STUDIES IN THE FIELD OF NATURAL PRODUCTS.

A thesis for the degree of Doctor of Philosophy
in the University of Glasgow.

Submitted by

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It is also a pleasure to thank Dr. P. de Mayo for his expert advice and assistance during a happy association.

Grateful acknowledgment is made to Professor H. Raistrick, F.R.S., who kindly provided the atrovenetin, herqueinone, and norherqueinone used in the work described in Part I of this thesis.

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SUMMARY.

The inter-relationship of the three mould metabolites atrovenetin, herqueinone, and norherqueinone has been elucidated by the discovery that deoxynorherqueinone (obtained by reduction of norherqueinone with zinc and acetic acid) is identical with atrovenetin. A study of the spectral properties of the three mould metabolites and their degradation products has been undertaken, from which it appeared that atrovenetin, deoxyherqueinone, xanthoherquein, and norxanthoherquein (the last two being obtained by acid hydrolysis of herqueinone and norherqueinone respectively[96]) are all derivatives of 9-hydroxyperinaphthenone. This has been confirmed by suitable degradations, among which may be mentioned the nitric acid oxidation of xanthoherquein, norxanthoherquein, and atrovenetin to nitrococussic acid; and the oxidation of atrovenetin with alkaline hydrogen peroxide to a derivative of 2:7-dihydroxynaphthalic anhydride. The available data have all been correlated in terms of unique structures for atrovenetin, and many of its derivatives and degradation products, and for xanthoherquein.

In particular, the compound, $C_{15}H_{14}N_2O_9 \cdot H_2O$, obtained by previous workers[92] by oxidising atrovenetin with concentrated nitric acid, has been reinvestigated, and assigned a phthalide type of structure consistent with all its chemical and physical properties (including its nuclear magnetic resonance spectrum). During the course of the work on atrovenetin, 2:7-dihydroxynaphthalic anhydride has been synthesised, and the spectra of this and seven other naphthalic anhydrides have been measured. A relationship between the amount of hydrogen-bonding in these compounds, and the positions of their infrared absorption bands in the carbonyl region has emerged.

The molecular skeletons of norherqueinone and herqueinone follow from the work on atrovenetin. The possible positions of the methoxyl group in herqueinone have been reduced to two, as a result of suitable degradations. Possible structures for herqueinone and its derivatives and degradation products have been discussed.

The structure of the interesting plant product, anisoxide[117,120], has been confirmed by the synthesis

of anisoxide itself, and of several of its degradation products. The biogenesis of C_5 isoprenoid side-chains attached to aromatic nuclei has been discussed, with special reference to atrovenetin and anisoxide.

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CONTENTS.

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PART I.

QUINONOID FUNGAL METABOLITES.

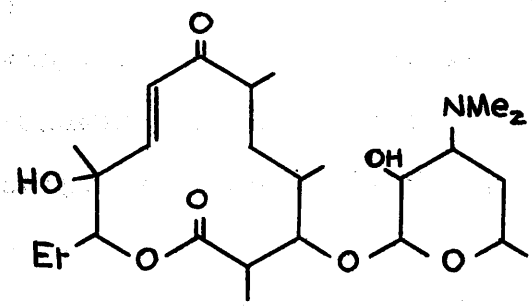
The diversity of chemical structure elaborated by the metabolism of fungi is unequalled elsewhere in the realm of natural products. To illustrate the immense range of types of structure embraced by the general term "mould metabolite", it may be mentioned that both ethylene[1] and methymycin (I)[2] qualify for admission to this class. An excellent review of those groups of mould products known in 1949 is provided by Raistrick's Bakerian Lecture, delivered in that year[19a].

It is proposed to deal here only with quinonoid fungal metabolites.

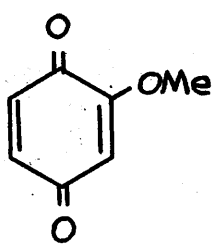
A considerable number of hydroxylated and methoxylated benzo- and toluquinones occur as mould products. Examples of these are 2-methoxybenzoquinone (II)[3], 2:6-dimethoxybenzoquinone (III)[3], gentisylquinone (IV)[4] and fumigatin (V)[6].

Both (IV) and (V) occur in the mould together with the corresponding quinol, a fact which lends support to the view that such quinones play a part in the oxidation-reduction processes of the moulds which produce them.

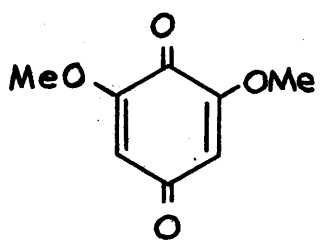
What are perhaps the two most interesting members



I



II



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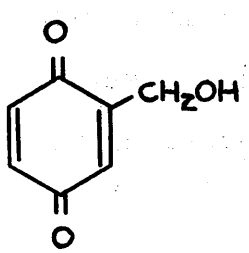
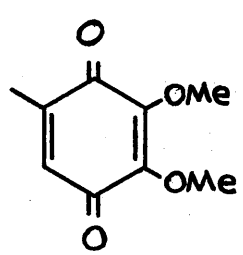
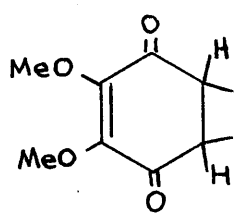


IV

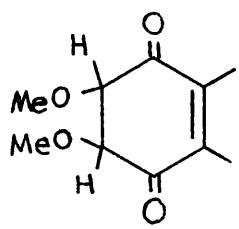


V

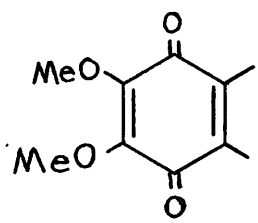
of this group, gliorosein and terreic acid, are not, strictly speaking, benzoquinones at all, but they are very closely related structurally to the benzoquinones already mentioned. Gliorosein (VI or VII) is the only naturally-occurring benzoquinol to be found in the enedione form. It was isolated from a species of Gliocladium[7] together with aurantiogliocladin (VIII), which occurs in the form of its quinhydrone.



VI



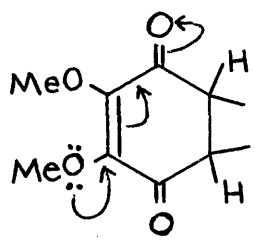
VII



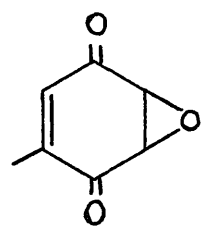
VIII

The structure of gliorosein was assigned by Visher[8] on the basis of the following evidence. Gliorosein is a colourless compound, which rapidly rearranges to aurantiogliocladin quinol when treated with base. It does not, however, form a quinhydrone with aurantiogliocladin, and it yields no colour with ferric chloride. Its infrared absorption spectrum exhibits strong bands at 1683 $cm.^{-1}$ and 1610 $cm.^{-1}$, attributable to a conjugated

ene-dione. A study of the ultraviolet absorption spectra of aurantiogliocladin and gliorosein reveal the same differences as are observed when the spectra of benzoquinone and benzoquinone dibromide are compared. Thomson[9] has expressed a preference for structure (VI) on the grounds that the vinylogous ester groups could account for the failure of gliorosein to form carbonyl derivatives, and the fact that no β-elimination of a methoxyl group is observed when the compound is treated with base. Structure (VI) might also be favoured because the mesomerism indicated in (IX) would have a stabilising influence.



IX

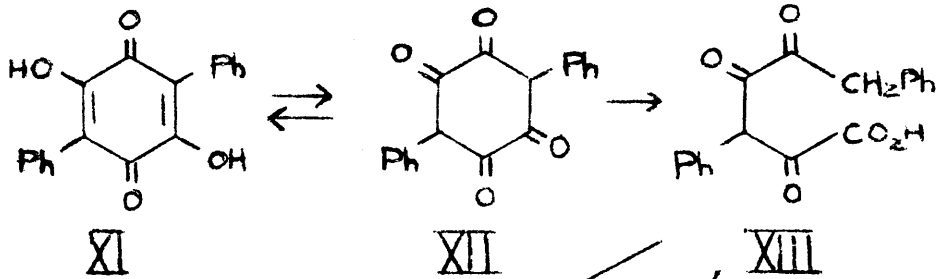


X

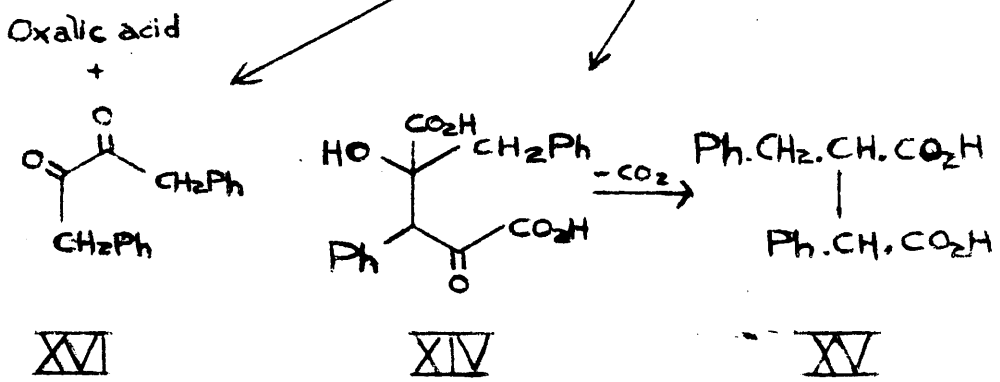
Structure (X) has only recently been suggested for terreic acid[10], and as yet no details of its chemistry have been published. This is the first example of an epoxide occurring in a simple quinonoid mould metabolite, although such a structural feature is well-known amongst other groups of natural products.

In the higher fungi there occurs a group of terphenyl derivatives related to 2:5-dihydroxybenzoquinone. These compounds undergo several very interesting reactions involving fission of the quinone ring. The simplest member of this group is polyporic acid (XI), the structure of which was elucidated by Kögl[11], and confirmed by synthesis[12].

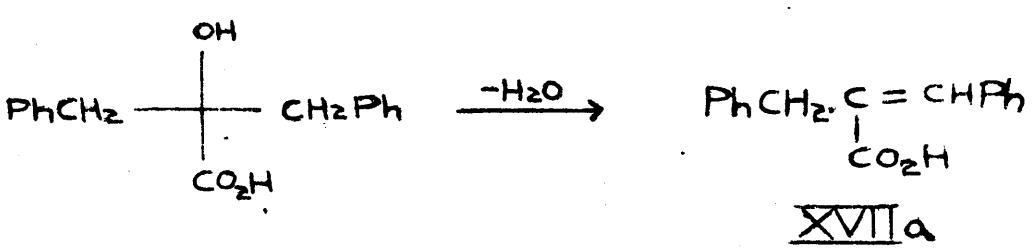
Hydrolysis of polyporic acid with aqueous caustic soda solution[13] yields 1-benzyl-2-phenylsuccinic acid (XV), cis- and trans-α-benzylcinnamic acids (XVIIa), and oxalic acid. By analogy with earlier work carried out by Fichter[12], Kögl explained these products by assuming hydrolytic fission of the tautomeric form (XII) of polyporic acid; the triketone (XIII) thus formed could then either be cleaved further to oxalic acid and the α-diketone (XVI) which by benzilic acid rearrangement and dehydration would afford the benzylcinnamic acids, or it could itself undergo a benzilic acid rearrangement to give (XIV). Ketolactones of type (XIVa) were known[12] to yield succinic acids (in this case, XV) and carbon dioxide, when refluxed with aqueous sodium hydroxide.



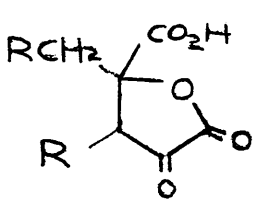
XI XII XIII



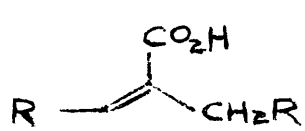
XVI XIV XV



XVIIa

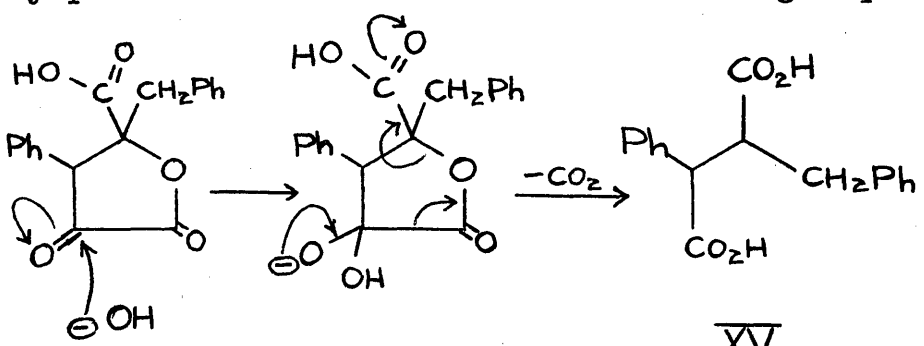


XIVa



XIVb

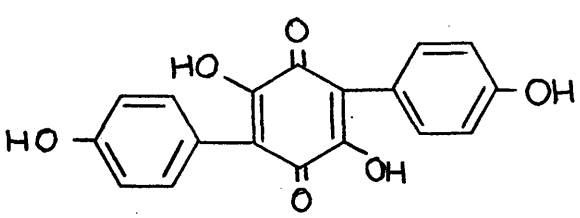
The mechanism of this last conversion requires some comment. The decarboxylative hydrolysis of the keto-lactone probably proceeds as indicated in the following sequence:



XV

In the case of the acid (XIV), it is likely that the reaction proceeds in the same way, since, even in basic solution, some of the lactone (XIVa: R = Ph) will exist in equilibrium with the acid.

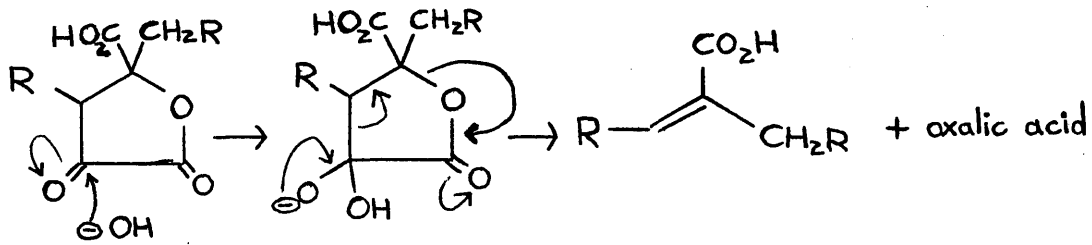
In the alkaline hydrolysis of atromentin (XVII) with hot 30% aqueous potassium hydroxide, the keto-lactone (XIVa: R = p-hydroxyphenyl) was actually isolated[13].



XVII

Further hydrolysis of the keto-lactone with 50% aqueous potassium hydroxide at 140-165°C. degrades it further to a mixture of the cis- and trans- isomers of (XIVb: R=

p-hydroxyphenyl). Apossible mechanism for the hydrolysis is the following:

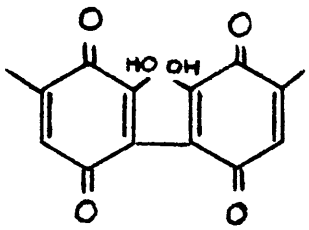


XIVa XIVb

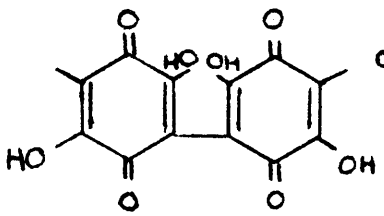
The α-benzylcinnamic acids (XVIIa) and the oxalic acid obtained in the alkaline hydrolysis of polyporic acid (XI)might also arise in the same way, rather than by direct alkaline cleavage of the triketone (XIII).

Two mould metabolic dibenzoquinones, phoenicin (XVIII)[14] and oosporein (XIX)[15] are known. It has been suggested by Friedheim[16] that phoenicin functions as a respiratory catalyst by virtue of the fact that several oxidation-reduction levels are possible in the molecule. Perhaps the main interest of these compounds to chemists lies in the possibility of their arising in Nature by the coupling of phenolic radicals (see next section).

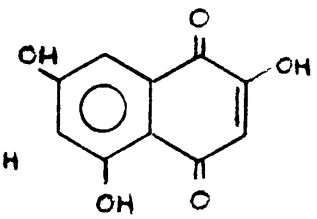
Relatively few naphthoquinones occur in fungi. One of them - flaviolin (XX) - has the distinction of



XVIII



XIX



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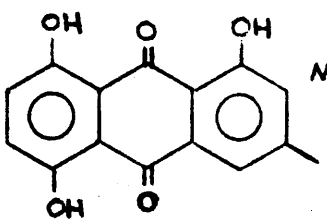
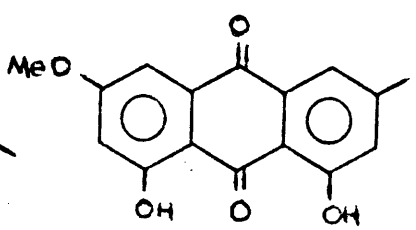
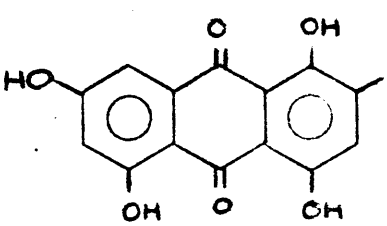


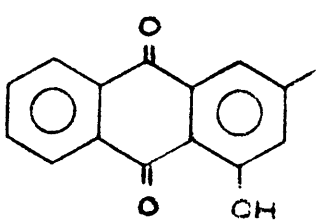
XXI



XXII



XXIII



XXIV

belonging to the select band of natural products whose structures have been correctly predicted on the basis of the acetate hypothesis[17]. The constitution of flaviolin was later confirmed by the synthesis of its trimethyl ether[18].

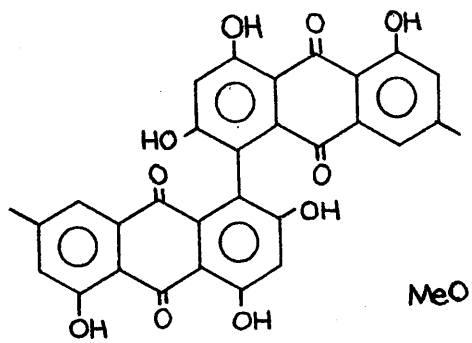
By far the largest group of fungal quinones is that of the anthraquinones. Raistrick and his co-workers have been particularly active in this field.[19a,19b]

Almost all fungal anthraquinones have the following structural features: oxygen functions in the 1- and 8-positions, and a one carbon side-chain at a β-position. Helminthosporin (XXI), the first of this group to be isolated[20,21,22], displays both these features. Other typical examples are physcion (XXII)[23] and catenarin (XXIII)[24].

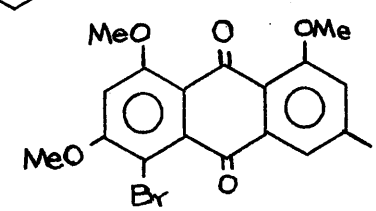
There are a few exceptions to the above generalisations, 4-hydroxy-2-methylanthraquinone (XXIV)[25] being one of them.

In recent years, a group of dianthraquinones and related compounds have been isolated as mould metabolic products. Skyrin (XXV) is one of these. Skyrin was

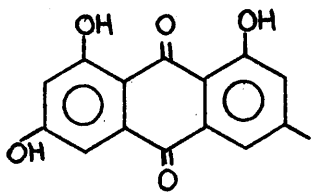
first obtained by Howard and Raistrick[26] who suggested (XXV) as a possible structure. This has been confirmed by later work by Shibata and his colleagues[27,28], and by the synthesis of skyrin hexamethyl ether from (XXVI) by the Ullmann reaction[29].



XXV



XXVI

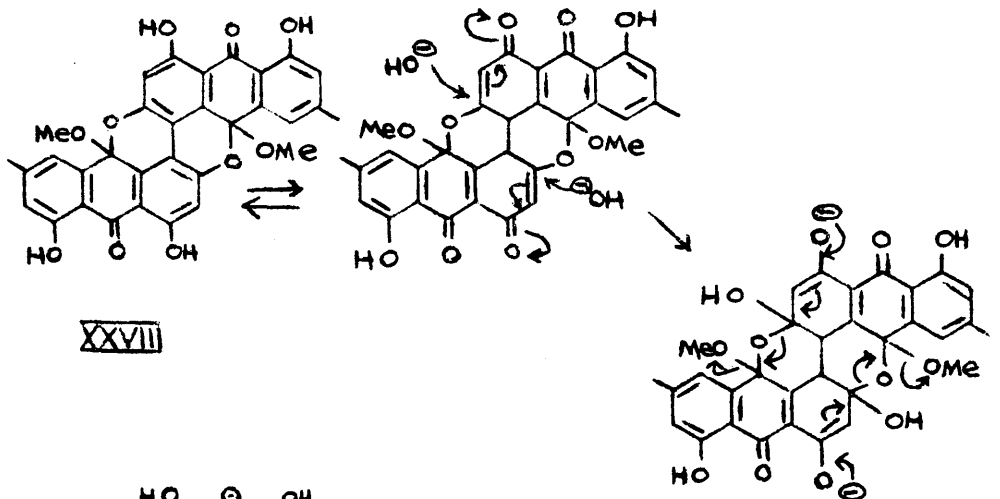


XXVII

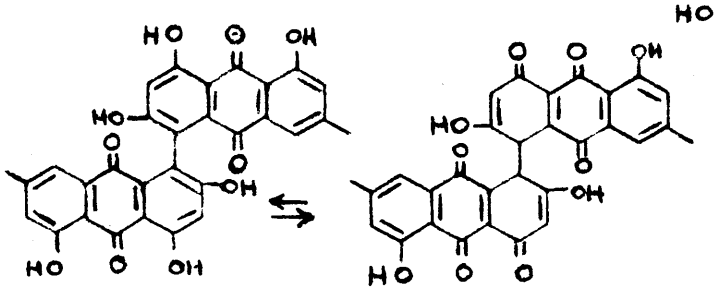
The principal feature of the chemistry of skyrin is the great ease with which the molecule can be cleaved to yield two molecules of emodin (XXVII). Another interesting reaction may be carried out by boiling a solution of skyrin hexa-acetate in methanol containing sulphuric acid. This results in the formation of a compound which analyses as a dimethyl ether of skyrin. Since this substance is insoluble in aqueous sodium

carbonate, it would appear that the β-hydroxyl groups are no longer free. The material is not, however, identical with skyrin ββ'-dimethyl ether, and Shibata and his co-workers have suggested structure (XXVIII) for the compound.[27] The fact that the "pseudo-dimethyl ether" reverts to skyrin on treatment with cold aqueous sodium hydroxide may at first appear to argue against a ketal structure, since such compounds are usually stable to alkali. However, the hydrolysis may be rationalised as shown on page 13.

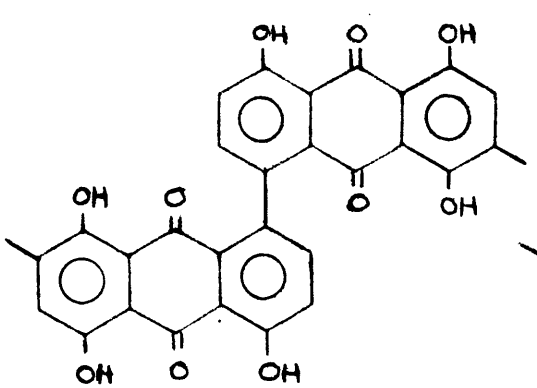
Iridoskyrin (XXIX)[30,31] is another example of a dianthraquinone produced by a mould. It occurs in Penicillium islandicum Sopp together with the interesting compound rubroskyrin[30]. Analysis indicates that the formula of rubroskyrin differs from that of iridoskyrin by two molecules of water, and, in fact, iridoskyrin is formed when rubroskyrin is dehydrated with concentrated sulphuric acid. To account for this ready dehydration, Shibata and his co-workers[32] have proposed structure (XXX), which is supported also by the spectral evidence. Evidence that the secondary alcoholic groups are situated β- to the carbonyl groups is provided by the isolation



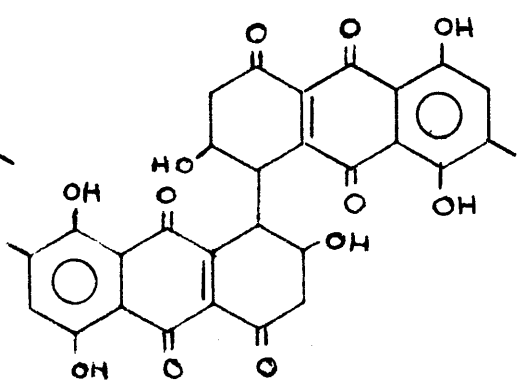
XXVIII



XXV



XXIX



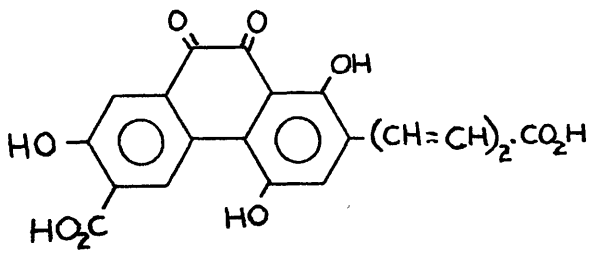
XXX

of catenarin (XXIII) as one of the products of pyrolysis.

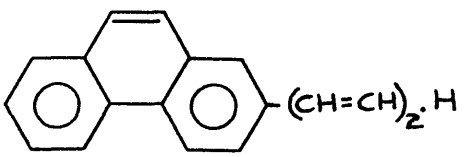
Howard and Raistrick have reported[30] that on treatment with zinc dust and acetic acid, rubroskyrin is converted to an amorphous reduction product which they suggest is rubroskyrin quinol. When a solution of this compound in normal caustic soda is shaken in air, then acidified, rubroskyrin is precipitated. If structure (XXX) is correct, then it is remarkable that, under such basic conditions, the secondary hydroxyl groups are not β-eliminated to yield iridoskyrin (XXIX), which is fully aromatic. The stability of dihydroherqueinone to similar treatment with base (see later) may provide a parallel for this.

Not many naturally-occurring phenanthroquinones are known; the only one which has been extensively investigated is the fungal pigment thelephoric acid. Kogl and his co-workers have proposed structure (XXXa) for this compound[45], based partly on the formation, in good yield, of a hydrocarbon, C_8H_{14}, on zinc dust distillation of thelephoric acid. This hydrocarbon could be oxidised to phenanthrene-2-carboxylic acid, and so was assigned structure (XXXb) by Kogl. Recently, Millward and Whiting

have synthesised[46] a compound possessing structure (XXXb), and have found that it differs considerably in melting-point from the substance obtained by Kögl *et alia*. It



XXXa

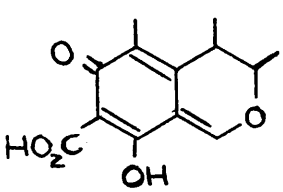


XXXb

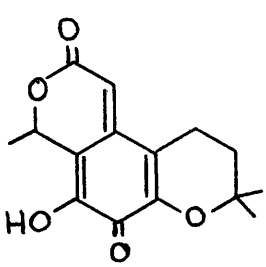
is possible, although unlikely, that the discrepancy can be accounted for by geometrical isomerism. Whatever the explanation, the situation at present is rather confused. Further work is desirable, but the inaccessibility of thelephoric acid renders this difficult.

Amongst the products of mould metabolism there are only a few methylene-quinones. The structures of both citrinin (XXXI)[33,34,35] and fuscin (XXXII)[36,37 38] have been firmly established by synthesis. The structure of purpurogenone (XXXIII), suggested by Roberts and Warren[39], rests on much flimsier evidence. Oxidation of

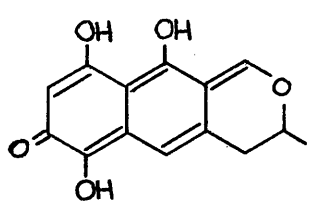
purpurogenone gives 3-hydroxybenzene-1:2:5-tricarboxylic acid. Alkaline hydrolysis yields formic acid, and a mixture of at least five coloured substances, one of which, obtained in minute yield, was classed as a derivative of 2-hydroxy-1:4-naphthoquinone solely on the results of various colour tests, and the ultraviolet absorption spectrum of its acetate. Such a degradation is, of course, in accord with a vinylogous lactone structure such as (XXXIII). The structure of the ether ring was put forward solely on the basis of biogenetic considerations. Accepting the evidence as presented, (XXXIV) fits all the data as adequately as (XXXIII), as indeed Roberts and Warren themselves have pointed out; further work is undoubtedly required to determine which, if either, of these structures is correct.



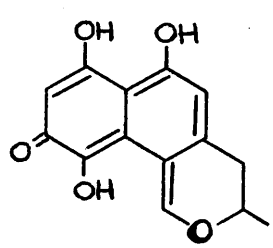
XXXI



XXXII

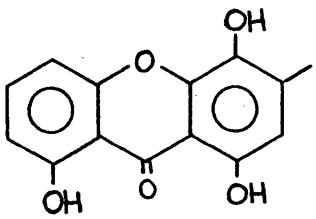


XXXIII

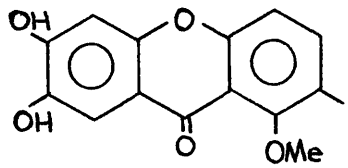


XXXIV

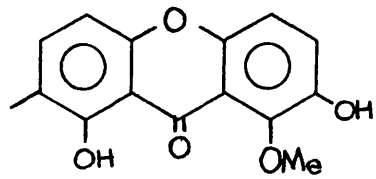
Finally, mention may be made of the small group of xanthones which occur as mould metabolic products. Ravenelin (XXXV) is the only one of established constitution.



XXXV



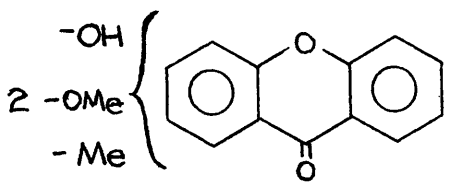
XXXVI



XXXVII

Its structure was deduced by Raistrick, Robinson, and White[40] from degradative evidence, and was confirmed when the compound was synthesised by Mull and Nord[41]. These latter workers have suggested[41] that rubrofusarin - another xanthone isolated by Raistrick and his collaborators[42] - might be represented by either (XXXVI) or (XXXVII). The only evidence presented for such structures is a set of empirical correlations of the ultraviolet absorption spectra of a limited number of hydroxyxanthones with the orientation of their substituents. More recently,

Lund, Robertson, and Whalley[42], in the course of investigations on another mould metabolic xanthone - asperxanthone, of partial structure (XXXVIII) - have found it impossible to correlate the ultraviolet absorption of eleven hydroxyxanthones of known constitution, with the orientation of the substituents. The conclusions reached by Mull and Nord regarding the structure of rubrofusarin must, therefore, be treated with reserve.

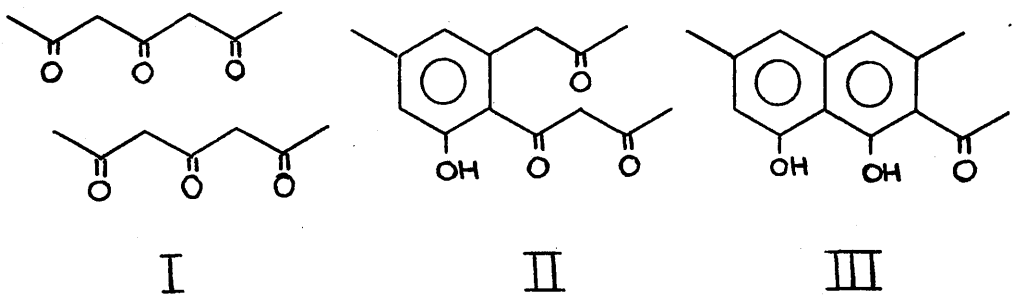


XXXVIII

--ooOoo--

THE BIOGENESIS OF MOULD METABOLITES.

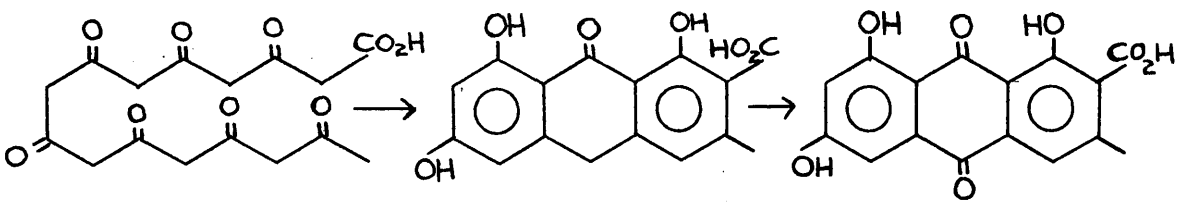
In 1907, J.N.Collie suggested[47] that many naturally-occurring compounds might arise by self-condensation of extended poly-β-diketones. He succeeded in carrying out several such condensations under mild conditions in the laboratory, such as the base-catalysed dehydration of two molecules of diacetylacetone (I) to yield first a benzenoid compound (II), then a naphthalenoid compound (III).



I II III

This early speculation has been greatly extended by Robinson[48], who has pointed out how a great many natural products may be considered as arising by condensations such as those described by Collie, followed by processes of oxidation, reduction, decarboxylation,

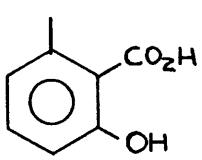
etc., all of which appear to take place in Nature with great ease. Endocrocin (V), a metabolite of Aspergillus amstelodami (Mangin) Thom and Church[49], for instance, may be formed, as shown in the diagram, from a hepta-ketopalmitic acid (IV), itself built up by condensation of acetic acid units.



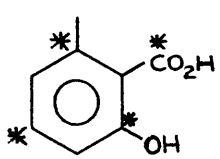
IV V

In a series of papers, the first of which was published in 1953[50a], Birch and his colleagues have stated the "acetate hypothesis" in much more definite terms[50b]. Their view is that many natural products are formed partially or wholly by the head-to-tail linkage of acetic acid units. In support of this, Birch and his co-workers have presented a considerable body of evidence. Some of it is based on tracer studies carried out on mould products, and will be briefly reviewed here.

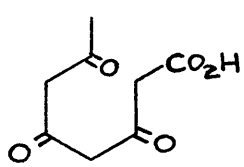
6-Methylsalicylic acid (VI) is produced by the mould Penicillium griseofulvum Diercx. By incorporating ^{14}C-carboxyl-labelled acetic acid in the growth medium, "labelled" 6-methylsalicylic acid can be isolated. By suitable degradations, it has been shown[51] that the distribution of radioactive carbon in the product is represented by (VII), in which asterisked carbon atoms are those of mass number 14. Such a distribution is, of course, in complete accord with a polyacetic acid precursor (VIII). It should, however, be emphasised here that at present the acetate hypothesis is a purely structural one. The nature of the intermediate compounds is not implied, though in most cases it is attractive and convenient to represent the process of biogenesis as the cyclisation of a straight-chain poly-β-kiketone.



VI



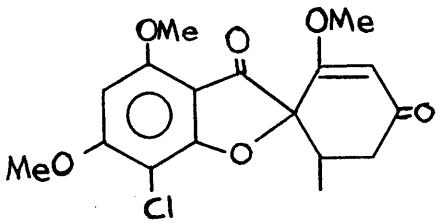
VII



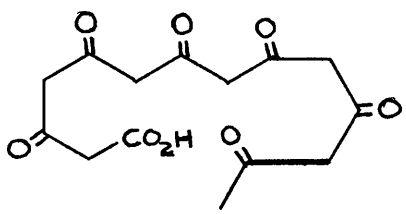
VIII

Recently[52], griseofulvin (IX), a metabolite of Penicillium griseofulvum[53], has been shown by similar

methods to be formed by the head-to-tail union of seven molecules of acetic acid, possibly through some such intermediate as (X).

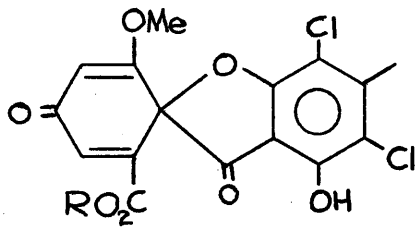


IX

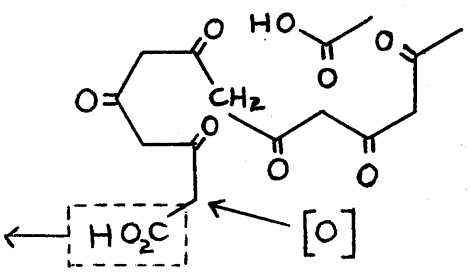


X

Experiments such as those described have proved beyond question that the acetate hypothesis contains a fair measure of truth, and on the basis of it, it is possible to suggest a plausible biogenesis for a great many natural compounds. As examples, the two mould metabolites, geodin (XI: R = Me) and erdin (XI: R = H) may be quoted. The chemistry of these compounds has been extensively studied by Raistrick and his co-workers ([54,55,56,57]). Their structures were finally established by recent work by Barton and Scott[58]. These structures may reasonably be considered as arising in the mould by condensation of a poly-β-keto-acid and a molecule of acetic acid, as shown in (XII).



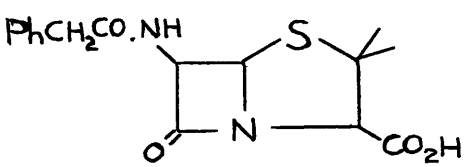
XI



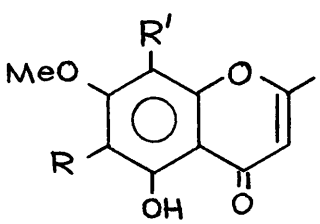
XII

On several occasions[17,50,59,60,61] the acetate hypothesis has been employed to predict which of several possible structures for various naturally-occurring compounds are the correct ones. Here, however, a note of caution might be sounded. There are many mould products with structures which, on the face of it, are impossible to account for by Birch's theory. A particularly well-known example is penicillin (XIII)[62].

Of recent years, it has become evident that acetic



XIII

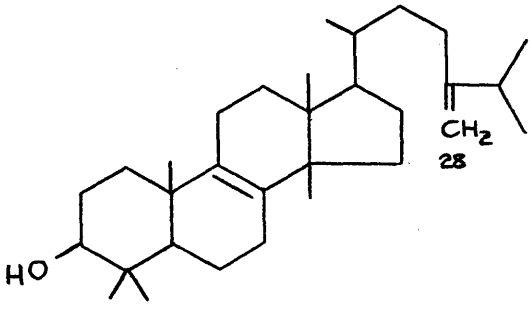


XIV

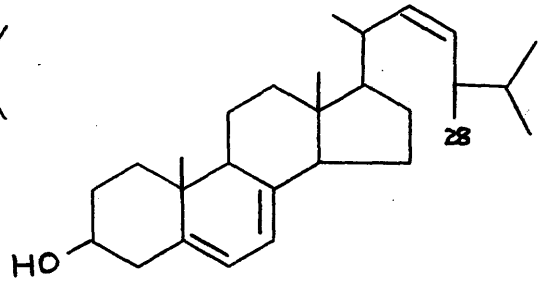
acid is not the only basic source of carbon atoms in

Nature. The compounds (XIV; R = R'= H), (XIV; R = Me, R'= H), (XIV; R =R'= Me), and the O-desmethyl derivative corresponding to (XIV; R = H, R'= Me) occur together in the plant Eugenia caryophyllata (L.) Thumb. These compounds represent all possible degrees of methylation of the phloroglucinol nucleus. Consideration of this led Birch, Elliott, and Penfold to propose[63] that many natural products contain extra C_1 units which are introduced at a different stage from the formation of the main skeleton, which in general is derived from acetic acid.

The possible sources of C_1 units (methionine, choline, etc.) have been reviewed[64]. More recently, it has been shown that the C_{28} carbon atom of eburicoic acid (XV)[65] and of ergosterol (XVI)[66] are not derived from acetic



XV

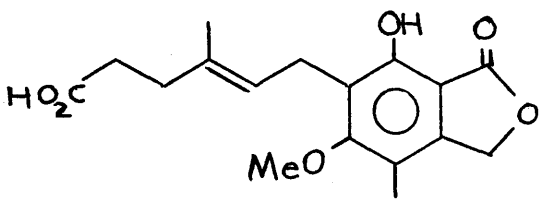


XVI

acid. In the case of ergosterol, Bloch and Danielsson

have shown[67] that formic acid is an efficient carbon source specifically for C_{28}.

As regards mycophenolic acid (XVII), also, the correctness of Birch's suggestion has now been demonstrated[68]. By suitable degradations it was shown[69] that the nuclear methyl group of the mycophenolic acid obtained when the mould was grown on a medium containing $Me^{14}CO_2H$ was non-radioactive. On the other hand, when the mould was grown on a medium containing [methyl-^{14}C]-methionine, both the O-Me and the C-Me groups were found to be labelled[68]: the remainder of the molecule was completely inactive. An interesting by-product of this work was the observation that not less than 75% of the methionine added was incorporated into the mycophenolic acid molecule. This would appear to indicate that the availability of methionine is a limiting factor in the synthesis.

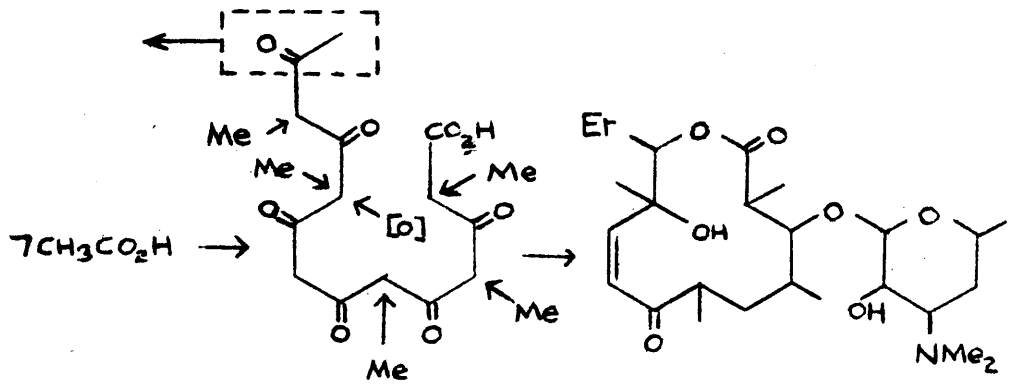


XVII

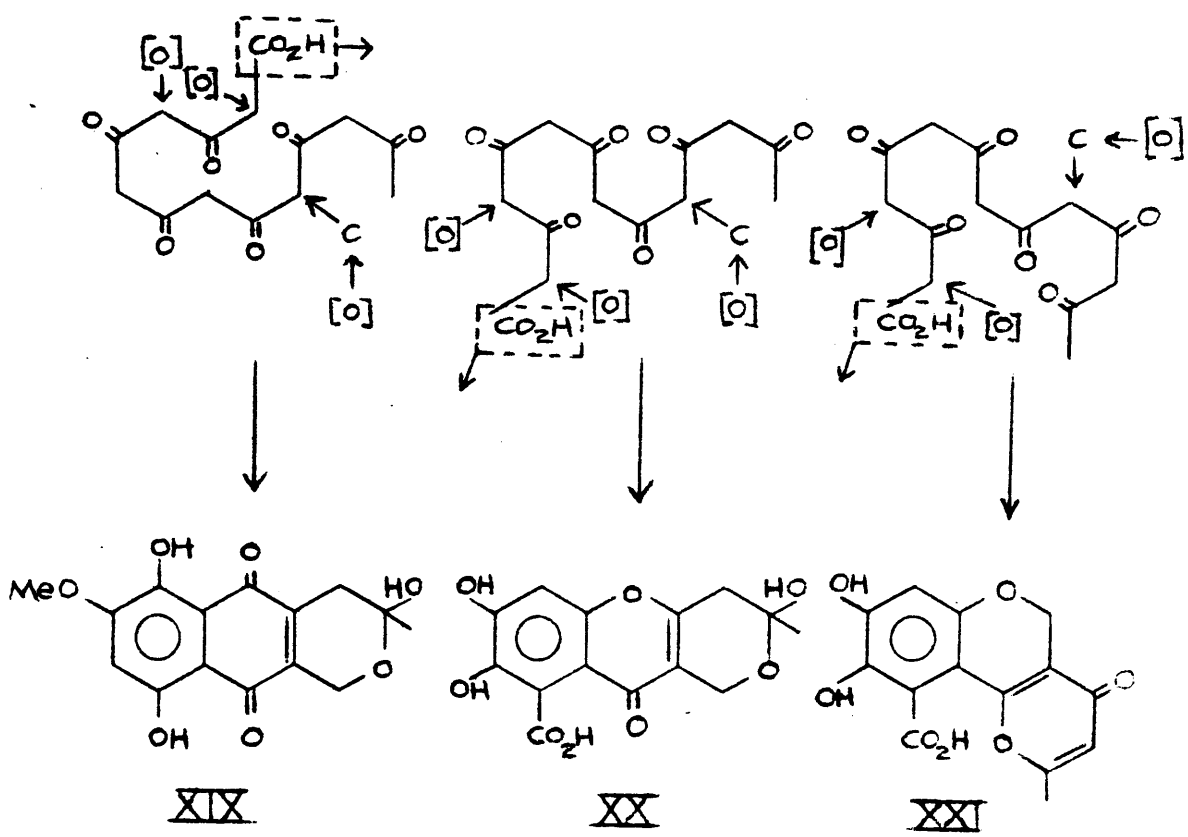
To account for the biosynthesis of the macrolides [e.g. methymycin (XVIII)], it has been suggested[70] that C_3 units are introduced during the formation of the main skeleton, by the intervention of propionic acid or its equivalent. Birch[68] has pointed out, however, that methymycin may be accommodated in the general biogenetic scheme, without the postulation of a C_3-donor, if it is assumed that C-methylation can occur readily. His scheme of biogenesis is illustrated for methymycin (XVIII) on page 27.

Birch has also drawn attention to the fact that, on the basis of the acetate hypothesis, three superficially quite dissimilar mould metabolites can be structurally correlated. These are fusarubin (XIX), fulvic acid (XX), and citromycetin (XXI), all of which may be regarded as being derived from the same skeleton composed of seven acetic acid units with an introduced C_1 unit and three introduced oxygen atoms. This is illustrated in the diagrams on page 27.

Two other theories advanced to account for the biosynthesis of aromatic compounds - one due to Seshadri and his collaborators, and the other to Davis and his



XVIII

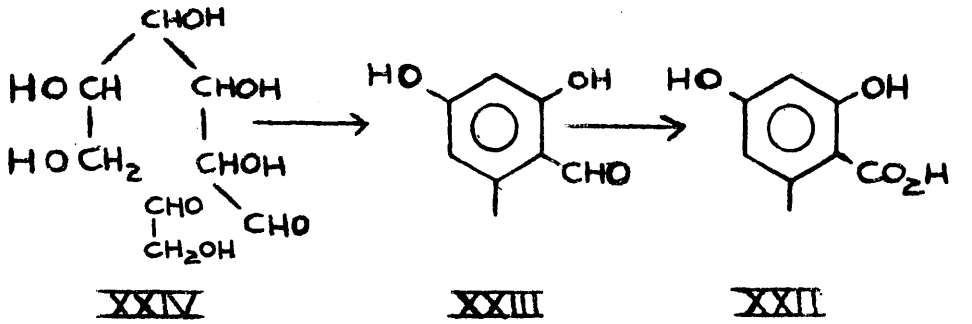


XIX XX XXI

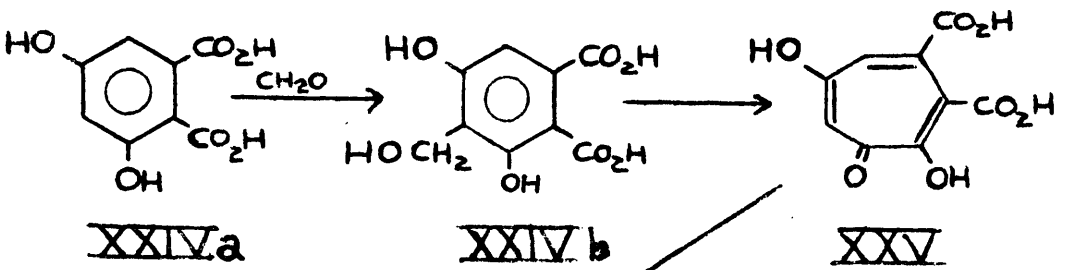
associates - are worthy of mention.

In 1944, Seshadri suggested[71] that orsellinic acid (XXII) might be a key intermediate in the biosynthesis of many lichen depsides and depsidones. The same author has since extended his scheme of biogenesis, based on the "orsellinic unit", to benzenoid mould metabolites.[72] Seshadri has suggested that the "orsellinic unit" may arise in Nature by an aldol-type condensation of a hexose and a biose. This is illustrated in the scheme (XXIV) to (XXII). The Indian workers have achieved the laboratory synthesis of 6-methylsalicylic acid (VI) by "nuclear reduction" of orsellinic acid. Birch's work[51], however, has clearly shown that 6-methylsalicylic acid is built up by head-to-tail linkage of acetate units and, while the two schemes are not irreconcilable, it may be considered an unnecessary complication at this stage to postulate hexose and biose intermediates.

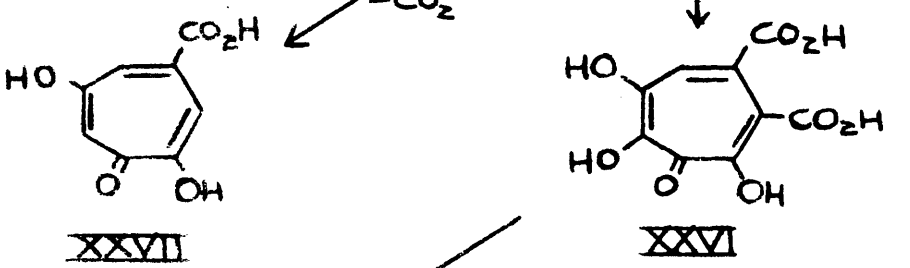
More recently, it has been suggested[73] that the "orsellinic hypothesis" provides a mode of biogenesis for the tropolone mould metabolites, stipitatic acid (XXVII), puberulic acid (XXVIII), and puberulonic acid (XXIX). The first stage is the ring enlargement of



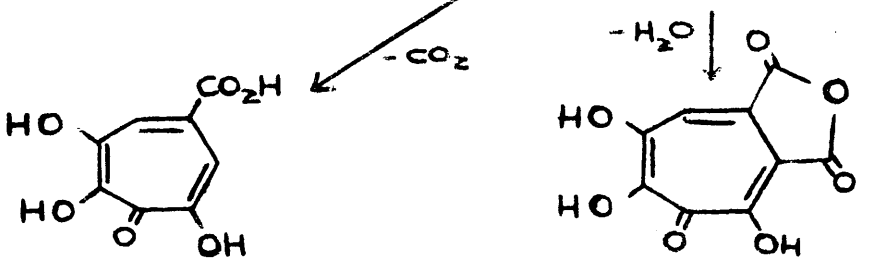
XXIV XXIII XXII



XXIV a XXIV b XXV



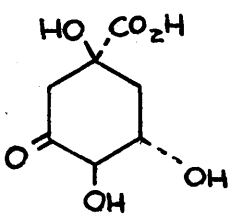
XXVII XXVI



XXVIII XXIX

3:5-dihydroxyphthalic acid (XXIVa) by means of formaldehyde or its equivalent, with oxidation of the intermediate 4-hydroxymethyl-3:5-dihydroxyphthalic acid (XXIVb) to yield 3:4-dicarboxy-6-hydroxytropolone (XXV). Decarboxylation of this leads to stipitatic acid (XXVII). Nuclear oxidation of (XXV) gives 3:4-dicarboxy-6:7-dihydroxytropolone (XXVI), which can be decarboxylated to afford puberulic acid (XXVIII), or dehydrated to give puberulonic acid (XXIX).

Davis and his co-workers have studied the biosynthesis of aromatic compounds in moulds, and, by employing enzymatic blocks, have clarified the mode of formation of such important compounds as phenylalanine, tyrosine, and tryptophan.[84] The earliest intermediate isolated is 5-dehydroquinic acid (XXX), which is converted, through



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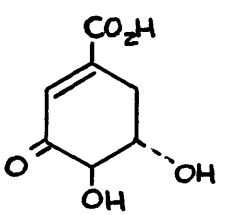
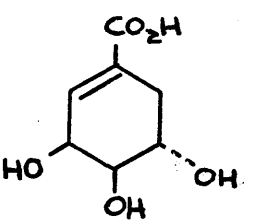


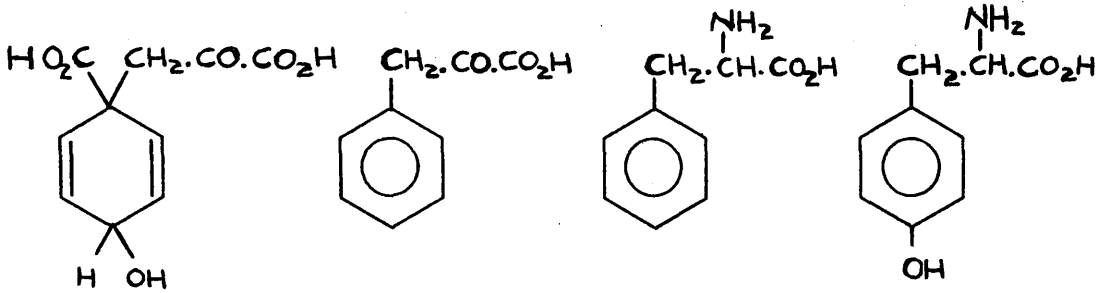
XXXI



XXXII

5-dehydroshikimic acid (XXXI) to shikimic acid (XXXII), and then, either directly, or through shikimic acid

5-phosphate, it is transformed, by a process as yet obscure, into prephenic acid (XXXIII). Prephenic acid



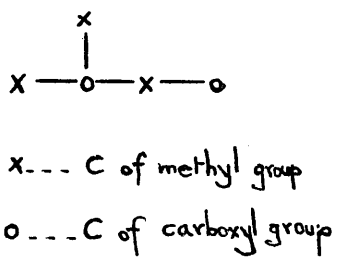
XXXIII XXXIV XXXV XXXVI

is very readily converted into phenylpyruvic acid (XXXIV)[85,86] which in turn may be converted into phenylalanine (XXXV) and tyrosine (XXXVI). An account of this work has been given in an excellent review by Dalgliesh[87].

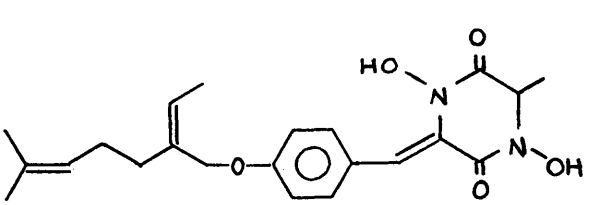
It is probable that the biogenetic route to phenylalanine, elucidated as a result of Davis' work, is involved also in the biosynthesis of the C_6-C_3 series of natural products, since, as Birch has observed[50b], phenylalanine or a hydroxylated derivative, is the logical precursor of this series. It is, in fact, known that both shikimic acid and phenylalanine can act as direct precursor for the C_6-C_3 polymer, lignin, when it is

formed as a product of the metabolism of wheat[88].

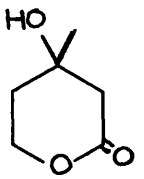
Work on steroid biosynthesis[74] first established that the manner in which the methyl and carboxyl carbon atoms of acetic acid are distributed in the isopentane unit in natural products is as shown in (XXXVII). It has now been shown[69] that, in the isoprenoid side-chains of the mould products mycelianamide (XXXVIII) and mycophenolic acid (XVII), the same pattern occurs.



XXXVII



XXXVIII

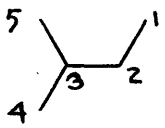


XXXIX

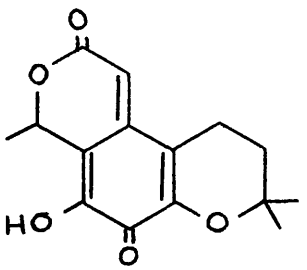
Various suggestions have been made as to the nature of the active intermediate which gives rise to the isopentane unit. In 1956, it was shown[75] that mevalonic lactone (XXXIX) is converted almost completely into cholesterol in rat-liver homogenates. Arigoni[76] has since found that mevalonic lactone can function as the isoprene precursor in the biosynthesis of soya-sapogenols A and D in germinating soya beans. It has now been established[69] that, in the biosynthesis of the

terpenoid side-chains of mycelianamide (XXXVIII) and mycophenolic acid (XVII), mevalonic lactone is again the active intermediate, or can give rise to it.

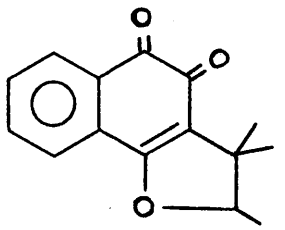
While mould metabolites with extended terpenoid side-chains are not of very wide occurrence, there are a great many examples of mould products which have a single isopentane unit attached to an aromatic or quinonoid nucleus. This structural feature, in fact, is common throughout the whole range of natural products. A survey of the literature reveals that in general the point of attachment of the isopentane unit is either C_1 or C_3 (XL), with attachment at C_1 greatly predominating. As typical examples of the two modes of attachment may be mentioned fuscin (XLI)[37] and the plant product dunnione(XLII)[77].



XL



XLI



XLII

Birch has suggested[63] that such isopentyl groups

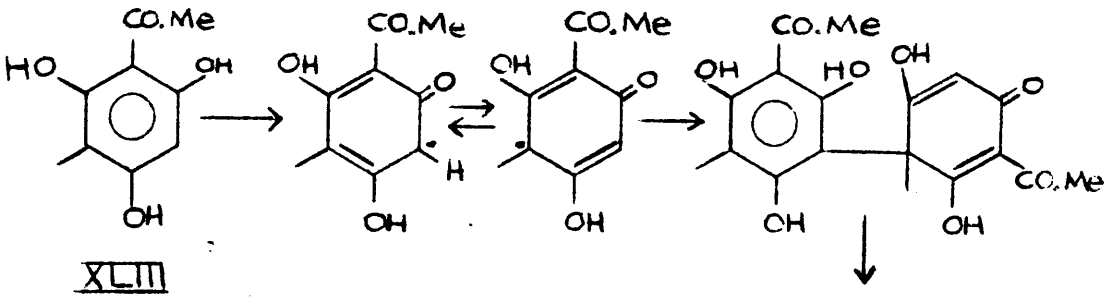
may have been introduced after the formation of the main skeleton (cf. the nuclear methyl groups in mycophenolic acid). It is interesting to speculate on the nature of the attacking species.

The fact that attachment is almost invariably at C_1 or C_3 suggests that the ion $[(CH_3)_2C{=}CH{=}CH_2]^+$, or its equivalent, might be the active intermediate. The precursor of such an ion might be the compound $HOCH_2{\cdot}CH{=}C(CH_3)_2$ (or an ester of it), which could arise from mevalonic acid by decarboxylation, followed by elimination of the tertiary hydroxyl group.

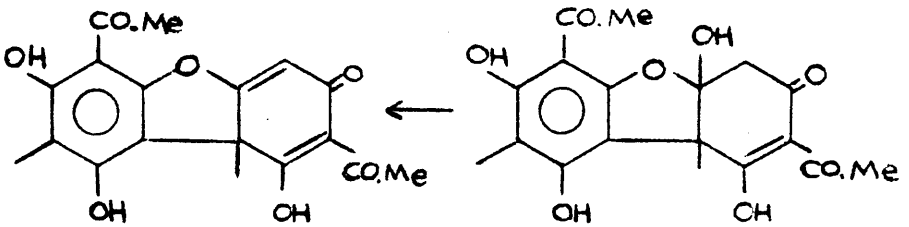
If the process involved is that suggested, then it is probable that the ether ring of the plant product anisoxide (see Part II) undergoes a methyl group migration during or after its formation.

In conclusion, something may be said about the role of phenolic radicals in the biogenesis of mould metabolites. Phenolic radicals are produced by the action of one-electron-transfer oxidising agents. By carrying out such an oxidation on methylphloroacetophenone (XLIII), Barton, Deflorin, and Edwards[78] obtained

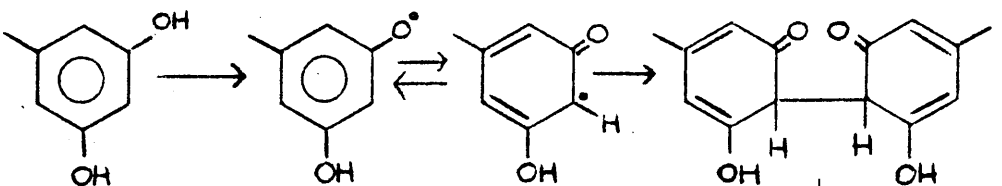
th



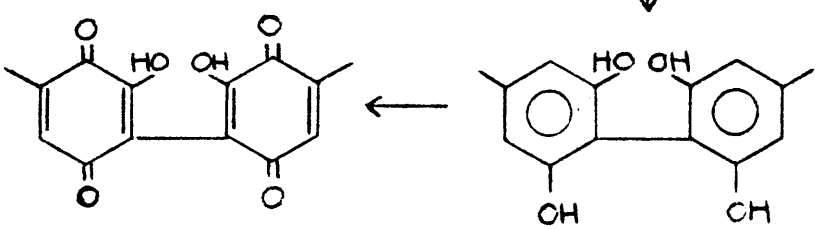
XLIII



XLV XLIV



XLVI



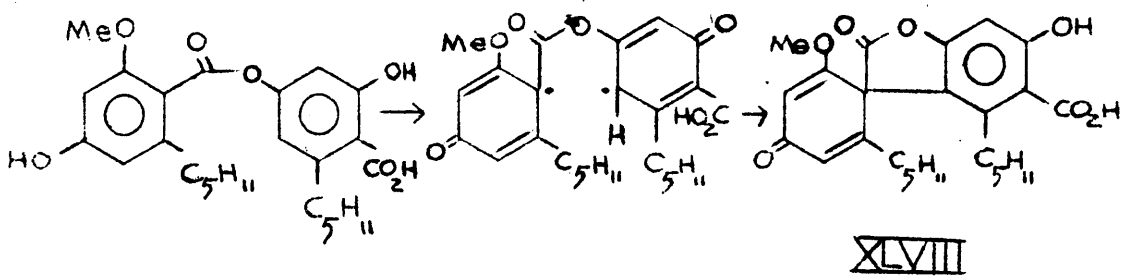
XLVII

the compound (XLIV), presumably by the mechanism indicated in the diagrams on page 35. Dehydration of (XLIV) gave the mould product, usnic acid (XLV).

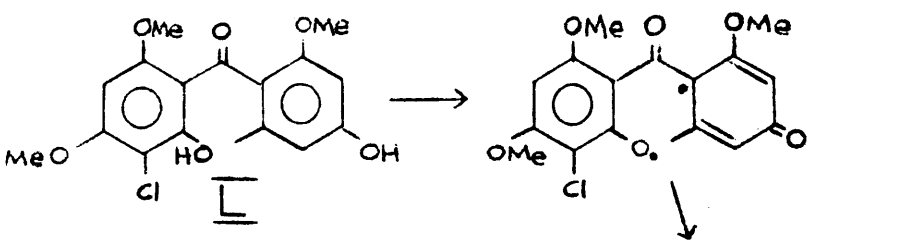
In recent reviews, Barton and Cohen[79], and Erdtman and Wachtmeister[80] have surveyed the part played by such oxidations in the biogenesis of natural products, and have suggested that the fungal diquinones might be formed in this way. The possible biosynthesis of phoenicin (XLVII) from orcinol (XLVI) is shown (page 35). It is probable that similar C· → C· coupling is involved in the biogenesis of picrolichenic acid (XLVIII), the structure of which has recently been elucidated by Wachtmeister[43].

Reference has already been made (page 21) to the tracer experiments which established that griseofulvin (IX) is formed by the head-to-tail union of acetate units. Barton and Cohen have proposed[79] that the final spiran formation is brought about by O·→C· radical coupling, as indicated on page 37, the intermediate (XLIX) being converted into griseofulvin by enzymic reduction.

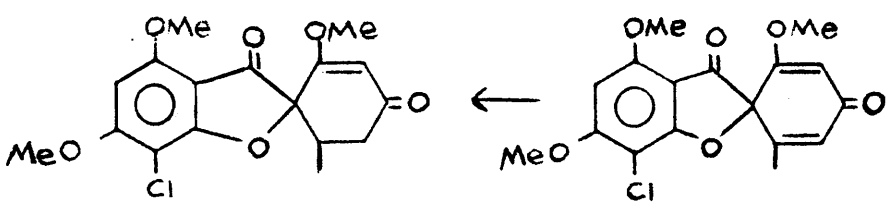
Support for these views has very recently been



XLVIII

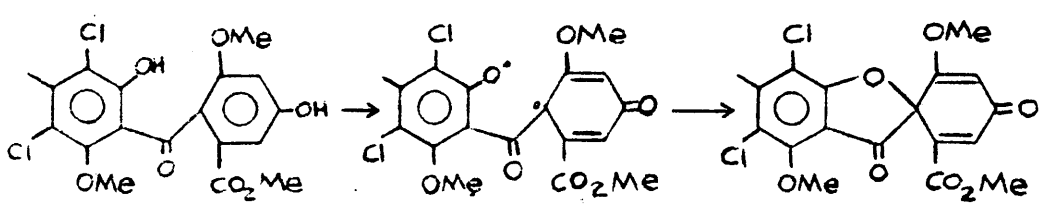


L



IX

XLIX



LII

LI

provided by Scott[44], who has succeeded in converting (L) into (XLIX) *in vitro*, using alkaline potassium ferricyanide as the one-electron-transfer oxidising agent. The same worker has also achieved[44] a partial synthesis of (±)-geodin methyl ether (LI), by an analogous oxidation of methyl 3':5'-dichloro-4:2'-dihydroxy-6:6'-dimethoxy-4'-methylbenzophenone-2-carboxylate (LII).

It is probable that phenolic radical coupling is widespread in Nature. It has been suggested as being operative in the biogenesis of the morphine alkaloids ([48,79,81,82]), and the results of recent tracer studies[83] are in agreement with such a scheme.

--ooOoo--

THE CHEMISTRY OF HAEMOCORIN.

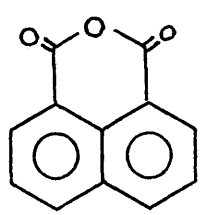
Haemocorin is a red crystalline glycoside, of molecular formula $C_{32}H_{34}O_{14}.H_2O$, extracted from the bulbous roots of Haemodorum corymbosum Vahl. Its chemistry has been investigated by Cooke and Segal[89,90].

Treatment of haemocorin with dilute mineral acid readily hydrolyses it to a sugar, which has been identified[89] as cellobiose, and a purple-red aglycone, $C_{20}H_{14}O_4$.

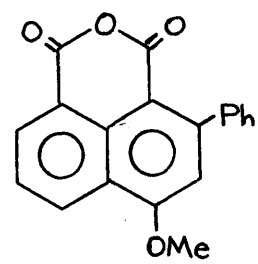
The aglycone contains one methoxyl group: it forms a diacetate, and two isomeric monomethyl ethers, which, on further methylation, give rise to a corresponding pair of dimethyl ethers. Hence, of the four oxygen functions, one is a methoxyl group, and two are hydroxyl groups. That the remaining oxygen atom is probably present as a highly conjugated carbonyl group is suggested by the presence of a band in the region 1620 $cm.^{-1}$ - 1635 $cm.^{-1}$ in the infrared absorption spectra of the aglycone and its methyl ethers. No carbonyl derivatives could be prepared, however.

Oxidation of the aglycone dimethyl ether A, $C_{22}H_{18}O_4$,

with potassium permanganate yields two products. One of these (anhydride A), $C_{20}H_{14}O_5$, has lost a methoxyl group. It was identified as a derivative of naphthalic anhydride (I) by its chemical properties, and by its infrared spectrum, which bears a very close resemblance



I



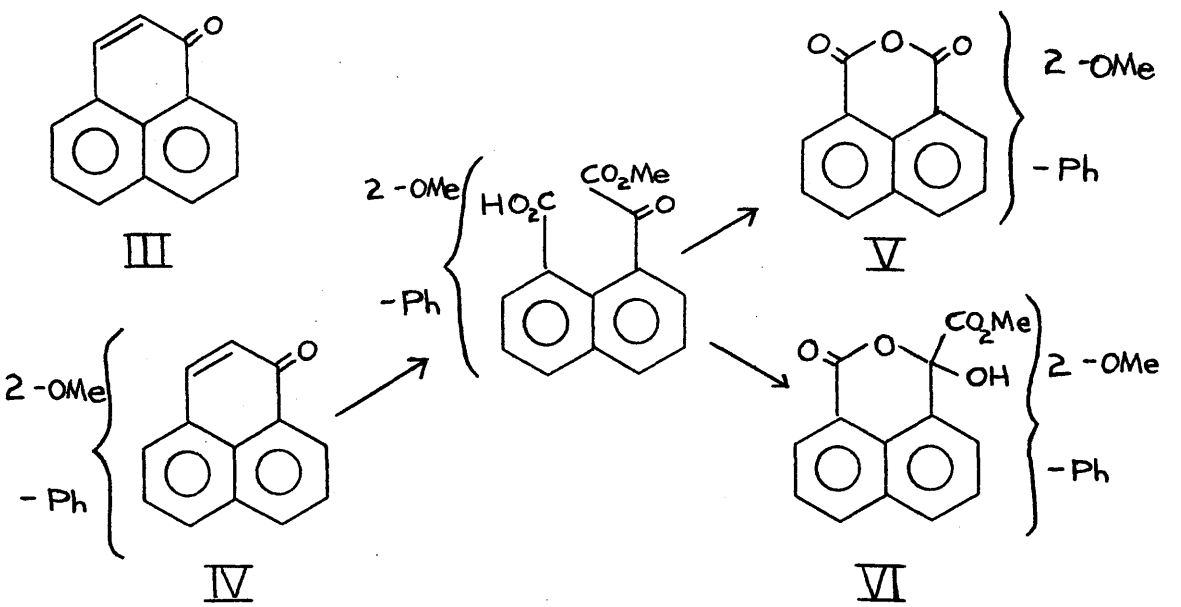
II

to that of 4-methoxy-2-phenylnaphthalic anhydride (II). The presence of a phenyl substituent in anhydride A was revealed by further oxidation to a gummy acid which yielded diphenyl when heated with soda-lime.

The other product, $C_{22}H_{18}O_7$, still contains the three original methoxyl groups. It is stable to diazomethane. When hydrolysed with alkali, it gives an acid, $C_{21}H_{16}O_7$, containing two methoxyl groups; this reacts with diazomethane to yield a compound, $C_{23}H_{20}O_7$, containing four methoxyl groups. If the oxidation

product is first boiled with caustic soda solution, then oxidised with silver oxide, anhydride A is obtained.

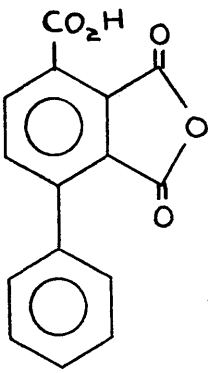
Cooke and Segal have explained the oxidation of dimethyl ether A by postulating that the aglycone is a derivative of perinaphthenone (III). On this basis, dimethyl ether A can be represented by partial structure (IV), and the two permanganate oxidation products by the partial structures (V) and (VI) respectively, these being formed from the dimethyl ether as shown in the diagrams.



The use of silver oxide to cleave the α-keto-acid

resulting from the alkaline hydrolysis of (VI) is noteworthy, since it appears to be the first time that this reagent has been used for such a purpose. It has, however, been suggested[93] that the carbon dioxide evolved when sugars are oxidised with silver oxide, arises by cleavage of intermediate α-keto-acids.

An isomeric phenylnaphthalic anhydride (anhydride B) is obtained by permanganate oxidation of the aglycone dimethyl ether B. Both of the isomeric anhydrides exhibit infrared absorption bands in the regions characteristic of the unsubstituted phenyl group (ca. 700 $cm.^{-1}$ and 750 $cm.^{-1}$). That the phenyl ring is, in fact, unsubstituted, was confirmed by the oxidation of each of the anhydrides to (VII), isolated as the corresponding mono-anilide phenylimide.

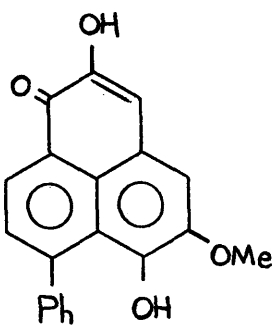


VII

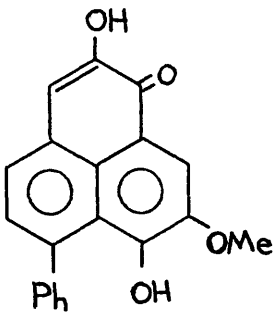
On the strength of the above evidence, and a study of various synthetic perinaphthenones, Cooke and Segal have proposed that the aglycone be represented by one of the structures (VIII),

(IX), or (X), or a tautomeric modification of any of these. [Structures based on 9-hydroxyperinaphthenone (XI) can be discounted, since the hydroxyl group in such compounds is resistant to acetylation and methylation under conditions which are effective for the aglycone.[90,91]]

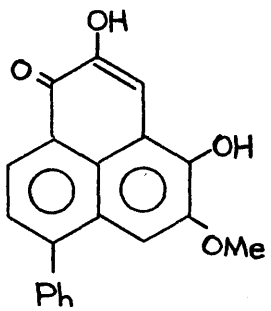
The existence of two isomeric dimethyl ethers can be rationalised by assuming that they correspond to different tautomers of the same structure.



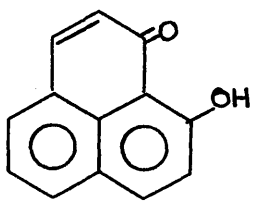
VIII



IX



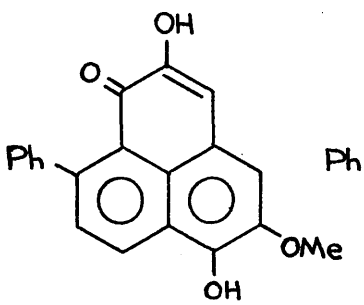
X



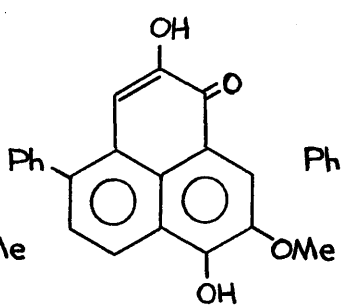
XI

The isolation of naphthalic anhydrides by suitable oxidations of the aglycone methyl ethers is compelling evidence for a perinaphthenone structure. Similarly, the formation of diphenyl-1:2:3-tricarboxylic acid by

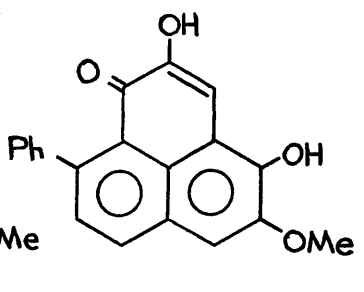
further oxidation of the naphthalic anhydrides, conclusively establishes that the phenyl group is attached to an otherwise unsubstituted ring of the perinaphthenone nucleus. However, the data presented can be accounted for equally well by structures in which the phenyl group occupies a position meta- to that shown in structures (VIII), (IX), and (X) respectively. Structures (XII), (XIII), and (XIV), and their tautomers, are therefore not excluded.



XII



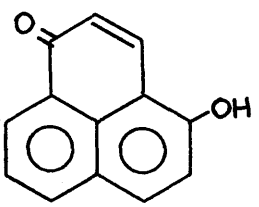
XIII



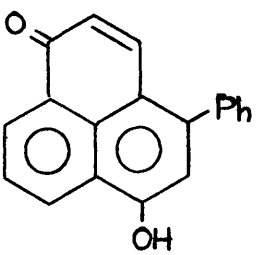
XIV

The arguments put forward by Cooke and Segal for the relative positions of the remaining substituents are also not wholly convincing. The assigned relationship of the methoxyl group to the carbonyl group is based on the following. The methyl ethers of

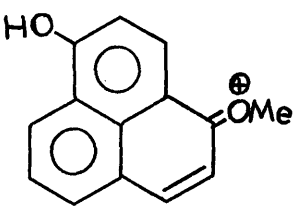
4-hydroxyperinaphthenone (XV), 6-hydroxy-4-phenyl-perinaphthenone (XVI), and the aglycone methyl ethers, can be very easily demethylated by refluxing with ethanolic sulphuric acid, probably through intermediates like (XVII). The fact that the original methoxyl group



XV



XVI

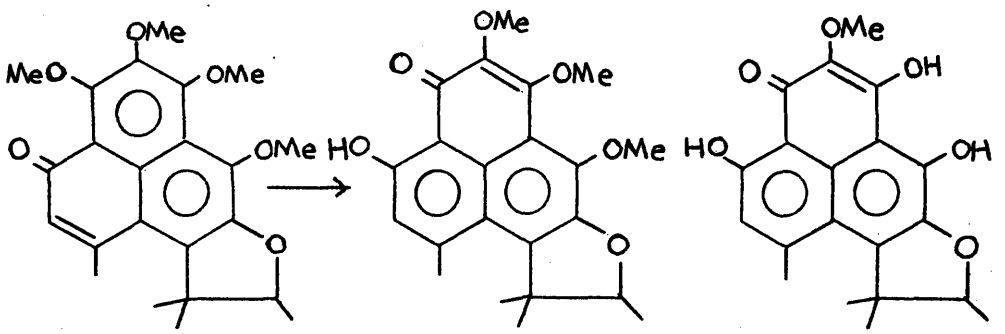


XVII

of the aglycone is unaffected under the same conditions is cited[90] as evidence that it is not related to the carbonyl group in the same way as the methoxyl groups in the compounds mentioned above, i.e. that the lone pairs on the methoxyl oxygen are not conjugated with the carbonyl group.

Reference to the chemistry of atrovenetin (see next section) suggests that this may be an over-simplification

of the situation. Prolonged refluxing of atrovenetin tetramethyl ether A (XVIII) with ethanolic hydrogen chloride affords atrovenetin orange trimethyl ether (XIX)[92]. On the tacit assumption of Cooke and Segal that the course of such a hydrolysis is determined solely by conjugative effects, the compound (XX), or a tautomer, would be the expected product.



XVIII XIX XX

Oxidation of the aglycone dimethyl ether A to the lactol ester (VI) proves conclusively that two of the oxygen substituents are ortho- to each other. The suggested contiguous arrangement of the second pair of oxygen substituents, however, is based only on the following infrared data. The aglycone exhibits two hydroxyl bands

in its infrared spectrum. One of these (at 3542 $cm.^{-1}$) is assigned[90] to the enolic α-diketone group in one ring; the other, a broader band at 3380 $cm.^{-1}$, is regarded[90] as suggestive of a guiacol type of grouping. These two bands also agree respectively with the single hydroxyl bands of the monomethyl ethers.

Clearly, further degradative evidence for the structure of haemocorin aglycone is desirable. Useful information might be obtained by oxidation of the aglycone itself to a naphthalic anhydride. From a study of the infrared spectrum of such a compound it would be possible to decide whether either of the anhydride carbonyl groups was hydrogen-bonded to a hydroxyl group, since work on atrovenetin (see next section) has shown that when hydrogen-bonding occurs the low-frequency carbonyl band is shifted to about 1660 $cm.^{-1}$ In the event of no hydrogen-bonding being apparent, the infrared spectrum of the demethylated anhydride might reveal that the methoxyl group is situated in the 2- or 7-position of the anhydride.

Haemocorin was the first naturally-occurring perinaphthenone to be reported in the literature. Recently,

it has been shown that the mould pigment atrovenetin is also a substituted perinaphthenone (see next section).

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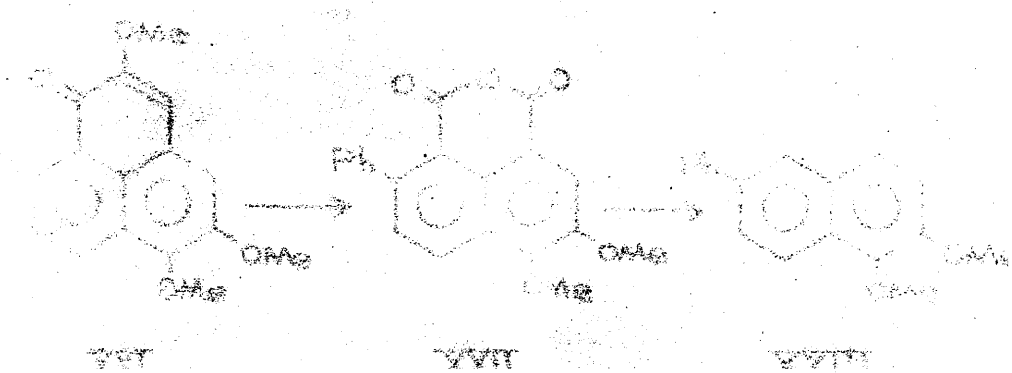
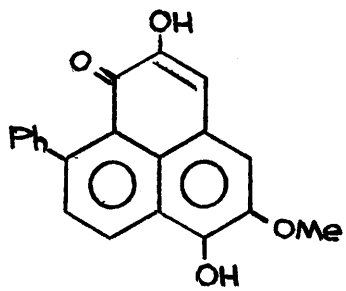
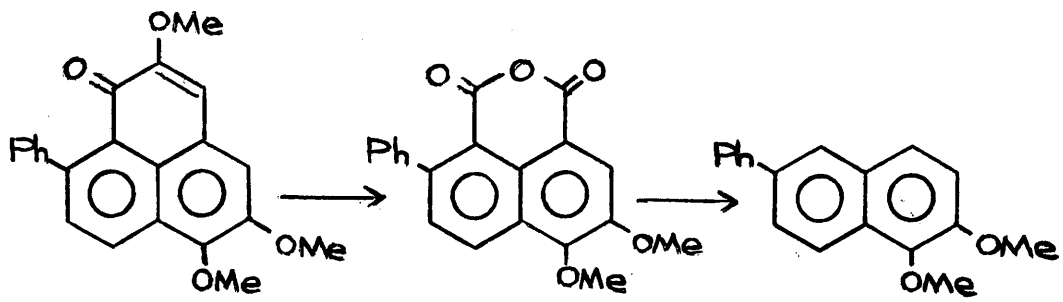


NOTE. Since the preceding section was written, a third paper on haemocorin has appeared.[132] In it, experiments are described which conclusively establish that the aglycone has structure (XII), or its tautomer.



XII

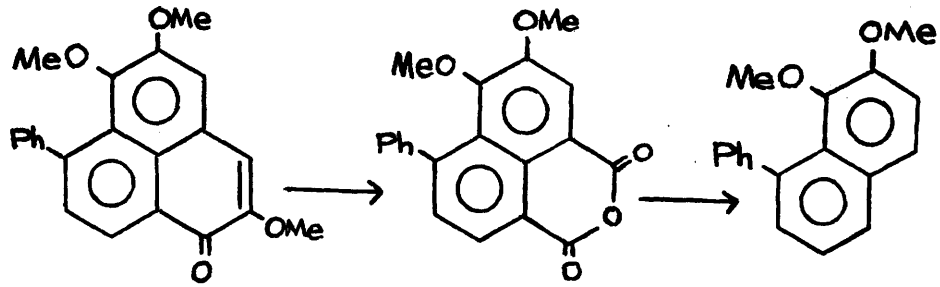
These experiments may be summarised as follows. Anhydride A was decarboxylated to afford 1:2-dimethoxy-6-phenylnaphthalene (XXIII). It follows from this that anhydride A possesses structure (XXII), while dimethyl ether A is to be represented as (XXI).



XXI XXII XXIII

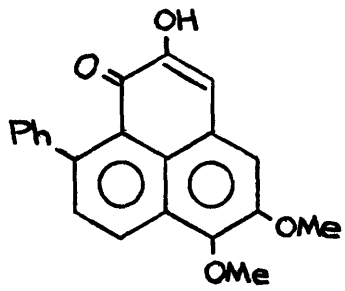
Similarly, anhydride B gave 1:2-dimethoxy-8-phenylnaphthalene (XXVI) on decarboxylation, thus establishing structures (XXV) and (XXIV) for anhydride B and dimethyl

ether B respectively.



XXIV XXV XXVI

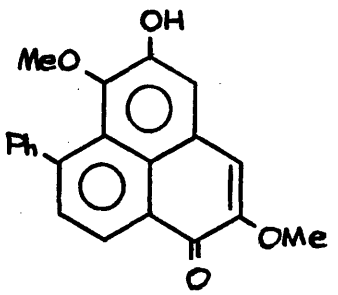
The structure of monomethyl ether A (XXVII) follows from the fact that it can be oxidised to anhydride A (XXII). The possible positions of the methoxyl group in the aglycone itself are thus reduced to two. Consideration of the structure of dimethyl ether B (XXIV) makes a final decision possible. The structure of the aglycone is thus fixed as (XII), or its tautomer.



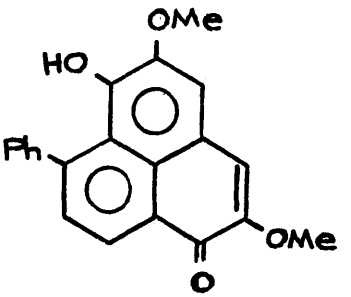
XXVII

Cooke, Johnson, and Segal have stated[132] that monomethyl ether B has structure (XXVIII). It has been reported that monomethyl ether B is demethylated under conditions which do not demethylate the aglycone itself.[90] However, as has already been pointed out (see p. 45), it

is dangerous to conclude from such evidence alone that the methoxyl groups involved are not conjugated with the carbonyl group in the same way. In the absence of any degradation products of monomethyl ether B, therefore, there remains the possibility of its having structure (XXIX), or its tautomer.



XXVIII



XXIX

--ooOoo--

ATROVENETIN, HERQUEINONE, and norHERQUEINONE

INTRODUCTION.

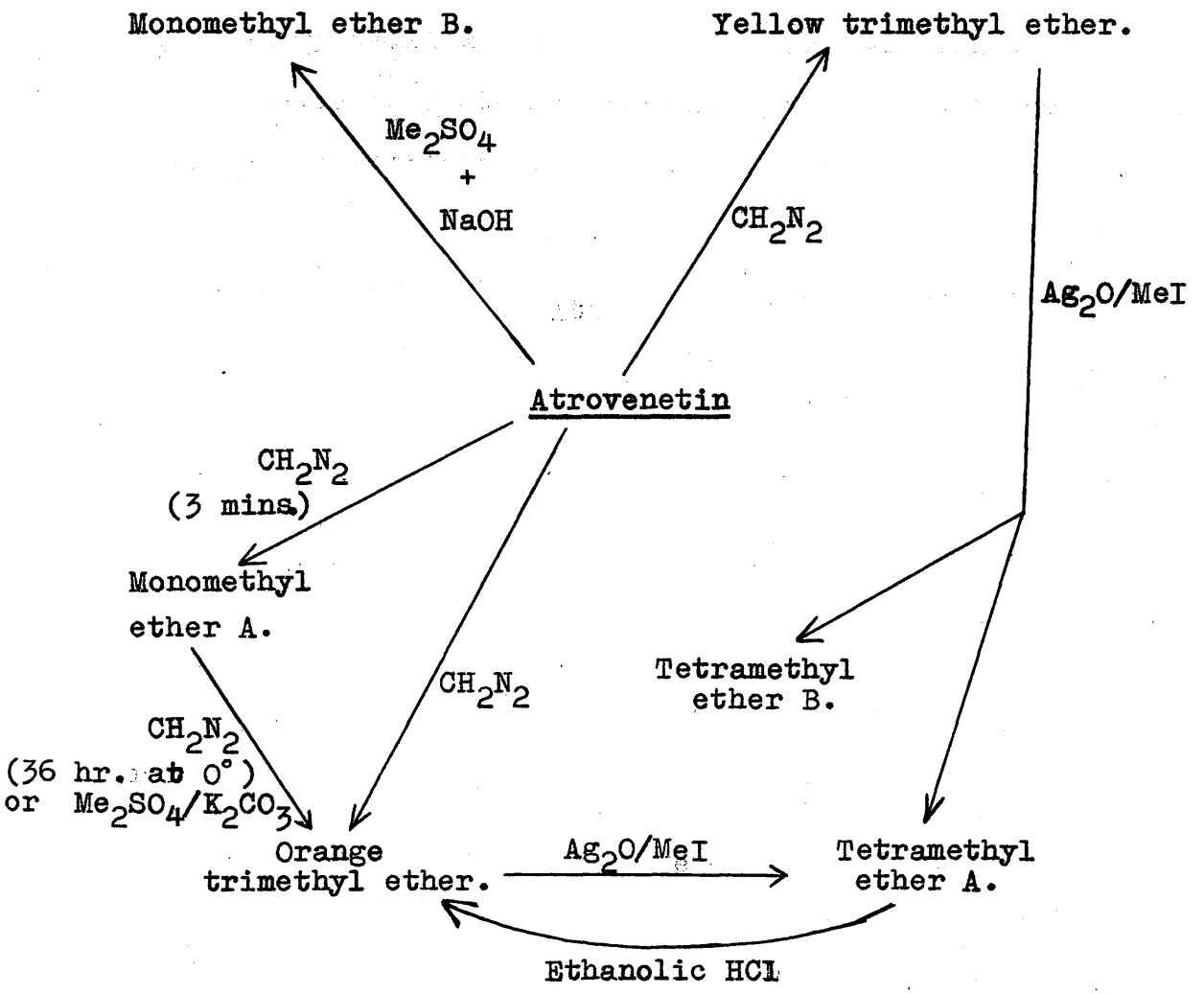
1.) Atrovenetin.

Atrovenetin was first isolated from Penicillium atrovenetum G. Smith by Neill and Raistrick.[92] It forms yellow-brown plates or prisms, solutions of which are strongly dextrorotatory and give a positive reaction for phenols with ferric chloride. Neill and Raistrick established the empirical formula of atrovenetin as $C_{19}H_{18}O_6$, and showed that, on Kuhn and Roth oxidation, it afforded between two and three molecules of acetic acid. Atrovenetin contains no alkoxyl groups.

A large number of functional derivatives of atrovenetin was prepared by these workers[92,94], among which may be mentioned a triacetate, a hydrochloride, $C_{19}H_{18}O_6 \cdot HCl$, and a perchlorate, $C_{19}H_{18}O_6 \cdot HClO_4 \cdot 2H_2O$. No fewer than six methyl ethers were prepared, and, since these have proved to be of considerable significance in the structural determination, a short account of them will be given.

Methylation of atrovenetin with diazomethane gives rise to a monomethyl ether (monomethyl ether A), and two trimethyl ethers (the "yellow" and "orange" trimethyl ethers). The orange trimethyl ether may also be obtained by prolonged treatment of monomethyl ether A with diazomethane. A second monomethyl ether (monomethyl ether B) is formed when atrovenetin is treated with methyl sulphate and caustic soda at room temperature. If a solution of the yellow trimethyl ether in dry benzene is refluxed with methyl iodide and silver oxide for twenty-four hours, a mixture of two tetramethyl ethers (A and B) is obtained. Similar treatment of the orange trimethyl ether affords only tetramethyl ether A, which is readily partially demethylated back to the orange trimethyl ether by treatment with ethanolic hydrogen chloride. These inter-relationships are summarised in the chart on page 51.

Each of the monomethyl ethers yields a diacetate, but the trimethyl ethers are resistant to acetylation under ordinary conditions: the yellow trimethyl ether, however, readily affords an acetate perchlorate when warmed with perchloric acid and acetic anhydride.



The Methyl Ethers of Atrovenetin.

A number of degradation experiments have also been reported by Neill and Raistrick[92]. Atrovenetin was found to be stable to prolonged refluxing with 2N sulphuric acid, and to refluxing in normal caustic potash for forty-eight hours. The yellow and orange trimethyl ethers were also stable to refluxing in methanolic potassium hydroxide. Atrovenetin itself was stable even to potash fusion.

Attempted hydrogenation of the yellow trimethyl ether, reduction of both trimethyl ethers with sodium and ethanol, and dehydrogenation of the yellow trimethyl ether led in all cases to the recovery of unchanged starting material.

Reduction of the yellow trimethyl ether with lithium aluminium hydride afforded an optically-active compound, $C_{22}H_{24}O_5$, which retained all three of the methoxyl groups. This compound, designated YA, resisted acetylation at 100° with acetic anhydride and sodium acetate. An isomeric product, also optically active, and also possessing three methoxyl groups, was obtained by reduction of the orange trimethyl ether. Neither of

the reduction products gave a positive ferric chloride reaction, nor was soluble in aqueous sodium hydroxide.

The oxidative degradations carried out on atrovenetin and its derivatives by Neill and Raistrick may be summarised as follows. Oxidation of atrovenetin with concentrated nitric acid gave an optically active compound, of empirical formula, $C_{15}H_{16}O_{10}N_2$ (i.e. $C_{15}H_{14}O_9N_2$ + H_2O). Between two and three molecules of acetic acid were obtained on Kuhn-Roth oxidation. A monomethyl derivative was obtained when the oxidation product was methylated with diazomethane.

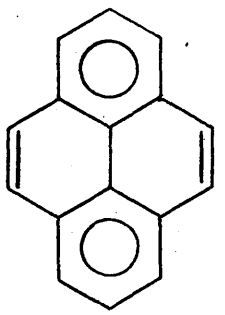
Two crystalline products were obtained by oxidising atrovenetin with hydrogen peroxide and sodium hydroxide overnight at room temperature. The compounds, designated "peroxide oxidation product A", and "peroxide oxidation product B", were assigned the empirical formulae $C_{15}H_{16}O_7$ and $C_{12}H_8O_7$ respectively. Both substances gave a positive ferric chloride reaction.

A compound, $C_{22}H_{22}O_8$, containing three methoxyl groups and yielding rather more than two molecules of acetic acid on Kuhn-Roth oxidation, was prepared by

oxidation of the yellow trimethyl ether with potassium permanganate. This substance gave a positive ferric chloride test and could be extracted from its ethereal solution with aqueous sodium bicarbonate.

Chromium trioxide oxidation of the yellow trimethyl ether gave a substance, of empirical formula $C_{19}H_{18}O_6$, which contained only one methoxyl group. This compound was reported[92] to yield an oxime when treated with hydroxylamine hydrochloride, and pyridine.

Finally, the production of pyrene (I) by zinc dust distillation of atrovenetin may be mentioned. This was obtained[92] in very small yield, and was characterised by forming its picrate, which had an ultra-violet absorption spectrum superposable on that of authentic pyrene picrate. The identity of the two samples was also established by melting-point and mixed melting-point.



I

--ooOoo--

INTRODUCTION.

2.) Herqueinone and norHerqueinone.

The first reference to the mould metabolic pigment herqueinone was made in 1951, when it was isolated from Penicillium herquei Bainier and Sartory by Stodola, Raper, and Fennell[95]. These workers established the empirical formula of herqueinone as $C_{20}H_{20}O_7$, and showed that it contained one methoxyl group. They also prepared an optically active dihydro derivative by catalytic hydrogenation.

Herqueinone was independently isolated by Raistrick and his co-workers[96], who found that it occurred in P. herquei Bainier and Sartory, together with a related pigment, norherqueinone, and the known compound, meso-erythritol, $CH_2OH.CHOH.CHOH.CH_2OH$. These workers carried out an extensive study of the chemistry of herqueinone and norherqueinone, the main results of which can be summarised as follows.

Herqueinone is a brick-red crystalline material, very strongly dextrorotatory ($[\alpha]_D$ = +440). It gives a positive ferric chloride test, and is soluble in

aqueous sodium hydroxide. Methylation with dimethyl sulphate and potassium carbonate affords two trimethyl ethers (A and B). Both of these trimethyl ethers are reported to be optically inactive, insoluble in aqueous sodium hydroxide, and to give no colour with ferric chloride. Trimethyl ether A is very pale yellow in colour, and trimethyl ether B is colourless.

Herqueinone is very smoothly reduced with zinc and acetic acid at room temperature to yield deoxyherqueinone, $C_{20}H_{20}O_6$. The deoxy compound is dextrorotatory, and still contains the original methoxyl group of herqueinone.

Prolonged hydrolysis of herqueinone with hot aqueous sulphuric acid yields methyl isopropyl ketone, characterised as its 2:4-dinitrophenylhydrazone and semicarbazone; and another compound, to which the name xanthoherquein has been given[96], which has the empirical formula, $C_{15}H_{12}O_7$. The hydrolysis may, therefore, be written stoichiometrically as:

$$\underset{\text{(Herqueinone)}}{C_{20}H_{20}O_7} + H_2O \longrightarrow \underset{\text{(Ketone)}}{C_5H_{10}O} + \underset{\text{(Xanthoherquein)}}{C_{15}H_{12}O_7}$$

Xanthoherquein gives a positive ferric chloride

test, and contains one methoxyl group. Raistrick and his colleagues have prepared from it a perchlorate[96], a tetramethyl ether[96], a tetra-acetate[92,94], and, under forcing conditions (silver oxide and methyl iodide in refluxing benzene for twenty-four hours), a pentamethyl ether[92,94]. Xanthoherquein and all its derivatives are optically inactive.

An isomer of herqueinone, isoherqueinone, was obtained during an unsuccessful attempt at methylation by refluxing in acetone with methyl iodide and potassium carbonate. The infrared absorption spectra of herqueinone and isoherqueinone were stated[96] to be almost identical. The specific rotation of isoherqueinone, as measured on the sodium D line, is zero.

By treatment of an ethanolic solution of herqueinone with bromine water, a white crystalline material, $(C_4H_4O_2Br)_n$, is obtained[96], which analyses for the equivalent of 8.2% of -OMe, by Zeisel determination.

Norherqueinone, $C_{19}H_{18}O_7$, contains no methoxyl groups, and, like herqueinone, is very strongly dextro-rotatory. It has been shown by Galarraga, Neill, and

Raistrick[96] to be demethylated herqueinone. On methylation, it affords herqueinone trimethyl ether A. Its other properties are analogous to those of herqueinone. Refluxing its solution in acetone with potassium carbonate affords isonorherqueinone, which is strongly laevorotatory, while hydrolysis with sulphuric acid cleaves the molecule into methyl isopropyl ketone and norxanthoherquein, $C_{14}H_{10}O_7$. Norxanthoherquein penta- and hexa- methyl ethers are identical with xanthoherquein tetra- and penta- methyl ethers respectively.

Herqueinone has also been investigated by Harman, Cason, Stodola, and Adkins[97], who also succeeded in cleaving the molecule into methyl isopropyl ketone and xanthoherquein. Dihydroherqueinone (named herqueinic acid by these workers) has been shown to have a pK_a of 4.2 (cf. benzoic acid, 6.0; and p-hydroxybenzoic acid, 6.7). One other degradation production, a methoxy-ketone, said to have the empirical formula $C_{10}H_{12}O_3$, is mentioned[97], but its properties are not described, and no experimental data are given.

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DISCUSSION.

1.) Atrovenetin.

Penicillium atrovenetum G. Smith was first described in 1956[98]: in his account of the species, Smith drew attention to its close morphological similarity to Penicillium herquei Bainier and Sartory. In view of this, and of the similarity in the empirical formulae of atrovenetin ($C_{19}H_{18}O_6$) and norherqueinone ($C_{19}H_{18}O_7$), it was expected that some structural relationship would be found. Atrovenetin, however, possesses considerable aromatic character (as exemplified by its stability to sulphuric acid and to potash fusion)[92], which herqueinone lacks.

Deoxyherqueinone, obtained by zinc and acetic acid reduction of herqueinone[96], does possess this stability. Moreover, it has an ultraviolet spectrum of the same general form as that of atrovenetin. It therefore appeared likely that both deoxyherqueinone and atrovenetin belong to the same structural group. This was established by reducing norherqueinone with zinc and acetic acid, to deoxynorherqueinone, which gave a tri-

acetate, identical with that obtained by acetylation of atrovenetin. Herqueinone must, therefore, have the same skeleton as atrovenetin: data obtained from experiments on herqueinone can thus be employed in elucidating the structure of atrovenetin.

The preparation of tetramethyl ethers of atrovenetin by Neill and Raistrick[92,94] means that, of the six oxygen atoms in the atrovenetin molecule, four are present as hydroxyl groups. An examination of the infra-red absorption spectrum of atrovenetin (which has a strong band at 1620 $cm.^{-1}$) reveals that, of the remaining two oxygen atoms, one is probably present as an inert (hydrogen-bonded) carbonyl group. The presence of hydrogen-bonding is borne out by the fact that, to methylate further either of the trimethyl ethers, very strong conditions must be employed. The formation of a triacetate (which, moreover, exhibits no free hydroxyl band in its infrared spectrum) is also consistent with one of the hydroxyl groups being very strongly hydrogen-bonded.

Since the tetramethyl ethers of atrovenetin are

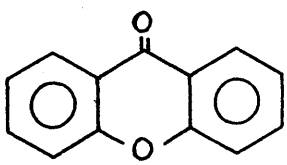
insoluble in alkali, and give negative ferric chloride reactions[94], it follows that the remaining oxygen function is probably ethereal. The hydrolytic cleavage of herqueinone and norherqueinone with sulphuric acid[96] gives xanthoherquein, $C_{15}H_{12}O_7$, and norxanthoherquein, $C_{14}H_{10}O_7$, respectively, as well as, in each case, methyl isopropyl ketone. Both xanthoherquein and norxanthoherquein contain one C-methyl group, and are optically inactive. It seemed reasonable, therefore, to conclude that norxanthoherquein represents the aromatic nucleus of atrovenetin, while the five carbon fragment expelled as methyl isopropyl ketone is derived from the ether ring, and incorporates the asymmetric centre from which the optical activity of atrovenetin derives.

Powerful evidence for the identity of the nuclei arose from a study of the ultraviolet spectra of the acetates of xanthoherquein, deoxyherqueinone, norxanthoherquein, and atrovenetin. These are recorded in Table I (page 135).

It follows from the functional group analysis

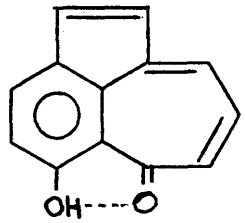
outlined above that the empirical formula of the nucleus is $C_{13}H_8O$; in the case of norxanthoherquein, for instance, the substituents are one C-methyl group, five free hydroxyl groups, and one strongly hydrogen-bonded hydroxyl group, making up the empirical formula of $C_{14}H_{10}O_7$.

The early stages of this work were carried out before it was known that norxanthoherquein could be induced to yield a hexamethyl ether. At that time it appeared that there was a total of only five hydroxyl groups in the molecule, and the possibility of the nucleus being xanthone (II) was considered. This was discounted for the following reasons. Firstly, the ultraviolet spectra of xanthoherquein, atrovenetin, etc. are quite dissimilar to that of xanthone[41] (see Table I, on page 135), and secondly atrovenetin and xanthoherquein were found to yield only monobromo- derivatives, thus indicating the presence of only one vacant position in the nucleus: an appropriately substituted xanthone nucleus would have two vacant positions.



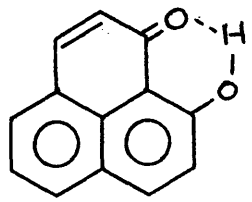
II

In order to account for the existence of only one vacant position in a C_{13} nucleus, it is necessary to postulate a system of three carbocyclic peri- fused rings. Structures involving the tropone nucleus (III) may be ruled out, since it has been found that xanthoherquein cannot be catalytically hydrogenated.



III

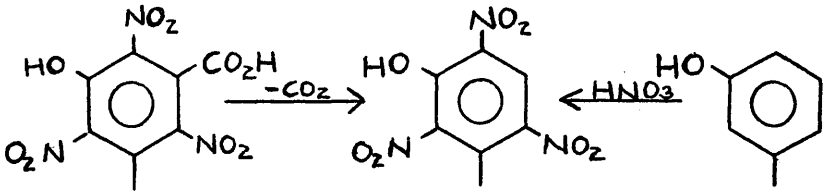
The 9-hydroxyperinaphthenone nucleus (IV) is in agreement with all the data so far mentioned. It is known[99] that the hydroxyl group in (IV) is resistant to acylation and alkylation under ordinary conditions. Moreover, its ultraviolet and infrared spectra (Tables I and II) are similar to those of compounds in the atrovenetin-xanthoherquein series. At the time this work was being carried out, no naturally-occurring perinaphthenone was known, but since then the structure of haemocorin, based on a perinaphthenone nucleus, has been published[89,90] (see earlier section).



IV

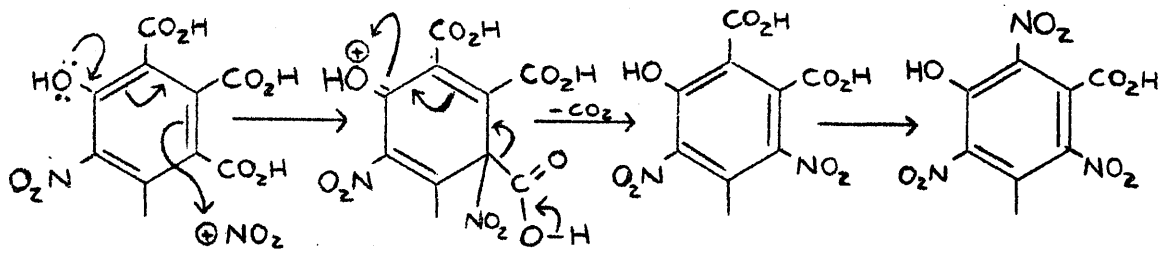
Attempts to degrade xanthoherquein to a naphthalic acid were made with the intention of establishing the hydroxyperinaphthenone nature of the nucleus. Oxidation with potassium permanganate under various conditions, ozonolysis, osmylation followed by periodate cleavage, all yielded indeterminate results.

A crystalline degradation product, of empirical formula $C_8H_5N_3O_9$, was finally obtained by heating xanthoherquein on the steam-bath with concentrated nitric acid. The compound was optically inactive, gave a positive ferric chloride test, and effervesced with sodium bicarbonate. On brief treatment with diazomethane, a dimethyl derivative was obtained. The properties of the degradation product agreed with those described for nitrococussic acid (V)[100,101,102]. That this was, in fact, the compound obtained was established by decarboxylation to 2:4:6-trinitro-m-cresol (VI), the latter and its methyl ether being compared with authentic specimens.



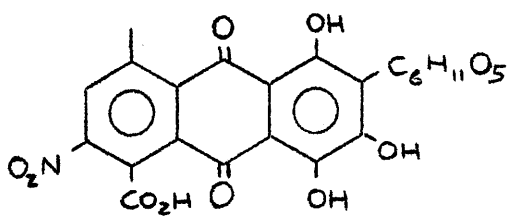
V VI

It is evident that nitrococussic acid could only have been formed in the nitric acid oxidation of xanthoherquein by decarboxylative nitration of an intermediate hemimellitic acid (VII), as shown.



VII V

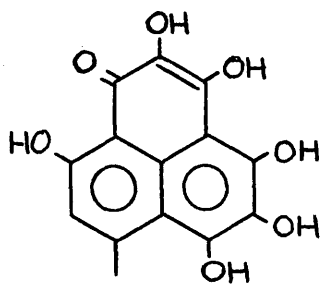
Such a process is the exact analogy of that occurring in the nitric acid oxidation of carminic acid (VIII), which also yields nitrococussic acid.[100,101]



VIII

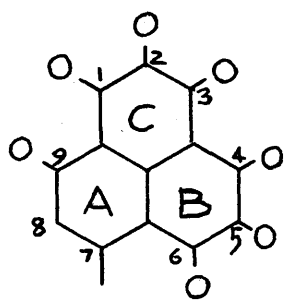
The vacant position in the perinaphthenone nucleus of xanthoherquein is clearly marked by the position of the nitro- group in the intermediate (VII). The survival of this ring is to be attributed to the deactivating influence of the nitro- group, which must be introduced with great facility.

All the positions on the perinaphthenone nucleus of norxanthoherquein, with the exception of two (the vacant one, and that occupied by the methyl group) are substituted with oxygen functions. The isolation of nitrococussic acid (which contains the two "odd" positions) therefore establishes the structure of norxanthoherquein as (IX), or a tautomer. Xanthoherquein is to be represented as a monomethyl ether of (IX). Confirmation of the structure of the nucleus in both atrovenetin and norxanthoherquein was obtained by oxidation of each of these compounds to nitrococussic acid.



IX

Attention was next directed to elucidating the points of attachment of the ether ring in atrovenetin. On the evidence so far presented, this could bridge any of the following positions (see skeleton structure X): C_1-C_2, C_2-C_3, C_3-C_4, C_4-C_5, C5-C_6, and C_9-C_1.

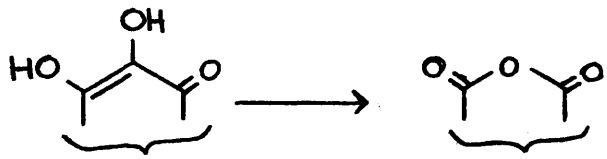


X

Some of these possibilities were eliminated as follows.

Atrovenetin was oxidised with alkaline hydrogen peroxide at 0°C. for a very short time. Partition chromatography of the gummy product on a cellulose column afforded a crystalline material of empirical formula, $C_{18}H_{16}O_6$. This compound was optically active (hence still contained the ethereal side-chain), gave a strongly positive ferric chloride test, and afforded a diacetate. It was formulated as a naphthalic anhydride, arising by oxidation of either ring B or ring C (see skeleton formula X), for the following reasons.

Firstly, the empirical formula is in agreement with an oxidation of the type (XI)⟶(XII). Secondly, the

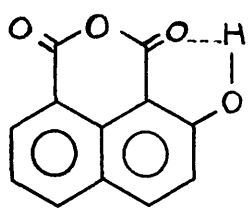


XI XII

compound is insoluble in sodium bicarbonate solution. Thirdly, solutions of both the oxidation product and its diacetate exhibit a vivid blue fluorescence under ultra-

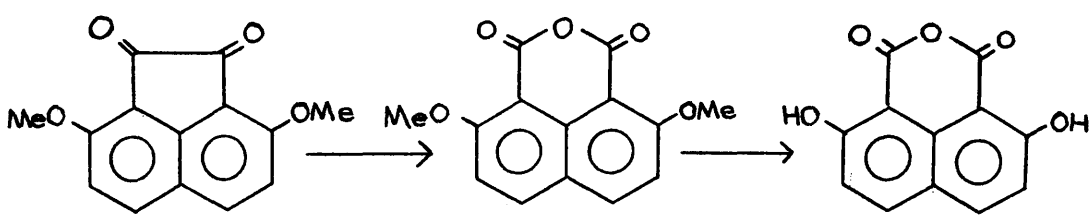
violet light, claimed[103] to be characteristic of a C - O - C bridge across the 1, 8 positions of naphthalene. Fourthly, the infrared spectrum of the diacetate exhibited carbonyl bands which corresponded well with those of naphthalic anhydride. (See Table III, page 137).

The infrared spectrum of the oxidation product itself had carbonyl bands of unusually low frequency (1703 $cm.^{-1}$ and 1663 $cm.^{-1}$). This was attributed to hydrogen-bonding of the type illustrated in (XIII). As a model for such hydrogen-bonding, 2:7-dihydroxynaphthalic anhydride was synthesised.



XIII

The starting material in the synthesis was 3:8-dimethoxyacenaphthenequinone (XVI), prepared[104] by condensation of diphenyloxalimide chloride[105] with



XVI XV XIV

2:7-dimethoxynaphthalene. The 3:8-dimethoxyacenaphthene-

quinone was cleaved with alkaline hydrogen peroxide to yield 2:7-dimethoxynaphthalic anhydride (XV), which, on demethylation with pyridine hydrochloride gave (XIV).

The infrared spectrum of the model compound (XIV) exhibited low-frequency carbonyl bands, as in the case of the oxidation product itself, which must be attributed to hydrogen-bonding. Moreover, the diacetate of (XIV) had an infrared spectrum which was in close agreement with that of the diacetate of the degradation product.

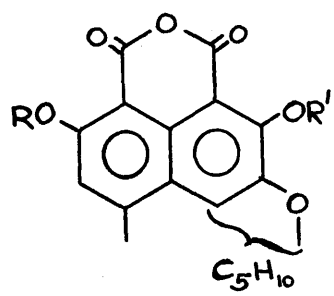
It could, therefore, be concluded that the compound obtained by mild oxidation of atrovenetin with hydrogen peroxide was indeed a naphthalic anhydride (anhydride A), with at least one of the anhydride carbonyl groups hydrogen-bonded to a hydroxyl group. This consideration limits the possible positions of attachment of the ether ring to the perinaphthenone nucleus of atrovenetin to C_1-C_2, C_4-C_5, C_5-C_6, or C_9-C_1. (See skeleton structure X, on page 66.)

A study of the oxidation products of the two trimethyl ethers of atrovenetin made a final decision

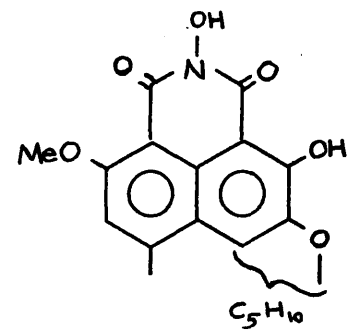
possible. The infrared spectrum of the compound, $C_{19}H_{18}O_6$ (i.e. $C_{18}H_{16}O_6 + CH_2$), obtained[92] by oxidation of the yellow trimethyl ether with chromium trioxide, suggested that it might also be a naphthalic anhydride (Anhydride B). This was established by demethylation with pyridine hydrochloride, when anhydride A was obtained.

In the infrared spectrum of anhydride B only one of the carbonyl bands has an abnormally low frequency. This suggested that in anhydride A both carbonyl groups were hydrogen-bonded, while in anhydride B only one of them was, the other being adjacent to a methoxyl group. Evidence that this was, in fact, the case, was obtained by chromium trioxide oxidation of the orange trimethyl ether, when an isomer of anhydride B, also containing one methoxyl group, was obtained. This compound was named anhydride C. It was optically active, gave a strongly positive ferric chloride test, and had an infrared spectrum identical in the carbonyl region to that of anhydride B. Demethylation of anhydride C, carried out in the same way as for anhydride B, led again to the production of anhydride A.

The inter-relationship of the three naphthalic anhydrides is now clear. Anhydrides A, B, and C may be represented by the partial structures (XVII; R = R'= H), (XVII; R = Me, R'= H), and (XVII; R = H, R'= Me) respectively. The orientations of the methoxyl groups in anhydrides B and C were established by experiments which will be described later.



XVII



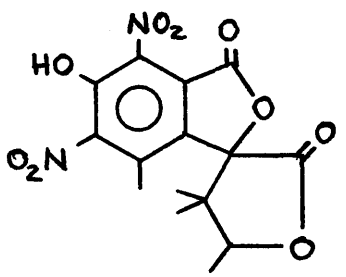
XVIII

The derivative of anhydride B obtained[92] by treating it with hydroxylamine hydrochloride is now to be formulated as the N-hydroxyimide (XVIII), and not as an oxime. The formation of N-hydroxyimides by treatment of naphthalic anhydrides with hydroxylamine hydrochloride is well-known[106].

The oxygen of the ethereal ring is attached at C_3 in (XVII) as a result of a study of the product,

$C_{15}H_{14}O_9N_2 \cdot H_2O$, obtained[92] by brief oxidation of atrovenetin with concentrated nitric acid at 100°C. This compound has been reinvestigated, and its empirical formula, and that of its methyl derivative, confirmed. Structure (XIX) is now proposed for this compound, on the basis of the following data.

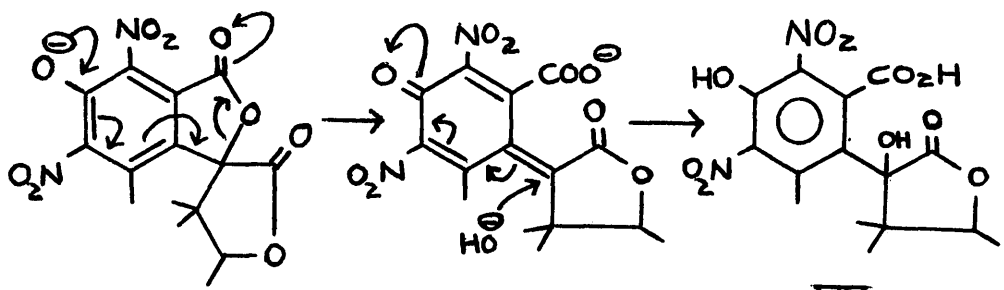
Firstly, the empirical formulae, both of the oxidation product, and of its monomethyl derivative, are in agreement with such a formulation. Secondly, the compound has been found to exhibit infrared bands at 3575 $cm.^{-1}$ (phenolic hydroxyl), 1780 $cm.^{-1}$ (γ-lactone), and 1740 $cm.^{-1}$ (phthalide)[107]: the methyl derivative has bands at 1780 $cm.^{-1}$ (γ-lactone) and 1740 $cm.^{-1}$ (phthalide), but no hydroxyl band. Thirdly, the degradation product is optically active[92], thus indicating that the asymmetric centre of the ethereal side-chain is still present.



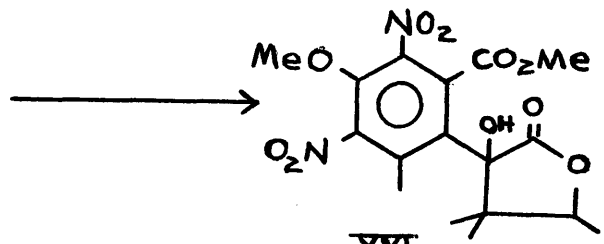
XIX

Structure (XIX) accounts also for the uptake of six molecules of hydrogen on micro-hydrogenation of the

methyl derivative. In addition, it provides a ready rationalisation for the extremely fast base-catalysed hydrolysis of the phthalide ring to yield a non-crystalline product (XX), which affords a dimethyl derivative (XXI) on treatment with diazomethane (see experimental). This may be represented in the following way.



XX

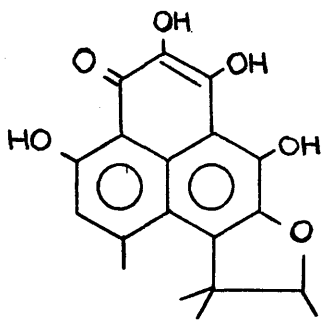


XXI

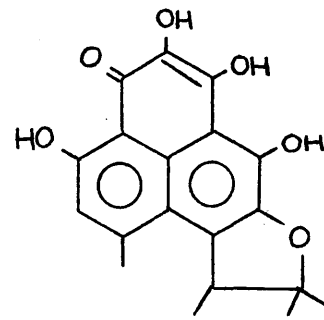
In agreement with structure (XXI), the dimethyl derivative exhibits carbonyl bands in its infrared spectrum at 1740 $cm.^{-1}$ (methoxycarbonyl, o-, p- to nitro- groups: cf. methyl nitrococussate, ν_{max} 1740 $cm.^{-1}$) and 1780 $cm.^{-1}$ (γ-lactone).

Finally, structure (XIX) accounts very well for the resistance of the degradation product to the vigorous conditions under which it is formed.

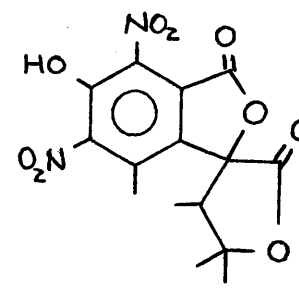
All the evidence so far presented may now be correlated in terms of structure (XXII), or a tautomeric modification, for atrovenetin. However, on the basis of what has so far been written, structure (XXIV) fits the properties of the nitric acid oxidation product as well as does (XIX), and so atrovenetin may equally well be represented by (XXIII).



XXII



XXIII

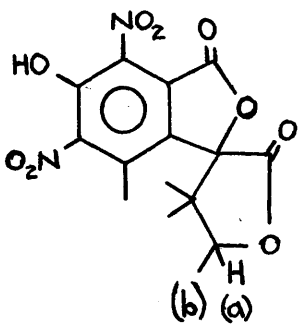


XXIV

A decision in favour of the structure (XIX) for the phthalide was made as the result of a study of its nuclear magnetic resonance spectrum, very kindly determined by Dr. Jackman of Imperial College, London.

The NMR spectrum was measured on a saturated solution

of the phthalide in pyridine. The compound possesses bands at 1222 c.p.s. and 1218 c.p.s. (gem-dimethyl group); and at 1154 c.p.s. (aromatic C-methyl group). In addition, there is a doublet (J = 6.5 c.p.s.) at 1209 c.p.s., and a weak multiplet (J = 6.5 c.p.s.) at 1066 c.p.s. The 1066 c.p.s. multiplet approximates to a quartet, although the expected intensity ratios were not observed, probably because the signal strength was too near the noise level. Nevertheless, the signal was genuine, as it was not observed with the solvent alone. The 1066 c.p.s. band may therefore be assigned to the proton (a) and the doublet to the adjacent methyl group (b) in structure (XIX). The shielded character of the proton (a) is in accord with structure (XIX), its resonance frequency being close to that of the equivalent proton (1068 c.p.s.) in isopropyl acetate[108]. Similarly, the resonance frequency of the adjacent methyl group has the correct value for a methyl group one carbon atom removed from oxygen. (Cf.



XIX

$\underline{CH_3}CH_2O.CO.CH_3$ at 1212 c.p.s.).[108]

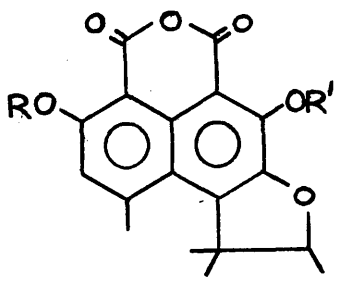
Structure (XIX) is therefore clearly to be preferred to (XXIV) for the nitric acid oxidation product. Furthermore, the fact that all the signals observed in the NMR spectrum can be accounted for by (XIX) provides additional valuable evidence for the correctness of the structure as a whole.

It follows from the evidence cited that the structure of atrovenetin is (XXII), or a tautomer. This is in accord with the observation that the yellow trimethyl ether is optically stable to refluxing in potassium tert.- butoxide. If the hydrogen atom attached to the asymmetric centre were benzylic, and, moreover, vinylogously α to a carbonyl group, racemisation would have been expected.

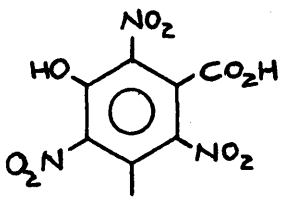
Structure (XXII) also accounts in a very simple way for the isolation of pyrene (I)[92] as a product of the zinc dust distillation of atrovenetin. This may now be seen to be formed by cleavage of the C - O bond of the cyclic ether, followed by recyclisation. The loss of methyl groups in this process finds analogy in the

chemistry of terramycin[109], and in several other well-authenticated cases, in which alkyl groups have been cleaved by distillation with zinc or selenium.[110]

The complete structures of anhydrides A, B, and C may now be written as (XXV; R = R'= H), (XXV; R = Me, R'= H), and (XXV; R = H, R'= Me) respectively.



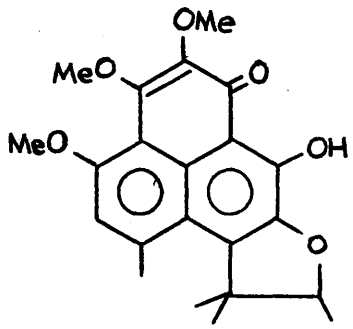
XXV



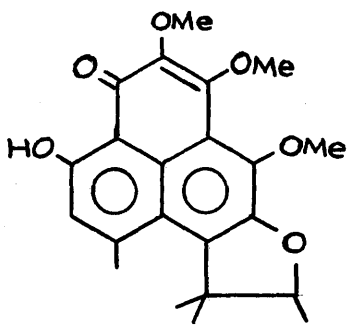
V

The orientation of the methoxyl groups in anhydrides B and C was determined by oxidising each of the anhydrides with concentrated nitric acid. Quantitative partition chromatography showed that anhydride C yielded more than 200 times as much nitrococussic acid (V) as did anhydride B. The nitrococussic acid produced in the oxidation was isolated by chromatography on a cellulose column, and was shown to be identical with an authentic specimen.

From these experiments, the positions of the methoxyl groups in anhydrides B and C, relative to the other substituents, readily follows. From these results, also, the structures of the two trimethyl ethers can be deduced. Anhydride B is derived from the yellow trimethyl ether; hence, since the hydroxyl group of the latter must be strongly hydrogen-bonded to the carbonyl group, structure (XXVI), or its tautomer, follows for this ether. By a similar argument, structure (XXVII), or its tautomer, may be assigned to the orange trimethyl ether.



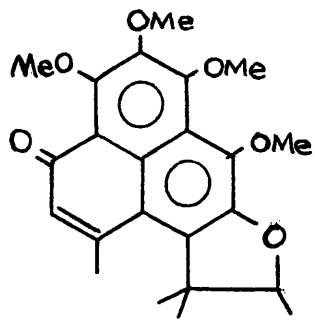
XXVI



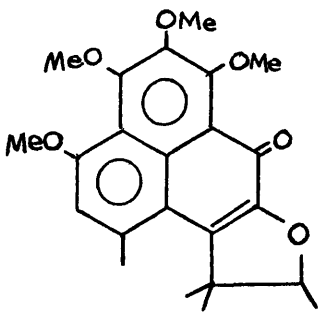
XXVII

Atrovenetin tetramethyl ether A is derived from the orange trimethyl ether, to which it may be hydrolysed with hydrogen chloride.[92] It has now been found that chromium trioxide oxidation of tetramethyl ether A

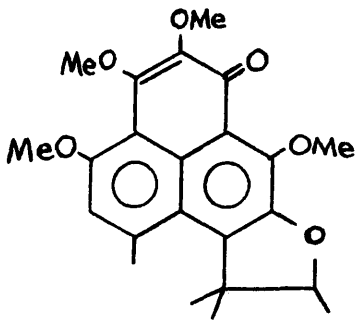
affords anhydride C, from which it follows that the former must possess structure (XXVIII). Tetramethyl ether B, which is derived from the yellow trimethyl ether[92], must have either structure (XXIX) or (XXX). Attempts to distinguish between these possibilities by chromium trioxide oxidation to the corresponding naphthalic anhydride led to inconclusive results.



XXVIII

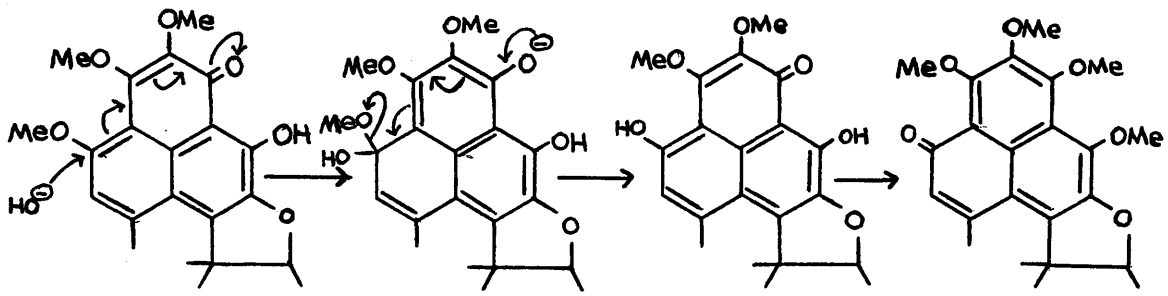


XXIX



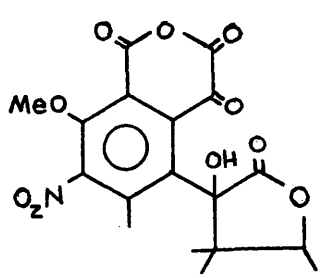
XXX

The formation of some tetramethyl ether A by methylation of the yellow trimethyl ether[92] may readily be rationalised as a vinylogous β-addition of a hydroxide ion, followed by elimination of a methoxyl group, and methylation of the appropriate tautomer:-



XXVI XXVIII

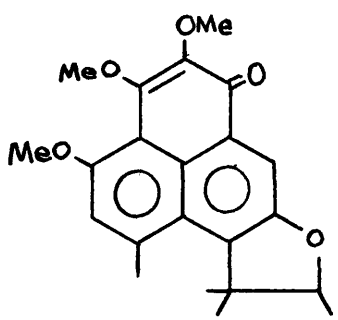
While nitric acid oxidation of the orange trimethyl ether gives nitrococussic acid, as already stated, oxidation of the yellow trimethyl ether affords a compound, $C_{18}H_{17}O_{10}N$, which contains one methoxyl group, and is optically active. The product does not react with sodium bicarbonate, and gives no colour with ferric chloride. The structure tentatively assigned to this compound is (XXXI). Structure (XXXI) is supported by the infrared spectrum of the material. In nujol, this has bands in the carbonyl region at 1803 $cm.^{-1}$ and 1735 $cm.^{-1}$ (anhydride); 1777 $cm.^{-1}$ (γ-lactone); and 1700 $cm.^{-1}$ (carbonyl



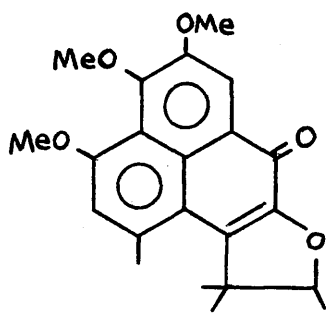
XXXI

group in a six-membered ring). The ultraviolet spectrum ($\lambda\lambda_{max}$ 231and 321 mμ, log ε 4.50 and 3.74 respectively) is also quite compatible with the compound being the methyl ether of a nitrophenol.

Most of the transformation products of atrovenetin and its derivatives described by Raistrick and Neill[92] are easily rationalised on the basis of structure (XXII) for atrovenetin. The product YA, obtained by the lithium aluminium hydride reduction of the yellow trimethyl ether may be formulated as either (XXXII) or (XXXIII), depending on which tautomeric form is reduced.

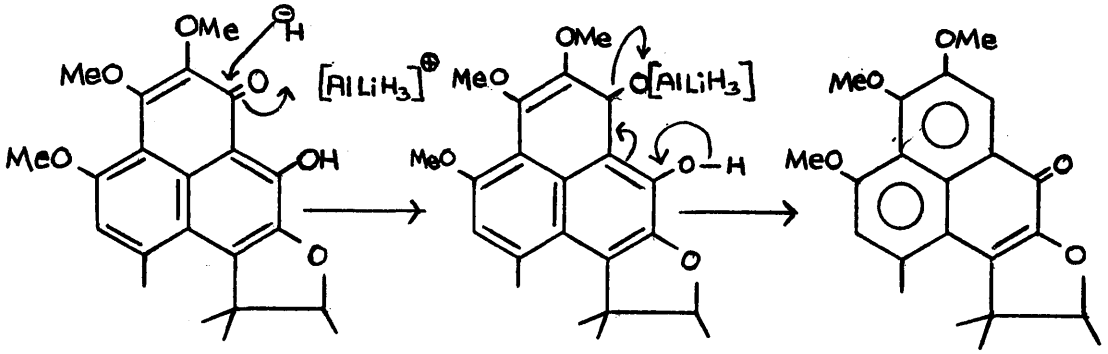


XXXII



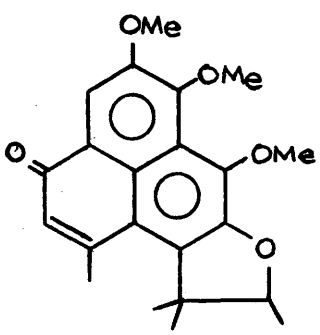
XXXIII

In support of such a structure, the ultraviolet[92] and infrared (Table II) spectra of YA are of the type associated with perinaphthenones. The genesis of (XXXIII), for example, may be expressed as follows:-

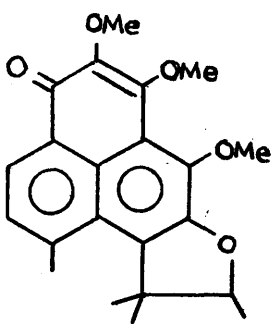


XXVI XXXIII

The compound OA (obtained by lithium aluminium hydride reduction of the orange trimethyl ether) also has the spectral properties of a perinaphthenone, and may be formulated as either (XXXIV) or (XXXV).



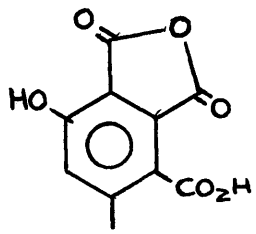
XXXIV



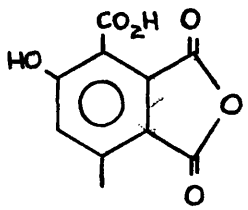
XXXV

The "peroxide oxidation product B" obtained by Neill

and Raistrick[92] was assigned the empirical formula $C_{12}H_8O_7$ by these workers. The analytical data quoted[92], however, fit the the formula $C_{10}H_6O_6$ equally well. A study of the physical properties of this compound has led to the adoption of structure (XXXVI) for it. Such



XXXVI

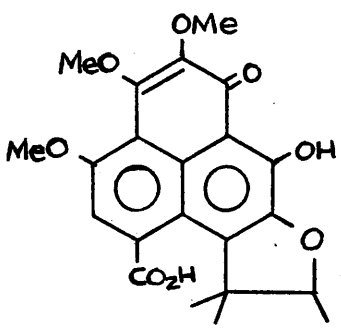


XXXVII

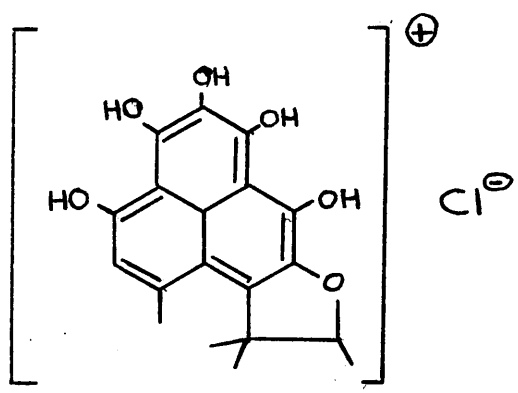
a structure is supported by the infrared spectrum (in nujol), which exhibits bands at 1840 $cm.^{-1}$ and 1748 $cm.^{-1}$ (phthalic anhydride), and at 1697 $cm.^{-1}$ (carboxyl group). In agreement with the proposed structure, the substance has been found to be optically inactive, to effervesce when treated with sodium bicarbonate solution, and to give a positive ferric chloride test. The ultraviolet absorption spectrum (λ_{max} 306-309 mμ, log ε, 3.56) is also quite compatible with structure (XXXVI). Structure (XXXVI) is preferred to the alternative formulation (XXXVII), because the absence of a free hydroxyl band

in the infrared spectrum of the compound, and the abnormally low frequency of one of the anhydride carbonyl bands are, as in the case of anhydrides B and C, suggestive of hydrogen-bonding.

Neill and Raistrick suggested[92] that the product which they obtained by oxidation of the yellow trimethyl ether with potassium permanganate was derived from the parent trimethyl ether by the oxidation of a C-methyl group to a C-carboxyl. The compound has been prepared again, and its ultraviolet spectrum ($\lambda\lambda_{max}$ 381 and 401-3mμ, log ε 4.30 at both maxima) found to be very closely similar to that of the yellow trimethyl ether itself. It is also optically active. It therefore appears that (XXXVIII), or a tautomer, is a satisfactory structure for this compound.



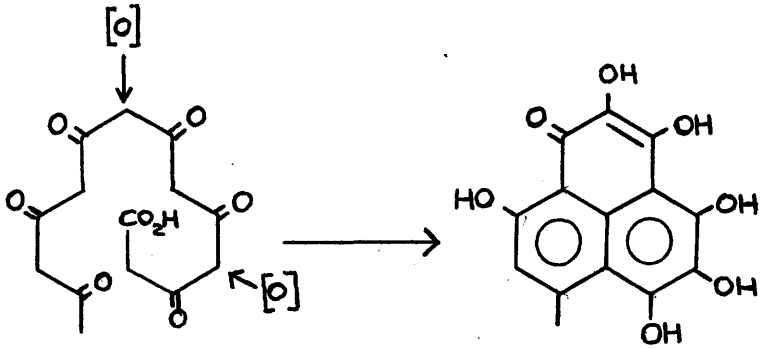
XXXVIII



XXXIX

Lastly, atrovenetin hydrochloride[92] may be formulated as a perinaphthenylium salt[111], as in (XXXIX). The perchlorates of atrovenetin and its methyl ethers may be represented by analogous structures.

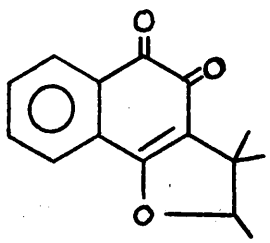
The probable biogenesis of atrovenetin is of some interest. Its nucleus can be regarded as being formed by condensation of a poly-β-diketone (XL), of the same general type as those proposed[48,50a] as precursors in the biogenesis of the fungal anthraquinones.



XL

The mode of attachment of the isoprenoid side-chain is unusual. The only other natural product possessing an isopentane unit $\left\{ {}^{4}C \atop {}^{5}C \right\} > {}^{3}C - {}^{2}C - {}^{1}C \Big\}$ attached to an aromatic nucleus at C_3 is dunnione (XLI).[77,112] The

possible mechanism of substitution of C_5 units into aromatic nuclei has already been discussed (pages 33-34).



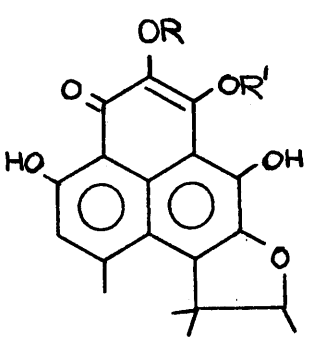
XLI

--ooOoo--

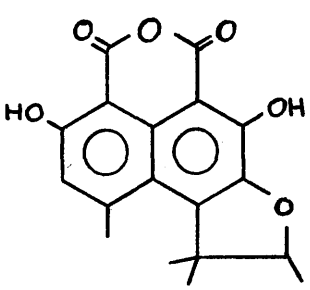
DISCUSSION.

2.) Herqueinone and norHerqueinone.

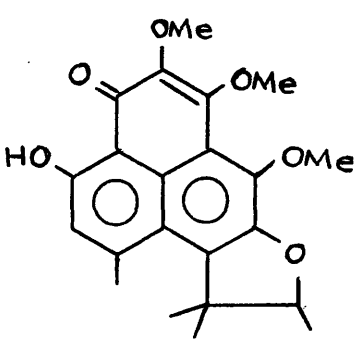
The two principal degradation products of norherqueinone are norxanthoherquein and deoxynorherqueinone (atrovenetin). Their structures have been determined as described in the preceding section. From the work of Galarraga, Neill, and Raistrick[96], it is known that herqueinone is the monomethyl ether of norherqueinone. It therefore follows that deoxyherqueinone bears the same relationship to atrovenetin. Deoxyherqueinone has now been shown to have either structure (XLII; R = H, R'= Me), or (XLII; R = Me, R'= H), or a tautomeric modification of one of these.



XLII

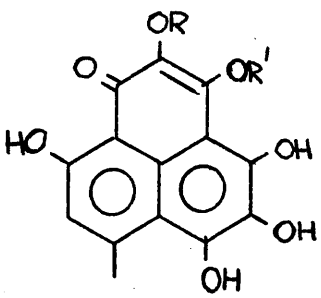


XLIII



XXVII

These possible structures follow from the oxidation of deoxyherqueinone with chromium trioxide to yield anhydride A (XLIII), and the fact that methylation with diazomethane affords atrovenetin orange trimethyl ether (XXVII). These experiments also establish the structure of xanthoherquein as (XLIV; R = H, R'= Me) or (XLIV; R = Me, R'= H), or a tautomer.

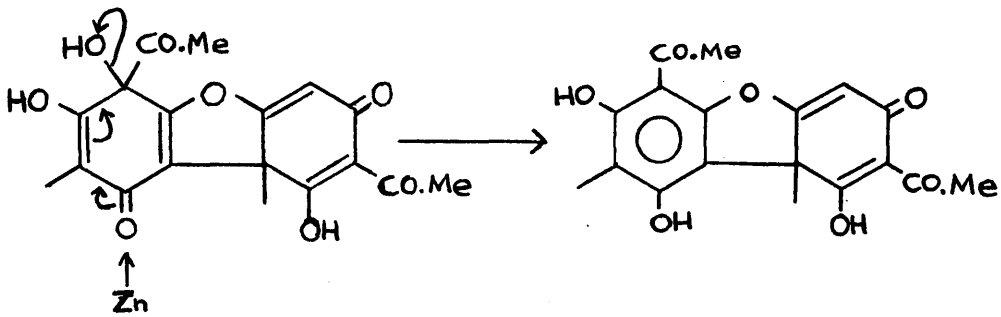


XLIV

Herqueinone itself contains the same basic skeleton as deoxyherqueinone, but has one extra oxygen function. Its ultraviolet spectrum differs greatly from those of deoxyherqueinone and xanthoherquein, and is indicative of extended conjugation in the molecule. The fact that its infrared spectrum has no carbonyl band of higher frequency than 1620 $cm.^{-1}$ provides evidence that any carbonyl groups contained in herqueinone are very highly conjugated, or strongly hydrogen-bonded, or both.

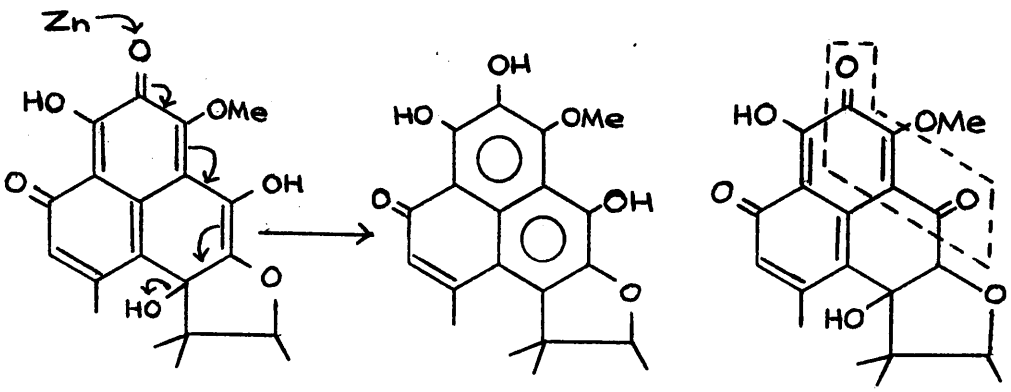
The greatly enhanced stability of deoxyherqueinone

relative to herqueinone suggests that, in the latter, the aromaticity of one or both of the benzene rings of the perinaphthenone nucleus is blocked by some kind of tertiary substituent. The ease with which the extra oxygen atom is removed by treatment with zinc and acetic acid calls to mind the similar reduction of usnonic acid (XLV, or equivalent structure) to usnic acid (XLVI)[113], which may be represented as follows:-



XLV XLVI

By analogy with this, herqueinone may be written as, for example, (XLVII), and its reduction to deoxy-herqueinone with zinc dust and acetic acid written in the following way:-



XLVII

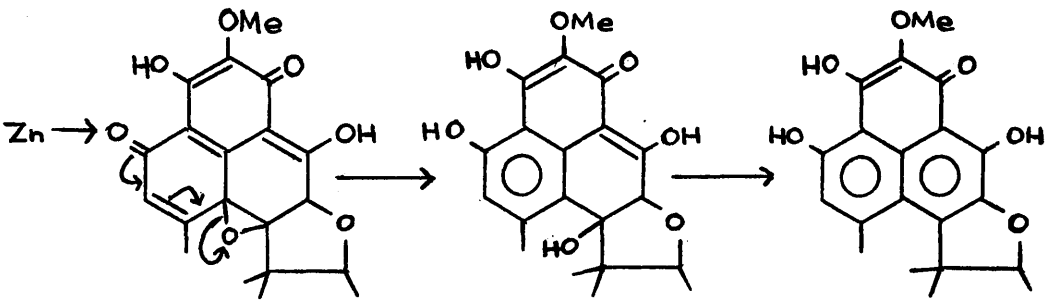
Deoxyherqueinone
(or equivalent structure)
(or equivalent structure)

XLVIII

Structures such as (XLVII) would, however, probably exist in the tautomeric form (XLVIII), the ene-dione system of which (enclosed by a broken line in the diagram) would be expected to absorb in the infrared at a rather higher frequency than 1620 $cm.^{-1}$

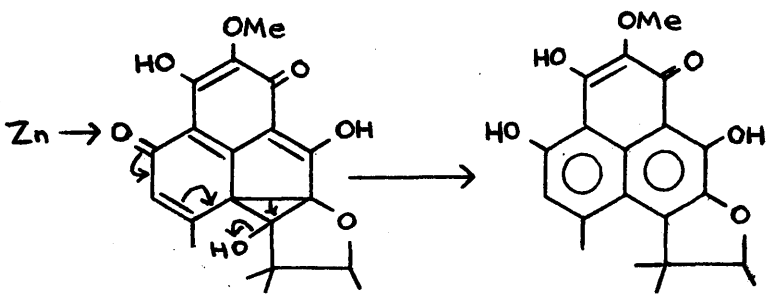
Structures in which the extra oxygen function is present as an epoxide might also be considered, e.g. (XLIX). Reduction of a compound with such a structure with zinc and acetic acid would give (L), which could afford deoxyherqueinone by β-elimination of the tertiary hydroxyl. Structures of this type, however, are rendered unlikely by the discovery that herqueinone is stable to treatment with a hot solution of hydrogen iodide

in acetic acid - conditions which would be expected to cleave any epoxides present.



XLIX L Deoxyherqueinone (or equivalent structure)

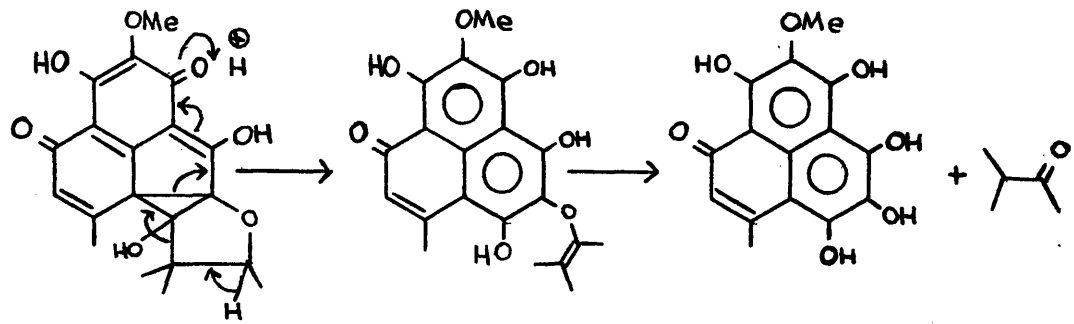
Structure (LI) is subject to neither of the objections raised for (XLVII) and (XLIX), and, like the others, it accounts very well for the formation of deoxyherqueinone.



LI Deoxyherqueinone (or equivalent structure)

The acid hydrolysis of herqueinone to yield xantho-herquein and methyl isopropyl ketone is readily rationalised

on the basis of any of the structures so far considered for herqueinone. In the case of structure (LI), a probable mechanism is protonation of a carbonyl group, with simultaneous loss of a proton from the carbon atom bearing the ethereal oxygen, to give the intermediate vinyl ether (LII). The driving force for such a transformation would be provided by the opening of the cyclopropane ring, and the aromaticity of the product. The vinyl ether (LII), of course, could give rise to the observed products by the usual mechanism of vinyl ether hydrolysis.

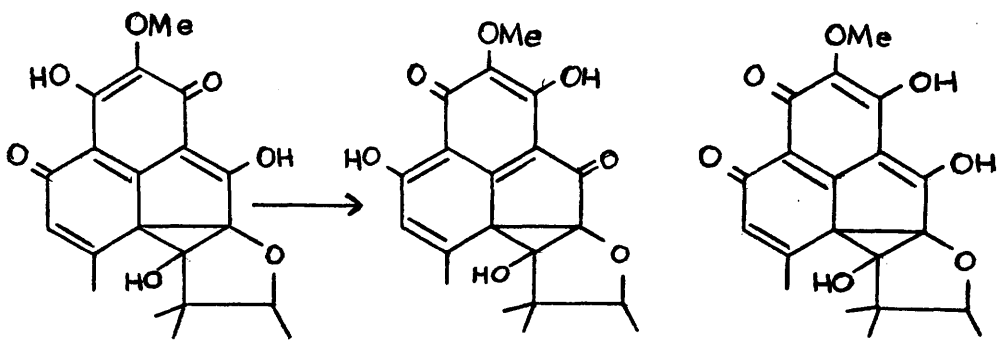


The remarkable isomerisation of herqueinone to isoherqueinone, reported by Galarraga, Neill, and Raistrick[96], has been re-investigated, and found to occur

under very much milder conditions than were employed by these workers. Isomerisation takes place even on merely refluxing an ethanolic solution of herqueinone. The reaction appears to be base-catalysed, and it has been found that the most convenient method for preparing isoherqueinone is to reflux a solution of herqueinone in alcohol containing a little sodium acetate. Isoherqueinone was reported by Raistrick and his co-workers to be optically inactive, and it has now been confirmed that $[\alpha]_D$ for this compound is zero. In view of the very large specific rotation of isonorherqueinone, which presumably has an analogous structure, the apparent optical inactivity of isoherqueinone was very surprising. However, the optical rotatory dispersion curve of isoherqueinone (very kindly determined by Professor C. Djerassi, of Wayne State University) shows that, at wavelengths of light other than the sodium D line, isoherqueinone is optically active.

It has also been shown in the present work that reduction of isoherqueinone with zinc and acetic acid gives deoxyherqueinone.

All these data point to isoherqueinone being simply a tautomer of herqueinone. The fact that the ultraviolet and infrared spectra of isoherqueinone are almost superposable on those of herqueinone supports this view. If herqueinone is supposed to (LI), then structure (LIII) for isoherqueinone would adequately account for the very similar spectral properties of the two compounds.



LI LIII LIV

However, since interconversion of these structures requires only a very small proton shift, it is doubtful if either would be capable of separate and independent existence. (LIV) might be a better formulation for isoherqueinone.*

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*NOTE. The complexity of the systems represented by (LI)

(continued at foot of next page)

Dihydroherqueinone is an important transformation product of herqueinone. It was first obtained by Stodola, Raper, and Fennell[95], who showed that the compound is optically inactive. A fuller investigation was carried out by Harman, Cason, Stodola, and Adkins[97]. These workers determined the pK_a value of dihydroherqueinone as 4.2, and reported that methylation with diazomethane gave a monomethyl derivative containing one more C-methyl group (by Kuhn-Roth analysis) than herqueinone itself. Dihydroherqueinone has been prepared in the course of the present work, and the formation of the monomethyl derivative confirmed. Kuhn-Roth analysis of dihydroherqueinone,

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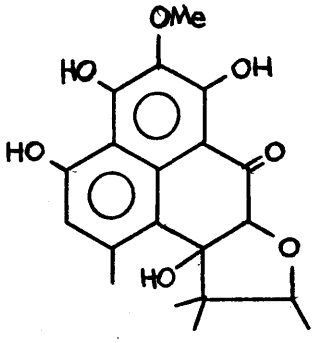
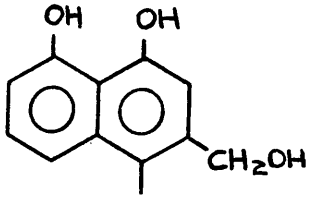
and (LIV) makes it difficult to decide which would possess the greater energy. Herqueinone must, of course, be assigned the structure of lesser stability. If this is considered to be (LIV), then isoherqueinone would have to be written as (LI). Were this the case, all the structural arguments advanced would still be valid, since herqueinone can be converted into isoherqueinone under extremely mild conditions.

however, has shown that only two C-methyl groups (excluding, of course, gem-dimethyl groups) are present in the molecule.

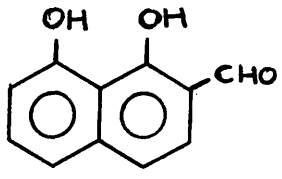
The ultraviolet spectrum of dihydroherqueinone ($\lambda\lambda_{max}$ 237, 289, and 398-402 mμ: log ε, 4.63, 4.16, and 4.09 respectively) is that of an aromatic compound. Its infrared spectrum (in nujol) has two bands in the hydroxyl region: one at 3400 $cm.^{-1}$ (free -OH), and one at 3230 $cm.^{-1}$ (hydrogen-bonded -OH). In the carbonyl region there is one strong band at 1600 $cm.^{-1}$, which is indicative of a very strongly hydrogen-bonded carbonyl group.



LV



LVI



LVII

Accepting the tentative structure (LI) put forward for herqueinone, (LV) is a satisfactory formulation for dihydroherqueinone. The low pK_a value of dihydroherqueinone

is predictable on the basis of such a structure. 1:8-Dihydroxy-3-hydroxymethyl-4-methylnaphthalene (LVI), for example, has a pK_a value of 7.5 (cf. pK_a of phenol, which is 9.94). The increased acidity in this case must be attributed to stabilisation of the phenolate anion by hydrogen-bonding with the hydroxyl group in the other peri- position. Introduction of an ortho- or para-carbonyl substituent increases the acidity still further. Thus, (LVII), which is a fairly good model for the situation in (LV) itself, has a pK_a value of 4.5.

A compound possessing the structure (LV) might be expected to lose the elements of water, by β-elimination of the tertiary hydroxyl group, to yield deoxyherqueinone. It was, therefore, surprising to find that dihydroherqueinone is quite stable to hot caustic soda solution. The tertiary hydroxyl group in skyrin, however, which has a similar environment to that of the one in dihydroherqueinone, also appears to possess resistance to base-catalysed β-elimination[30].

Treatment of dihydroherqueinone with perchloric acid in acetic acid solution resulted, even at room temperature,

in a rapid spectral change. The new spectrum resembled that of deoxyherqueinone. It was not found possible to obtain a crystalline acetate, but the ultraviolet spectrum of the gum obtained on acetylation was very similar to that of deoxyherqueinone diacetate, which suggests that the structure (LV) proposed for dihydroherqueinone contains a fair measure of truth.

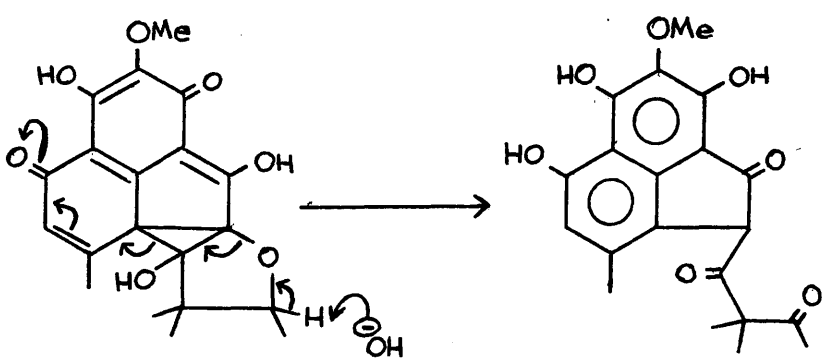
The white bromo-compound obtained from herqueinone by treatment of an ethanolic solution with bromine water[96] has been re-investigated. The compound is optically active, and has ultraviolet and infrared spectra which indicate that it is not a simple derivative of herqueinone, but a complex product formed by addition of bromine to the conjugated system, followed perhaps by rearrangement. Approximately half of the bromine in the molecule can be reduced out with sodium thiosulphate or by catalytic hydrogenation. Attempts to prepare the compound by bromination in solvents other than aqueous ethanol yielded no crystalline material, and it would appear that the incorporation of ethanol into the molecule is essential for its formation.

When herqueinone is brominated in glacial acetic acid, a red substitution product, monobromoherqueinone, is obtained. This yields the white material when brominated further in aqueous ethanol. No useful purpose would be served at this stage by speculations as to the structure of the white bromo-compound, but the analytical data can all be expressed in terms of the molecular formula, $C_{19}H_{11}O_9Br_6(OEt)_2(OMe)$. [See experimental section.]

The two trimethyl ethers of herqueinone[96] also appear to be rather complex rearrangement products, and in the absence of molecular weight determinations it is impossible to be certain that no carbon atoms have been lost. Their ultraviolet and infrared absorption spectra are quite different from those of herqueinone. Trimethyl ether A has sharp infrared absorption bands (in nujol) at 3240 $cm.^{-1}$ (hydrogen-bonded -OH), 1672 $cm.^{-1}$, and 1642 $cm.^{-1}$ Trimethyl ether B has infrared bands at 1742 $cm.^{-1}$ and 1690 $cm.^{-1}$

Perhaps the most noteworthy feature of these trimethyl ethers is their reported[96] optical inactivity. It may be that, as in the case of <u>iso</u>herqueinone and its bromo-

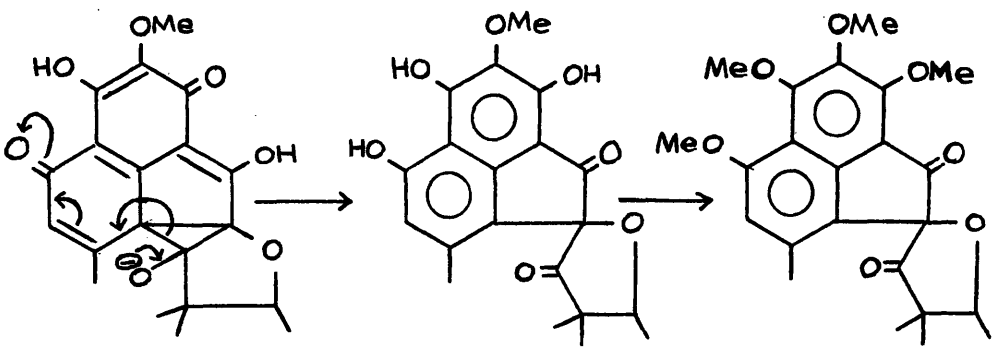
derivative, the observed specific rotations of zero are purely fortuitous. It is interesting, however, that it is possible to suggest a plausible mechanism for racemisation on the basis of the structure (LI) tentatively put forward for herqueinone. This involves direct attack of a hydroxyl ion on the hydrogen atom attached to the asymmetric centre of the ether ring. Formation of the intermediate (LVIII), which could undergo further transformations in the basic methylating medium, would be facilitated by the cyclopropane cleavage and aromatisation envisaged in the mechanism shown.



LI LVIII

If trimethyl ether B is, in fact, optically active (cf. isoherqueinone) then a possible structure for it

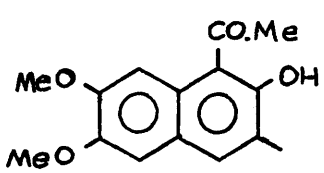
might be (LIX), arising from (LI) as indicated by the arrows. Here again, cyclopropane cleavage and aromatisation may provide the driving force for the transformation.



LI LIX

Structure (LIX) accounts for the absence of any hydroxyl bands in the infrared spectrum of trimethyl ether B, while the carbonyl group in the ether ring would account for the band at 1742 $cm.^{-1}$, and the other carbonyl group for the band at 1690 $cm.^{-1}$

1-Acetyl-2-hydroxy-3-methyl-6:8-dimethoxynaphthalene (LX) has substituents of roughly the same type as in (LIX). Support for (LIX) as the structure of trimethyl ether B is provided by



LX

the similarity of the ultraviolet spectra of the latter and the naphthol (LX).[114] The maxima of the two compounds are as follows. (Wavelengths are given in mμ, and log ε values are in parenthesis.)

Trimethyl ether B.	236 (4.58)	268 (4.27)	355 (3.92)
1-Acetyl-2-hydroxy-3-methyl-6:8-dimethoxynaphthalene.	233 (4.65)	260 (4.35)	343 (3.81)

A major objection to the adoption of structure (LIX) for trimethyl ether B is that, under the alkaline conditions employed[96] during the methylation and subsequent working-up, the β-diketone system might be expected to be cleaved. In connection with this, however, it must be borne in mind that the ether was obtained from herqueinone in only about 5% yield. It therefore appears likely that much of the material initially formed during the methylation is, in fact, further transformed into intractable products. Structure (LIX) is thus consistent with most of the observed facts: in particular, the observed specific rotation of zero may be explained if it is assumed that the two asymmetric centres in the molecule mutually compensate

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EXPERIMENTAL.

Melting-points were taken on the Kofler block, unless specified to the contrary. Ultraviolet spectra were determined in ethanol solution, with the Unicam SP500 Spectrophotometer. Infrared spectra were determined by Dr. G. Eglinton and his colleagues for nujol suspensions, except where otherwise qualified. $[\alpha]_D$ are in chloroform unless stated otherwise. Light petroleum refers to the fraction of b.p. 60-80. Micro-analyses were carried out by Mr. J. M. L. Cameron and his associates. X-ray powder diffraction photographs were taken by Dr. J. C. Speakman, using copper K_α-radiation, and a 9.0 cm. diameter camera.

The nuclear magnetic resonance spectra were determined by Dr. L. M. Jackman on a Varian 4300B nuclear resonance spectrometer using a 40 Mc. oscillation. The spectrum was calibrated against cyclohexane as an internal standard, using the usual side-band technique. Cyclohexane at infinite dilution in carbon tetrachloride gives a single line at 1204 c.p.s. relative to external toluene, the aromatic line of the latter being given the arbitrary

value of 1000 c.p.s.

Bromoherqueinone. Herqueinone (50 mg.) in acetic acid (100 ml.) was treated with a solution of bromine in acetic acid (1.03N, 1 ml.). Titration of an aliquot indicated the uptake of 0.97 molecules of bromine in 10 minutes, no further uptake occurring over a further hour. Isolation of the product in the usual way gave, after crystallisation from chloroform and light petroleum, bromoherqueinone, m.p. 235°(dec.), $[\alpha]_D$ +460° (c, 0.054), $\lambda\lambda_{max}$ 222, 254-57, 266, 322-23, 410-14 mμ (log ε, 4.49, 4.25, 4.27, 4.53, 3.72 respectively). (Found: C, 52.95; H, 4.55; Br, 17.5; OMe, 6.5; C-Me, 7.35. $C_{20}H_{19}O_7Br$ requires C, 53.25; H, 4.25; Br, 17.7; OMe, 6.9; 2 C-Me, 6.65%).

Bromoisoherqueinone. A solution of bromine in acetic acid (0.88N, 1 ml.) was added to isoherqueinone (102 mg.) dissolved in acetic acid (40 ml.). The titration of aliquots indicated the uptake of 0.95 molecules of bromine. The red needles separating from the reaction medium were collected and crystallised from chloroform and light

petroleum to give bromoisoherqueinone, decomposing slowly from 240 - 260° (capillary), $[\alpha]_D$ 0° (c, 0.065). (Found: C, 53.35; H, 4.55; Br, 18.1. $C_{20}H_{19}O_7Br$ requires C, 53.25; H, 4.25; Br, 17.7%). The infrared spectrum showed small differences from that of bromoherqueinone in the finger-print region.

Bromoxanthoherquein. Xanthoherquein (53 mg.) in acetic acid (100 ml.) was treated with a solution of bromine in acetic acid (1.0N, 1 ml.). The titration of aliquots indicated the cessation of uptake after the consumption of 1.3 molecules of bromine. Isolation of the product in the usual way, followed by crystallisation from acetic acid, gave bright yellow needles of bromoxanthoherquein, decomposing slowly above 200°, $\lambda\lambda_{max}$ 220 and 426-429 mμ (log ε, 4.64 and 4.23 respectively). (Found: C, 46.8; H, 3.05; Br, 20.65. $C_{15}H_{11}O_7Br$ requires C, 47.05; H, 29; Br, 20.85%).

Bromoatrovenetin. Atrovenetin (51 mg.) in acetic acid (50 ml.) was treated with a solution of bromine in acetic acid (0.1N, 5 ml.). The titration of aliquots indicated

that uptake of bromine had ceased after 40 minutes, with the consumption of one molecule. On being set aside overnight, the product crystallised from the solution to give, after crystallisation from acetic acid, bromoatrovenetin, m.p. 232-5°. (Found: C, 54.45; H, 4.35; Br, 19.4. $C_{19}H_{17}O_6Br$ requires C, 54.15; H, 4.05; Br, 18.9%).

Polybromoherqueinone. (i) From herqueinone.[96] Herqueinone (102 mg.) was dissolved in ethanol (25 ml.), and saturated bromine water (25 ml.) was added. The colour of the solution immediately faded to yellow, and cream needles slowly separated. The reaction mixture was left in the refrigerator for two hours, and the crystals (39 mg.) were then removed by filtration and washed with methanol. The product was recrystallised from chloroform and light petroleum to a constant m.p. of 167°dec., $[\alpha]_D$ +105°(c, 0.41), λ_{max} 252 mμ, λ_{infl} 312 mμ (log ε, 3.93 and 3.48 respectively), ν_{max} 1777, 1750, 1727, and 1643 $cm.^{-1}$ (Found: Br, 48.70; OR[calculated as OMe], 7.15). The compound gave a very faint brown colour with ferric chloride, but did not react with sodium bicarbonate. It

appeared to decompose when an attempt was made to crystallise it from methanol.

No crystalline material was obtained when the above preparation was repeated using tert-butanol, acetic acid, ethylene bromohydrin, or chloroethanol in place of ethanol.

(ii) From bromoherqueinone. Monobromoherqueinone (47 mg.) was dissolved in ethanol (18 ml.) and treated with saturated bromine water (15 ml.) in the manner already described for herqueinone. Polybromoherqueinone (17 mg.) was obtained, and shown to be identical with an authentic specimen by m.p. and mixed m.p.; $[\alpha]_D$; and ultraviolet and infrared spectra.

Reduction of Polybromoherqueinone. (i) with potassium iodide. The iodine liberated when a chloroform solution of polybromoherqueinone was treated with sulphuric acid and excess potassium iodide was titrated with standard sodium thiosulphate, and found to be equivalent to 53.2% of the bromine content of the molecule being labile.

(ii) by catalytic hydrogenation. A solution of the polybromo- compound (30 mg.) in ethyl acetate (10 ml.) was

hydrogenated over 10% palladised calcium carbonate (64 mg.). Uptake of hydrogen (2.09 ml.) was complete after 30 minutes. Assuming that all the hydrogen absorbed displaced labile bromine, then 51.0% of the bromine content of polybromoherqueinone is labile.

Formula of Polybromoherqueinone. The analytical data can best be accommodated by the formula, $C_{19}H_{11}O_9Br_6(OEt)_2(OMe)$. Found: C, 29.1[96]; H, 2.6[96]; Br, 48.6[96], 48.7; OR (calculated as OMe), 8.2[96], 7.2. $C_{19}H_{11}O_9Br_6(OEt)_2(OMe)$ requires C, 29.3; H, 2.5; Br, 48.7; OR (calculated as OMe), 9.5%.

Xanthoherquein Tetra-acetate. In our hands, the following procedure was found to yield a purer product than that described in the literature.[92] Xanthoherquein (213 mg.) was heated on the steam-bath for two hours with acetic anhydride (2 ml.) and pyridine (4 drops). The solution was cooled and the acetate (136 mg.) filtered off. Recrystallisation from ethyl acetate afforded the product (125 mg.) as yellow rods, m.p. 217-19°, undepressed on admixture with an authentic specimen of m.p. 218-19°.

Deoxyherqueinone Diacetate. (i) Herqueinone (520 mg.) was

dissolved in glacial acetic acid (125 ml.), and the solution was shaken with zinc dust (4 gm.) at room temperature, under nitrogen, for fifteen minutes. After filtration, the acetic acid was evaporated under reduced pressure, and acetic anhydride (15 ml.) and pyridine (50 drops) were added.

The mixture was warmed on the steam-bath for six hours. The reaction mixture was worked up by pouring into water, extracting with chloroform, and washing the chloroform extract with N hydrochloric acid, then with water. Evaporation of the chloroform under reduced pressure gave, after recrystallisation from methanol, deoxyherqueinone diacetate, (265 mg.), m.p. 170-72°. Further recrystallisations raised the m.p. to a constant value of 174-75°. $[\alpha]_D$ +57° (c, 0.463), ν_{max}, 1770, 1613, 1593 $cm.^{-1}$ (Found: C, 65.26; H, 5.65; CH_3CO, 20.22. $C_{24}H_{24}O_8$ requires C, 65.44; H, 5.49; $2CH_3CO$, 19.5%).

(ii) Deoxyherqueinone (34 mg.) was treated overnight with acetic anhydride (1 ml.) and pyridine (0.5 ml.) at room temperature. Working-up in the usual way afforded deoxyherqueinone diacetate, identical in all respects

with the material obtained as described in (i) above.

(iii) Isoherqueinone (109.5 mg.) was reduced with zinc dust (1 gm.) in acetic acid (25 ml.), as described for herqueinone in (i) above. To the gum obtained on evaporation of the acetic acid was added pyridine (20ml.) and acetic anhydride (10 ml.). The mixture was left overnight at room temperature, then worked up in the usual way to yield deoxyherqueinone diacetate, m.p. 170-71°, undepressed on admixture with the material obtained in (i) and (ii) above.

Atrovenetin Triacetate.[92] (i) As described in the literature.[92]

(ii) Atrovenetin (305 mg.) was left overnight at room temperature with acetic anhydride (4 ml.) and pyridine (2 ml.). Working-up in the usual way yielded atrovenetin triacetate as yellow prisms, m.p. 185-87°(from methanol), undepressed on admixture with an authentic specimen of the same m.p. obtained by method (i). $[\alpha]_D$ +73° (c, 0.70).

(iii) Norherqueinone (1 gm.) was dissolved in pyridine (30 ml.) and glacial acetic acid (80 ml.), and the solution was shaken for fifteen minutes at room temperature with

zinc dust (8 gm.). The reaction was followed by observing the falling-off of the absorption band at 320 mμ. The The reaction mixture was then filtered, poured into water, and extracted with chloroform. The chloroform extract was repeatedly washed with water, and evaporated to yield a sticky red solid. When this was washed with acetone, an orange-red amorphous material (942 mg.) was obtained. This material (which could also be obtained by similar treatment of atrovenetin) was a nitrogen-containing complex. It was acetylated, without further purification, by treatment with acetic anhydride and pyridine overnight at room temperature, to yield atrovenetin triacetate as orange prisms, mp. 185-87° (from methanol), undepressed on admixture with an authentic specimen. $[\alpha]_D$ +70° (c, 0.57), ν_{max}, 1777, 1633, 1597 $cm.^{-1}$ X-ray powder diffraction photographs of this material and of an authentic specimen were identical, as were their Infrared and ultraviolet spectra. (Infrared spectra were taken in chloroform solution.) (Found: C, 63.77; H, 5.38; CH_3CO, 27.38. Calculated for $C_{25}H_{24}O_9$ C, 64.10; H, 5.16; $3CH_3CO$, 27.57%).

Attempted Hydrogenation of Xanthoherquein Tetramethyl Ether.

Xanthoherquein tetramethyl ether (10.6 mg.) was dissolved in ethanol (8 ml.), and hydrogenated at atmospheric pressure, using platinum oxide (6.6mg.) as catalyst. After 52 hours, the uptake of hydrogen was only 0.2 molecules. Working-up afforded unchanged starting material, identified by m.p. and mixed m.p.

Nitrococussic Acid (V). (i) To xanthoherquein (120 mg.) concentrated nitric acid (5 ml.) was added, and the mixture was heated on the steam-bath for one hour. The reaction mixture was poured into water, and repeatedly extracted with ether. Evaporation of the ether extract gave rosettes of pale yellow needles. These were dissolved in 10% ether-benzene and chromatographed on silica (3 gm.) to yield, after recrystallisation from benzene and light petroleum, nitrococussic acid, m.p. 179-180° dec. (sealed capillary), λ_{max} 380-83 mμ (log ε, 3.91), ν_{max} 3480, 1720, 1617, 1593 $cm.^{-1}$ (Found: C, 33.20; H, 2.00; N, 14.32. Calculated for $C_8H_5N_3O_9$: C, 33.46; H, 1.76; N, 14.64.) The material effervesced with sodium bicarbonate, and gave a red-brown colouration with ferric chloride. It was fairly soluble in water, from which it crystallised as square plates on long standing.

(ii) Norxanthoherquein (235 mg.) was heated on the steam-bath for 70 minutes with concentrated nitric acid (10 ml.). The reaction mixture was worked up in the usual way, and the product chromatographed on silica (4 gm.), using 10% ether-benzene to yield very pale yellow needles (68 mg.). These were recrystallised from dioxan and cyclohexane to a constant m.p. of 180-181° dec. (sealed capillary), undepressed on admixture with an authentic sample of nitrococussic acid.

Dimethyl Nitrococussate. (i) Xanthoherquein (112 mg.) was heated on the steam-bath for ten minutes with concentrated nitric acid (5 ml.). The product was worked up in the usual way, then treated with excess ethereal diazomethane for five minutes. The yellow needles so obtained were dissolved in light petroleum and filtered through a short column of neutral alumina (2 gm.) to yield, after recrystallisation from aqueous methanol, dimethyl nitrococussate (24 mg.) as white needles, m.p. 135-36°, λ_{max} 298-301 mμ (log ε, 3.24), ν_{max} 1740, 1593 $cm.^{-1}$ (Found: C, 38.11; H, 3.31; N, 13.00; OMe, 19.27. $C_{10}H_9O_9N_3$ requires C, 38.10; H, 2.88; N, 13.32; 2 OMe, 19.69.)

(ii) Atrovenetin (103 mg.) was heated on the steam-bath for two hours with concentrated nitric acid (5 ml.). Working-up in the usual way gave an oily product, from which a partially crystalline material was obtained by brief treatment with ethereal diazomethane. The methyl derivative was dissolved in light petroleum and filtered through a short column of neutral alumina (2 gm.) to yield, after crystallisation from methanol, dimethyl nitrococussate, as white needles, m.p. 132-35°, undepressed when mixed with an authentic specimen, m.p. 133-35°.

2:4:6-Trinitro-m-Cresol (VI).[115] (i) By direct nitration. m-Cresol (1 gm.) was heated on the steam-bath for 1.5 hours with concentrated nitric acid (50 ml.). Working-up in the usual way afforded, after recrystallisation from ether, pale yellow crystals of 2:4:6-trinitro-m-cresol, m.p. 106-107°, λ_{max} 350-52 mμ (log ε, 4.02), ν_{max} (KCl disc), 3170-3280, 1625, 1593 $cm.^{-1}$ (Found: C, 34.68; H, 2.18; N, 17.27. Calculated for $C_7H_5O_7N_3$: C, 34.58; H, 2.07; N, 17.28%.)

Trinitro-m-cresol methyl ether was prepared by treating trinitro-m-cresol with ethereal diazomethane for about

ten minutes. The methyl ether was crystallised from methanol to a constant m.p. of 89-90°, λ_{max} 241-42 mμ (log ε, 4.22). (Found: N, 16.06. Calculated for $C_8H_7O_7N_3$: N, 16.34%.)

(ii) By decarboxylation of nitrococussic acid. This has been described by Liebermann and van Dorp[101], but these workers have not left full experimental details. In the present work, the following procedure was employed. Nitrococussic acid (54 mg.) was heated for five hours at 170° in a sealed tube with water (5 ml.) containing a little dissolved sodium acetate (5 mg.). The reaction mixture was poured into water, and the product isolated by extraction with ether. Chromatography on silica (2 gm.) using benzene as eluent then afforded 2:4:6-trinitro-m-cresol (19 mg.), m.p. 104-106°, undepressed on admixture with an authentic specimen prepared as described in (i). The ultraviolet and infrared (KCl discs) spectra of the sample obtained by decarboxylation were identical with those of the authentic sample.

Methylation with diazomethane afforded the methyl derivative, which was shown by m.p. and mixed m.p. to be

the methyl ether of 2:4:6-trinitro-m-cresol.

Anhydride A. (XXV, R = R'= H).(i) Atrovenetin (100 mg.) was dissolved in boiling ethanol (100 ml.). The solution was cooled to 0°, and sodium hydroxide (4N, 18 ml.) was added, followed by hydrogen peroxide (30%, 18 ml.), then water (20 ml.). The solution was left at 0° for five minutes, then excess peroxide was destroyed by the addition of a little Adams' catalyst. The solution was acidified (pH 3-4) with hydrochloric acid, poured into saturated ammonium chloride solution, and thoroughly extracted with ether. Evaporation of the ether under reduced pressure gave a red oil. This was chromatographed on a column of cellulose (35 cm. × 4 cm.) using as solvent the upper (organic) layer of the system obtained by shaking together n-butanol (1), benzene (49), and an aqueous solution of ammonium carbonate and ammonium hydroxide, 1.5N with respect to each (50). Those fractions which fluoresced blue under ultraviolet light were combined (450 ml.) and evaporated to yield anhydride A (37 mg.) as fine needles, faintly discoloured pink: purification was effected by crystallisation from benzene and light petroleum and from chloroform and light petroleum

to a constant m.p. of 255-56°(with sublimation). $[\alpha]_D$ +76° (c, 0.173), $\lambda\lambda_{max}$ 256, 297-98, 360-61 mμ (log ε, 4.47, 3.94, 4.12 respectively). (Found: C, 65.89; H, 4.99. $C_{18}H_{16}O_6$ requires C, 65.85; H, 4.91%.) The compound gave a deep brown-red ferric chloride colour and in solution it fluoresced bright blue under ultraviolet light. It did not, however, react with sodium bicarbonate, and it gave a negative reaction in the Thomas test for catechols.[116]

Treatment of anhydride A with acetic anhydride and pyridine at room temperature overnight afforded anhydride A diacetate, which crystallised from ethyl acetate and light petroleum as very pale yellow hexagonal plates, m.p. 176-181°, $\lambda\lambda_{max}$ 264, 377-78 mμ (log ε, 4.59, 4.07 respectively). (Found: C, 64.37; H, 4.86; CH_3CO, 20.59. $C_{22}H_{20}O_8$ requires C, 64.07; H, 4.89; $2CH_3CO$, 20.87%.) The diacetate gave a negative ferric chloride test, but like anhydride A itself, its solutions exhibited a blue fluorescence under ultraviolet light.

(ii) Herqueinone (292 mg.) was dissolved in pyridine (5 ml.) and acetic acid (25 ml.) and after the addition of zinc dust (3 gm.) the mixture was shaken for twenty minutes.

The zinc was then removed by filtration, the mixture acidified with 6N sulphuric acid, and the precipitated zinc salts filtered off. The filtrate was heated on the steam-bath, and a solution of chromium trioxide (590 mg.) in aqueous acetic acid (85%, 23 ml.) was added dropwise over thirty minutes. Heating was continued for a further 1.5 hours. Excess oxidant was reduced by the addition of sodium metabisulphite solution, and the mixture was poured into water, and acidified with dilute hydrochloric acid. The product was isolated by extraction with chloroform. Chromatography on silica, followed by crystallisation from chloroform and light petroleum, gave anhydride A, m.p. 250-252° (undepressed when mixed with an authentic specimen), $[\alpha]_D$ +72° (c, 0.18). The ultraviolet and infrared spectra of the material were superposable on those of an authentic specimen.

(iii) Anhydride B (36 mg.) was added to refluxing pyridine hydrochloride (ca. 1 gm.), and refluxing was continued for a further four minutes. After cooling, the reaction mixture was suspended in water, and the product was isolated by extraction with chloroform. The crystalline solid obtained by evaporation of the chloroform was dissolved

in benzene and filtered through a short column of silica (6 gm.), to give, after crystallisation from chloroform and light petroleum, anhydride A, as white needles, m.p. 254-55°, undepressed on admixture with an authentic sample of the same m.p. $[\alpha]_D$ +73° (c, 0.505). The ultraviolet and infrared spectra of this material were identical with those of the authentic sample.

(iv) Anhydride C (31 mg.) was added to boiling pyridine hydrochloride (1 gm.), and the mixture refluxed for a further three minutes. Working-up in the usual way gave anhydride A (16 mg.) as white needles, m.p. 255-56°, undepressed on admixture with an authentic sample of m.p. 254-55°. $[\alpha]_D$ +78° (c, 0.563). The ultraviolet and infrared spectra of this material were identical with those of an authentic sample.

Anhydride B.[92] This was prepared as described by Neill and Raistrick[92], except that the material was purified by chromatography. In this way, anhydride B was obtained as very pale yellow needles (from chloroform and light petroleum), of m.p. 269-279°, undepressed when mixed with an authentic sample, of m.p. 260-273°. $[\alpha]_D$ +62°

(c, 0.501), $\lambda\lambda_{max}$ 255, 351, 366 mμ (log ε, 4.67, 4.17, 4.11 respectively), $\lambda\lambda_{infl}$ 290-296, 379-385 mμ (log ε, 3.87, 4.00 respectively).

Anhydride C. (i) Atrovenetin orange trimethyl ether (510 mg.) was dissolved in glacial acetic acid (3 ml.), heated on the steam-bath, and a solution of chromium trioxide (572 mg.) in aqueous acetic acid (85%, 23 ml.) was added dropwise during thirty minutes. Heating was continued for a further 1.5 hours. After working-up in the usual way, the product was chromatographed on silica (30 gm.) using 1% ether-benzene as eluent, to give, after crystallisation from chloroform and light petroleum, anhydride C (98 mg.) as very pale yellow needles, m.p. 235-36°, depressed to 212° when mixed with anhydride B. $[\alpha]_D$ +75° (c, 0.576), $\lambda\lambda_{max}$ 233-4, 258, 341, 370-71, 386 mμ (log ε, 4.26, 4.51, 4.07, 4.11, 4.07 respectively), $\lambda\lambda_{infl}$ 290-295 mμ (log ε, 3.63). (Found: C, 66.52; H, 5.49; OMe, 9.16. $C_{19}H_{18}O_6$ requires C, 66.66; H, 5.30; 1 OMe, 9.07%.) The compound gave a red-brown colour with ferric chloride, and its solution in methanol fluoresced blue under ultraviolet light.

(ii) Atrovenetin tetramethyl ether A (99 mg.) was dissolved in glacial acetic acid (2 ml.), and the solution was heated on the steam-bath. A solution of chromium trioxide (110 mg.) in aqueous acetic acid (85%, 6 ml.) was added dropwise during thirty minutes, and heating was continued for a further 1.5 hours. After working-up in the usual way, the product was chromatographed on silica (6 gm.), using 1% ether-benzene as eluent, to yield, after crystallisation from chloroform and light petroleum, anhydride C, identified by m.p. and mixed m.p. $[\alpha]_D$ +79° (c, 0.443). The ultraviolet and infrared spectra of the product were superposable on those of an authentic sample.

3:8-Dimethoxyacenaphthenonequinone (XVI).[104] 2:7-Dimethoxy-naphthalene (1.99 gm.) were stirred with carbon disulphide (15 ml.). Diphenyloxalimide chloride (4.5 gm.), prepared as described by Bauer[105], and anhydrous aluminium chloride (4 gm.) were added with cooling and stirring. The reaction mixture was stirred at room temperature for ten minutes, then refluxed, with stirring, for four hours. The solvent was evaporated under reduced pressure, and the residue

was warmed on the steam-bath for thirty minutes with 10% hydrochloric acid, in order to decompose the intermediate Schiff's base. The suspended solids were removed by filtration, and extracted with aqueous sodium bicarbonate. After washing with water, the solid residue was extracted with warm saturated sodium metabisulphite solution. Acidification of the bisulphite afforded 3:8-dimethoxy-acenaphthenequinone (624 mg.) as yellow needles, m.p. 278-79° (lit.[104] 273°), $\lambda\lambda_{max}$ 232-33, 342-43 mμ (log ε, 4.87, 4.06 respectively), ν_{max} 1710, 1620, 1600 $cm.^{-1}$

2:7-Dimethoxynaphthalic Anhydride (XV). 3:8-Dimethoxy-acenaphthenone (504 mg.) was dissolved in ethanol (50 drops) and aqueous caustic soda (4N, 25 ml.). Hydrogen peroxide (30%, 25 ml.) and water (35 ml.) were added. The solution was allowed to stand at room temperature for 30 minutes, during which time the initial yellow colour faded away. The solution was acidified with dilute hydrochloric acid, warmed gently, and stirred. The white crystalline precipitate (486 mg.) which was formed, was removed by filtration, and recrystallised from ethylene glycol to yield 2:7-dimethoxynaphthalic anhydride, m.p. 340-44°, $\lambda\lambda_{max}$ 245, 283-84, 365 mμ,

(log ε, 4.58, 3.65, 4.26 respectively). (Found: C, 65.06; H, 4.07; OMe, 23.88. $C_{14}H_{10}O_5$ requires C, 65.12; H, 3.90; 2 OMe, 24.03%.). The compound was almost insoluble in methanol, benzene, and chloroform. A suspension in methanol exhibited the familiar blue fluorescence under ultraviolet light.

Dimethyl-2:7-dimethoxynaphthalate. 2:7-Dimethoxynaphthalic anhydride (55 mg.) was dissolved in aqueous caustic soda (4N, 1.5 ml.) and water (9.5 ml.). The solution was acidified at 0° and the precipitate collected immediately. The wet precipitate was suspended in methylene chloride, and methylated with ethereal diazomethane. Isolation of the product and crystallisation from chloroform and light petroleum gave dimethyl 2:7-dimethoxynaphthalate, m.p. 128-30°, $\lambda\lambda_{max}$ 239, 308-13, 320, 334 mμ (log ε, 4.79, 3.74, 3.74, 3.74 respectively). (Found: C, 62.75; H, 5.05; OMe, 40.7. $C_{16}H_{16}O_6$ requires C, 63.15; H, 5.3; OMe, 40.8%.)

2:7-Dihydroxynaphthalic Anhydride (XIV). Pyridine hydrochloride (1200 mg.) was heated until refluxing gently.

2:7-Dimethoxynaphthalic anhydride (40 mg.) was added, and the refluxing continued for a further three minutes. The reaction mixture was cooled, suspended in water, and the product isolated by extraction with chloroform. The residue on evaporation of the chloroform under reduced pressure was recrystallised from chloroform and light petroleum to give 2:7-dihydroxynaphthalic anhydride (21 mg.) as white needles, slightly discoloured grey, m.p. 283-84° (with preliminary sintering), $\lambda\lambda_{max}$ 225, 240, 285, 335, 355, 365, 400 mμ (log ε, 4.39, 4.39, 3.73, 3.97, 4.03, 4.09, 3.33 respectively.) (Found: C, 62.40; H, 2.87; $C_{12}H_6O_5$ requires C, 62.62; H, 2.63%.) The compound gave a deep-red colour with ferric chloride, and its solution in methanol fluoresced blue under ultraviolet light.

When 2:7-dihydroxynaphthalic anhydride was dissolved in pyridine, and acetic anhydride added, 2:7-diacetoxynaphthalic anhydride crystallised from the reaction mixture as white plates, which were recrystallised from chloroform and light petroleum to a constant m.p. of 265-69°, $\lambda\lambda_{max}$ 238, 330-31, 343 mμ (log ε, 4.64, 4.09, 4.11 respectively). (Found: C, 60.85; H, 3.4; CH_3CO, 33.0. $C_{16}H_{10}O_7$ requires C, 61.15; H, 3.2; $2CH_3CO$, 27.3%). The

compound gave no colour with ferric chloride. Like all the other naphthalic anhydrides encountered in the course of the present work, its solutions exhibited a vivid blue fluorescence under ultraviolet light.

The Phthalide (XIX).[92] Atrovenetin (1 gm.) was heated on the steam-bath for ten minutes with concentrated nitric acid (5 ml.). Working-up as usual yielded a semi-crystalline product, which was recrystallised three times from ethyl acetate and ether to yield the phthalide (XIX) as very pale yellow needles, of m.p. 230-32° dec., $[\alpha]_D$ = +469° (c, 0.061, in ethanol), $\lambda\lambda_{max}$ 263, 344-45 mμ (log ε, 4.07, 4.09 respectively). (Found: C, 47.3; H, 4.35; N, 7.3. Calculated for $C_{15}H_{14}N_2O_9.H_2O$: C, 46.9; H, 4.15; N, 7.3%.) The compound gave no colour with ferric chloride, and did not react with saturated sodium bicarbonate.

Treatment of a solution of the phthalide (XIX) in a mixture of methanol and ether with an ethereal solution of diazomethane results in the formation of the methyl derivative[92] of the phthalide, which crystallises from the reaction mixture within five minutes. It can be crystallised from methanol as cream needles, m.p. 241-42° dec., $[\alpha]_D$ +343° (c, 0.545), λ_{max} 309 mμ (log ε, 3.26),

λ_{infl} 240 mμ (log ε, 3.91), ν_{max} 1780, 1740, 1603 $cm.^{-1}$ (Found: C, 50.72; H, 4.20; N, 7.09; OMe, 7.51; mol. wt. [ebullioscopic in benzene], 370. Calculated for $C_{16}H_{16}N_2O_9$: C, 50.53; H, 4.24; N, 7.37; 1 OMe, 8.15; mol. wt., 380.) The compound gave no colour with ferric chloride. When the methyl derivative (5.01 mg.) was hydrogenated in ethyl acetate solution (10 ml.), using Adams' catalyst, uptake of hydrogen was complete after ten minutes, with the absorption of 6.2 molecules.

Base Treatment of the Phthalide (XIX). The phthalide (104 mg.) in ethanol (5 ml.) was added to water (5 ml.) containing caustic soda solution (4N, 30 drops). After ten minutes, the solution was acidified (hydrochloric acid) and the product was isolated by extraction with ether. The yellow gum obtained on evaporation of the ether was methylated, in ethyl acetate solution, with excess ethereal diazomethane. Evaporation and crystallisation from dry ether gave the methyl ester methyl ether (XXI) (51 mg.) as almost colourless square plates, m.p. 125-30°, $[\alpha]_D$ -89°(c, 1.08), λ_{max} 295 mμ (log ε, 3.23). (Found: C, 49.75; H, 5.0; N, 6.65; OMe, 15.05. $C_{17}H_{20}O_{10}N_2$

requires C, 49.5; H, 4.9; N, 6.8; 2 OMe, 15.05%.)

Nitric Acid Oxidation of Anhydrides B and C. (a) Anhydride B (22.7 mg.) and anhydride C (20.8 mg.) were each heated for one hour with concentrated nitric acid (1 ml.) on the steam-bath. After isolation of the products in the usual way, the yellow gums so obtained were examined by ascending paper partition chromatography using the upper (organic) layer of the system n-butanol - acetic acid - water (4:1:5). The section of the chromatograms in the region of R_f 0.4 (that of nitrococussic acid) was cut into strips and eluted with boiling ethanol. With an appropriate blank, and using the optical density at 380 mμ, it was found that anhydride C gave more than 200 times as much nitrococussic acid as did anhydride B.

(b) Anhydride C (123 mg.) was heated on the steam-bath with concentrated nitric acid (6 ml.) for one hour. The gum obtained by isolation in the usual way was added in isopropanol-ammonia (0.15N) (4:1) to a column of cellulose powder (21 cm. × 2.5 cm.) previously washed with ethanol and with isopropanol-ammonia. The crystalline

fractions were combined and recrystallised from chloroform and light petroleum to give nitrococussic acid, identified by m.p., mixed m.p., ultraviolet and infrared spectra.

(c) Anhydride B (160 mg.) was oxidised on the steam-bath in concentrated nitric acid (5 ml.) for one hour. Isolation of the product gave a gum which crystallised on trituration with methanol. Crystallisation from chloroform and light petroleum gave the anhydride (XXXI) (60 mg.) as white needles, m.p. 215-225° (gas evolution), $[\alpha]_D$ +161° (c, 0.29), $\lambda\lambda_{max}$ 231, 321 mμ (log ε, 4.50 and 3.74 respectively.) (Found: C, 52.9; H, 4.45; N, 3.3; OMe, 7.2%. $C_{18}H_{17}O_{16}N$ requires C, 53.05; H, 4.2; N, 3.45; 1 OMe, 7.6%.)

Methylation of Deoxyherqueinone. Deoxyherqueinone (153 mg.) was treated in methylene dichloride with an excess of diazomethane in methylene dichloride solution and set aside for twenty hours. The red oil obtained on evaporation of the solvent was chromatographed on silica to give, after crystallisation from chloroform and light petroleum, atrovenetin orange trimethyl ether, m.p. 176-

179°, identical in all respects with an authentic specimen. The m.p. was depressed on admixture with the yellow trimethyl ether (m.p. 163-169°).

Base Treatment of Atrovenetin Yellow Trimethyl Ether. Atrovenetin yellow trimethyl ether (80.1 mg.) was dissolved in anhydrous potassium tert.-butoxide solution (0.1N in tert.-butanol, 15 ml.), and the solution was refluxed for two hours in an atmosphere of nitrogen. The trimethyl ether was recovered, and recrystallised from methanol. $[\alpha]_D$ +97° (c, 1.78). The authentic yellow trimethyl ether had $[\alpha]_D$ +99° (c, 0.958).

Treatment of Herqueinone with Hydriodic Acid. Herqueinone (5 mg.) was dissolved in glacial acetic acid (6.5 ml.) at 55-60° (water-bath), and hydriodic acid (55% aq., 1 ml.) was added. The solution was maintained at 55-60° for 3.5 hours. Free iodine was removed by the addition of sodium thiosulphate solution. The reaction mixture was poured into water, and extracted with chloroform. Evaporation of the chloroform extract and crystallisation of the residue from chloroform and light petroleum gave red needles

of unchanged herqueinone, identified by m.p. and mixed m.p.

Isomerisation of Herqueinone.

1.) In Alcohol. (i) Herqueinone (19.4 mg.) was dissolved in ethanol (25 ml.), and the solution was refluxed. After approximately 2.5 hours' refluxing, the specific rotation of the solution had fallen from +440° to ca. 0°. No further change in $[\alpha]_D$ was observed during a further 3 hours' refluxing. Working-up yielded a crop of short yellow needles of isoherqueinone[96], m.p. 237-238°, undepressed on admixture with authentic isoherqueinone, of m.p. 238-39, $[\alpha]_D$ 0° (c, 0.057, in ethanol).

(ii) Herqueinone (1 gm.) was dissolved in absolute alcohol (300 ml.). The solution was filtered, and sodium acetate (58 mg.) was added. After refluxing the solution for thirty minutes, the specific rotation of an aliquot, suitably diluted, was zero. Working-up yielded isoherqueinone, identical in all respects with an authentic specimen.

2.) In Dioxan. (i) Pure, dry dioxan. No change

in specific rotation was observed when a solution of herqueinone (6.23 mg.) in redistilled dioxan (10 ml.) was refluxed for 1.5 hours, and then left at room temperature for 3 hours.

(ii) Aqueous dioxan. No significant change in specific rotation was observed when a solution of herqueinone (6.07 mg.) in redistilled dioxan (10 ml.) was added to water (0.8 ml.) and refluxed for two hours.

(iii) Aqueous dioxan, containing dissolved sodium acetate. Herqueinone (5.9 mg.) was dissolved in dioxan (10 ml.), and this solution was mixed with a solution of sodium acetate (1 mg.) in water (0.8 mg.). After ten minutes' reflux, the specific rotation had fallen off to zero. No further change in $[\alpha]_D$ was caused by refluxing for a further 1.5 hours.

3.) In Acetone. No significant change in specific rotation was observed when a solution of herqueinone (2.06 mg.) in acetone (5 ml.) was refluxed for eight hours.

Dihydroherqueinone.[95,97] Hydrogenation of recrystallised herqueinone (188 mg.) in ethyl acetate solution (15 ml.)

using 10% palladised charcoal (345 mg.) as catalyst, resulted in the uptake of one molecule of hydrogen within an hour. Working-up yielded dihydroherqueinone (101 mg.), which crystallised from ether and benzene as yellow needles, becoming opaque at 243°, and completely liquid, with decomposition, at 270°. (Found: OMe, 8.23; C-Me, 8.62. Calculated for $C_{20}H_{22}O_7$: OMe, 8.28; 2 C-Me, 8.02%.)

Treatment of dihydroherqueinone with diazomethane in methylene dichloride solution for two hours at 0° gave the methyl derivative[97] of dihydroherqueinone as yellow needles, which crystallised from chloroform and light petroleum. M.p. 171-171.5° (lit.[97], 179-180°), ν_{max} 1667, 1620, 1567 $cm.^{-1}$ (Found: C, 65.2; H, 6.15; OMe, 15.75. Calculated for $C_{21}H_{24}O_7$: C, 64.95; H, 6.2; OMe, 16.0%.)

Base Treatment of Dihydroherqueinone. Dihydroherqueinone (5.1 mg.) was warmed on the steam-bath for ten minutes with caustic soda solution (N, 1 ml.). The amber-coloured solution was acidified with a few drops of 6N hydrochloric acid, to precipitate a yellow solid. This was separated on the centrifuge, washed with water, and dried. From

its ultraviolet spectrum, it was concluded that the material was unchanged dihydroherqueinone.

Perchloric Acid Treatment of Dihydroherqueinone.

(i) Dihydroherqueinone (4.2 mg.) was dissolved in perchloric acid and acetic acid (10 ml. of a mixture prepared from 2 ml. 72% aq. perchloric acid and 48 ml. glacial acetic acid). After ten minutes, the ultraviolet spectrum of the solution (as measured for an aliquot, suitably diluted) was profoundly changed. In place of the maximum at 289 mμ (log ε, 4.16) in dihydroherqueinone, there was an inflexion at 280 mμ (log ε, 3.71): the new spectrum also possessed the plateau of heavy absorption (log ε ca. 4.0) in the region 400-430 mμ, which is characteristic of polyhydroxyperinaphthenones (cf. bromoxanthoherquein, λλ 400-430 mμ, log ε 4.20-4.23). No further spectral change was observed when the solution was left for a further 1.5 hours at room temperature.

(ii) Dihydroherqueinone (31 mg.) was dissolved in perchloric acid and acetic acid (10 ml. of the solution described in parenthesis in (i)), and left at room temperature for ten minutes. The reaction mixture was poured

into water, and the product isolated by extraction with ethyl acetate. The gum thus obtained was treated overnight with pyridine (1 ml.) and acetic anhydride (0.5 ml.). Working-up in the usual way afforded a yellow gum, which could not be induced to crystallise, even after chromatography on silica. The acetylated material had the following ultraviolet absorption spectrum: $\lambda\lambda_{max}$ 370, 420, 445 mμ (log ε, 3.77, 3.60, 3.51 respectively), λ_{infl} 230-240 mμ (log ε, 4.14), λ_{min} 295 mμ (log ε, 3.15). This bears a very close resemblance to the spectra of the various acetylated polyhydroxyperinaphthenones examined. E.g. xanthoherquein tetra-acetate has the following ultraviolet spectrum: $\lambda\lambda_{max}$ 240, 267, 375, 420, 445 mμ (log ε, 4.39, 4.00, 4.28, 4.03, 3.97 resp.), λ_{min} 298 mμ (log ε, 2.93).

The Acid (XXXVIII). Atrovenetin yellow trimethyl ether (507 mg.) was oxidised with a saturated solution of potassium permanganate in acetone in the manner described by Neill and Raistrick[92]. The product (16 mg.) crystallised from methanol as yellow needles, m.p. 290-293°, $[\alpha]_D$ +80° (c, 0.464), ν_{max} 2650, 1747, 1610, 1590 $cm.^{-1}$ The material gave a red-brown colour with ferric chloride.

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ULTRAVIOLET SPECTRA OF HYDROXYPERINAPHTHENONES.

	$\lambda\lambda_{max}$ (mμ). Intensity (log ε) in parentheses.						λ_{min} (mμ) log ε in parenthesis.
9-Hydroxyperinaphthenone.	235 (4.38)	260 (3.92)	352 (4.26)	395(infl.) (3.68)	415 (3.96)	438 (4.05)	280 (2.97)
Deoxynorherqueinone triacetate.	247(infl.) (4.28)	276 (4.19)	348-51 (4.07)	390(infl.) (4.00)	407-10 (4.18)	433 (4.27)	298 (3.25)
Atrovenetin triacetate.	247(infl.) (4.23)	275 (4.23)	347-50 (4.08)	390(infl.) (4.01)	409 (4.20)	433 (4.29)	298 (2.93)
Deoxyherqueinone diacetate.	ca.250(infl.) (4.27)	278 (4.10)	358 (4.20)	389 (4.09)	410 (4.18)	433 (4.23)	294 (2.54)
Xanthoherquein tetra-acetate	240 (4.39)	267 (4.00)	375 (4.28)		420 (4.03)	445 (3.97)	292 (3.08)
norXanthoherquein penta-acetate.	242 (4.44)	266(infl.) (4.12)	370 (4.28)		415 (4.03)	440 (4.03)	298 (2.93)
Perinaphthenone.		248 (4.34)	315 (3.58)	360 (4.02)	384 (3.95)		275 (2.95)
Atrovenetin.	222 (4.52)	250-60(infl.) (4.26)			385 (4.21)	410-20 (4.10)	310 (2.74)
Deoxyherqueinone.		250-65(infl.) (4.26)			385 (4.22)	405 (4.27)	300 (3.17)
Xanthoherquein.	216 (4.57)	255(infl.) (4.19)			395 (4.19)	410(infl.) (4.17)	305 (3.22)
$LiAlH_4$ reduction product YA.	220 (4.31)	239 (4.36)	272 (4.40)	340 (3.96)		422 (4.14)	300 (2.90)
$LiAlH_4$ reduction product OA.	220 (4.48)		275 (4.56)	342 (3.97)		438 (4.27) 462 (4.25)	295 (3.00)

TABLE II.

INFRARED ABSORPTION SPECTRA OF PERINAPHTHENONES.

Compound	Bands ($cm.^{-1}$) (Nujol)		
Perinaphthenone.	1637	(1620)	1582
9-Hydroxyperinaphthenone.		1625	1585
Xanthoherquein.			1600 (broad)
Atrovenetin.		1620	1570
Atrovenetin orange trimethyl ether.		1613	1557
Atrovenetin yellow trimethyl ether.		1607	1590
Atrovenetin tetramethyl ether A.		1630	1577
Atrovenetin tetramethyl ether B.		1613	1600
$LiAlH_4$ reduction product OA.		1630	1582
$LiAlH_4$ reduction product YA.		1620	1577

--ooOoo--

TABLE III.

INFRARED SPECTRA OF NAPHTHALIC ANHYDRIDES.

Compound	Bands ($cm.^{-1}$) (Nujol)		
Naphthalic anhydride.	1765	1735	
2:7-Diacetoxynaphthalic anhydride.	1760 (broad)	1720	
2:7-Dimethoxynaphthalic anhydride (XV).	1750	1720	
*2:7-Dihydroxynaphthalic anhydride (XIV).		1720	1685
Anhydride A (XXV; R=R'=H).		1703	1660
Anhydride A diacetate (XXV; R=R'=CH_3CO).	(1773) 1750	1720	
Anhydride B (XXV; R=Me, R'=H).	1750		1663
Anhydride C (XXV; R=H, R'=Me).	1747		1670

--ooOoo--

* Also measured in dilute solution in chloroform in order to establish that the frequency shift of the carbonyl bands was not due to intermolecular hydrogen-bonding.

PART II.

THE CONSTITUTION OF ANISOXIDE.

Anisoxide is a constituent of star aniseed oil (from Illicium verum), where it occurs to the extent of about 0.2%. It was first isolated by Jackson and Short by protracted fractionation of the oil[117].

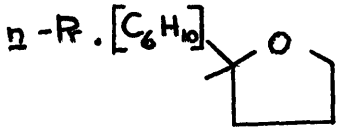
These workers established the empirical formula of the compound as $C_{14}H_{18}O$. Since it contains no methoxyl or ethoxyl groups, and is inert to all the usual reagents for alcohols and carbonyl compounds, they concluded that the oxygen atom was present in an ether ring.

Hydrogenation of anisoxide under a pressure of 40 lb./sq. inch in acetic acid solution, using platinum oxide as catalyst, resulted in the uptake of four moles of hydrogen, and the formation of perhydroanisoxide, $C_{14}H_{26}O$. This established that anisoxide is bicyclic.

Perhydroanisoxide was stable to treatment with phenylmagnesium bromide at 100°, thus rendering it unlikely that a 1:2 or 1:3 oxide ring is present in the molecule.[118,119] The oxide ring in perhydroanisoxide was opened by heating with hydrogen bromide. The unstable dibromide, $C_{14}H_{26}Br_2$,

thus obtained gave an unsaturated hydrocarbon, $C_{14}H_{24}$, when digested with aniline. Ozonolysis of the hydrocarbon afforded a ketone, $C_{11}H_{20}O$. The ketone gave a positive iodoform test, thus indicating that in perhydroanisoxide a C-Me group is attached to the cyclic ether.

To account for the above series of results, Jackson and Short advanced partial structure (I) for perhydroanisoxide.[117]



I

Kuhn-Roth oxidation of anisoxide showed the presence of two C-methyl groups. The experiments already described established that one of these is attached to the ether ring. The other one was shown to be present in an ethylidene group by the ozonolysis of anisoxide to give acetaldehyde. The double bond of the ethylidene group could be smoothly reduced with sodium and ethanol to yield dihydroanisoxide: it could therefore be presumed to be conjugated.

Further evidence for a conjugated system of double bonds was provided by the formation of an addition compound of anisoxide and maleic anhydride.

Potassium permanganate oxidation of anisoxide gave three important degradation products. The first product was an acid, $C_{12}H_{14}O_3$, (acid A), produced by oxidation of the ethylidene group. Further oxidation of acid A gave acid B, to which the formula $C_{11}H_{12}O_3$ was attributed[117], and further oxidation of this compound gave acid C, $C_{11}H_{10}O_4$.

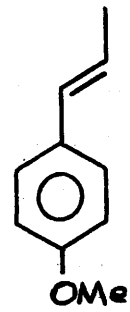
One further compound obtained by Jackson and Short may be mentioned. Oxidation of dihydroanisoxide with potassium permanganate or ozone gave a ketone, to which the formula $C_{14}H_{18}O_2$ was assigned. This compound was characterised as its semicarbazone, m.p. 191.5-192.5°.

Recently, the constitution of anisoxide has been elucidated by Barton and Bhati at the University of Glasgow, on the basis of the following evidence.[120]

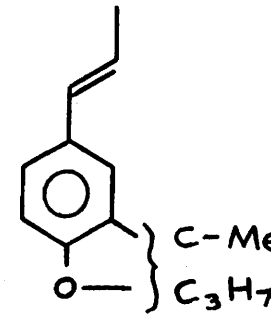
The ultraviolet spectrum of anisoxide, with absorption bands at 265 and 302 mμ (ε, 18,740 and 3,700 respectively), suggested a close structural relationship to anethole (II), which has ultraviolet absorption bands at 258 and 300 mμ (ε, 14,000 and 1,000 respectively).[121] A direct comparison of the ultraviolet spectra of dihydroanisoxide ($\lambda\lambda_{max}$ 231 and 286 mμ, ε 6,700 and 3,200 respectively)

and dihydroanethole ($\lambda\lambda_{max}$ 224, 278, and 284 mμ, ε 9,500, 1,800, and 1,500) confirmed this.

The infrared spectrum of anisoxide exhibited bands at 741 $cm.^{-1}$, 784 $cm.^{-1}$, 826 $cm.^{-1}$, 852 $cm.^{-1}$, and 1100$cm.^{-1}$ which suggested a 1:3:4-trisubstituted benzene ring. This would imply partial structure (III) for anisoxide.



II



III

On the basis of such a structure, however, it is extremely difficult to account for the loss of one carbon atom during the formation of acid B from anisoxide. Accordingly, the oxidation of anisoxide with potassium permanganate was reinvestigated.

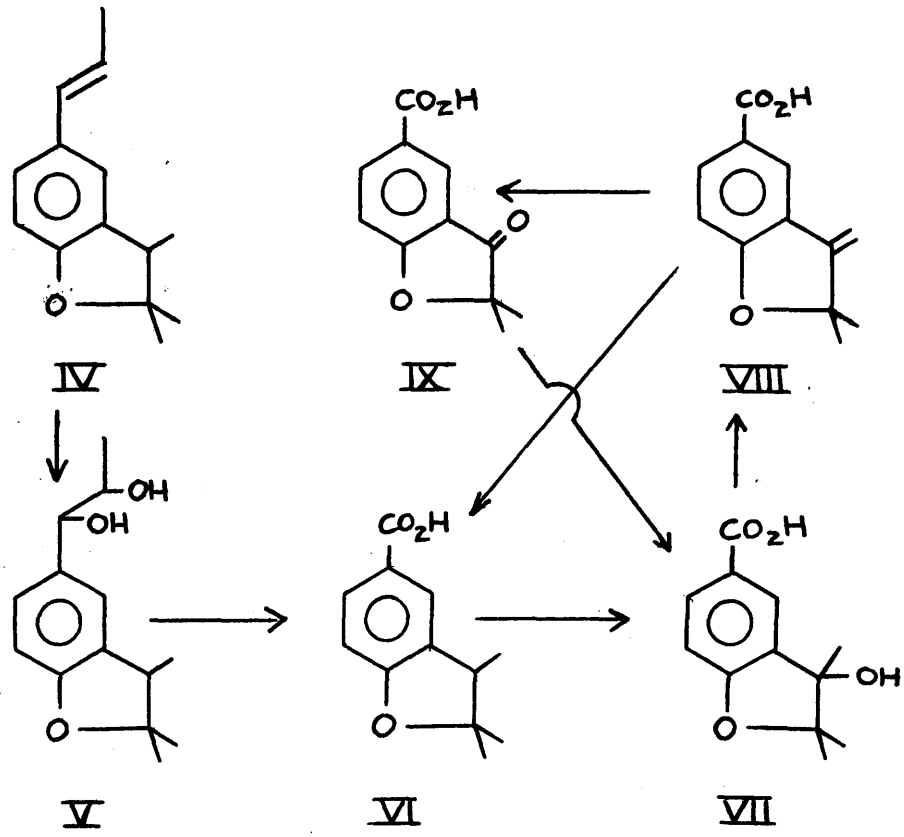
When the oxidation was carried out in pyridine solution at 0° the first product obtained was not acid A, but a neutral glycol, $C_{14}H_{20}O_3$, presumably formed by the hydroxylation of the styrenoid double bond. Further

oxidation of the glycol gave acid **A**. This was found to have λ_{max} 264 mμ (ε, 12,000), which is fully in accord with the carboxyl group being formed by oxidation of the ethylidene side-chain. Its infrared spectrum was that of an aromatic acid, and it had bands at 1388 $cm.^{-1}$ and 1374 $cm.^{-1}$, characteristic of a gem-dimethyl group.

Further oxidation of acid A with alkaline potassium permanganate afforded a hydroxy-acid, $C_{12}H_{14}O_4$, and not acid B. It was found that the hydroxy-acid was readily dehydrated with warm acetic acid to yield acid B. Since the acid B obtained by Jackson and Short[117] was re-crystallised from acetic acid, it is probable that the hydroxy-acid initially formed was dehydrated during purification.

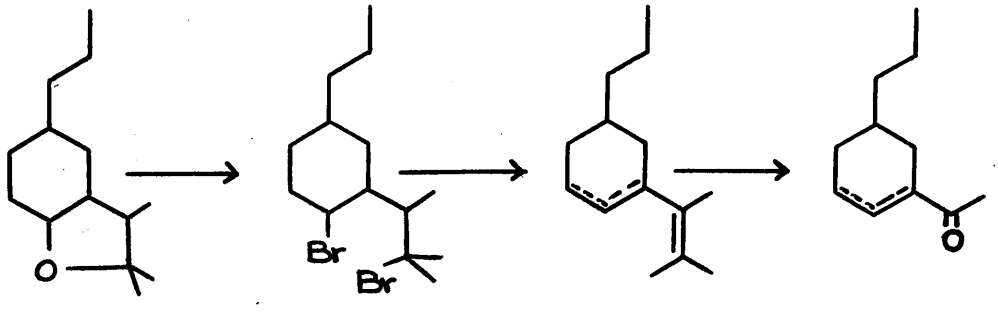
Acid B has the empirical formula, $C_{12}H_{12}O_3$, not $C_{11}H_{12}O_3$ as originally reported.[117] It has a band at 1640 $cm.^{-1}$ in its infrared spectrum, characteristic of a double bond conjugated with a benzene ring. Ozonolysis of acid B gave formaldehyde and acid C, which has the ultraviolet and infrared spectra of an acetophenone derivative.

All these data can be expressed in terms of structure (IV) for anisoxide. The glycol may then be formulated as (V), acid A as (VI), the hydroxy-acid as (VII), acid B as (VIII), and acid C as (IX). These inter-relationships were confirmed by the hydrogenation of acid B (VIII) to give back acid A (VI), and by the conversion of acid C (IX) to the hydroxy-acid (VII) by reaction with methylmagnesium iodide. Acid A (VI) has the same m.p. as a compound of the same structure synthesised by Lauer and Moe.[122]



All the experiments carried out by Jackson and Short[117] can be satisfactorily explained on the basis of structure (IV) for anisoxide. The Diels-Alder adduct with maleic anhydride, although analysing as a 1:1-adduct, has all the properties (doubtful crystallinity, very low solubility, very high melting-point) associated with the "heteropolymers" described by Hudson and Robinson[123], and probably belongs to this category.

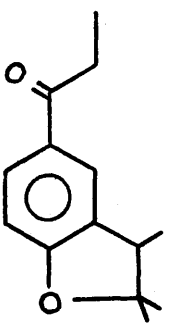
The dibromide obtained by treating perhydroanisoxide (X) with hydrogen bromide in acetic acid solution may be formulated as (XI), and the unsaturated hydrocarbon, $C_{14}H_{24}$, obtained by digesting the dibromide with aniline, may be represented as (XII). Ozonolysis affords the methyl ketone (XIII).



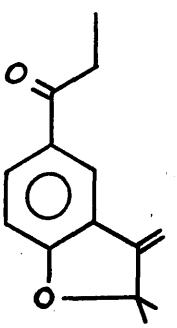
X XI XII XIII

The ketone obtained by oxidation of dihydroanisoxide

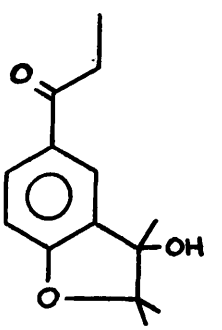
with potassium permanganate or ozone was assigned the empirical formula, $C_{14}H_{18}O_2$, by Jackson and Short.[117] If this formula were correct, then (XIV) might be considered a satisfactory structure for this compound. However, a compound possessing structure (XIV) has now been synthesised (see next section), and its semicarbazone has a different melting-point from that recorded in the literature for the semicarbazone of the oxidation product. It appears likely, therefore, that the ketone obtained by Jackson and Short has the empirical formula, $C_{14}H_{16}O_2$, and the structure (XV), arising probably by dehydration during working-up of the primary reaction product (XVI).



XIV



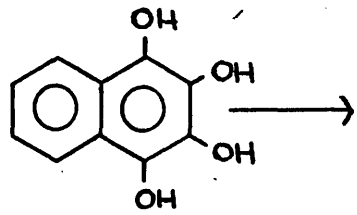
XV



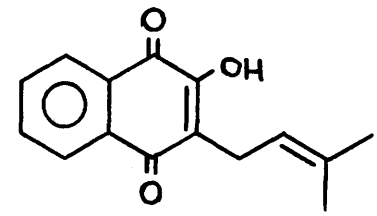
XVI

Two features of anisoxide are worthy of comment. One is the method of attachment of the isoprenoid residue.

This is unique in Nature. It has already been mentioned (see p. 34) that isoprenoid residues are in general attached to aromatic rings at C_1 or C_3 (of $\overset{1}{C} - \overset{2}{C} - \overset{3}{C} \leq \overset{4}{C} \overset{5}{C}$), corresponding to the electrophilic carbon atoms of a postulated carbonium ion $[(CH_3)_2C{=}CH{=}CH_2]^+$. Lapachol (XVIII) has, in fact, been synthesised[124] in the laboratory by reacting leucoisonaphthazarin (XVII) with isoprene, in the presence of oxalic acid, a process which presumably involves a carbonium ion of the type suggested.

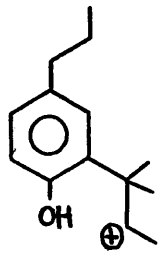


XVII



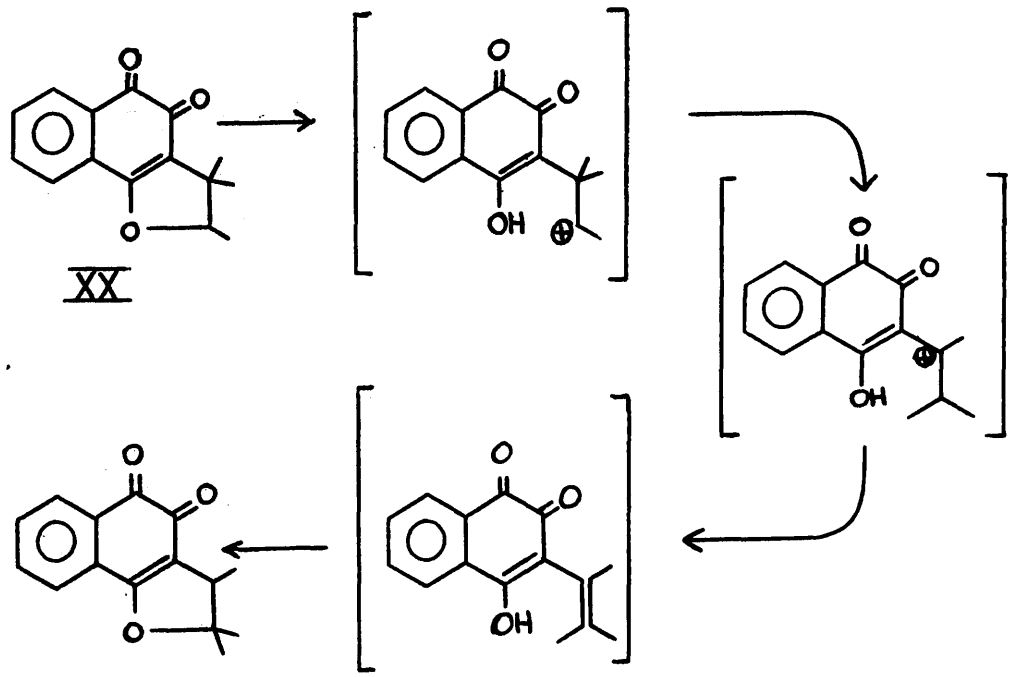
XVIII

In the case of anisoxide, however, the attachment of the isoprene unit is at C_2. If the same ionic attacking species is involved in the biogenesis, then it is necessary to assume a rearrangement of, for instance, the carbonium ion (XIX) at some stage. A rearrangement of this type is observed when dunnione (XX) is treated with hot concentrated sulphuric acid[77], the



XIX

isomer (XXI) being formed.



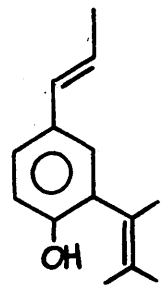
XX

XXI

It is, of course, possible that the isoprenoid residue in anisoxide arises from a different ionic precursor. The attacking species in this case might be the ion $[CH_3.CH{=}{=}C{<}^{CH_2}_{CH_3}]^+$, or its equivalent. This could arise by protonation of isoprene, which could itself be formed from mevalonic acid by decarboxylation, followed by dehydration.

The other interesting point about anisoxide is that, although it possesses an asymmetric centre, it is

optically inactive. This may be due to racemisation during working-up of the oil, since this involved heating with an alloy of sodium and potassium to destroy oxygenated constituents.[117] On the other hand, it is possible that anisoxide is formed by cyclisation of an unsaturated precursor (e.g. XXII), which lacks asymmetry. Anisoxide is by no means unique among natural products in lacking optical activity, while possessing an asymmetric centre. The interesting lichen product picrolichenic acid is a recently discovered example of such a compound.[43]



XXII

--ooOoo--

THE SYNTHESIS OF ANISOXIDE.

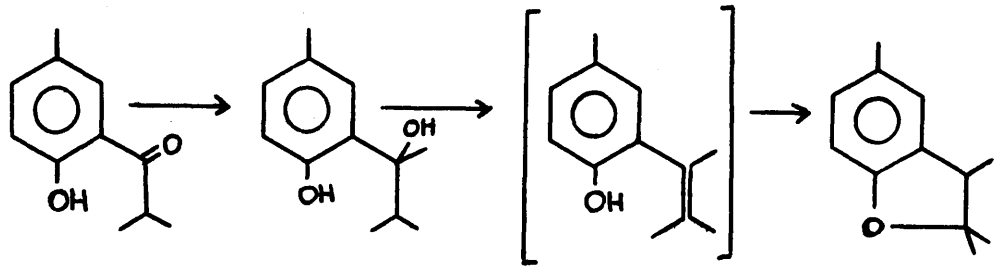
DISCUSSION.

Owing to the unique mode of attachment of the C_5 ether ring in anisoxide, it was thought desirable to synthesise the compound, and its principal degradation products.

The starting material for the synthesis was 2-hydroxy-5-methyl-*iso*butyrophenone (I). This was prepared by reacting together *p*-methyl-anisole and *iso*butyryl chloride in carbon disulphide solution, in the presence of anhydrous aluminium chloride. The liquid thus obtained was a mixture of (I) and its methyl ether, which was entirely converted into (I) by treatment with pyridine hydrochloride. The phenolic ketone was characterised by its ultraviolet and infrared spectra, and by the analysis of its 3:5-dinitrobenzoate. The ketone (I) has been prepared previously by other methods.[125,126]

2-Hydroxy-5-methyl-*iso*butyrophenone (I) was treated with methylmagnesium iodide to yield the corresponding phenolic alcohol (II), characterised by its infrared

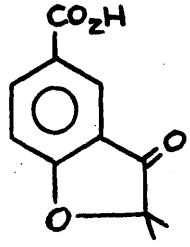
spectrum. The phenolic alcohol (II) yielded only a mono-dinitrobenzoate when the derivative was prepared by Brewster's method of benzoylation.[127] That it was the phenolic hydroxyl group which had acylated was borne out by the infrared spectrum of the 3:5-dinitrobenzoate, which had a band at 1742 $cm.^{-1}$ (nujol).



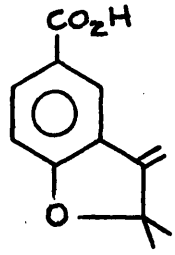
I II III

The carbinol (II) was dehydrated and cyclised smoothly, using perchloric acid as catalyst, to yield 2:2:3:5-tetramethyl-2:3-dihydrobenzofuran (III), which had the expected ultraviolet spectrum ($\lambda\lambda_{max}$ 228-30 and 287 mμ; ε, 5,720 and 3,100 respectively). Oxidation of (III) with chromium trioxide afforded anisoxide acid C (IV), identical in all respects with the material obtained by degradation of anisoxide. When (III) was oxidised with potassium permanganate, and hydrochloric

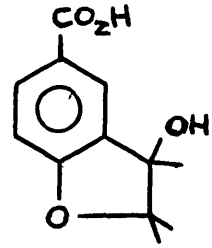
acid was employed during working-up, acid B (V), identical with the authentic degradation product of anisoxide, was obtained: its identity was further confirmed by catalytic hydrogenation to acid A (VI).



IV



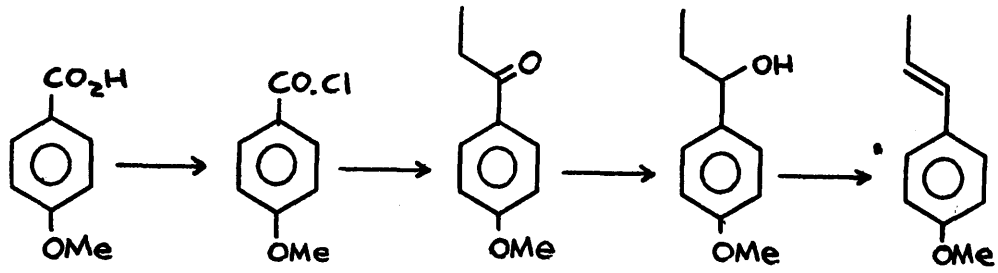
V



VI

The remainder of the synthesis comprised the elaboration of the styrenoid side-chain. The synthesis of anethole (XI) from anisic acid (VII) was carried out as a model. Anisic acid was converted to the corresponding acid chloride (VIII) and this was treated with diethylcadmium to yield p-methoxypropiophenone (IX), which was reduced to the corresponding alcohol (X). This was dehydrated by distillation from iodine to give anethole (XI), which was identical with a sample of the natural product.

However, diethylcadmium was not used in the synthesis

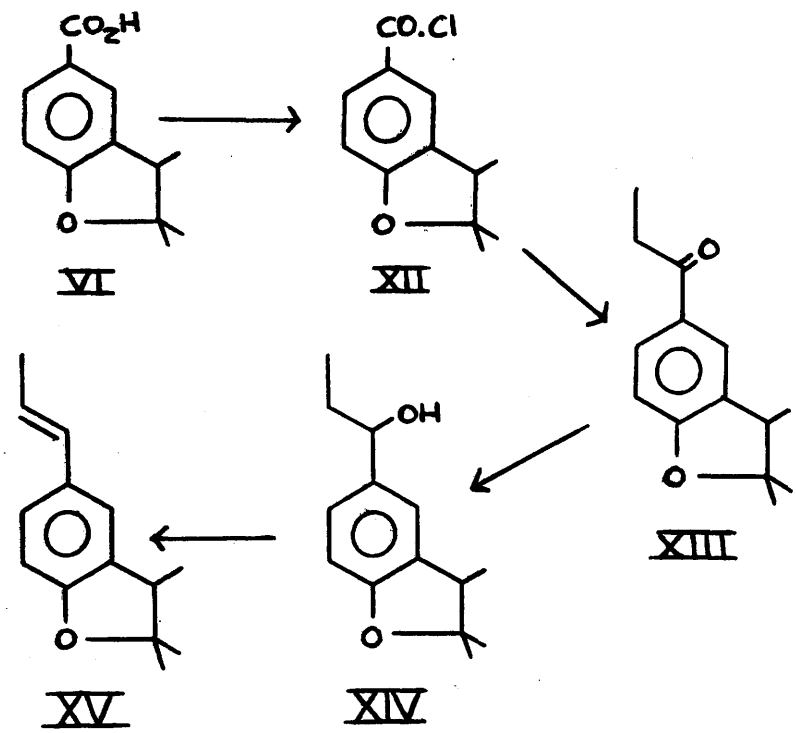


VII VIII IX X XI

of anisoxide, because the employment of this reagent in small-scale experiments was found to give rise to substantial quantities of ester impurities. Instead, acid A (VI) was converted into its acid chloride (XII). 2:2:3-Trimethyl-5-propionyl-2:3-dihydrobenzofuran (XIII) was obtained by reacting (XII) with diazomethane, and reducing the intermediate diazo-ketone with hydriodic acid, and with zinc and acetic acid.

Reduction of the ketone (XIII) with potassium borohydride led smoothly to the corresponding carbinol (XIV). Difficulties were encountered in the dehydration of this alcohol,[128] due to the great ease of polymerisation of anisoxide itself. Distillation of (XIV) from a crystal of iodine gave anisoxide (XV) in very small yield, but

hot aqueous ethanolic hydrochloric acid proved to be the reagent of choice. By taking advantage of the emergence of a band in the ultraviolet at 265 mμ, optimum conditions for the dehydration were determined. In this way, anisoxide was obtained from (XIV) in a yield of about 20%. The material thus obtained was identical in all respects with an authentic specimen of anisoxide.



--ooOoo--

EXPERIMENTAL.

Melting-points were taken on the Kofler block, unless specified to the contrary. Ultraviolet absorption spectra were determined in ethanol solution, with the Unicam SP500 Spectrophotometer. Infrared spectra were determined in liquid film, unless stated otherwise. Light petroleum refers to the fraction of boiling point 60-80. Micro-analyses were carried out by Miss J. Cuckney, and her staff.

2-Hydroxy-5-methyl-isobutyrophenone (I). isoButyryl chloride (50 gm.; 0.47 moles)[129] and methyl p-cresyl ether (60 gm.; 0.49 moles) were dissolved in carbon disulphide (600 ml.) and the solution was cooled in ice. Anhydrous aluminium chloride (70 gm.; 0.53 moles) was added in portions to the cooled solution, with stirring.

The reaction mixture was then warmed, with stirring, on a 60° water-bath for 2.5 hours, and set aside at room temperature overnight. The carbon disulphide was removed at the pump, and the mixture remaining was

hydrolysed by the addition of ice and hydrochloric acid.

An ether extract was made. This was washed successively with dilute hydrochloric acid, saturated sodium bicarbonate, and water. After drying, the ether was evaporated to leave a pale yellow liquid (49.3 gm.), b.p. 162-172° at a pressure of 40 mm. The liquid exhibited infrared absorption bands at 1640 $cm.^{-1}$ and 1680 $cm.^{-1}$ and was therefore a mixture of the desired product and its methyl ether. The mixture was fractionated, using a 30 cm. Vigreux column, and those fractions containing at least some of the free phenol (total weight, 36 gm.) were refluxed for one hour with pyridine hydrochloride (130 gm.) to demethylate the methyl ether.

Further fractionation then afforded 2-hydroxy-5-methyl-<u>iso</u>butyrophenone (25 gm.), b.p. 157-160° at a pressure of 40 mm., which was shown to be homogeneous by vapour-phase chromatography. $\lambda\lambda_{max}$ 217, 256, and 334 mμ, (ε 17,200, 11,250, and 3,610 respectively), ν_{max} 1640 $cm.^{-1}$

The <u>3:5-dinitrobenzoate</u> (prepared by the general method of Brewster and Ciotti[127]) crystallised from

benzene and light petroleum as long rods, m.p. 190-93°. (Found: C, 58.12; H, 4.57; N, 7.19. $C_{18}H_{16}N_2O_7$ requires C, 58.06; H, 4.33; N, 7.52%.)

The carbinol (II). 2-Hydroxy-5-methyl-isobutyrophenone (21 gm.) was dissolved in dry ether and added slowly, with stirring, to an ethereal solution of methylmagnesium iodide (prepared from 100 gm. of methyl iodide, 17 gm. of magnesium, and 300 ml. of dry ether). The reaction mixture was refluxed and stirred for one hour.

The complex was hydrolysed with ice-water and hydrochloric acid, and the ether extract washed successively with dilute hydrochloric acid and saturated sodium bicarbonate solution. The product was extracted with caustic soda solution, and worked up in the usual way. The yield of crude product was 12 gm. This was fractionated, using a 30 cm. Vigreux column, and the fraction boiling at 142-46° at 25 mm. pressure (10.5 gm.) was shown by vapour-phase chromatography to be homogeneous carbinol (II), ν_{max}, 3520 $cm.^{-1}$ and 1612 $cm.^{-1}$ methanol as white needles, m.p. 116-117°, ν_{max}, 3106 $cm.^{-1}$ and 1742 $cm.^{-1}$ (nujol). (Found: C, 58.52; H, 5.42; N, 7.61.

methanol as white needles, m.p. 116-117°, ν_{max}, 3106 $cm.^{-1}$ and 1742 $cm.^{-1}$ (nujol). (Found: C, 58.52; H, 5.42; N, 7.61.

$C_{19}H_{20}N_2O_7$ requires C, 58.76; H, 5.19; N, 7.21%.)

2:2:3:5-Tetramethyl -2:3-dihydrobenzofuran (III). The carbinol (II) (10.3 gm.) was added to an anhydrous 4% solution of perchloric acid in acetic acid (prepared by mixing together 10 ml. 60% aq. perchloric acid, 35.5 ml. acetic anhydride, and 105 ml. acetic acid). The reaction mixture was set aside at room temperature for 16 hours, then poured into water, and extracted with ether. The ether extract was washed successively with dilute caustic soda solution, dilute hydrochloric acid, and water. After drying, the ether was evaporated to leave a pleasant-smelling yellow liquid (10 gm.). This was fractionated and the fraction of b.p. 132-133°/25 mm. was collected to give 2:2:3:5-tetramethyl-2:3-dihydrobenzofuran (III), n_D^{28} 1.5100, $\lambda\lambda_{max}$ 228-30, and 287 mμ (ε, 5,700 and 3,100 respectively). (Found: C, 81.62; H, 9.21. $C_{12}H_{16}O$ requires C, 81.77; H, 9.15%.)

Anisoxide Acid C (IV). The dihydrobenzofuran (III) (255 mg.) was dissolved in glacial acetic acid (5 ml.), and a decinormal solution of chromic acid in acetic acid (50 ml.) was added. By titration, it was found that, after

3.5 hours, the equivalent of 6.25 atoms of oxygen had been consumed. No further uptake occurred during the following hour.

Excess chromium trioxide was reduced by the addition of sodium metabisulphite solution. The mixture was then acidified and extracted with chloroform. The chloroform extract was washed with water and evaporated to yield a small quantity of white crystals of acid C. These were recrystallised once from benzene and light petroleum, and washed with benzene. M.p. 177-80°, undepressed on admixture with the authentic degradation product of m.p. 178-80°. The synthetic specimen had ultraviolet and infrared spectra superposable on those of the authentic material. (Infrared spectra in nujol.)

Anisoxide Acid B.(V). 2:2:3:5-Tetramethyl-2:3-dihydrobenzofuran (III) (163 mg.) was heated on the steam-bath with potassium permanganate (4% aq. solution, 30 ml.), for 8.5 hours. The reaction mixture was cooled, and sulphur dioxide was bubbled through to dissolve the manganese and reduce the excess potassium permanganate. The mixture was acidified with 6N hydrochloric acid, and the white crystalline precipitate of acid B (23 mg., m.p.

209-13°) was recrystallised from chloroform and light petroleum as white needles, m.p. 218-20°, undepressed on admixture with a sample of the authentic degradation product of the same m.p. The identity of the two samples was further confirmed by their superposable ultraviolet and infrared (nujol) spectra.

Anisoxide Acid A (VI). Acid B (44mg.) was hydrogenated in acetic acid (5 ml.) in the presence of platinum oxide (18 mg.) as catalyst. After 7 minutes, 1.02 mol. of hydrogen had been consumed. The solution was filtered and the product was isolated by evaporation of the solvent. Recrystallisation from benzene afforded shiny white crystals of acid A, m.p. 180-82°, undepressed on admixture with an authentic specimen, of m.p. 180-83°. The ultraviolet and infrared (nujol) spectra of the two samples were superposable.

The methyl ester of acid A was prepared, using diazomethane. It was a colourless liquid, n_D^{22} 1.5325. (Found: C, 70.55; H, 7.4. $C_{13}H_{16}O_3$ requires C, 70.9; H, 7.3%.)

2:2:3-Trimethyl-5-propionyl-2:3-dihydrobenzofuran (XIII). Acid A (2.15 gm.) was suspended in dry benzene (6 ml.) and oxalyl chloride (4.5 ml.) was added. The mixture was set aside at room temperature overnight, by which time all the acid had gone into solution. Excess oxalyl chloride was removed by evaporation on the steam-bath under reduced pressure. The product was dissolved in dry benzene (7 ml.) and added to a solution of diazoethane (1 gm.) in ether (700 ml.). The reaction mixture was left in the refrigerator for two hours, then evaporated to dryness at room temperature under reduced pressure. The residual oil was taken up in chloroform and shaken for five minutes with hydriodic acid (60% aq. solution, 2 ml.). The chloroform solution was washed with sodium thiosulphate solution and saturated aqueous sodium bicarbonate. Evaporation of the solvent left a red oil. The oil was dissolved in glacial acetic acid, and shaken for one hour with zinc dust (10 gm.). A very viscous red oil was obtained on working-up. This exhibited infrared absorption bands at 1680 $cm.^{-1}$ (ketone conjugated with an aromatic ring) and 1715 $cm.^{-1}$ (weak), due to a small amount of acid A ethyl ester, present as

an impurity. The ester impurity was removed by dissolving the reaction product in a mixture of dioxan (80 ml.), caustic soda (N solution, 20 ml.), and water (40 ml.), and leaving at room temperature for 40 hours. A very dark-coloured tar (1.4 gm.), having only one carbonyl band in the infrared (at 1680 $cm.^{-1}$), was obtained on working-up. Distillation of this gave 2:2:3-trimethyl-5-propionyl-2:3-dihydrobenzofuran (XIII), b.p. 128-30°/0.4 mm., n_D^{29} 1.5363. (Found: C, 76.72; H, 8.42. $C_{14}H_{18}O_2$ requires C, 77.03; H, 8.31%.)

The semicarbazone, crystallised from chloroform and light petroleum, had m.p. 179-80°. (Found: N, 15.61. $C_{15}H_{21}O_2N_3$ requires N, 15.26%.)

The Alcohol (XIV). The ketone (XIII) (590 mg.) and potassium borohydride (475 mg.) were dissolved in aqueous methanol (50%, 60 ml.) and the solution was left overnight at room temperature. Most of the methanol was evaporated under reduced pressure. The product was isolated with ether and distilled to yield the alcohol (XIV). (Bath temperature 180°, pressure 0.5 mm.). n_D^{25} 1.5223, $\lambda\lambda_{max}$ 231 and 284 mμ (ε, 5,850 and 2,440).

(Found: C, 76.33; H, 9.15. $C_{14}H_{20}O_2$ requires C, 76.32; H, 8.9%).

Anisoxide. (a) The alcohol (XIV) (200 mg.) was distilled under reduced pressure from a small crystal of iodine. A red oil (23 mg.) was obtained. This was dissolved in light petroleum, and filtered through a short column of Grade III alumina (700 mg.). Sublimation under high vacuum at 40-50° then yielded a colourless oil, which, on cooling in a Drikold-methanol bath, afforded crystalline anisoxide, m.p. 34-37°, undepressed on admixture with an authentic sample of the same m.p. The identity of the two samples was confirmed by their superposable ultraviolet and infrared spectra.

(b) Optimum conditions for the dehydration of the alcohol (XIV) with aqueous ethanolic hydrochloric acid were determined spectrophotometrically as follows. The alcohol (XIV) (7.6 mg.) was dissolved in absolute alcohol, (10 ml.), and aqueous hydrochloric acid (6N, 10 drops) were added. Aliquots (0.1 ml.) were removed from time to time, diluted (to 5 ml.) with ethanol, and the optical density of the solution measured. The progress

of the dehydration was indicated by the emergence of an absorption maximum at 265 mμ. It was found that the extinction coefficient at 265 mμ reached its maximum value of 10,000 (ε_{265} for anisoxide = 18,740) after the reaction mixture had been refluxed for 20 minutes. This remained unchanged for about an hour, after which time the value of ε diminished gradually.

The dehydration was carried out on a preparative scale as follows. The alcohol (XIV) (100 mg.) was dissolved in absolute ethanol (100 ml.), and aqueous hydrochloric acid (6N, 70 drops) was added. The reaction mixture was refluxed for 20 minutes, by which time a peak of 10,500 (ε value) had appeared at 265 mμ. The intensity of this peak was unchanged after a further 10 minutes' reflux. The solution was cooled, neutralised by the addition of saturated aqueous sodium bicarbonate solution, and most of the ethyl alcohol removed by evaporation at room temperature under reduced pressure. The reaction mixture was poured into water, and extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated <u>in vacuo</u>. The residual oil (90 mg.) was dissolved in light petroleum, and filtered

through a short column of Grade III alumina (4 gm.). White crystals of anisoxide (20 mg.) were obtained on cooling the product in a Drikold-methanol bath. The identity of the product was established by m.p. and mixed m.p., and by its infrared and ultraviolet spectra, which were superposable on those of an authentic sample.

p-Methoxypropiophenone (IX). Anhydrous cadmium chloride (15.3 gm.) was added to a well-stirred solution of ethylmagnesium bromide (prepare from 2.3 gm. magnesium, excess ethyl bromide, and 60 ml. dry ether), with ice-cooling. The mixture was then stirred and refluxed until it gave a negative test for Grignard reagent[130] (i.e. for about 15 minutes). The ether was then evaporated off, dry benzene (60 ml.) added, and evaporation continued until a further 40 ml. of solvent had been removed. More benzene (20 ml.) was then added.

Anisoyl chloride was obtained by refluxing together for one hour anisic acid (5 gm.) and oxalyl chloride (10 gm.), then removing excess oxalyl chloride at the pump. The anisoyl chloride prepared in this way was added to the diethylcadmium solution, and the mixture

was refluxed and stirred for 1.75 hours. The reaction mixture was then cooled and hydrolysed by the addition of 10% aqueous sulphuric acid. The benzene layer was separated and washed successively with 10% sulphuric acid, saturated sodium bicarbonate solution, and water. Removal of solvents left a yellow liquid (5.1 gm.). p-Methoxypropiophenone (IX) (4.2 gm.) was distilled from this as a colourless liquid, b.p. 116-20°/1.8 mm. On cooling, the product crystallised as white needles, m.p. 20-24°, ν_{max} 1685 $cm.^{-1}$

The semicarbazone of p-methoxypropiophenone crystallised from chloroform and light petroleum as white needles, m.p. 173-74° (lit. 173°)[131].

1-(p-Methoxyphenyl)-n-Propanol (X). p-Methoxypropiophenone (3.8 gm.) and potassium borohydride (4 gm.) were dissolved in aqueous methanol (50%, 250 ml.), and the solution was set aside at room temperature for 16 hours. Most of the methanol was then evaporated, and the remaining solution was poured into water, and extracted with benzene. The benzene extract was washed with water, dried, and the solvent was then removed to yield a colourless liquid (2.9 gm.

This was distilled to yield 1-(p-methoxyphenyl)-n-propanol (X), b.p. 118-20°/1 mm., which was shown to be homogeneous by vapour phase chromatography. $\lambda\lambda_{max}$ 224, 275, and 281 mμ (ε 10,120, 1,580, and 1,330 respectively).

Anethole (XI). The alcohol (X) (1.5 gm.) was distilled under reduced pressure from a small crystal of iodine, to yield anethole (XI) (700 mg.) as a colourless liquid. The synthetic material had ultraviolet and infrared spectra superposable on those of an authentic specimen.

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