonyl) $cm.^{-1}$

τ (100 Mc./s.; $CDCl_3$) : 5.31 (2H, d, J = 11 c.p.s. >C= $C\underline{H}_2$), 6.33 (3H, S, $-CO_2C\underline{H}_3$), methyl contour 8.93, 8.97, 8.99, 9.06, 9.19.

m/e 496 (20%), 478 (32%), 464 (26%), 449 (13%), 435 (12%)
421 (8%), 419 (5%), 407 (7%), 403 (4%), 395 (13%)
382 (26%), 381 (29%), 367 (15%), 349 (10%), 335 (8%)
328 (15%), 327 (49%), 309 (60%), 295 (15%), 293 (20%)
285 (12%), 283 (15%), 271 (12%), 269 (15%), 258 (15%)
257 (30%), 255 (12%), 245 (15%), 241 (12%), 231 (18%)
229 (12%), 223 (13%), 215 (15%), 211 (15%), 209 (15%)
201 (14%), 199 (16%), 197 (18%), 187 (18%), 185 (20%)
183 (19%), 173 (24%), 171 (36%), 169 (24%), 161 (18%)

159 (34%), 157 (36%), 147 (23%), 145 (38%), 143 (40%) 135 (20%), 133 (26%), 131 (30%), 123 (20%), 121 (36%), 119 (48%), 109 (24%), 107 (40%), 105 (52%), 97 (24%), 95 (40%), 83 (34%), 81 (55%), 69 (80%), 67 (52%) 57 (46%), 55 (100%).

Literature m.p. 141 - 143°C.

ν_{max} (KBr) : 1740, 1710, 1640 $cm.^{-1}$

(b) Partial Jones oxidation of methyl sulphurenate; methyl 15-ketosulphurenate :

This compound, R_f 0.2, was obtained by repeating the Jones oxidation on L/2 (36 mg.) until t.l.c. showed a preponderance of the required product; the reaction mixture was treated as in (a) and the concentrated chloroform solution adsorbed onto one p.l.c. plate (20x20x0.5 m.m. Kieselgel HF_{254}), which was developed in chloroform. This method gave unreacted starting material (13 mg.), the diketo compound (1 mg.) , and the required keto-alcohol (14 mg., 39%) as a white solid which crystallised from methanol as colourless needles, m.p. 124 - 126°C., $R_f(CHCl_3)$ 0.2: O.R.D. (CH_3OH; c 0.875) ; $(\alpha)_{400}$ + 40°, $(\alpha)_{326}$ + 355°, $(\alpha)_{304}$ + 245°, $(\alpha)_{258}$ + 685°, $(\alpha)_{241}$ + 1375°.

(Found : C, 76.98%; H, 9.90%; M^+ at m/e 498:

calculated for $C_{32}H_{50}O_4$: C, 77.06%; H, 10.10%; m.w. 498.8

υ max (KBr) : 3474 (broad, hydroxyl), 2966, 2890, 1742 (ring D, carbonyl), 1717 (infl., ester carbonyl), 1648 (C = C), 1462, 1442, 1388, 1348, 1270, 1220, 1196, 1160, 1100, 1045, 893 (>C = CH_2) $cm.^{-1}$

υ max (CCl_4) : 1735 (E = 1032) $cm.^{-1}$ (ring D carbonyl + ester)

τ (100 Mc./s., $CDCl_3$) : 5.31 (2H, d, J = 10 c.p.s., >C= $C\underline{H}_2$); 6.33 (3H, S, $-CO_2C\underline{H}_3$); 6.80 (1H, m, - C<u>H</u>OH ring A), methyl contour 8.93, 8.98, 9.02, 9.05, 9.20.

m/e : 498 (58%), 496 (32%), 484 (37%), 480 (47%), 466 (53%), 465 (53%), 451 (37%), 433 (21%), 397 (16%), 384 (21%), 383 (20%), 381 (10%), 369 (11%), 365 (16%), 351 (10%), 329 (53%), 311 (58%), 295 (32%), 293 (32%), 287 (20%), 277 (26%), 259 (32%), 241 (40%), 239 (20%), 225 (21%), 223 (20%), 215 (29%), 199 (26%), 197 (26%), 187 (37%), 185 (39%), 183 (34%), 161 (29%), 159 (26%), 157 (40%), 147 (34%), 145 (53%), 143 (37%), 135 (37%), 133 (39%), 131 (37%), 121 (53%), 119 (34%), 109 (40%), 107 (53%), 105 (55%), 97 (34%), 95 (34%), 93 (50%), 91 (45%), 83 (37%), 81 (53%),

79 (47%), 69 (79%), 67 (53%), 55 (100%).

M* for m/e 498 - 480, m/e 484 - 466.

Literature m.p. 130 - 132°C.

υ max (KBr) : 1735, 1640 cm.$^{-1}$.

(7) The compound L/4. "Methyl lenzitate"; obtained as described (section 1), this compound crystallised from ethyl acetate as colourless needles, m.p. 139.5 - 140°C.,

R_f 0.4 (3% CH_3OH/97% $CHCl_3$)

$(\alpha)_D$ ($CHCl_3$, C, 1.30) + 123°.

(Found : C, 70.77%; H, 9.51%; M^+ at m/e 530.

After 4 hours at 110°C. in vacuo, C, 71.10%; H, 9.51%.

$C_{32}H_{50}O_6$ requires C, 72.42%; H, 9.50%; m.w. 530.8.).

υ max (KBr) : 3420(broad, hydrogen - bonded hydroxyl), 2930, 2880, 2830, 1722 (ester carbonyl), 1704 (α,β-unsaturated carbonyl), 1644 (C = C), 1435, 1412, 1336, 1296, 1272, 1192, 1155, 1122, 1114, 1092, 1046, 1020, 990 cm.$^{-1}$

υ max ($CHCl_3$) : 1723 (E = 889); 1707 (E = 864) cm.$^{-1}$

λ max (EtOH) : 215 (E = 20,900)nm.

τ (60 Mc./s.; $CDCl_3$) : 3.30 (1H, t, J = 7 c.p.s. - C$\underline{H}$ = C.CO_2CH_3), 5.80 (1H, m, C$\underline{H}$ - OH, ring D), 6.39 ((3H, S, $C\underline{H}_3CO_2$-), 6.46 (3H, S, $C\underline{H}_3CO_2$-) 6.77 (2H,m, - C$\underline{H}$-OH, ring A); 8.22 (3H,S, $C\underline{H}_3$ - C = C-),

methyl contour 9.04, 9.05, 9.10, 9.20, 9.26 (15H)

m/e : 530 (90%), 528 (12%), 515 (24%), 512 (38%), 497 (100%) 479 (57%), 465 (29%), 447 (20%), 437 (24%), 419 (19%), 405 (20%), 311 (24%), 297 (19%), 289 (33%), 279 (24%), 275 (19%), 259 (24%), 257 (38%), 241 (33%), 229 (24%), 199 (24%), 187 (57%), 173 (38%), 171 (32%), 161 (24%), 159 (38%), 157 (29%), 147 (33%), 145 (43%), 135 (43%), 133 (38%), 121 (48%), 119 (62%), 109 (38%), 107 (52%), 105 (52%), 95 (62%), 93 (48%), 91 (38%), 81 (60%), 79 (38%), 69 (48%), 67 (48%), 55 (57%),

M* for m/e 530 - 515; m/e 530 - 512; m/e 515 - 497; m/e 497 - 479.

(8) Jones oxidation of L/4: bis-dehydro methyl lenzitate.

A solution of L/4 (50 mg.) in ice-cooled acetone (5ml) was treated with Jones reagent in an exactly analagous manner to that described in (section 6). Again t.l.c. showed conversion, firstly to two less polar species of $R_f(CHCl_3)$ 0.2 and 0.6, and then, after complete oxidation, wholly to the compound of R_f 0.6. This was isolated as described previously and crystallised from methanol as colourless prisms (35 mg., 71%), m.p. 151 - 153°C.

(Found : C, 73.06%; H, 8.69%; M^+ at m/e 526.

$C_{32}H_{46}O_6$ requires C, 72.97%; H, 8.80%; m.w. 526.7).

υmax (KBr) : 2940, 2871, 1738 (ester and ring D carbonyl) 1706 (α,β- unsaturated ester and ring A carbonyl), 1652 (C = C), 1458, 1434, 1386, 1346, 1278, 1262, 1220, 1190, 1160, 1120, 1019 $cm.^{-1}$

υmax ($CHCl_3$) : 1736 (E = 1082); 1711 (E = 951) $cm.^{-1}$

λmax (EtOH) : 215 (E = 16,720) nm.

τ100Mc./s : $CDCl_3$) : 3.32 (1H,t, J = 7 c.p.s. HC = C-CO_2CH_3), 6.24, 6.26 (6H, 2 CH_3-CO_2-); 8.13 (3H,S, CH_3-C = C-); methyl contour 8.82, 8.86 (12H), 9.08 (3H).

m/e : 526 (42%), 508 (89%), 493 (42%), 476 (32%), 461 (21%), 449 (21%), 433 (16%), 382 (32%), 335 (26%), 327 (53%), 309 (100%) 293 (32%), 283 (16%), 271 (21%), 269 (22%), 257 (37%), 245 (21%), 231 (22%), 223 (26%), 215 (26%), 211 (27%), 209 (32%), 201 (21%), 197 (33%), 185 (42%), 171 (58%), 159 (53%), 157 (58%), 145 (52%), 133 (48%), 131 (48%), 125 (42%), 119 (68%), 107 (64%), 105 (68%), 95 (78%), 91 (58%), 79 (53%), 55 (74%).

M* for m/e 526 - 508.

(9) Methyl leptoporate from methyl tyromycate.

To a solution of methyl tyromycate (44 mg.) in ether (50 ml.) was added diazomethane (0.04 mole) in ether (150 ml.) T.l.c. analysis of the solution after three days at room temperature showed approximately 40% conversion to a more polar substance with R_f and t.l.c. staining characteristics identical to methyl leptoporate. After further treatment with the same quantity of diazomethane under the same conditions, the mixture of starting material and product was separated by p.l.c. on Kieselgel HF_{254} nach Stahl, (one 20 x 20x 0.5 mm.), to give methyl leptoporate (28 mg. 58%) which crystallised from methanol as microcrystalline plates, m.p. 176 - 177°C.

R_f ($CHCl_3$) 0.3; $(\alpha)_D$ ($CHCl_3$, C, 0.70) + 95.5°.
(Found : C, 75.24%; H, 9.67%; N, 5.36%; M^+ at m/e 508. $C_{32}H_{48}O_3N_2$ requires C, 75.55%; H, 9.51%; N, 5.51%; m.w. 508.8).

νmax (KBr) : 3018, 2957, 2943, 2924, 2880, 1741 (ester carbonyl), 1710 (ring A carbonyl), 1556 (-N = N-), 1448, 1432, 1373, 1303, 1256, 1244, 1204, 1167, 1136, 1173, 1003, 991, 977, 901, 814 $cm.^{-1}$

νmax (CCl_4) : 1737 (E = 444), 1704,(E = 479) $cm.^{-1}$

λmax (EtOH) : 227 (infl., E = 12,220), 235 (E = 18,120), 243 (E = 20,770), 252 (E = 13,850), 324 (E = 310)nm.

τ(100 Mc./s., $CDCl_3$): 4.60 (2H,m, H-7 and H-11) ABX

system, τ_A 5.2, τ_B 5.96, τ_X 8.09, J_{AB} = 17 c.p.s., J_{AX} = J_{BX} = 8 c.p.s. ($C\underline{H}$ - $C\underline{H}_2$ - N = N), 6.25 (3H, S, - $CO_2C\underline{H}_3$) 8.34 (3H, S, $C\underline{H}_3$.C.N = N), methyl contour 8.80, 8.86, 8.91, 9.11, 9.40.

m/e : 508 (19%), 493 (4%), 480 (30%), 449 (100%), 311 (25%), 269 (50%), 256 (23%), 244 (19%) 229 (13%), 213 (13%), 199 (19%), 185 (28%), 173 (30%), 171 (32%), 169 (30%), 159 (25%), 157 (40%), 145 (28%), 143 (40%), 133 (40%), 123 (50%), 119 (30%), 109 (28%), 107 (26%), 105 (26%), 95 (60%), 83 (50%), 81 (40%), 69 (60%).

m* for m/e 508 - 449.

The melting point of a mixture of the methyl tyromycate obtained as described above, and a sample previously isolated from L. stipticus by column chromatography, was undepressed.

(10) Extraction and separation of the metabolites of Daedalea quercina

Fresh sporophores collected from an oak stump on a field research station in the Lake District were superficially cleaned, homogenised, and extracted into cold methanol. After removal of light-petroleum soluble

material, the methanolic solution of the terpenoid acids (23g.) was treated with an excess of ethereal diazomethane at room temperature over a period of twenty-four hours. Removal of solvent gave a golden-coloured oil (22.5g.), which was adsorbed onto Mallinckrodt silicic acid (30g.) and placed on top of a column of silicic acid (450g.) packed in 20% ethyl acetate / 80% light petroleum.

Fractions (20 ml.) were eluted from the column at the rate of three per hour, while the polarity of the eluting solvent was slowly increased to 80% ethyl acetate/20% light petroleum. Analysis of the fractions indicated the groupings of table below:

CODE	FRACTIONS	WT.(g.)
DQ1	20 - 31	2.1
DQ1 + DQ4	32 - 35	1.5
DQ4	35 - 56	0.1
DQ7	53 - 73	1.9

Later fractions, previously isolated by Campbell[163] and Adam,[146] were not further examined here.

(11) <u>The fraction DQ7</u>

Isolated as described above, this fraction was a light

yellow oil, $R_f(CHCl_3)$ 0.43 on Keiselgel G. nach Stahl. t.l.c. on Kieselgel impregnated with silver nitrate showed that DQ7 consisted of two components of R_f ($CHCl_3$) 0.44 and 0.42, which were separated in the manner described below.

Mallinckrodt silicic acid (35g.) was thoroughly mixed with a solution of silver nitrate (1.5g.) in aqueous methanol (100 ml.). Removal of solvent by rotary evaporation was followed by overnight drying in a dessicator. The silver nitrate impregnated silicic acid thus prepared was packed as a column in 20% ethyl acetate/80% light petroleum and the fraction DQ7 introduced on to the top of the column in a little of the same solvent. Fractions (5ml.) were eluted, without changing solvent polarity, at the rate of six per hour: the separation achieved is shown in the table below:

NAME	FRACTIONS	WT.(g.)
PYRAZOLINE-A	8 - 15	0.451
PYRAZOLINE A + B	16 - 19	0.290
PYRAZOLINE-B	20 - 50	0.401.

(12) Pyrazoline-A :

Isolated as described (section11), this compound was a white solid, which crystallised from ethyl acetate/light petroleum as needles, m.p. 133 - 135°C.

R_f ($CHCl_3$) 0.44.

(Found : C, 69.28%; H, 8,80%; N, 4.39%;
$C_{37}H_{56}O_7N_2$ requires C, 69.35%; H, 8.81%; N, 4.37%;
m.w. 640.9).

O.R.D.: (CH_3OH; C 0.027): $(\alpha)_{589}$ - 106.5°, $(\alpha)_{500}$ 0°, $(\alpha)_{336}$ - 4750°, (trough), $(\alpha)_{308}$ + 4425° (peak), $(\alpha)_{263}$ + 735°, $(\alpha)_{227}$ 0°.

υmax (KBr) : 2940, 2870, 1730 with inflexions at 1745 and 1718 (malonate ester, aliphatic ester and ketonic carbonyl), 1550 (-N = N-), 1453, 1433, 1405, 1335, 1270, 1200, 1163, 1057, 1030, 1010, 975, 885 $cm.^{-1}$

υ max (CCl_4) : 1755 (E = 1,077), 1737 (E = 1362), 1719 (E = 837) $cm.^{-1}$

λmax (EtOH) : 210, 329 (E = 420)nm.

γ (100 Mc./s.; $CDCl_3$) : 5.27 (1H, m, C - 3 proton under malonate ester), 5.46 (2H, m, - $C\underline{H}_2$-N = N -), 6.24 (3H, S,-$CO_2C\underline{H}_3$), 6.30 (3H, S, -$CO_2C\underline{H}_3$), 6.65 (2H, S, MeOCO.$C\underline{H}_2$-COO-), 7.30, 8.20(m,-$C\underline{H}_2$-CH_2-N = N-), methyl contour, 8.89, 8.96, 9.01, 9.16.

m/e : 612 (23%), 597 (49%), 553 (7%), 512 (7%), 497 (16%), 495 (11%), 494 (18%), 480 (40%), 479 (98%), 461 (9%), 447 (14%), 427 (37%), 383 (9%), 327 (16%), 325 (18%), 324 (18%),

310 (37%), 309 (100%), 197 (71%), 189 (36%), 187 (51%), 165 (61%), 155 (63%), 127 (49%), 121 (60%),

M* for m/e 612 ⟶ 597; m/e 597 ⟶ 479; m/e 597 ⟶ 427 m/e 479 ⟶ 309; m/e 427 ⟶ 309.

(13) Pyrazoline -B:

A white crystalline solid, isolated as described (section 11), this crystallised from ethyl acetate/ light petroleum as needles, m.p. 99 - 101°C.

(Found : C, 69.33%; H, 8.77%; N, 4.32%

$C_{37}H_{56}O_7N_2$ requires C, 69.35%; H, 8.81%; N, 4.37% m.w. 640.9).

O.R.D. (CH_3OH; C, 0.031) : $(\alpha)_{589}$ + 77°, $(\alpha)_{500}$ + 325°, $(\alpha)_{334}$ + 2770° (peak), $(\alpha)_{306}$ - 2770°(trough), $(\alpha)_{276}$ - 585°, $(\alpha)_{233}$ 0°.

υ max (KBr) : 2940, 2870, 2830, 1727 with inflexions at 1745 and 1715 (malonate ester, aliphatic ester, and ketonic carbonyl), 1550 (N=N), 1452, 1433, 1368, 1310, 1262, 1198, 1150, 1055, 1030, 971, 881 $cm.^{-1}$

υ max (CCl_4) : 1755 (E = 941), 1736 (E = 1180), 1716 (E = 739) $cm.^{-1}$

λ max (EtOH) : 208, 330 (E = 350)nm.

γ(100 Mc./s., $CDCl_3$) : 5.28 (1H, m, C - 3 proton under malonate ester), 5.50 (2H, m, $-C\underline{H}_2-N{+}N-$), 6.25 (3H, S, $-CO_2C\underline{H}_3$), 6.31 (3H, S, $-CO_2C\underline{H}_3$), 6.64

(2H, S, Meo-CO.$\underline{CH_2}$.COO-), 7.32, 8.25(m, $\underline{CH_2}$-$\underline{CH_2}$-N = N-), methyl contour 8,78, 8.86, 8.88, 8.96, 8.98, 9.01, 9.11, 9.14.

m/e : 612 (12%), 597 (33%), 553 (7%), 512 (4%), 497 (13%), 495 (11%), 494 (18%), 480 (52%), 479 (98%), 461 (10%), 447 (28%), 427 (30%), 383 (11%), 327 (17%), 325 (22%), 324 (19%), 310 (57%), 309 (100%),197 (98%), 189 (62%), 187 (79%), 165 (98%), 155 (98%), 127 (71%), 121 (86%).

m* for m/e 612 - 597; m/e 597 - 479; m/e 597 - 427; m/e 479 - 309; m/e 427 - 309.

SECTION III.

FUNGAL TERPHENYLS.

INTRODUCTION

SHIKIMIC ACID DERIVED METABOLITES FROM FUNGI

The "shikimic acid pathway", typified by the important products, phenylalanine and tyrosine is outlined in Table22. This biosynthetic route, initiated by the condensation of phosphoenol-pyruvate (180) and erythrose-4-phosphate (181) is of primary importance in the higher plants and is the means of formation of such secondary metabolites as the alkaloid precursors anthranilic acid and tryptophane, quinic acid, produced from 5-dehydroquinic acid (182), protocatechuic and gallic acids from 5-dehydroshikimic acid (183), the hydroxybenzoic acids thought to derive from chorismic acid (184) and the cinnamic acid derivatives, or "C_6C_3" metabolites, formed from phenylalanine and tyrosine.

Although secondary metabolites derived by this pathway are by no means rare in the fungi, they are less common than in the plant kingdom; in view of the close link of the shikimic pathway with the pathways of carbohydrate metabolism, this may be connected with the ability of plants to carry out photosynthesis.

The simple "C_6C_3" metabolites such as methyl trans-

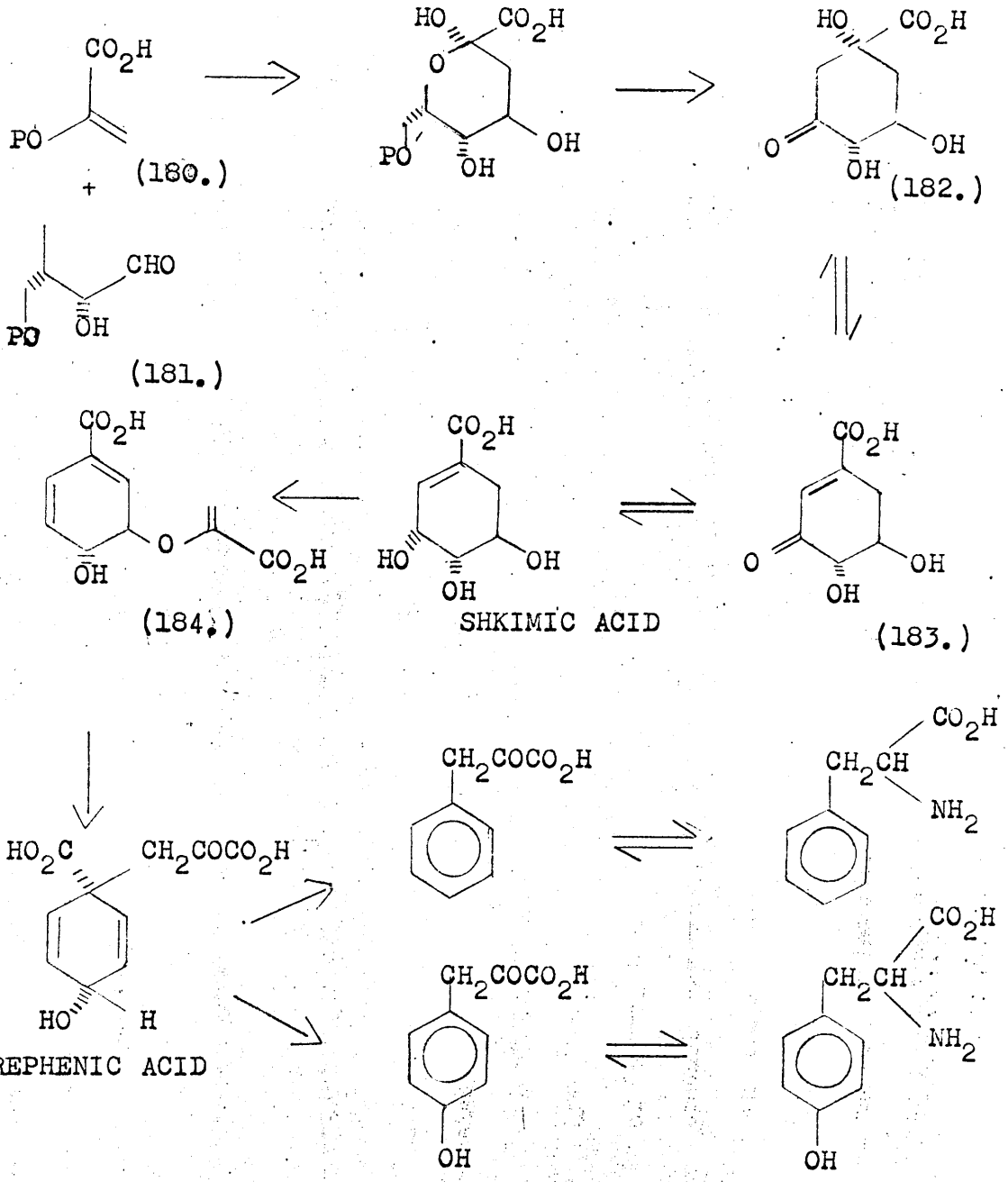
cinnamate (185)[166] and methyl p-coumarate (186)[167] have been isolated from Lentinus lenideus, while the occurrence with methyl p-methoxycinnamate of anisaldehyde and anisic acid suggests that the latter may be degradation products of the former. The phenylalanine nucleus is evident in picrorocellin (187)[168] and viridicatin (188)[169] , and fermentation experiments with (1-C^{14})-phenylalanine and phenylalanine-(H^3) have demonstrated that this amino acid is incorporated as a whole into the indole ring of gliotoxin (189), a metabolite of Trichoderma viride.[170] The entire skeleton of tyrosine is incorporated into the coumarin nucleus of the antibiotic, novobiocin (190)[171] , while both tyrosine and alanine are thought to form the benzenoid and hetero-cyclic rings of mycelianamide (112).[172]

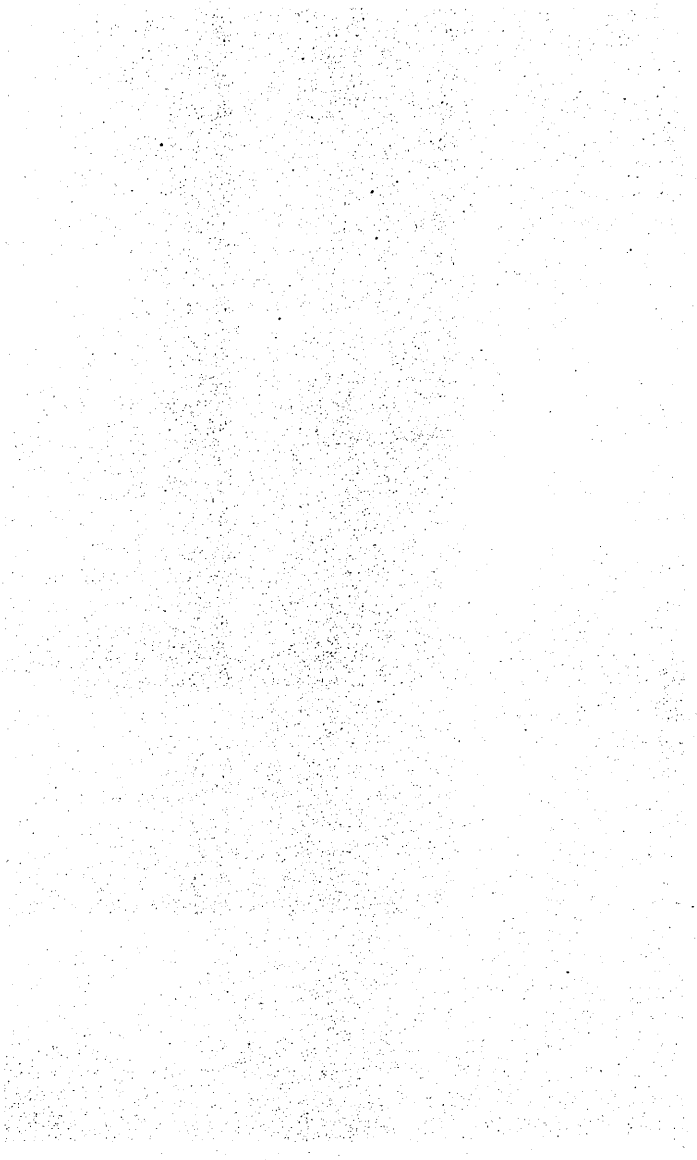
The suggestion has been made that the terphenyl nucleus might arise in nature by autocondensation of a phenylpyruvic ($ØC_3$) type of molecule as indicated in Table 23.[173] Such structures are indeed common in nature, the main source being wood-rotting fungi (basidiomycetes); a common structural feature is the presence of a para quinone function in the central ring, which in nearly every case also carries other oxygen functions. Perhaps the best known is polyporic acid

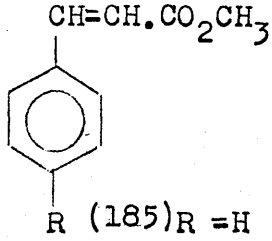
(191), the main pigment of the fungus Peniphora filamentosa[174], while atromentin (192)[175] shows an analagous structure presumably derived from p-hydroxyphenylpyruvate. The dibenzoate (193) of atromentin, named aurantiacin[176], has been isolated from Hydnum aurantiacum, and this fungus is also the source of the "leucobenzoate" (194)[177] of aurantiacin; phlebiarubrone (195)[178], possessing a methylenedioxy group on the central ring, represents a recent addition to this group. In experiments with labelled substrates[179], shikimic acid, phenylalanine, phenyllactic acid, and metatyrosine were found to be the precursors of the terphenylquinone volucrisporin (196) indicating the expected biogenesis from autocondensation of two meta-hydroxylated phenylpyruvate moieties. The structure of thelephoric acid (197)[180] suggests that it is formed in nature from two 3,4-dihydroxyphenylpyruvate molecules, while leucomelone (198)[181] and its leucoacetate (199), metabolites of Polyporus leucomelas, could be derived either from condensation of two p-hydroxyphenylpyruvate molecules with subsequent hydroxylation, or by a mixed condensation of one p-hydroxy and one 3,4-dihydroxyphenylpyruvate precursor. The biosynthetic pathway to the terphenylquinones could also include a rearrangement of the

$\emptyset C_3$ carbon chain, in which the carboxyl group migrates to the β-carbon, so that the condensing species has a branched C_3 chain; such a migration is known in the biosynthesis of tropic acid (200)[182].

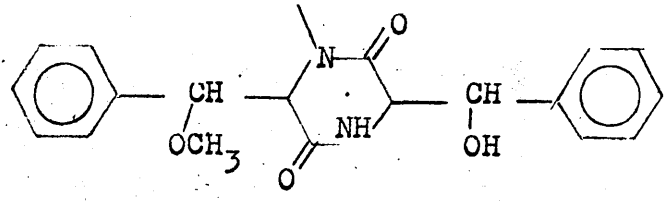
TABLE 22. SHKIMIC ACID PATHWAY.



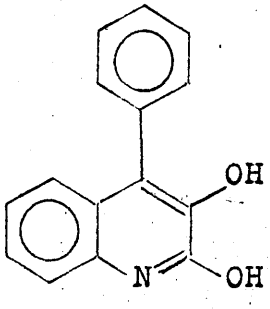




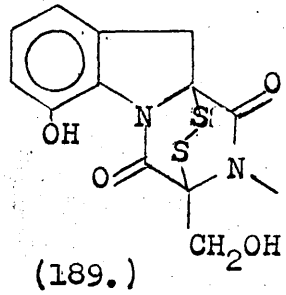
(185) R = H
(186) R = OH



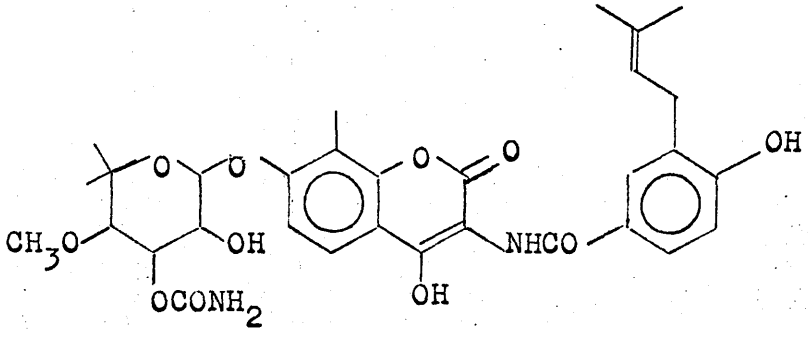
(187.)



(188.)

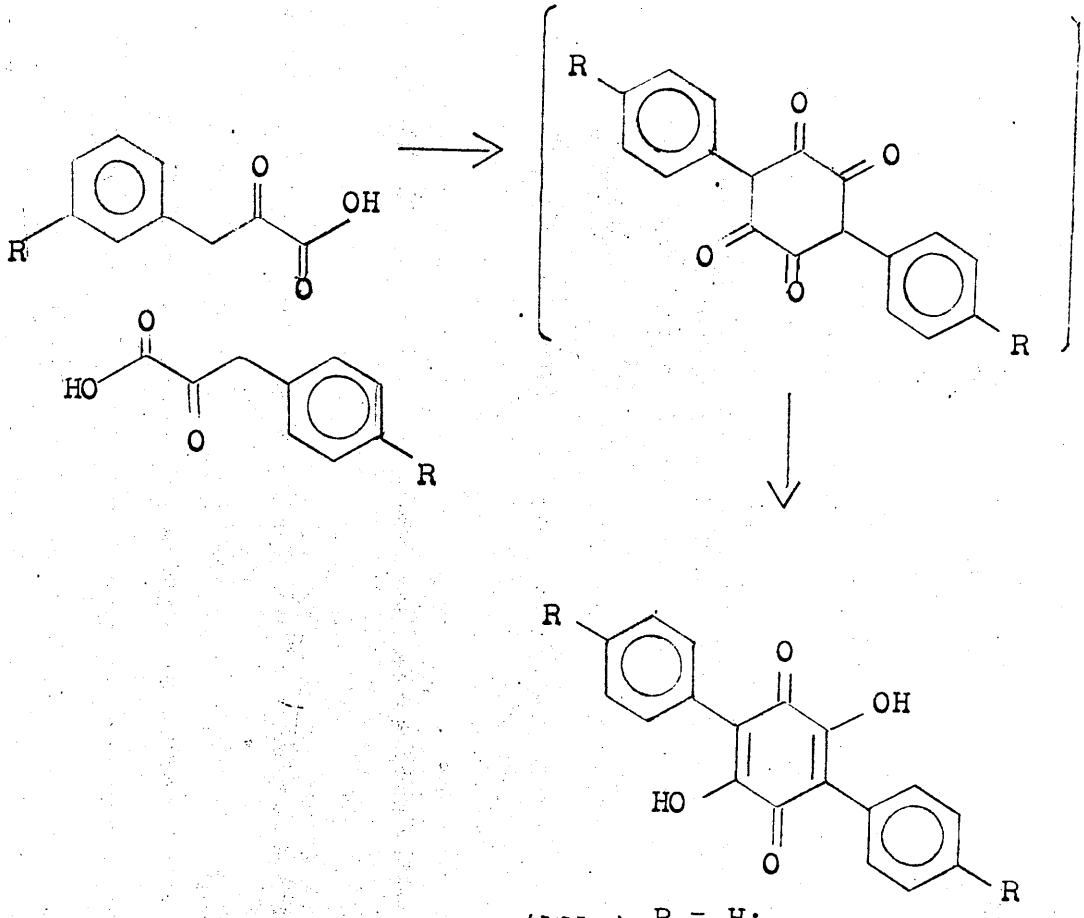


(189.)

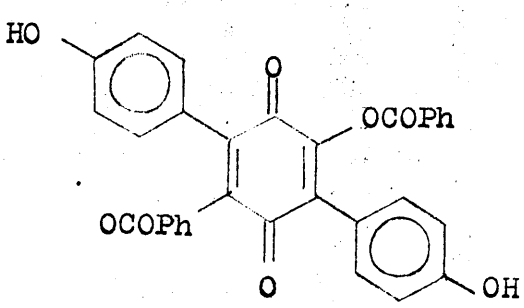


(190.)

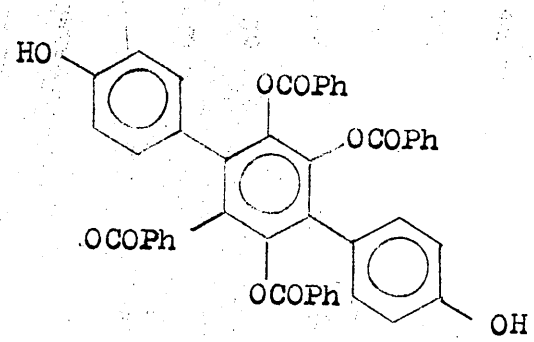
TABLE 23.



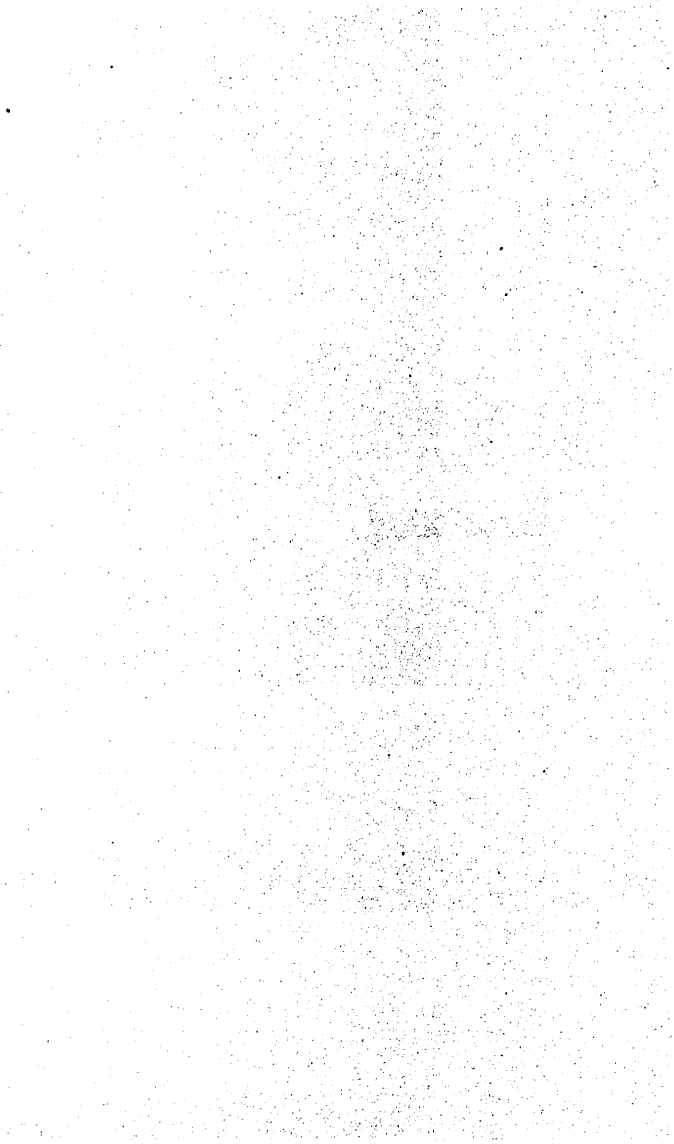
(191.) R = H;
(192.) R = OH.

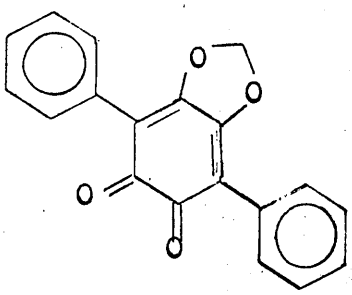


(193.)

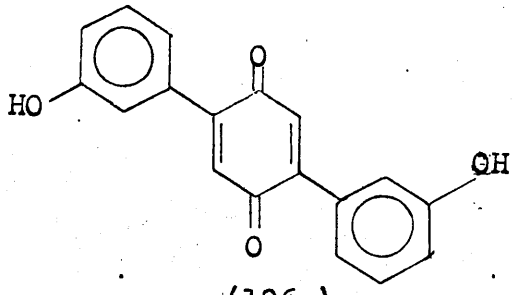


(194.)

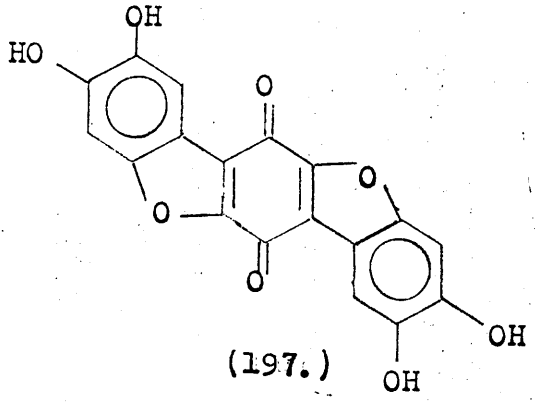




(195.)



(196.)



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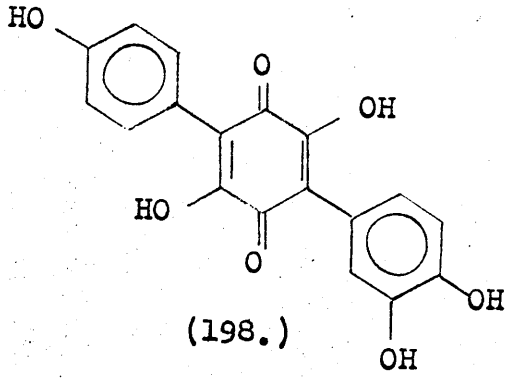
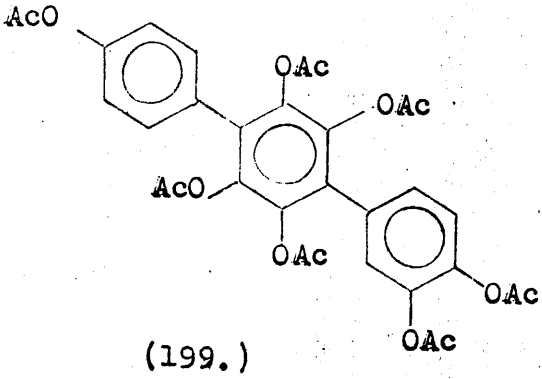


(198.)



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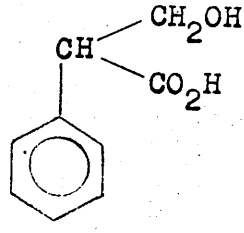


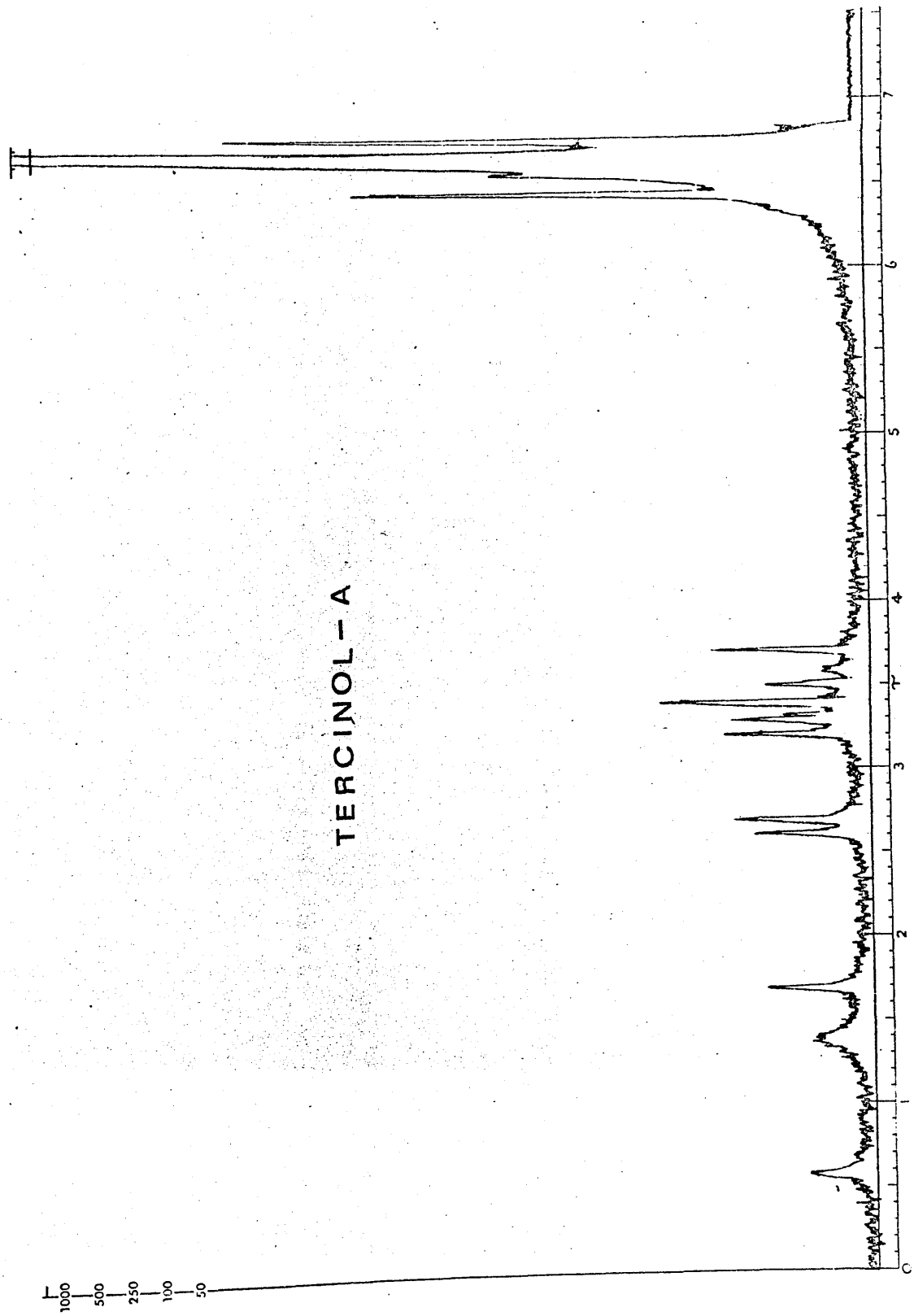
(200.)

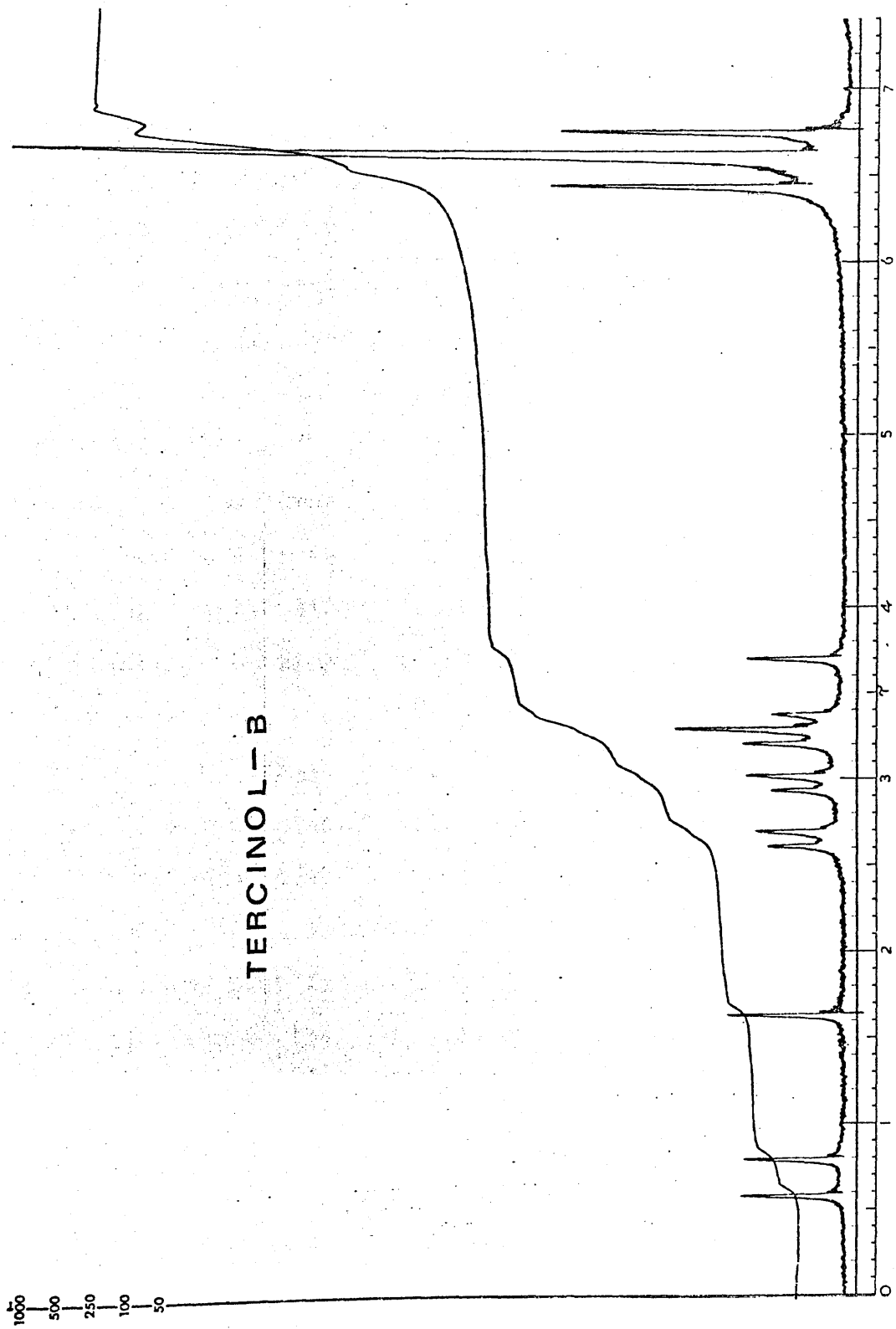
DISCUSSION.

SECTION I

A recent examination in this laboratory of the chemical constituents of the mould, Aspergillus candidus,[183] revealed the presence of two related substances showing anti-keratinophilic activity; the following evidence led to partial structures for these compounds, "tercinol - A" and "tercinol - B".[183] These both showed i.r. absorption typical of phenolic systems and exhibited u.v. maxima at 272 and 276 nm., respectively, which underwent bathochromic shifts of 23 and 25 nm., respectively, upon basification. Molecular formula $C_{20}H_{18}O_6$, (M^+ at m/e 354) was found for tercinol-A and $C_{20}H_{18}O_5$, (M^+ at m/e 338) for tercinol-B. The extra oxygen atom in the former was present as a hydroxyl group from examination of the low field singlets in the n.m.r. spectra; thus, tercinol-A (p.168) showed four hydroxylic protons at 0.58τ (1H), 1.39τ (2H) and 1.70τ (1H), exchangeable by D_2O, while tercinol-B (p.169) showed that only three such protons at 0.57τ (1H), 0.78τ (1H) and 1.62τ (1H), again exchangeable by D_2O.

The simpler of the two compounds, tercinol-B, was examined first; possible structures (201), (202) and (203) (R = OH or OCH_3), were proposed for this substance on the following evidence. The n.m.r. spectrum showed the presence of two AA'BB' systems as doublets at 2.65, 3.24τ


TERCINOL-A
1000
500
250
100
50
0
1
2
3
4
5
6
7


TERCINOL-B
1000
500
250
100
50
0
1
2
3
4
5
6
7

(J_{AB} = 9 c.p.s.) and 2.97, 3.32 τ (J_{AB} = 8 c.p.s.); the remaining low field signal at 3.69 τ (1H) was assigned to an aromatic proton H_A, ortho or para to two oxygen substituents. Two methoxyl singlets were also observed at 6.44 τ (3H) and 6.75 τ (3H).

Two explanations seemed possible for the upfield position of the methoxyl groups; in all three structures, where R_1 = OCH_3, this methoxyl group might be shielded by the ortho aromatic ring A, ("explanation A"); alternatively, in the structures (201) and (202) where R_4 (or R_5) = OCH_3, a preferred conformation (204), as a result of ortho substitution, would lead to shielding of the substituent R_4 by ring C, ("explanation B"). This shielding of a methoxyl group by an aryl ring is analagous to that found in the benzylisoquinolines[184, 185]; for example, in (205) the methoxyl group at C-7 appears in the n.m.r. as a singlet at 6.48 τ.

Tercinol-B formed a tribenzyl ether, the n.m.r. spectrum of which showed one high field benzylic methylene resonance (5.47 τ) in addition to that corresponding to the remaining benzylic protons at 5.08 τ (4H). From this, it was concluded that one hydroxyl group in tercinol-B was situated either ortho to an aryl substituent, ("explanation A")

or at a terminal position, i.e. R_4 or R_5,("explanation B").

Tercinol-A was next examined; as previously observed, this compound possessed a hydroxyl function additional to tercinol-B, the position of which became evident from comparison of the n.m.r. spectra. Thus, tercinol-A showed only one AA'BB' system (doublets at 2.66 τ, 3.26τ, J_{AB} = 8 c.p.s.), irradiation at 2.66 τ causing a collapse of the doublet at 3.26 τ to a singlet and vice-versa. This suggested the possible terphenyl structures(206),(207) and (208)for tercinol-A.; the alternative structures to these in which rings A and C are interchanged could not be ruled out at this stage.H-5 and H-6 were observed as a doublet at 3.36τ and a broadened doublet at 3.56τ, respectively, and H-2 as a broadened singlet at 3.40τ ; the remaining low-field singlet at 3.51τ was assigned to the aryl proton H_A which, as in tercinol-B, was ortho or para to two oxygen substituents. Of the two methoxyl singlets in the n.m.r. spectrum of tercinol-A, one occurred at 6.44τ and the other at 6.76τ, the possible reasons for this shielding being similar to those invoked for tercinol-B.

In the tetra-methyl ether of tercinol-A, one additional high-field methoxyl singlet was present (6.56τ, 3H), again suggesting that one free hydroxyl function in the natural

product was located ortho to an aryl ring system ("explanation A") or in a terminal position R_4 or R_5 ("explanation B"). The n.m.r. spectrum of o-hydroxydiphenyl consists of a sharp 5H singlet at 2.60 τ and two 2H multiplets centred at 2.75 τ and 3.05 τ. This seems to provide evidence against "explanation A" since there is no apparent deshielding of ortho protons in the phenyl ring. To test whether a ring A methoxyl group might be shielded by ring C, (cf.204), a synthesis of ortho-terphenyl systems related to (201), in which $R_4 = R_5 = OCH_3$, was undertaken, such studies being encouraged by the potential biological usefulness of analogues of the natural product.

A variety of synthetic routes to substituted terphenyls, ortho, meta and para, have been established and these have been adequately reviewed elsewhere[186]. They include union of aryl rings by the Ullman and related reactions, substitution reactions of the preformed terphenyl nucleus and condensation of quinones, the latter being particularly useful for the synthesis of the naturally occurring terphenylquinones.

Two main approaches to the required o-terphenyls were examined here and are discussed in detail in Section II (p.183). The first of these, outlined in scheme (2,)

gave the o-terphenyl (209) the n.m.r. of which showed an AA'BB' quartet (doublets at 2.90 τ and 3.21 τ), and 6H singlet at 6.22 τ, due to the p-methoxyphenyl groupings, as well as signals corresponding to the ethyl ester groupings (6H triplet at 8.60τ; 4H quartet at 5.60τ) and a two proton singlet at 2.24τ, assigned to the protons of the central ring deshielded by the ortho carbonyl functions. The aryl methoxy protons thus resonated at a quite unexceptional τ value and the chemical shift remained unchanged even when the rate of rotation about the diaryl linkages was retarded by cooling to ca. - 50°C.

This seems to rule out shielding by ring C of a ring A methoxyl group ("explanation B") as the explanation of the high τ value of the methoxyl group in the tercinols. However, since the o-terphenyl (209), unlike the tercinols, lacked central ring substituents ortho to the other aryl groups, the effect could conceivably be diminished by a freer rotation about the diaryl single bonds.

As indicated in section II , several attempts to introduce 3'- substituents in the o-terphenyl (209) by modifications of the Diels-Alder route were unsuccessful. A more recent synthesis of o-terphenyls, in which the central ring is formed via Michael and Dieckmann

condensations was applied as outlined in scheme 4 to give the two isomeric o-terphenyls (210) and (211).

Each compound showed a 2H singlet (3.48 τ in (210) and 3.57 τ in (211) assigned to H - 4' and H - 6' and also showed an octet of peaks in the region 2.9 - 3.5 τ (8H) corresponding to two overlapping AA'BB' systems. The methoxyl signals appeared in the 6.3 τ region, the highest resonating at 6.34 τ . This again seems to indicate the absence of significant shielding of a ring A methoxyl by ring C in the o-terphenyl system.

Evidence for the correct explanation for the methoxyl group shielding was obtained by bromination of the o-terphenyls (210) and (211). In both cases, a dibromo derivative was obtained showing the parent molecular ion as a doublet (m/e 496 and 494). The dibromo derivative (212) like its parent o-terphenyl (210) showed an octet of peaks (8H) in the n.m.r. region 3 - 3.4 τ but lacked the higher field 2H singlet shown by (210); the hydroylic proton was identified as a singlet (4.53 τ) exchangeable by D_2O. Examination of the methoxyl signals showed one 3H singlet at 6.05 τ assigned to the methoxyl group at C - 5', ortho to the two bromine atoms, and a 6H singlet at 6.27 τ for the remaining methoxyl groups. The unexceptional τ value

of this latter signal reinforces the argument against the theory of a ring A methoxyl group being shielded by ring C. The dibromo derivative (213) showed in the n.m.r. the 8H multiplet (2.98 - 3.45 τ) of two AA'BB' systems as well as a hydroxylic proton as a broad singlet (7.9τ), exchangeable by D_2O. In this case, however, two methoxyl groups were observed as a 6H singlet at 6.23τ, and one other at 6.62τ (3H singlet). This shows that, if a sufficiently bulky substituent is located ortho to a methoxyl group of ring B, then that group can be shielded by the aryl ring system ortho to it, i.e. "explanation A."

At this stage, further supplies of the tercinols became available and new derivatives were prepared. Examination of the n.m.r. spectrum of tercinol-A tetra-acetate showed signals corresponding to three acetoxyl groups at 7.68τ, and one acetoxyl group at the higher value of 7.93τ, confirming the conclusion already drawn from the spectra of tercinol-A tetramethyl ether and the synthetic model compounds (209) - (213) that one hydroxyl group was located ortho to an aryl group. Tercinol-B triacetate also showed one acetoxyl group at high field, paralleling the observations on its tribenzyl ether derivative.

In addition, comparison of the τ values (Table 24),

of the AA'BB' system, H - 5, H - 6, and H - 2 in tercinol-A and its tetraacetate showed down-field shifts explicable only if $R_4 = R_5 = OH$ i.e. the methoxyl groups are both located in ring B. Further to this, since H_A in the tetraacetate was also shifted downfield this proton must be ortho or para to OH, and possible structures for tercinol-A could be narrowed to (214), (215) or (216) (R' = OH). Similar observations for tercinol-B and its triaacetate also suggested three possible analagous structures (R' = H)

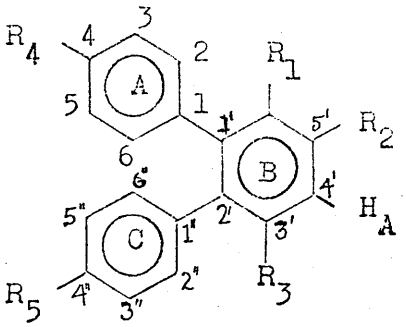
Nuclear Overhauser studies[187] on tercinol-A tetraacetate allowed elimination of the structure (216; R' = OH) as a possibility: thus, irradiation of the high field (methoxyl) signal gave no integral change for the remaining protons, but irradiation at the low-field (methoxyl) signal (6.26τ) resulted in a 14% enhancement of the integral of the H_A proton, an increase which could only be accommodated by either the o-terphenyl (214) or p-terphenyl (215). Again, the alternative structures to (214) and (215) for tercinol-A in which rings A and C are interchanged could not be eliminated at this stage .

Recent observations[188] of the Nuclear Overhauser effect between a phenolic hydroxyl group and an ortho aryl proton

suggested a similar experiment for the tercinols. As a preliminary, the synthetic *p*-terphenyl(210) was examined for this effect; irradiation at 4.86τ, (OH) resulted in a 7% integral enhancement in the signal corresponding to H - 4'. The results obtained for tercinol-B, chosen because it possessed fewer OH groups, are summarised in Table(25); these allowed the allocation of the OH signal at 1.62τ to the ring B hydroxyl and the absence of any integral enhancement at H_A on irradiation of this OH signal indicated that these two groups were not situated *ortho* to each other. Thus, the only possible remaining structure for tercinol-B is (215), R' = H). Of the two remaining possible structures for tercinol-A i.e. (215); R' = OH) or (217), R' = OH) the former seemed the more likely since the protons of the AA'BB' system *ortho* to the central ring were observed as a deshielded doublet attributed to the proximity of both the C - 3' OH and C - 5' OCH_3 groups. This deshielding is not observed when only one oxygen function is located in the *ortho* position to an AA'BB' system e.g. in the dibromo terphenyl (213), and thus structure (217) seems less likely.

A synthesis of p-terphenyls of the required substitution is at present in hand which it is hoped will finally

confirm the correctness of the structures deduced for tercinols-A and B.



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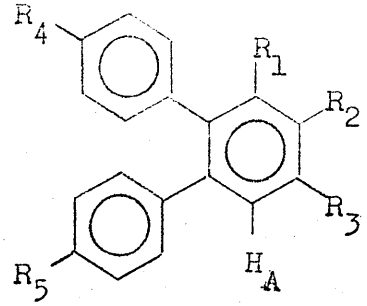

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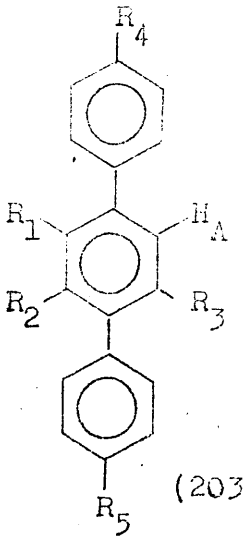

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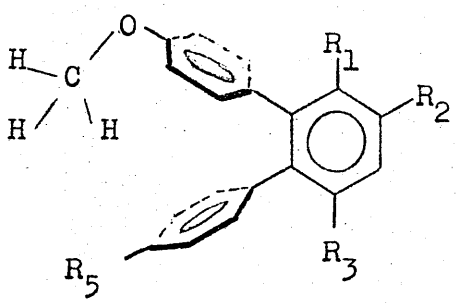

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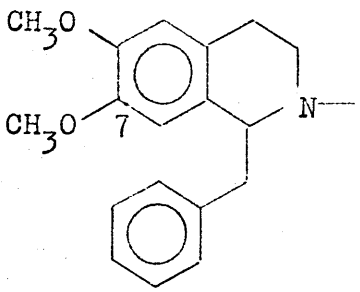

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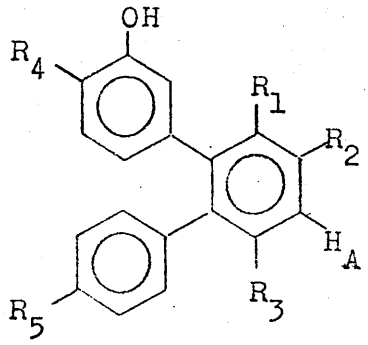

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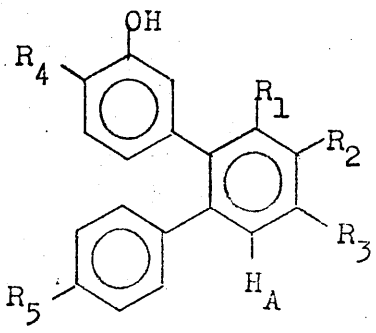

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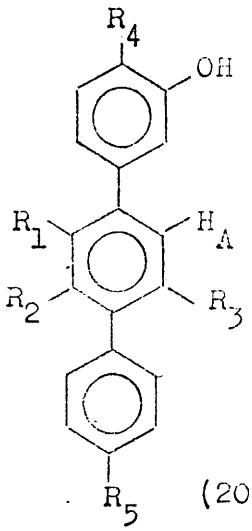

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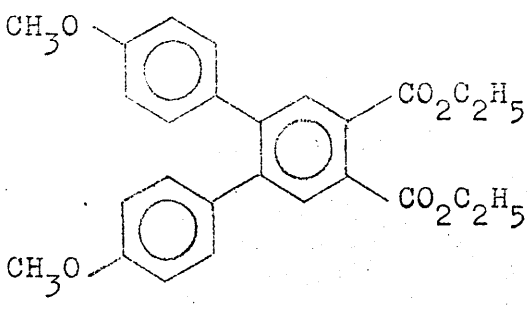

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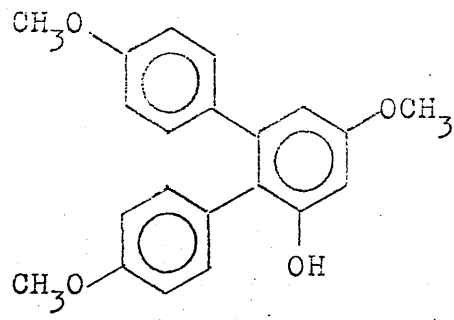

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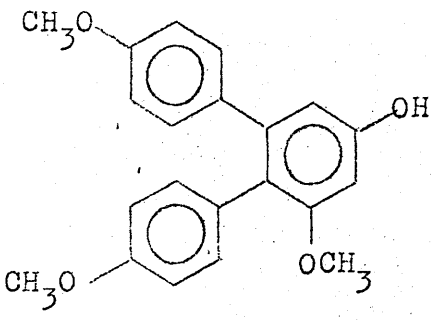

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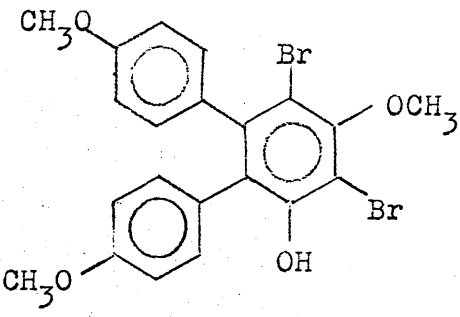

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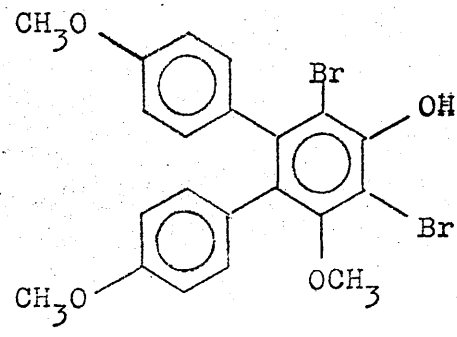

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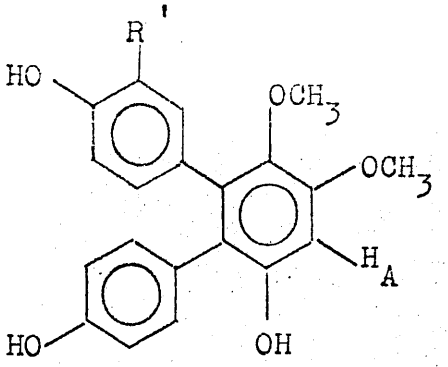

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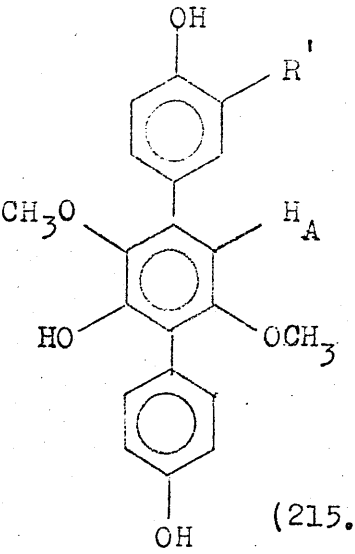
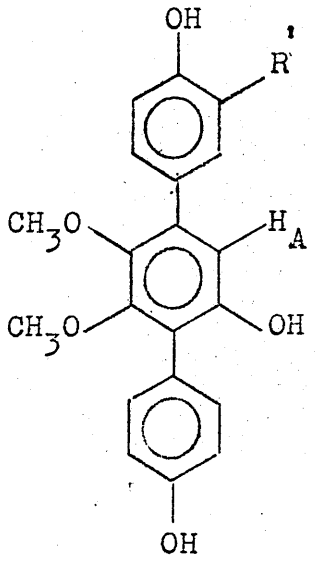


(215.)

R' = OH, tercinol A R' = H, tercinol B



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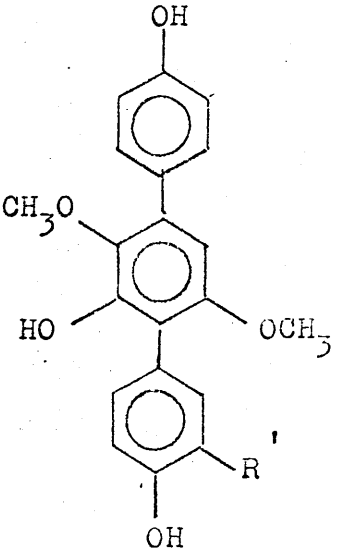


(217.)

Table 24.

τ values (D.M.S.O.)

	Tercinol-A	Tercinol-A Tetraacetate	Δ p.p.m.
AA'BB'system.	2.66,3.26	2.31, 2.71	0.35,0.55
H_5	3.36	2.74	0.62
H_6	3.56	2.82	0.74
H_2	3.40	2.88	0.52
H_A	3.51	2.97	0.54

Table 25.

Nuclear Overhauser Studies on Tercinol-B.

% Integral Enhancement.

τ	3.24(AA'BB' ,d.)	3.32(AA'BB' ,d.)	3.69(H_A,S)
0.57(OH)	15%	---	---
0.78(OH)	---	10%	---
1.62(OH)	---	---	---

SECTION II.

The following section describes the synthetic approaches to the o-terphenyls required for the n.m.r. studies discussed in Section I.

(1) Synthesis of 4,4"-dimethoxy - 4',5'-dicarbethoxy o-terphenyl (209)

This was synthesised as outlined in scheme [2] : p-methoxy acetophenone underwent reductive condensation on treatment with either aluminium amalgam in an ethanol/benzene mixture[189] or more conveniently zinc dust in alkali[190] This was the required diol(218) since it gave molecular formula $C_{18}H_{22}O_4$, determined by elemental analysis and showed in the n.m.r. spectrum two broad one-proton singlets (7.45, 7.70τ), exchangeable by D_2O, indicative of hydroxylic protons and confirmed by the i.r. absorption at 3560 $cm.^{-1}$ The tertiary methyl groups (CH_3-C-OH) appeared as two singlets at 8.43 and 8.52τ and the presence of a typical AA'BB' aryl system (doublets at 2.83, 3.25τ), together with two closely spaced singlets (6.20, 6.23τ) confirmed the presence of two p-methoxyphenyl groupings. It may be noted that the mass spectral parent molecular ion (m/e 302) constituted only 2% of the base peak which appeared at m/e 241; if this diol undergoes pinacol-pinacolone type of rearrangement in the mass spectrometer, subsequent loss

of the CH_3CO grouping thus formed could account for this base peak.

Dehydration of the diol (218) was effected by heating under vacuum in the presence of potassium bisulphate; the solidified reaction product proved to be a mixture of two chromatographically separable compounds present in approximately equal amount and low yield. The first crystalline compound had molecular formula $C_{18}H_{18}O_2$ (analysis and mass spectrum) and gave a positive reaction with tetranitromethane. This was identified as 2,3 di-(p-methoxyphenyl)-1,3 - butadiene (219) by the presence in the n.m.r. spectrum of two doublets at 4.52τ and 4.75τ (J = 2c.p.s.) and the i.r. absorption at υ_{max} 865 $cm.^{-1}$ indicative of the groupings $>C=CH_2$: the AA'BB' quartet (doublets at 2.64, 3.20τ) and 6H singlet at 6.27τ of the p-methoxyphenyl system were also present. The conjugated diene was typified by strong absorption in the u.v. spectrum at λ_{max} 305 nm. (E = 44,380), and i.r. absorption at υ_{max} 1610 $cm.^{-1}$ The second product of the sodium bisulphate reaction was characterised as the pinacolone-type rearrangement product, 3,3-di-(p-methoxyphenyl)-butan-2-one (221) from the following data: the compound had molecular formula $C_{18}H_{20}O_3$, a 3H singlet in the n.m.r.

at 8.20τ, assigned to the tertiary methyl group (β to CO) and a three-proton singlet at 7.93τ corresponding to methyl α to carbonyl group. Carbonyl absorption was present in the i.r. spectrum at ν max 1710 cm.$^{-1}$. The highest ion of the mass spectrum occurred at m/e 241 corresponding to loss of CH_3CO from the parent molecular ion.

A better technique for the dehydration of the diol (218) was to reflux in a mixture of acetic anhydride and pyridine[191], the diene being formed in much improved yield, while the pinacolone rearrangement product was now a minor reaction product.

The Diels - Alder condensation reaction of the diene (219) and acetylene dicarboxylic ester proceeded smoothly in refluxing xylene giving a 49% yield of the crystalline adduct (220), $C_{26}H_{28}O_6$. The n.m.r. spectrum of this compound showed the triplet (8.65τ) and quartet (5.72τ) of the ethyl ester groupings, and carbonyl absorption corresponding to these in the i.r. (ν max 1725, 1703 cm.$^{-1}$); a 4H singlet (6.54τ) in the n.m.r. spectrum was assigned to the allylic methylene groupings of the 1,4-cyclohexadiene system. This Diels-Alder adduct was readily dehydrogenated on treatment with 2,2-dichloro-5,6-dicyanobenzoquinone (D.D.Q.) to the crystalline

o-terphenyl(209) $C_{26}H_{26}O_6$. This structure was supported by the u.v. spectrum (λ_{max} 271nm) and also by the n.m.r. spectrum discussed in detail in section I.

(2) Attempted synthesis of 3'-substituted o- terphenyls

A number of modifications to the route described above were examined. For example, treatment of the 1.,4-cyclohexadiene(220) with chromium trioxide in aqueous acetic acid in a manner analagous to that used in a new preparation of naphthazarin[192], resulted only in formation of the o-terphenyl(209). Treatment of the cyclohexadiene with N -bromosuccinimide was also undertaken with the object of introducing two bromine atoms into allylic positions, one of which might be lost on aromatisation to give the bromo-terphenyl(222), but again the only product obtained was the o-terphenyl(209).

In an attempt to obtain C -3' oxygenated o-terphenyls, a route (scheme 3) was envisaged, analagous to that in scheme 2, whereby reductive condensation of the ketoacetate (223) to the dioldiacetate (224) might upon dehydration afford the dienoldiacetate, which upon Diels-Alder condensation with a dienophile and subsequent loss of acetic acid might be converted to o-terphenyl (226).

The starting material, p-methoxy-ω-acetoxyacetophenone,

was conveniently prepared via reaction of anisoyl chloride with dry ethereal diazomethane[193]; the intermediate yellow crystalline diazoketone which was formed in good yield, showed the expected n.m.r. spectrum including a one-proton singlet at 4.14τ assigned to the grouping $COCH - \overset{+}{N} - \overset{-}{N}$ Carbonyl absorption was also present in the i.r. spectrum at 1610 $cm.^{-1}$; typical of an α-diazoketone.[194] The required ketoacetate (223) was obtained by reaction of the diazoketone with glacial acetic acid and showed the expected n.m.r. spectrum. including a 3H singlet (7.80τ) assigned to the $\underline{CH}_3CO\cdot$ group, and a 2H singlet (4.72τ) due to the grouping $\cdot COC\underline{H}_2.OAc$. Two absorption peaks in the carbonyl region of the i.r. spectrum were assigned to the ester grouping (ν max 1735 $cm.^{-1}$) and an aryl carbonyl group (ν max 1690 $cm.^{-1}$).

The reaction of the ketoacetate with aluminium amalgam proceeded vigorously with formation of a solid product which showed no carbonyl absorption in the i.r. spectrum; comparison of i.r. spectra and t.l.c. properties showed that this compound was 2,3-di-(p-methoxyphenyl)-2,3-butanediol (218). No other more polar products were detected. The formation of the diol (218) from the ketoacetate probably occurs by preliminary reduction to p-methoxyacetophenone which self-condenses on the aluminium surface by the accepted diradical mechanism. The keto-alcohol (227) formed by treatment of the

above diazoketone with dilute mineral acid[195] did not react under the conditions of the aluminium condensation reaction.

Another substrate submitted to aluminium catalysed condensation reaction was p-methoxyphenylglyoxylic ester (228), prepared by alkaline permanganate oxidation of p-methoxyacetophenone[196] and esterification of the resultant acid with diazomethane.

Treatment of this ester with aluminium amalgam, gave a low melting solid, $C_{11}H_{14}O_4$; this was shown to be the mandelic ester (229) from the following spectroscopic evidence; hydroxylic adsorption was evident in the i.r. spectrum at ν max 3500 cm.$^{-1}$ and also in the n.m.r. spectrum as a 1H singlet (5.91τ) exchangeable by D_2O. The typical p-methoxyphenyl grouping was observed in the n.m.r. as a AA'BB' system (4H, doublets at 2.68, 3.16τ) and singlet (6.21τ), while ester carbonyl absorption was noted in the i.r. spectrum (ν max 1725 cm.$^{-1}$); this was identified as an ethyl ester from the presence of a quartet (5.82τ) and triplet (8.80τ) in the n.m.r. spectrum. The mass spectral parent molecule ion was observed at m/e 210. Evidently, reduction of the keto ester (228) had occurred with concurrent transesterification by the ethanol solvent.

Although reductive condensation of glyoxylic esters in

sunlight is known,[197] the same reaction could not be successfully repeated on the p-methoxyglyoxylic ester (228). Prolonged treatment of this compound in either iso-propanol or butan-2-ol with white light at 40°C resulted in a complex mixture of reaction products which was not further examined.

(3) Synthesis of 4,4",5'-trimethoxy-3'-hydroxy o-terphenyl (210) and 3',4,4"-trimethoxy-5'-hydroxy o-terphenyl (211).

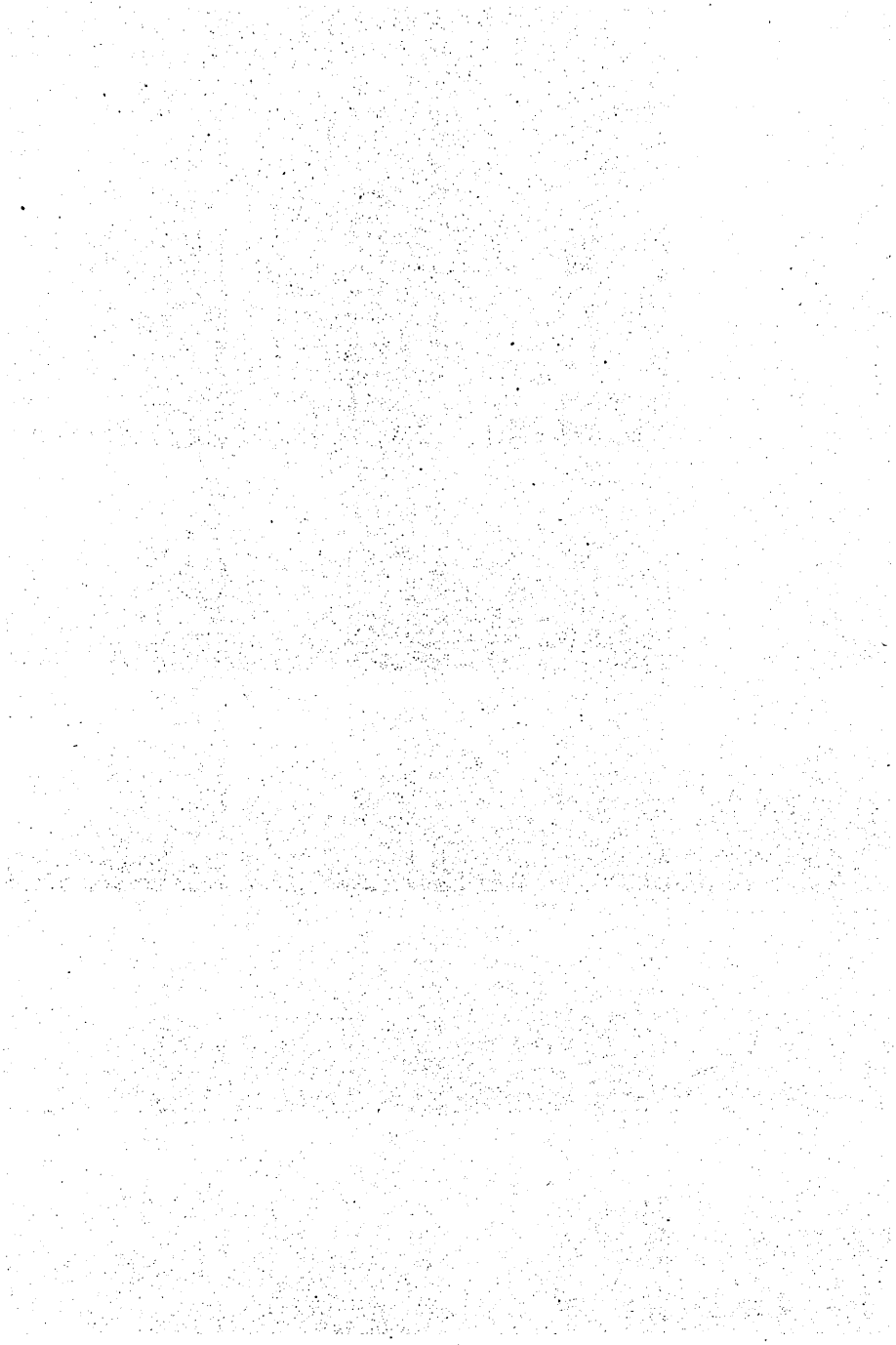
This synthetic route, outlined in Scheme 4, involved initial condensation[198] of anisalacetone and p-methoxyphenylacetic ester in the presence of sodium hydride. The product of this combined Michael and Dieckmann condensation had molecular formula, $C_{20}H_{20}O_4$, and was assigned the structure (230) from the following evidence; the presence of a β-dicarbonyl system was deduced from the broad absorption between ν_{max} 3500 and 3000 $cm.^{-1}$ and also at ν_{max} 1620 $cm.^{-1}$ in the i.r. spectrum, while the u.v. maximum at λ_{max} 258 n.m was in close agreement with the calculated value (257 n.m.) for a six membered enone system of the type (230). The n.m.r. spectrum showed an 8H multiplet (3.20 τ, aryl -H) and a 6H singlet at 6.26τ(aryl methoxyl groups): a hydroxylic proton was observed as a multiplet at 3.53τ, exchangeable by D_2O, and the vinyl proton as a singlet at 4.22 τ; the remaining protons were located as a multiplet between 6.98 and 7.33 τ ($-CH_2$ and 2CH groups.) The initial

loss in the mass spectrum, (M^+ at m/e 324), of 84 mass units was rationalised by a retro-Diels Alder cleavage (Scheme 5) .

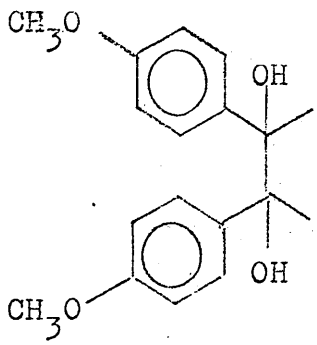
Final confirmation of the structure (230) was obtained by treatment of the above compound with N-bromosuccinimide whereby a mono-bromo derivative was obtained, $C_{20}H_{19}O_4Br$, as confirmed by the parent molecular ion, a doublet at m/e 404, 402. The u.v. absorption maximum had increased to λ max 272 n.m., of the order of the calculated value (282 n.m.) for the α-bromo enone system (231).

Treatment of the 1,3-diketone (230) with excess of ethereal diazomethane resulted in a two-component mixture of the methyl ethers (232), used without further purification for the next stage of the synthetic route: this involved sulphur dehydrogenation[199] of the methyl ethers, the product of which was also a mixture of two-components, R_f(3% CH_3OH/ 97% $CHCl_3$) 0.5 and 0.2, which were separated by column chromatography. Both crystalline compounds had molecular formula $C_{21}H_{20}O_4$ and the same mass spectral fragmentation pattern, with parent ion at m/e 336. The less polar terphenyl was assigned the structure (210), that is, 4,4",5'-trimethoxy-3'-hydroxy-ortho-terphenyl, and the more polar the structure 211,i.e. 3',4,4"-trimethoxy-5'-hydroxy-ortho-terphenyl ; this assignment was based on comparison of the R_f, i.r. and n.m.r. spectra of the o-terphenyls.

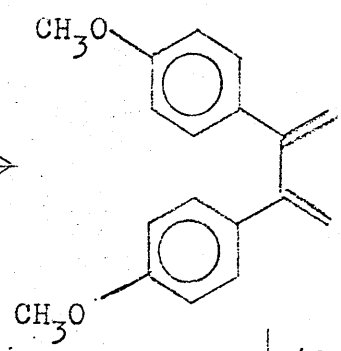
Thus, terphenyl (211) would be more polar than that of structure (210) since the C-3' hydroxyl group of the latter occupies a more hindered position and is less likely to participate in hydrogen-bonding; this was also reflected in the hydroxyl absorption in the i.r. spectrum of (211) which was a broad peak at ν max 3360 cm.$^{-1}$ compared to that of (210), a sharp peak at νmax 3515 cm.$^{-1}$. Also, the hydroxylic proton of (210) was observed in the n.m.r. as a singlet at 4.86 τ (exchangeable by D_2O) while that of (211) was a singlet at 4.34 τ (exchangeable by D_2O which may be indicative of a greater tendency in the latter to hydrogen bond. The n.m.r. spectra of these o-terphenyls is discussed in detail in Section I (p.174).



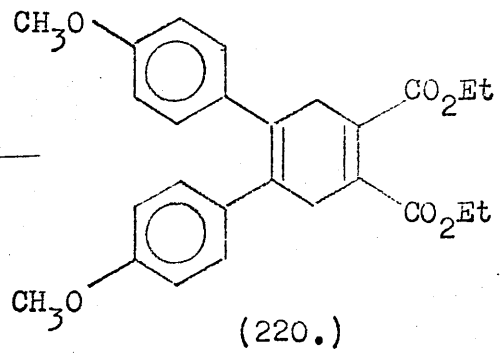
Scheme 2.



(218.)



(219.)



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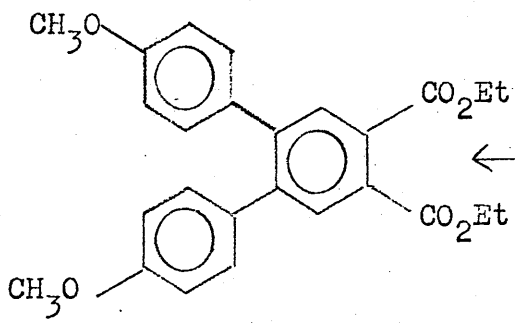
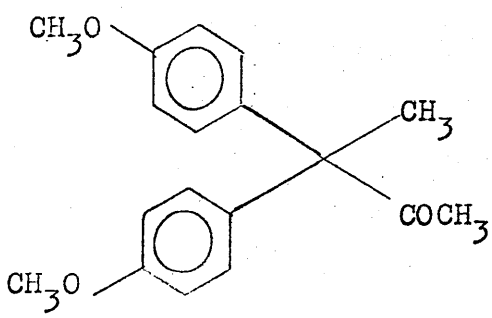
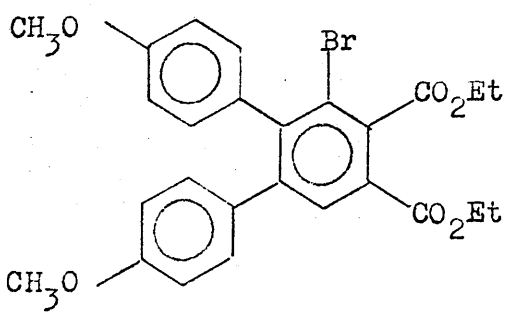


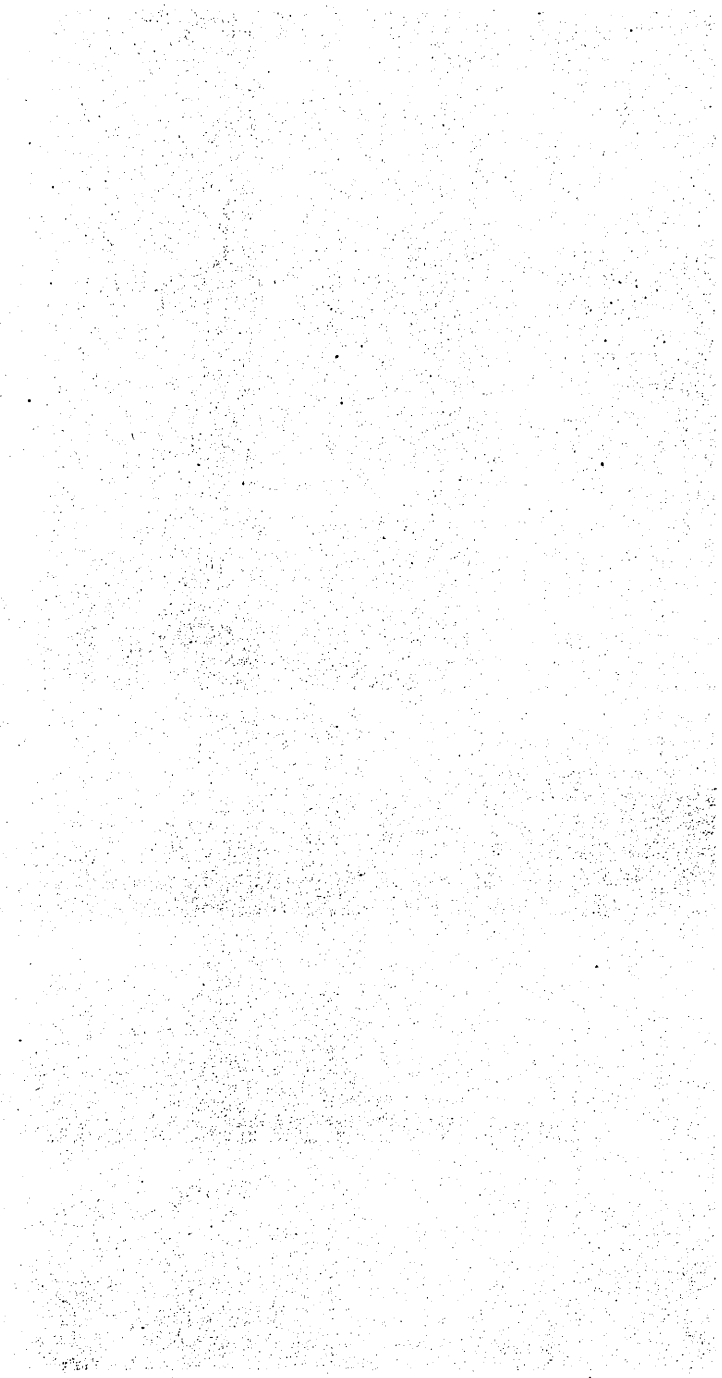
(209.)



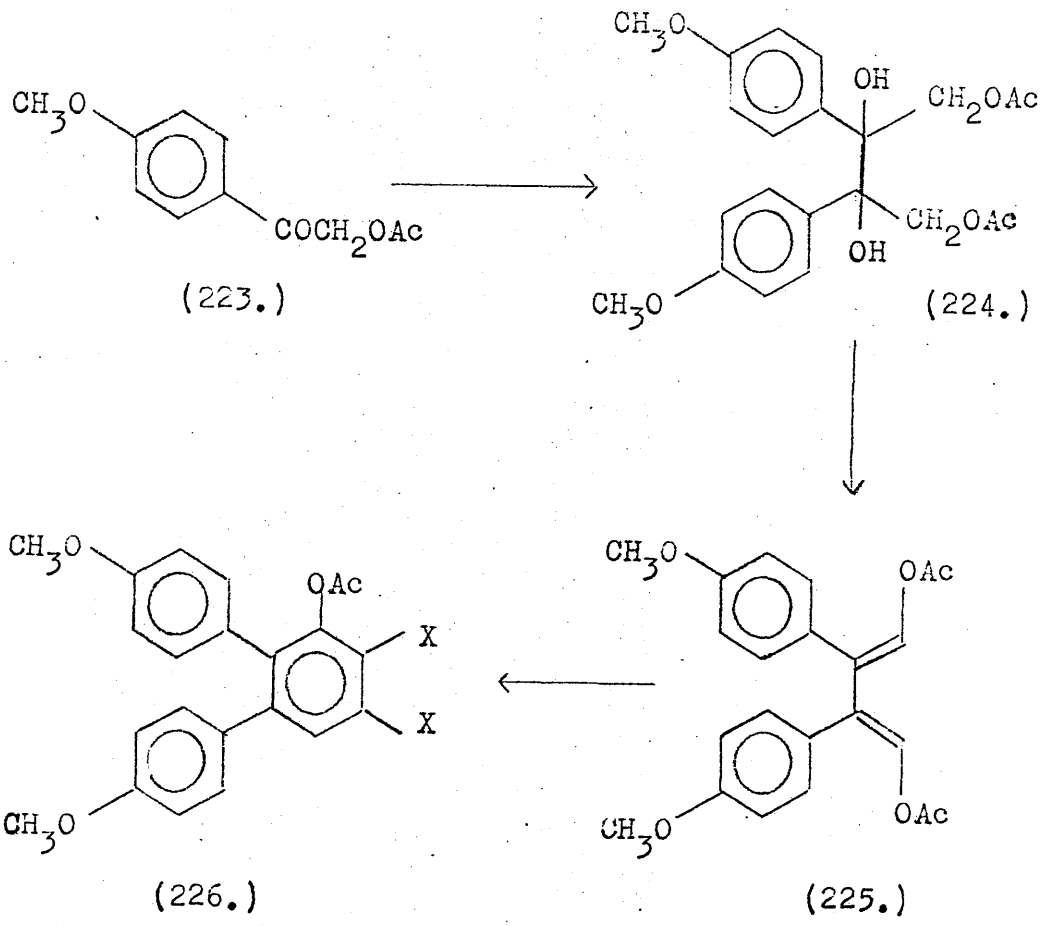
(221.)

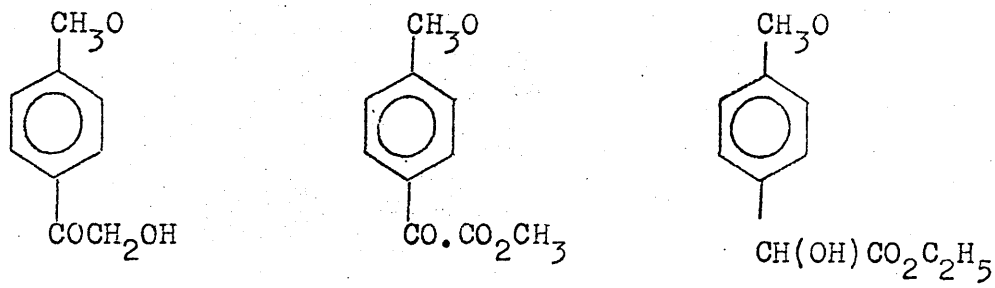


(222.)

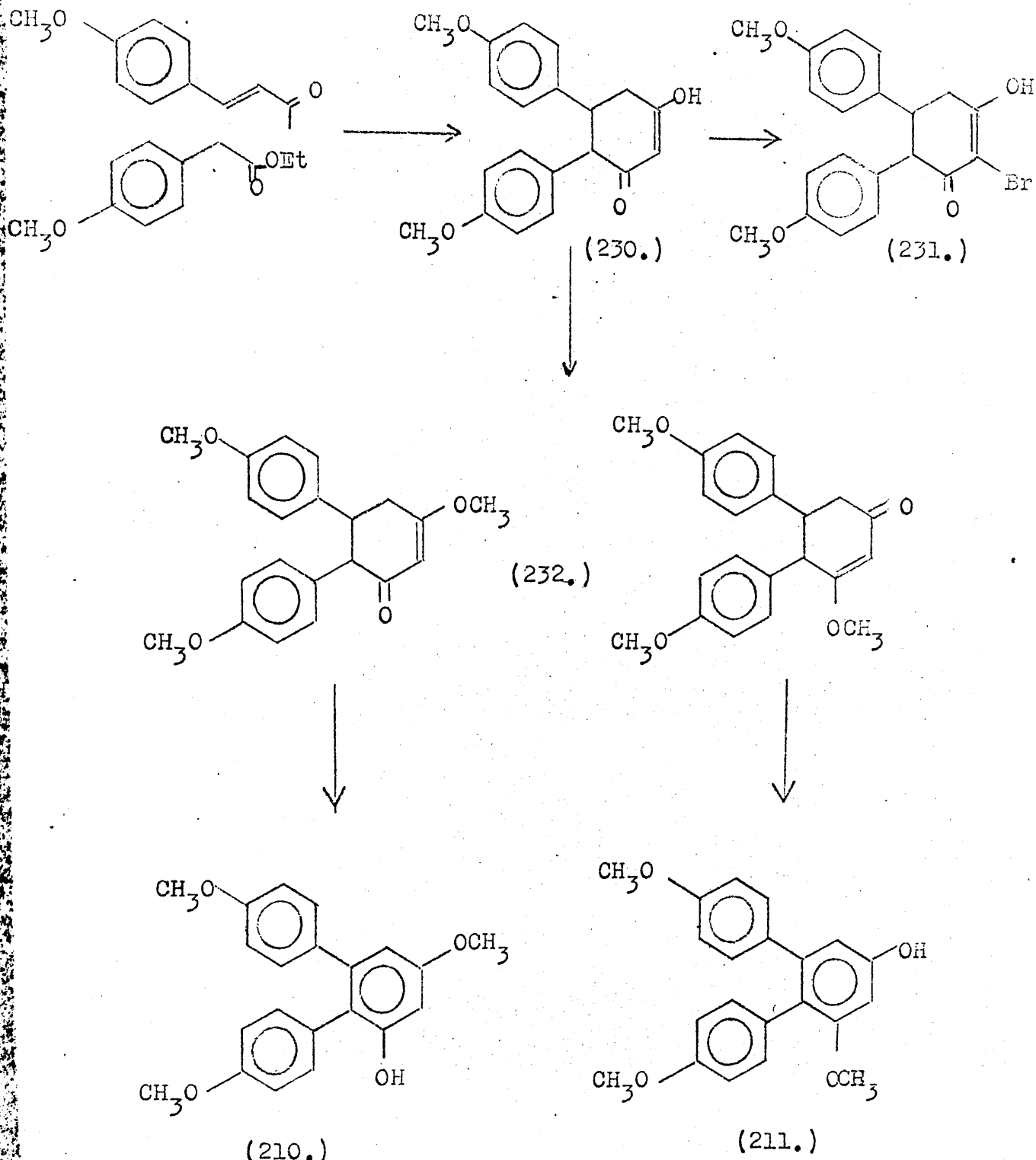


Scheme 3.


CH3O
COCH2OAc
(223.)
CH3O
OH
CH2OAc
CH2OAc
OH
CH3O
(224.)
CH3O
OAc
OAc
CH3O
(225.)
CH3O
OAc
X
X
CH3O
(226.)


CH3O
COCH2OH
(227.)
CH3O
CO.CO2CH3
(228.)
CH3O
CH(OH)CO2C2H5
(229.)

Scheme 4.


CH3O
O
OEt
CH3O
CH3O
OH
O
CH3O
(230.)
CH3O
OH
Br
O
CH3O
(231.)
CH3O
OCH3
O
CH3O
(232.)
CH3O
O
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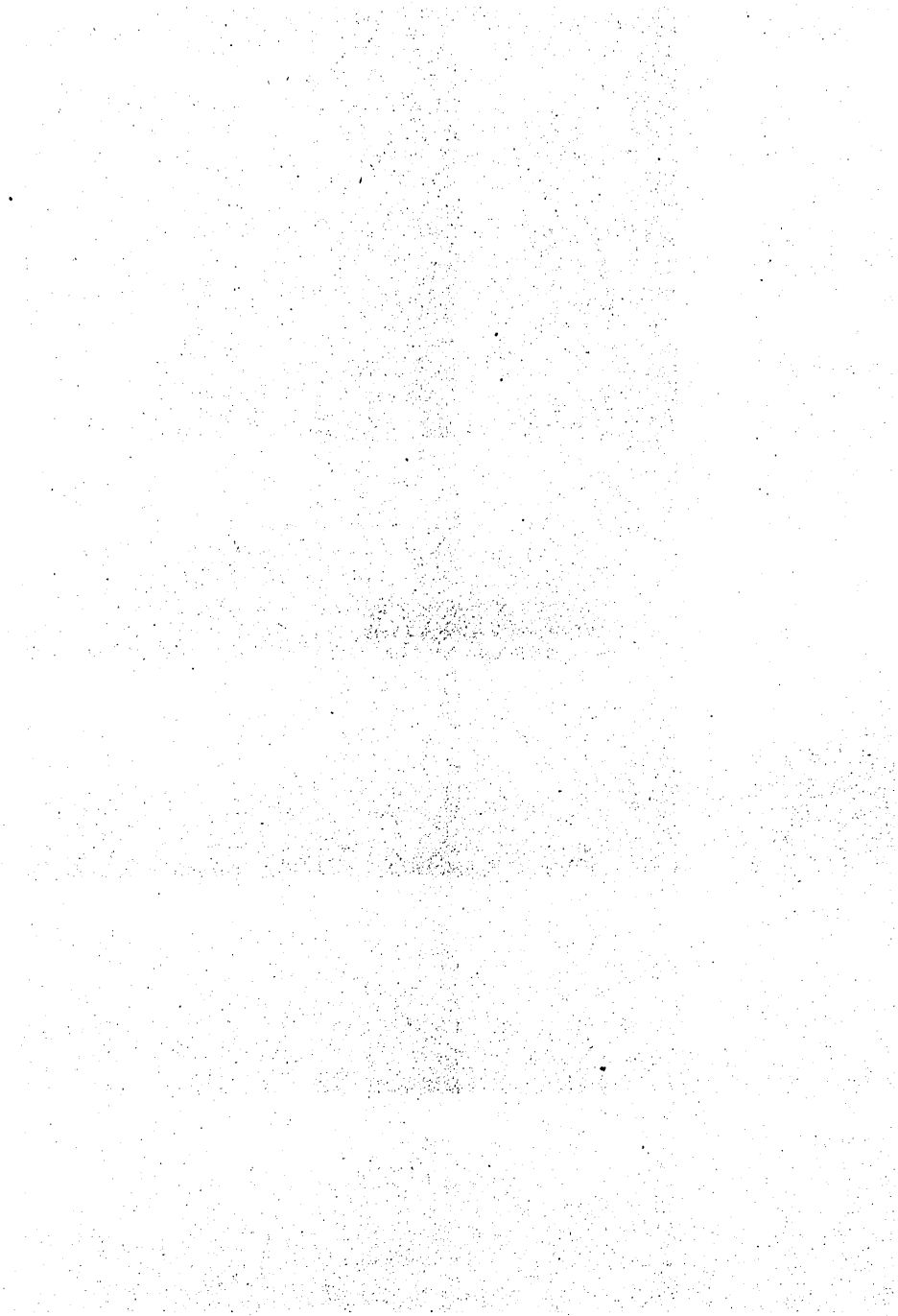
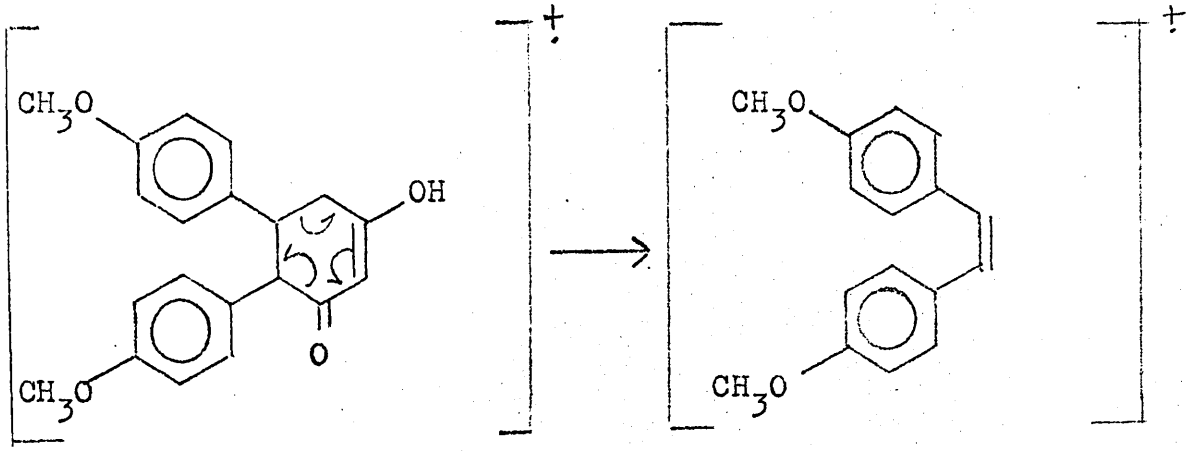
CH3O
OCH3
OH
CH3O
(210.)
CH3O
OH
CH3O
CCH3
(211.)



Scheme 5.

Proposed Fragmentation of Diketone(230)



m/e 324 m/e 240

EXPERIMENTAL.

(1) 2,3- di- (p-methoxyphenyl) -,2,3-butanediol :

(a) from aluminium amalgam condensation [189].

P-methoxyacetophenone (30g.) in a mixture of absolute alcohol (130 ml.) and dry benzene (130 ml.) was treated with mercuric chloride (0.5g.) and aluminium foil (8.0g.), sandpapered immediately beforehand and cut into one-inch squares. The reaction began spontaneously and stirring and refluxing was maintained on the steam bath for 2 hr; after cooling and addition of dilute aqueous HCl (75 ml.), the mixture was extracted into benzene, then washed with more dilute aqueous HCl, followed by saturated aqueous Na_2CO_3 and then saturated aqueous NaCl.

The benzene solution was then dried over magnesium sulphate. Removal of solvent gave the diol as a white crystalline solid, (12g., 40%), which crystallised from ethyl acetate/light petroleum as prisms, m.p. 168-170°C.

R_f (1% CH_3OH/ 99% $CHCl_3$) 0.4.

(Found: C, 71.24%; H, 7.14%, M^+ at m/e 302;

$C_{18}H_{22}O_4$ requires C, 71.50% ; H, 7.33% m.w. 302.4).

ν max (nujol): 3560 (hydroxyl), 1608, 1580, 1520, 1300, 1250, 1190, 1100, 1040, 920, 850, 812, 760 $cm.^{-1}$

λ max (EtOH) : 228 (E = 16, 830), 274 (E= 2,750), 281 (E = 2,419) nm.

τ(60 Mc./s.: $CDCl_3$) : 2.83, 3.25 (two 4H doublets AA'BB' system, J_{AB} = 9 c.p.s.), 6.20 (3H, S, $\underline{CH_3}$- OAr), 6.23 (3H,S, $\underline{CH_3}$ - OAr), 7.45 (1H, broad S., exchanged by D_2O, R - O$\underline{H}$), 7.70 (1H, broad S, exchanged by D_2O, R-O$\underline{H}$), 8.43, 8.52 (6H, 2$\underline{CH_3}$ -C-O-).

m/e : 302 (2%), 284 (1%), 268 (10%), 241 (100%), 189 (10%), 165 (10%), 151 (58%), 135 (44%), 133 (30%), 77 (34%), 43 (84%).

(b) from zinc-alkali condensation [190] :

In a typical reaction, p-methoxyacetophenone (100g.) was dissolved in a mixture of water (400 ml.) and 95% ethanol (2 l.) containing sodium hydroxide (70g.) and a slurry of zinc dust (300g.). After 36 hr. reflux, the hot solution was filtered, and the pH adjusted to 6. Extraction into chloroform gave, after drying and evaporation of solvent, an oil which slowly crystallised. The total yield of product, identical in spectroscopic properties and t.l.c. behaviour to that described in section (a), was 41g. (41%).

(2) 2,3- di - (p-methoxyphenyl) - buta -1,3- diene :

(a) by potassium bisulphate dehydration of 2,3-di-(p-methoxyphenyl) -2,3-butanediol :

The above diol (10.0g.) was mixed with anhydrous

potassium bisulphate (3.0g.) and heated under vacuum until a melt formed. After 15 min., the cooled product, in ethyl acetate, was adsorbed onto silica gel and added to a column of silica gel (30.0g.) packed in 2% ethyl acetate /98% light petroleum; fractions (50 ml.) were collected from the column at the rate of three per hour. Fractions 1 - 6 yielded pure 2,3-di-(p-methoxyphenyl)-buta-1,3 diene (0.20g., 2.3%), which crystallised from ethyl acetate in lustrous plates, m.p. 105-107°C,

R_f ($CHCl_3$) 0.9.

(Found: C,80.95%; H,6.88%; m^+ at m/e 266.

$C_{18}H_{18}O_2$ requires C, 81.17%; H,6.81%; m.w. 266.3).

νmax (nujol): 1610, 1580, 1520, 1295, 1255, 1185, 1123, 1040, 933, 920, 856, 843, 817 $cm.^{-1}$

λmax (EtOH) : 230 (infl), 305 (E = 44,380)nm.

τ(60Mc./S.; $CDCl_3$): 2.64, 3.20 (two 4H doublets, aryl, AA'BB' system, J_{AB}= 9c.p.s.), 4.52 (2H,d, J = 2 c.p.s.) and 4.75 (2H, d, J = 2 c.p.s; 2 Ar-C = CH_2), 6.27 (6H, S, 2 CH_3OAr).

m/e: 266 (100%), 251 (29%), 235 (66%), 145 (27%), 121 (39%).

Later fractions yielded 2, 3-di(p-methoxyphenyl)-butan-2-one, m.p. 67 - 68°C. R_f($CHCl_3$) 0.7.

(Found: C, 75.98%; H, 7.09%, highest ion at m/e 241.
Calculated for $C_{18}H_{20}O_3$: C, 76.03%; H,7.09%, m.w. 284.4).

νmax (nujol) : 1710, 1610, 1515, 1300, 1250, 1185, 1123, 1082, 1040, 865, 850 cm^{-1}.

λmax (EtOH) : 229 (E = 17,400), 277 (E = 4,014)nm.

τ(60 Mc./S.; $CDCl_3$) : 2.88, 3.16 (two 4H doublets, aryl, AA'BB' system; J_{AB} = 9 c.p.s.), 6.21 (6H, S, 2 $\underline{CH_3}$ OAr), 7.93 (3H, S, $\underline{CH_3}$ CO.) , 8.20 (3H, S, $\underline{CH_3}$ - C - Ar_2)

m/e : 241 (100%), 226 (22%), 211 (19%), 183 (21%), 165 (21%) , 152 (19%), 133 (97%), 77 (19%), 43 (21%).

(b) <u>from treatment of 2,3-di- (p-methoxyphenyl)- 2,3-butanediol with acetic anhydride /pyridine.</u>[191]

A solution of the diol (10.0 g.) in freshly distilled acetic anhydride (75 ml.) and dry pyridine (75 ml.) was refluxed for 20 hr., after which time excess solvent was removed by rotary evaporation. The residue was adsorbed onto silica gel (5 g.) and introduced on top of a column of silica gel (30 g.) in 5% ethyl acetate / 95% light-petroleum; fractions (50 ml.) were collected at the rate of three per hour. Fractions 1 - 5 yielded pure 2, 3-di-(p-methoxyphenyl)-buta- 1,3-diene (2.2g, 25%) and

fractions 6 - 10 contained a mixture of this diene and 3, 3-di-(p-methoxyphenyl)- butan- 2 - one; this latter compound was obtained pure from later fractions (0.5g.).

(3) 1,2-di-(p-methoxyphenyl) -4, 5-dicarbethoxy- cyclohexa - 1, 4-diene.

2,3-di-(p-methoxyphenyl)-buta-1,3-diene (1.0g.) and acetylenedicarboxylic acid diethyl ester (0.63g.) were refluxed in xylene (15 ml.) for 2 hr. Removal of xylene by rotary evaporation gave a red oil which solidified on standing and crystallised from acetone in colourless needles (0.80g., 49%), to m.p. 127.5 - 129°C. $R_f(CHCl_3)$ 0.5. (Found : C, 71.66%; H, 6.59%; M^+ at m/e 436. $C_{26}H_{28}O_6$ requires C, 71.54%; H, 6.47%, m.w. 436.5).

νmax (nujol) : 1725, 1703, 1675, 1605, 1575, 1516, 1414, 1310, 1250, 1175, 1150, 1107, 1075, 1050, 1038, 870, 844, 793 $cm.^{-1}$

λmax (EtOH) : 271 (E = 23,900) nm.

τ(100 Mc/s.; $CDCl_3$) : 3.06, 3.34 (two 4H doublets, aryl, AA'BB' system J_{AB} = 9 c.p.s.), 5.72 (4H, q , 2 CH_3 $\underline{CH_2}$ -OCO -), 6.29 (6H,2 $\underline{CH_3}$ - OAr), 6.54 (4H, 2 -C:C-$\underline{CH_2}$ -C:C), 8.65 (6H, t, 2 $\underline{CH_3}$.$\underline{CH_2}$. OCO -)

m/e : 436 (61%), 434 (23%), 389 (35%),

360 (68%), 316 (100%), 254 (42%), 214 (29%), 201 (29%), 120 (23%).

(4) 4,4"-dimethoxy, -4',5'-dicarbethoxy o-terphenyl

(a) Using D.D.Q.

A solution of D.D.Q. (79 mg.) and 1,2-di-(p-methoxyphenyl),4,5-dicarbethoxy - cyclohexa - 1,4-diene (100 mg) in benzene (5ml) was refluxed under nitrogen for 4 hr. The solution was filtered and the product(60 mg. 60%) isolated by p.l.c. on Kieselgel HF_{254} (two 20x20x0.5 m.m.) using chloroform as developing solvent. Recrystallisation from methanol yielded colourless needles, m.p. 105-106°C.

R_f ($CHCl_3$) 0.5.

(Found : C, 72.01%; H, 6.09%; m^+ at m/e 434.

$C_{26}H_{26}O_6$ requires C, 71.87%; H, 6.03%; m.w. 434.5).

νmax (nujol) : 1728, 1708, 1605, 1583, 1520, 1418, 1370, 910, 850, 820, 816, 800 $cm.^{-1}$

λmax (EtOH) : 271 (E = 23,900)nm.

τ(100 Mc./S.; $CDCl_3$) : 2.24 (2H, S, H-3' and H-6'), 2.90, 3.21 (two 4H doublets, aryl, AA'BB' system, J_{AB} = 9 c.p.s.), 5.60 (4H, q, 2 $CH_3\underline{CH_2}$-OCO-), 6.22 (6H, S, 2 $\underline{CH_3}$-OAr), 8.60 (6H, t, 2 $\underline{CH_3}$-CH_2-OCO.).

m/e 434 (100%), 406 (7%), 389 (13%), 361 (54%), 288 (14%), 201 (20%).

(b) Using Chromium trioxide .[192]

Chromium trioxide (0.15g.) was dissolved in 80% aqueous acetic acid (10 ml.) and the solution cooled to 0°C. A solution of 2,3-di-(p-methoxyphenyl)-4,5-dicarbethoxy cyclohexa-1,4-diene (0.15g.) in glacial acetic acid (15 ml.) was added dropwise and the solution stirred at 0°C. for a further 2½ hr.: dilution with water (10 ml.) and extraction into chloroform gave, after washing with brine and drying, a product (30 mg.) showing identical t. l.c. properties and superposable infra-red spectrum to the ortho-terphenyl obtained as described in section 4 (a).

(c) Using N-bromosuccinimide :

To a solution of 2,3-di-(p-methoxyphenyl)-4,5-dicarbethoxycyclohexa-1,4-diene (43.7 mg., 0.1 m.mole) in carbon tetrachloride (5 ml.) was added N-bromosuccinimide (37 mg., 0.2 m.mole) and the solution stirred at room temperature for 2 days. After washing with water, the solution was dried and concentrated to give a crystalline product (40 mg.), with identical R_f and infra-red absorption to the o-terphenyl described in section 4(a).

(5) Anisoyl diazoketone (p-methoxy-ω-diazoacetophenone):[193]

An excess of diazomethane in dry ether was added to a solution of anisoyl chloride (8.5g.) in dry ether (25 ml.)

cooled in ice. Evolution of nitrogen occurred for about 2 hr. during which time the solution was allowed to warm to room temperature. Concentration of the solution after a further 14 hr., gave the yellow crystalline diazoketone (8.0g. 100%), m.p. 65 - 67°C, which gave a positive sodium-fusion test for nitrogen.

ν max (nujol); 3130, 2120, 1610 (α -diazoketone carbonyl) 1590, 1565, 1517, 1420, 1310, 1270, 1233, 1180, 1120, 1030, 883, 855, 800, 760, 695 cm.$^{-1}$

τ(60 Mc./S.; $CDCl_3$): 2.24, 3.07 (two 2H doublets aryl, AA'BB' system, J_{AB} = 9 c.p.s.), 4.14 (1H, S, -CO-CH = $N^+ = N^-$), 6.16 (3H, S, CH_3OAr.).

(6) P-methoxy-w-acetoxyacetophenone:[193]

Anisoyl diazoketone (4.0g.) was dissolved in glacial acetic acid (5.6 g.) and the solution warmed to 80°C. for 30 min. During the course of the reaction, the solution changed from yellow to dark - red with accompanying evolution of nitrogen. Removal of acetic acid by rotary evaporation gave the ketone which crystallised from diethyl ether in needles (4.5g., 87%), m.p. 55 - 57°C.

(Found : C, 63.19%; H, 5.69%:
calculated for $C_{11}H_{12}O_4$; C, 63.45%; H, 5.81%.)

ν max (nujol): 1735 (acetate), 1690 (-aryl ketone),

1610, 1588, 1515, 1426, 1250, 1183, 1115, 1085, 1030, 980, 846, 815 $cm.^{-1}$

τ(60 Mc./S.; $CDCl_3$) : 2.08, 3.04 (two 2H doublets, aryl, AA'BB' system, J_{AB} = 9 c.p.s.), 4.72 (2H,S, -CO-$\underline{CH_2}$ - OAc), 6.14 (3H, S, $\underline{CH_3}$ OAr), 7.80 (3H,S, $\underline{CH_3}$ CO.)

(7) Treatment of p-methoxy-w-acetoxyacetophenone with aluminium amalgam.

The acetate (4.0g.) obtained as described in section (6), in a mixture of dry benzene (20 ml.) and absolute ethanol (20 ml.) was treated with mercuric chloride (0.15g.) and aluminium foil (1.1g.) as described in section (1a) for p-methoxyacetophenone. The solid product (0.30g.) was identified as 2,3-di-(p-methoxyphenyl),-2,3-butanediol (section 1a) by comparison of the t.l.c. properties and i.r. spectra.

The aqueous solution remaining after extraction of the reaction mixture was subjected to a 24-hour liquid-liquid extraction using ethyl acetate. T.l.c. analysis showed that no more-polar material had been formed.

(8) P-methoxy-w-hydroxyacetophenone.[195]

The diazoketone (4.0g.) obtained as described in section (5), in dioxan (4 ml.) was treated with 2N. sulphuric acid (5 ml.). A vigorous exothermic reaction

began immediately and lasted for 10 min: dilution with water (10 ml.) gave a yellow precipitate which crystallised from methanol/light-petroleum in prisms(2.4g.,58%). m.p. 100 - 102°C.

(Found : C, 64.78%; H, 6.07%; calculated for $C_9H_{10}O_3$; C 65.05%; H, 6.07%).

νmax (nujol) : 3,500 (hydroxyl), 1683 (α-aryl ketone), 1610, 1580, 1523, 1415, 1300, 1252, 1235, 1186, 1020, 1005, 990, 850, 842 cm.$^{-1}$

τ(60 M.c./S.; $CDCl_3$) : 2.08, 3.04 (two 2H doublets aryl, AA'BB' system, J_{AB} = 9 c.p.s.), 5.21 (2H, S, CO-$\underline{CH_2}$-OH), 6.15 (3H, S, $\underline{CH_3}$OAr), 6.73 (1H, broad S, exchanged by D_2O, - O$\underline{H}$).

(9) Treatment of p-methoxy-ω-hydroxyacetophenone with aluminium amalgam.

The keto alcohol (2.0g.) was dissolved in dry benzene (20 ml.) and absolute ethanol (20 ml.) containing mercuric chloride (0.60g.) and treated with aluminium (0.3g.) as described in section(1a). No spontaneous reaction occurred in this case; after 3 hr. refluxing, the reaction mixture was treated in the manner previously described. A quantitative return of starting material was obtained.

(10) P-methoxyphenylglyoxylic acid[196] :

A solution of potassium permanganate (32g.) and potassium hydroxide (12g.) in water (600 ml.) was added dropwise with stirring during 4 hr. to p-methoxyacetophenone (12g.) dissolved in pyridine (100 ml.) maintained at 5°C. After stirring for a further 60 min., excess permanganate was destroyed by adding sodium bisulphite. The precipitated manganese dioxide was removed and the solution washed with water (2 x 100 ml.). After acidification with conc. HCl (200 ml.), the solution was allowed to stand overnight. After filtration of precipitated anisic acid, the solution was extracted repeatedly with ether, and the combined extracts (1.5L.) evaporated to dryness. The residual oil solidified on trituration and crystallised from benzene/light petroleum as colourless plates (7.6 g., 53%), m.p. 84 - 86°C, (literature yield 7g., m.p. 87°C.)

νmax (nujol) : 1730 (ester), 1660 (α-aryl, - α carbethoxy carbonyl), 1603, 1515, 1260, 1205, 1165, 1025, 980, 863, 810 cm.$^{-1}$

τ(60 Mc./S.; $CDCl_3$): 1.55, 3.02 (two 2H doublets aryl, AA'BB' system, J_{AB} = 9 c.p.s.), 6.05 (3H, S, CH_3OAr).

(11) Methyl p-methoxyphenylglyoxylate:

A solution of p-methoxyphenylglyoxylic acid (1.0g.) in ether (25 ml.) was treated with an excess of ethereal diazomethane until effervescence ceased. Excess diazomethane was destroyed by addition of a few drops of glacial acetic acid, and the ether solution concentrated to give colourless needles (0.7g., 65%) m.p. 48 - 49°C. (literature m.p. 50°C.)

νmax (nujol) : 1725 (ester), 1670 (α-aryl, α-carbethoxy carbonyl), 1603, 1515, 1428, 1325, 1270, 1220, 1165, 1035, 925, 860, 810 cm.$^{-1}$

τ (60 Mc./S., $CDCl_3$) : 1.98, 3.03 (two 2H doublets, aryl AA'BB' system, J_{AB} = 9 c.p.s.), 6.03 (3H, S, CH_3OAr), 6.11 (3H, S, $-CO_2CH_3$).

(12) Attempted reductive condensation of methyl p-methoxyphenylglyoxylate.

(a) Using aluminium amalgam:

In an experiment similar to that described in section 1(a), the ester (0.8g.) was dissolved in a mixture of dry benzene (15 ml.), abolute ethanol (15 ml.) containing mercuric chloride (0.06 g.), and freshly sandpapered aluminium foil (0.4g.). The reaction again began spontaneously with a colour change to green. The product of the reaction was an oil which solidified on cooling

in ice. Crystallisation from ethyl acetate/light petroleum gave ethyl p-methoxymandelate (0.5g. 58% as colourless needles, m.p. 37 - 39°C.

(Found: C, 63.30%; H, 6.75%; M^+ at m/e 210: calculated for $C_{11}H_{14}O_4$; C, 62.85%; H, 6.71%. m.w. 210.2).

ν_{max} (nujol): 3500 (hydroxyl), 1725(ester), 1610, 1585, 1519, 1430, 1250, 1180, 1115, 1085, 1030, 985, 920, 845, 830, 805 $cm.^{-1}$

τ (60 Mc/S.; $CDCl_3$): 2.68, 3.16 (two doublets, aryl, AA'BB' system, J_{AB}= 9 c.p.s.), 4.91 (1H, S, - CH.OH), 5.82 (2H, q, $CH_3\underline{CH_2}$-OCO-); 5.91 (1h, S, exchanged by D_2O, -OH), 6.21(3H, S, CH_3OAr), 8.08(3H, t, $\underline{CH_3}CH_2$-OCO-).

m/e: 210 (45%), 148 (14%), 137 (100%), 135 (98%), 121 (28%), 109 (98%), 94 (91%), 77 (98%).

(b) by white light irradiation:[197]

In two separate experiments, ethyl p-methoxyphenylglyoxylate (0.4g.) was dissolved in isopropanol 3.3g.) and also in butan - 2 - ol (1.4g.) contained in test tubes, through which nitrogen was continually passed, and irradiated with white light. After 15 days, t.l.c. analysis showed that approximately 50% of the starting material had reacted to give a complex mixture of more polar products which was not further examined.

(13) Anisalacetone:

This was prepared after the method of Drake and Allen[200], by base-catalysed condensation of anisaldehyde and acetone. 100g. anisaldehyde gave 103.5 g. anisalacetone (80%).

(14) Ethyl p-methoxyphenylacetate

P-methoxyphenylacetic acid (99.6 g., 0.6 mole) was dissolved in absolute ethanol (55.2g., 1.2 mole) and to this solution was added sodium-dried benzene (180 ml.) and conc. H_2SO_4 (18.0 g.). After 36 hours at reflux temperature, the solution was cooled, diluted with ether (250 ml.), then washed successively with sodium bicarbonate, and water. After drying, removal of solvent and distillation under reduced pressure gave the ester as a colourless liquid, (100g., 93%), b.p. 86 - 89°C./0.25 mm.

(15) 5, 6-di-(p-methoxyphenyl)-cyclohexa-1,3-dione.[198]

To a one-litre three necked flask equipped with condenser, mechanical stirrer, and pressure-equilibrating dropping funnel was added a solution of anisalacetone (25.4 g., 0.14 mole) and ethyl p-methoxyphenylacetate (42.0 g, 0.22 mole), in sodium-dried toluene (120 m.l). The flask was flushed with nitrogen and the solution temperature raised to 40°C. To this stirred solution was added sodium hydride (28.8 g. of a 50% dispersion;

0.6 mole) suspended in dry toluene (60 ml.), dropwise over a period of one hour. The solution quickly became dark-brown in colour with accompanying effervescence. After a further two hours stirring, the solution was allowed to cool to room temperature and stirred overnight. Excess sodium hydride was destroyed by careful addition of ethanol (5 ml.), the solution was diluted with ether (200 ml.), and poured onto ice (500 g.) and acetic acid (75 ml.). The organic layer was separated and the aqueous layer extracted several times with ether; the combined ether extracts (approx. 1 l.) were then washed with sodium bicarbonate solution, followed by water, and dried. Concentration of the solution and cooling in ice, gave the required diketone as a yellow crystalline solid which crystallised from ethyl acetate as yellow rods (8g., 17%) m.p. 131 - 134°C. R_f (10% CH_3OH/90% $CHCl_3$) 0.4. (C, 73.86%; H, 6.34%; M^+ at m/e 324. $C_{20}H_{20}O_4$ requires C, 74.06%; H, 6.21%. m.w. 324.4).

ν max (nujol) : 3500 - 3000 (broad absorption) 1620 (broad), 1550, 1520, 1415, 1215, 1205, 1180, 1160, 1112, 1090, 1040, 890, 865, 840, 823, 798 $cm.^{-1}$

λ max (EtOH) : 258 (E = 17,590), 284 (infl., E = 6,631) n.m.

τ (100 Mc./S.; $CDCl_3$) : 3.20 (8H, m, aryl), 3.53 (1H, m, exchanged by D_2O, C = C - O<u>H</u>), 4.22 (1H, m, vinyl H), 6.26 (6H, S, 2 <u>CH_3</u>OAr), 6.98 - 7.33 (4H, m, - <u>CH_2</u> and 2 C<u>H</u>).

m/e : 324 (25%), 240 (13%), 225 (8%), 190 (2%), 176 (40%), 165 (16%), 161 (50%), 148 (100%), 145 (20%), 134 (40%), 120 (39%), 91 (40%).

(16) <u>5,6-di-(p-methoxyphenyl)-2-bromo-cyclohexa-1,3-dione</u>

To a solution of the diketone (200 mg.) in carbon tetrachloride (7ml.) was added freshly crystallised N-bromosuccinimide (90 mg.) and the solution refluxed for 90 min. P.l.c. of the reaction mixture on Kieselgel HF_{254} (three 20x20x0.5 mm.), gave the <u>bromodiketone</u> as a solid (135 mg., 54%), which crystallised from carbon tetrachloride as prisms, m.p. 100 - 102°C. R_f (10% CH_3OH/90% $CHCl_3$) 0.2.

(Found: C, 59.39%; H, 4.56%; M^+ at m/e 403. $C_{20}H_{19}O_4Br$ requires C, 59.53%; H, 4.73%, m.w. 403.2).

νmax (nujol) : 1612, 1594, 1515, 1353, 1290, 1248, 1215, 1180, 1119, 1040, 1012, 970, 840, 800 $cm.^{-1}$

λmax (EtOH) : 272 (E = 16,380), 300(infl.) n.m.

τ (100 Mc./S., $CDCl_3$) : 2.97 - 3.33 (8H, m, aryl H), 6.24 (6H,S <u>CH_3</u>OAr), 7.05 - 7.25 (4H,m, 2C<u>H</u> and .<u>CH_2</u>).

m/e : 404 (17%), 402 (17%), 324 (34%), 322 (34%), 270 (28%), 268 (28%), 255 (15%), 253 (15%), 240 (20%), 225 (17%), 215 (20%), 190 (24%), 134(57%), 121 (68%), 99 (43%), 91 (63%).

(17) Enol ethers of 5,6-di-(p-methoxyphenyl)-cyclohexa -1,3-dione.

An ethereal solution of diazomethane, prepared in three-fold molar excess, was added to a solution of the diketone (1.2g.) in methanol (100 ml.) cooled to 0°C. After 12 hours, the solution was filtered to remove polymeric material, and evaporated to give a yellow oil (1.0g.) which by t.l.c. was observed to be a two-component mixture, $R_f(CHCl_3)$ 0.21 and 0.22. This mixture was used without further purification for the next synthetic stage.

(18) Aromatisation of the enol ethers of 5,6,-di-(p-methoxyphenyl)-cyclohexa -1, 3-dione.[199]

The mixture of ethers (1.0g.), obtained as described in section (17), was mixed with sulphur (0.4g.) and heated under a brisk current of nitrogen to 220 - 230°C. After 2 hours at this temperature, the melt was allowed to cool, dissolved in ethyl acetate, then adsorbed onto silicic acid (1.5g.) and placed on top of a column of silicic acid (80g.)

packed in 10% ethyl acetate/90% light-petroleum. Fractions (15 ml.) were collected from the column at the rate of two per hour, as the polarity of the eluting solvent slowly increased. By this method, two distinct crystalline compounds were isolated:

(a) 4,4",5'-trimethoxy- 3'-hydroxy- o-terphenyl:

Isolated from fractions 38-46 as a pale yellow solid this compound crystallised from benzene/light-petroleum as prisms (226 mg., 23%) m.p. 142-142.5°C. Rf (3% CH_3OH/97% $CHCl_3$) 0.5.

(Found: C, 75.24%; H, 6.03%; M^+ at m/e 336.
$C_{21}H_{20}O_4$ requires C, 74.98%; H, 5.99%; m.w. 336.4).

ν max (KBr) : 3515 (hydroxyl), 3060, 3000, 2930, 2900, 2825, 1604, 1578, 1510, 1470, 1463, 1431, 1402, 1393, 1300, 1284, 1236, 1198, 1170, 1151, 1109, 1100, 1050, 1030, 995, 974, 844, 830, 795 $cm.^{-1}$

λ max (EtOH) : 246 (E = 28,258), 280 (infl.) nm.

τ (60 Mc./S., $CDCl_3$) : 3.00, 3.05, (two 2H doublets, aryl AA'BB' system, J_{AB} = 9 c.p.s.), 3.21, 3.35 (two 2H doublets, aryl AA'BB' system, J_{AB} = 9 c.p.s.), 3.48 (2H,S,H-4' and H-6'), 4.86 (1H, S, exchanged by D_2O, - O<u>H</u> 6.19 (3H,S, CH_3O-), 6,20 (3H,S, CH_3O-), 6.23 (3H,S, CH_3O-).

m/e : 336 (100%), 321 (16%), 305 (11%), 293 (27%), 275 (9%), 261 (18%), 189 (22%), 178 (16%), 165 (13%), 152 (16%).

(b) 3',4',4",-trimethoxy- 5'-hydroxy- o- terphenyl.

Isolated from fractions 58 - 70 as an oil, which, after purification by p.l.c. on Kieselgel HF_{254} (five 20x20x0.5 mm.) in 10% CH_3OH/ 90% $CHCl_3$ as developing solvent, solidified and crystallised from benzene/light-petroleum as plates (190 mg., 19%) m.p. 142.5-143.5°C.

R_f (3% CH_3OH/97%$CHCl_3$) 0.2.

(Found : C, 74.83%; H, 6.02%; M^+ at m/e 336).

ν max (KBr) : 3360 (hydrogen - bonded hydroxyl), 3030, 3,000, 2935, 2835, 1597, 1573, 1517, 1455, 1430, 1403, 1345, 1285, 1238, 1188, 1170, 1104, 1099, 1083, 1015, 1000, 983, 825, 798 $cm.^{-1}$

λ max (EtOH) : 245 (E = 28,870), 280 (infl.) nm.

τ (60 Mc./S., $CDCl_3$) : 3.06, 3.09 (two 2H doublets, aryl AA'BB' system, J_{AB} = 8 c.p.s.), 3.36, 3.40 (two 2H doublets, aryl AA'BB' system, J_{AB} = 8 c.p.s.) 3.57 (2H,S, H-4' and H-6'), 4.34 (1H, S, exchanged by D_2O, - O<u>H</u>), 6.23 (6H,S, 2 $C\underline{H}_3O$-), 6.34 (3H,S, $C\underline{H}_3$ -O)

m/e :identical fragmentation pattern to that described in section (18)(a).

(19) 4,4",5'-trimethoxy,-3'-hydroxy,-4'6'-dibromo-o-terphenyl.

A solution of 4,4",5'-trimethoxy,-3'-hydroxy-o-terphenyl (20 mg.) in excess bromine/carbon tetrachloride was refluxed for 2 hr. when t.l.c. showed virtually complete conversion to a less-polar product. Evaporation of solvent gave the required dibromo derivative which crystallised from benzene/light petroleum as prisms, (20 mg. 68%) m.p. 144 - 146°C., $R_f(CHCl_3)$ 0.4.

νmax (KBr): 3476, 3005, 2940, 2840, 1608, 1578, 1536, 1515, 1461, 1445, 1417, 1392, 1348, 1292, 1250, 1212, 1181, 1112, 1076, 1059, 1026, 963, 839, 809, 756 cm.$^{-1}$

τ(60 Mc./S., $CDCl_3$) : 3.08, 3.12 (two 2H doublets, aryl AA'BB' system, J_{AB} = 8 c.p.s.), 3.22, 3.38 (two 2H doublets, aryl AA'BB' system, J_{AB} = 8 c.p.s.), 4.53 (1H,S,exchanged by D_2O, - O<u>H</u>), 6.05 (3H,S,C<u>H</u>$_3$O-), 6.27 (6H,S, 2 C<u>H</u>$_3$O-).

m/e : 496 ((54%), 494 (100%), 492 (54%), 481 (4%), 479 (5%), 477 (4%), 400 (24%), 398 (24%), 291 (13%), 248 (9%), 205 (10%), 189 (15%), 176 (16%).

m* for m/e 494 - 479.

(20) 3', 4,4" - trimethoxy,-5'-hydroxy,-4',6'-dibromo-o-terphenyl .

A solution of 3', 4, 4"-trimethoxy-5'-hydroxy-o-terphenyl (21 mg.), in excess bromine carbon tetrachloride, was refluxed for one hour when t.l.c. showed complete conversion to a less polar product. Evaporation of solvent gave the required dibromo derivative (25 mg.,85%) which crystallised from benzene/light petroleum, m.p. 162 - 165°C.

R_f (3%CH_3OH/ 97% $CHCl_3$) 0.4.

ν max (KBr) : 3443, (broad), 3007, 2960, 2834, 1612, 1580, 1544, 1518, 1456, 1428, 1400, 1350, 1290, 1248, 1210, 1178, 1111, 1082, 1055, 1030, 966, 838, 810, 756 $cm.^{-1}$

τ(60 Mc./s., $CDCl_3$) : 3.06, 3.12 (two 2H doublets, aryl AA'BB' system, J_{AB} = 10 c.p.s.), 3.34, 3.39 (two 2H doublets, aryl AA'BB' system, J_{AB} = 8 c.p.s.), 6.23 (6H, S, 2$\underline{CH_3}$O-), 6.62 (3H, S, $\underline{CH_3}$ O-), 7.91 (1H, broad, O$\underline{H}$).

m/e : parent ion at m/e 496, 494, 492: fragmentation similar to that described in section (19).

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