THE SCLEROTIORIN GROUP OF FUNGAL METABOLITES: THEIR STRUCTURE AND BIOSYNTHESIS

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INTRODUCTION

This lecture is concerned with a group of closely related fungal metabolites which has been extensively investigated in our Laboratories during recent years. These metabolites include sclerotiorin[1–3], $C_{21}H_{23}O_5Cl$ (I), rotiorin[4,5], $C_{23}H_{24}O_5$ (VI), rubropunctatin[6], $C_{21}H_{22}O_5$ (VII), monascorubin[7], $C_{23}H_{26}O_5$ (VIII) and monascin[8], $C_{21}H_{26}O_5$ (IX). Since sclerotiorin is the most readily available member of this group its chemistry has been more extensively investigated than that of its associates. Further, the elucidation of the novel constitution of sclerotiorin greatly facilitated the structural determinations of the less accessible pigments. Thus this new class of compounds which are derivatives of the hitherto unknown, and as yet unsynthesized pyrono-system (X), is termed the sclerotiorin group of pigments†.

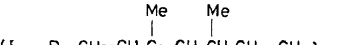
SCLEROTIORIN

Sclerotiorin (I), $[\alpha]_D$ +493° was first isolated from the mycelium of *Penicillium sclerotiorum* van Beyma by Curtin and Reilly[10] who proposed the empirical formula, $C_{20}H_{19}O_5Cl$ or $C_{21}H_{21}O_5Cl$. Subsequently Watanabe[11] and Birkinshaw[12] obtained the metabolite from *P. multicolor* Grigorieva-Manoilova and Poradielova and revised the formula to $C_{21}H_{25}O_5Cl$. Our work confirmed the molecular formula $C_{21}H_{23}O_5Cl$ and defined the constitution as (I).

Sclerotiorin is a yellow compound devoid of quinonoid properties and is a member of the relatively select group of natural products containing halogen. Although moderately stable towards dilute, mineral acids it is extremely sensitive to the action of alkali (*cf.* Watanabe[11] and Birkinshaw[12]). Degradation with aqueous sodium hydrogen carbonate or with dilute sodium hydroxide solution (*cf.* Watanabe[11] and Birkinshaw[12]) readily yields hydrochloric, formic and acetic acid together with 4,6-dimethylocta-2,4-dienoic acid (XI) and 2,4-dimethylhexa-2-enal (XII). The conclusive identification of acetic acid in the presence of formic acid caused considerable difficulties in our preliminary investigations[3]. The constitution of the dienoic acid (XI)

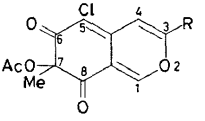
† In an earlier communication[9] the avidity of these compounds for ammonia was recognized in the generic term, azaphilone. However, it has since become apparent that this term is too restrictive in its connotation, so that *e.g.* monascin, although obviously a member of this group, cannot be classed as an azaphilone since it is inert towards ammonia.

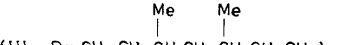
was established[11, 12] by ozonolysis to (+)-α-methylbutyraldehyde, pyruvaldehyde and 2,4-dimethylhexa-2-enal (XII), whilst hydrogenation furnished (+)-4,6-dimethyl-n-octanoic acid. The constitution of this



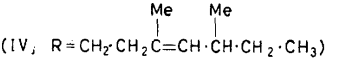
(I; R = CH=CH·C(Me)=CH·CH(Me)·CH$_2$·CH$_3$)

(II; R = CH=CH·COCH$_3$)



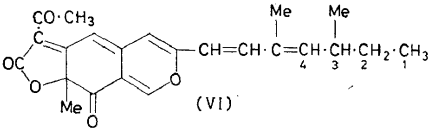


(III; R = CH$_2$·CH$_2$·CH(Me)·CH$_2$·CH(Me)·CH$_2$·CH$_3$)

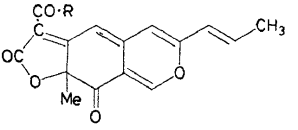


(IV; R = CH$_2$·CH$_2$C(Me)=CH·CH(Me)·CH$_2$·CH$_3$)

(V; R = CH$_2$·CH$_2$·CO·CH$_3$)

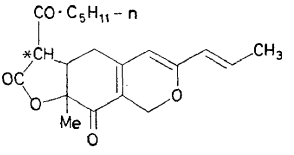


(VI)



(VII; R = n-C$_5$H$_{11}$)

(VIII; R = n-C$_7$H$_{15}$)



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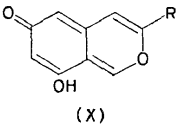
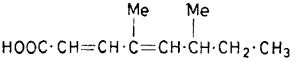
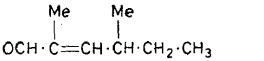


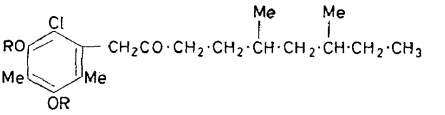
(X)



(XI)

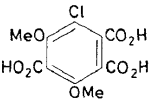


(XII)

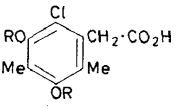


(XIII; R = H)

(XIV; R = Me)



(XV)



(XVI)

n-octanoic acid has been confirmed by synthesis[13]. The infrared absorption of (XI) at 988 cm^{-1} indicates a *trans*-arrangement of the $\alpha\beta$-disubstituted double bond.

Ozonolysis of sclerotiorin forms (+)-α-methylbutyraldehyde together with the ketone, pentanorsclerotiorone, $C_{16}H_{13}O_6Cl$ (II), which retains the intact nucleus of the metabolite, and is identical with the product obtained in low yield by the oxidation of sclerotiorin with chromic oxide.

Hydrogenation of sclerotiorin is complex[1, 14], but under carefully defined conditions tetrahydrosclerotiorin (III) is the principal product. Oxidation of tetrahydrosclerotiorin with chromic oxide produces (+)-4,6-dimethyl-n-octanoic acid thereby establishing that in tetrahydrosclerotiorin hydrogenation is confined to the C_9 side-chain. Since the formation of tetrahydrosclerotiorin produced a new asymmetric centre at C* in formula (III), tetrahydrosclerotiorin is a mixture, m.p. 142–144°, $[\alpha]_D$ +213°, of two diastereoisomers. These have been separated[1] into α-tetrahydrosclerotiorin, m.p. 118°, $[\alpha]_D$ +206° and β-tetrahydrosclerotiorin, m.p. 159°, $[\alpha]_D$ + 230°. However, for degradative purposes the mixture of m.p. 142–144° was always used. Hence in the subsequent discussion it will be understood that all derivatives of sclerotiorin containing the tetrahydro-side-chains are mixtures of the appropriate pair of diastereoisomers.

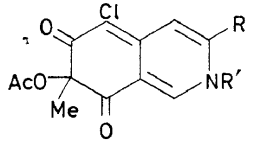
Hydrogenation of sclerotiorin (or of tetrahydrosclerotiorin) under more vigorous conditions causes hydrogenolysis of the α-acetoxy ketone residue and of the vinyl ether system with the production of a resorcinol derivative, sclerotinol, $C_{19}H_{29}O_3Cl$ (XIII). The constitution of this has been defined[14] by oxidation of the di-*O*-methyl ether (XIV) with potassium permanganate to 6-chloro-3,5-dimethoxytrimellitic acid (XV). Oxidation of di-*O*-acetylsclerotinol with chromic oxide gave 3,5-diacetoxy-2-chloro-4,6-dimethylphenylacetic acid, (XVI; R = Ac) which after saponification and conversion to the di-*O*-methyl ether gave 2-chloro-3,5-dimethoxy-4,6-dimethylphenylacetic acid (XVI; R = Me), identical with a synthetic specimen[14]. The definition of the orientation of sclerotinol provided collateral, unequivocal support for the orientation of sclerotiorin which had been derived initially from investigations concerning aposclerotioramine (see later).

Reduction of sclerotiorin with hydriodic acid, under strictly defined conditions gives dihydrosclerotiorin (IV), the constitution of which was established by ozonolysis to (+)-α-methylbutyraldehyde and pentanordihydrosclerotiorone, $C_{16}H_{15}O_6Cl$ (V).

The most characteristic reaction of the bicyclic pyrono-quinone nucleus of sclerotiorin is the facile production of derivatives of type (XVII)[12, 15, 16] by the action of ammonia and primary amines (hence the term azaphilone—see footnote on p. 565). Thus with ammonia the metabolite yields sclerotioramine, $C_{21}H_{24}O_4NCl$ (XVII), whilst with *e.g.* methylamine, the product is *N*-methylsclerotioramine, $C_{22}H_{26}O_4NCl$ (XX). Sclerotioramine is a natural product since it has been isolated, together with sclerotiorin, from the mycelium of *P. multicolor* grown under special conditions[1]. That the conversion of sclerotiorin into *e.g.* sclerotioramine, involves only the replacement of the heterocyclic oxygen by $\rangle$NH, is completely compatible with the extensive chemistry of sclerotiorin and its derivatives.

Although the formation of *e.g.* sclerotioramine and *N*-methylsclerotioramine proceeds rapidly to completion, interaction of sclerotiorin with aniline yields an intermediate, stable, yellow adduct, $C_{27}H_{30}O_5ClN$, which is readily transformed into *N*-phenylsclerotioramine, $C_{27}H_{28}O_4ClN$ (XXII) by acids or bases. The formation of this adduct is typical of pyrones. In accord with its spectral properties the adduct is represented by structure (XXIV). That sclerotiorin is soluble in dilute sodium hydroxide and is regenerated (at least in part) on immediate acidification is to be attributed not to the presence of a lactone ring as was at one time believed[16], but to the fission of the heterocyclic ring to yield the acidic enol (XXIII). This interpretation is in accord with the proposed mechanism[3] for the formation of the aniline adduct (XXIV).

The amphoteric, weakly basic sclerotioramine (XVII) was a key intermediate in the elucidation of the structure of sclerotiorin. In agreement with its structure sclerotioramine is decomposed by boiling acid or alkali



(XVII ; R = CH=CH·C(Me)=CH·CH(Me)·C$_2$H$_5$; R′ = H)

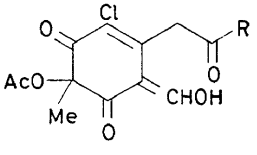
(XVIII ; R = CH$_2$·CH$_2$CH(Me)·CH$_2$·CH(Me)·C$_2$H$_5$; R′ = H)

(XIX ; R = CH$_2$·CH$_2$C(Me)=CH·CH(Me)·C$_2$H$_5$; R′ = H)

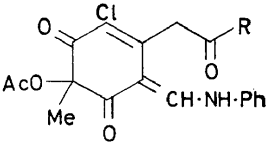
(XX ; R = CH=CH·C(Me)=CH·CH(Me)·C$_2$H$_5$; R′ = Me)

(XXI ; R = CH$_2$·CH$_2$·CH(Me)·CH$_2$·CH(Me)·C$_2$H$_5$; R′ = Me)

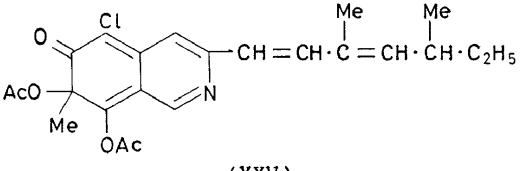
(XXII ; R = CH=CH·C(Me)=CH·CH(Me)·C$_2$H$_5$; R′ = Ph)



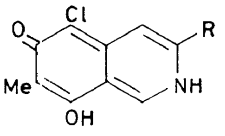
(XXIII)



(XXIV)



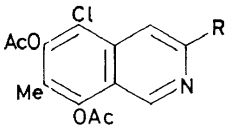
(XXV)



(XXVI; R = CH=CH·C(Me)=CH·CH(Me)·C$_2$H$_5$)

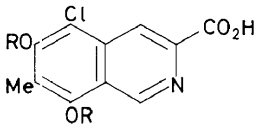
(XXVII; R = CH$_2$CH$_2$C(Me)=CH·CH(Me)·C$_2$H$_5$)

(XXVIII; R = CH$_2$·CH$_2$·CH(Me)·CH$_2$·CH(Me)·C$_2$H$_5$)



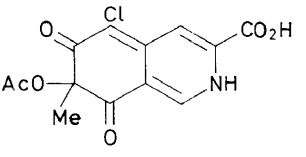
(XXIX; R = CH=CH·C(Me)=CH·CH(Me)·C$_2$H$_5$)

(XXX; R = CH$_2$·CH$_2$C(Me)=CH$_2$·CH(Me)·C$_2$H$_5$)

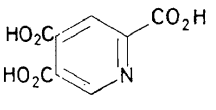


(XXXI; R = H)

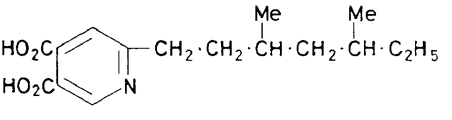
(XXXII; R = Ac)



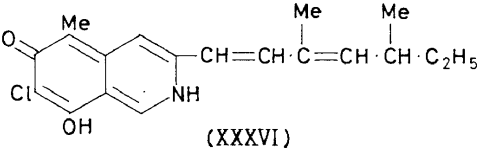
(XXXIII)



(XXXIV)



(XXXV)



(XXXVI)

to yield acetic acid but without the liberation of ammonia or (+)·4,6-dimethylocta-2,4-dienoic acid (XI). Methylation furnishes *N*-methylsclerotioramine (XX) identical with the product from the interaction of sclerotioramine and methylamine.

This *N*-methyl derivative is insoluble in sodium hydroxide solution, cannot be acetylated and shows no absorption in the 3μ region of the infrared spectrum. Acetylation of sclerotioramine yields *O*-acetylsclerotioramine, $C_{21}H_{23}O_3ClN{\cdot}OAc$, (XXV), which is insoluble in dilute alkali, readily forms a yellow solution in dilute hydrochloric acid and may be de-acetylated to the parent base. That this acetyl derivative is an *O*- and not an *N*-acetate is demonstrated by, *inter alia*, (*a*) that the melting point is considerably (100°) below that of the parent base: *N*-acetylation usually gives a product with a melting point higher than that of the parent base whilst *O*-acetylation exerts a converse effect, (*b*) that acetylsclerotioramine

is a stronger base than sclerotioramine: *N*-acetylation would reduce the basicity, and (*c*) that the acetate shows the anticipated, additional infrared absorption band at 1779 cm^{-1}.

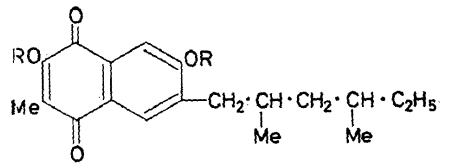
In accord with its formulation as an α-acetoxy-ketone the reduction of sclerotioramine with zinc and acetic acid or zinc and sodium hydroxide proceeds with the extrusion of the acetoxyl group as acetic acid (approximately one mol) and the formation of the aromatic, amphoteric, aposclerotioramine, $C_{19}H_{22}O_2NCl$ (XXVI). Aposclerotioramine forms a diacetate, $C_{19}H_{20}NCl(OAc)_2$ which is formulated as a di-*O*-acetate (XXIX) for reasons similar to those used to deduce the acetylation pattern of *O*-acetylsclerotioramine (XXV). The general properties of di-*O*-acetylaposclerotioramine together with the bathochromic shift of the ultraviolet absorption spectrum of the hydrochloride by 20–50 mμ for the various peaks indicated a quinoline or isoquinoline nucleus. Moreover, since the ultraviolet spectrum of aposclerotioramine did not show an appreciable shift in acidic or alkaline medium, it was deduced that the nucleus was a quinolone or isoquinolone in accord with the pyridone character of the parent sclerotioramine.

The action of ammonia upon dihydrosclerotiorin (IV) furnishes dihydrosclerotioramine, $C_{21}H_{26}O_4NCl$ (XIX) which is strictly analogous to sclerotioramine. Reduction of (XIX) with zinc and alkali furnishes dihydroaposclerotioramine (XXVII) which yields a di-*O*-acetyl derivative (XXX) analogous to di-*O*-acetylaposclerotioramine (XXIX).

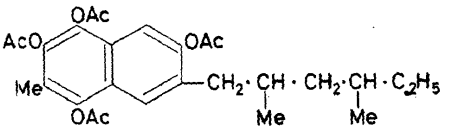
The ultraviolet spectrum of di-*O*-acetyldihydroaposclerotioramine (XXX) in neutral solution is very similar to that of isoquinoline (but not that of quinoline) in intensity and in the relative position of the peaks but as anticipated at wavelengths longer by about 20 mμ. Since the absorption of aromatic systems is not influenced by the presence of acetoxyl groups and since in (XXX) the conjugated side-chain is insulated from the aromatic chromophore these results provided compelling circumstantial evidence for the presence of an isoquinolone nucleus in the aposclerotioramines.

The non-participation of the alkyl side-chain in the formation of sclerotioramine and its analogues is indicated by *e.g.* the preparation of tetrahydrosclerotioramine, $C_{21}H_{28}O_4NCl$ (XVIII) from tetrahydrosclerotiorin (III) and ammonia, or by the hydrogenation of sclerotioramine. Tetrahydrosclerotioramine is aromatized with loss of two carbon atoms (as acetic acid) to tetrahydroaposclerotioramine (XXVIII) analogous to aposclerotioramine. Ozonolysis of di-*O*-acetylaposclerotioramine (XXIX) removes eight carbon atoms [five as (+)-α-methylbutyraldehyde] with the production of di-*O*-acetylaposclerotaminic acid (XXXII). The red, ferrous sulphate reaction of (XXXII) indicated that the conjugated carboxyl group and hence the C_9 side-chain are situated in the *ortho*-position to the heterocyclic nitrogen atom. Collateral evidence for the non-participation of the alkyl side chain in these transformations is provided by the ozonolysis of *O*-acetylsclerotioramine (XXV) to yield sclerotaminic acid (XXXIII) [the pyridone-type *O*-acetoxyl-residue is hydrolysed during decomposition of the ozonide] together with 2:4-dimethylhexa-2-enal (XII). Aromatization of sclerotaminic acid yields aposclerotaminic acid (XXXI).

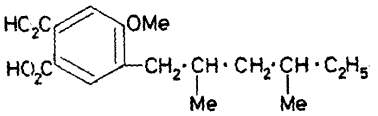
The orientation of the substituents in the isoquinoline nucleus of *e.g.*



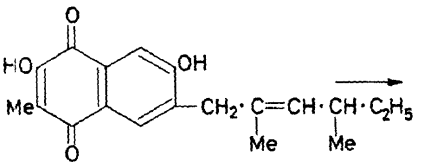
(XXXVII; R = H)
(XXXVIII, R = Me)



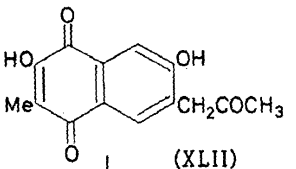
(XXXIX)



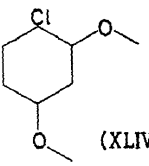
(XL)



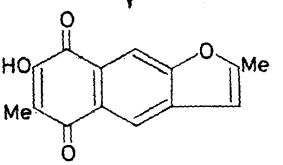
(XLI)



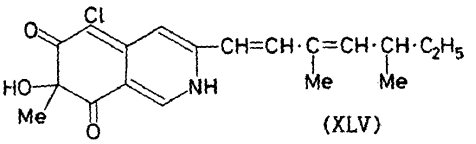
(XLII)



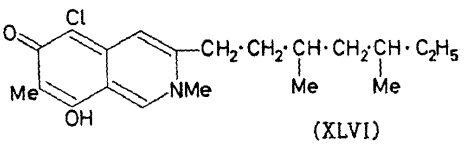
(XLIV)



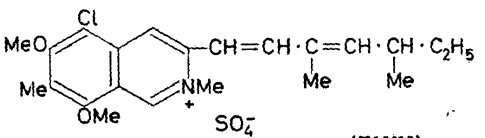
(XLIII)



(XLV)



(XLVI)



(XLVII)

aposclerotioramine, was elucidated as follows[16]. Oxidation of sclerotioramine or of its tetrahydro-derivative, with nitric acid formed berberonic acid (pyridine-2,4,5-tricarboxylic acid) (XXXIV), whilst oxidation of tetrahydroaposclerotioramine (XXVIII) with potassium permanganate or with alkaline hydrogen peroxide gave (+)-2-(3,5-dimethyl-n-heptyl)-pyridine-4,5-dicarboxylic acid (XXXV) which has a negative ferric reaction, and furnished berbonic acid upon further oxidation. Not only does the production of (XXXV) support the isoquinolone nature of tetrahydroaposclerotioramine (XXVIII) but it conclusively establishes that the nucleus is not an α-isoquinolone but of type (XXVIII). Further, since aposclerotioramine and tetrahydroaposclerotioramine exhibit negative ferric reactions and are not easily oxidized it follows that the hydroxyl functions in the isoquinoline system have the *m*- rather than *o*- or *p*-orientations. Hence, aposclerotioramine may be represented by (XXVI) or (XXXVI) and their appropriate tautomers. The behaviour of aposclerotioramine and its derivatives on methylation is in accord with the isoquinolone structure.

A decision between these two alternatives was originally made from a study of the behaviour of dihydro-(IV) and tetrahydro-(III) sclerotiorin upon degradation with alkali[2, 16]. Thus whilst alkali degradation of sclerotiorin gives formic, acetic, hydrochloric and 4:6-dimethyl-2,4-dienoic (XI) acids, tetrahydrosclerotiorin yields acetic, formic and hydrochloric acids but no (+)-4,6-dimethyl-n-octanoic acid. In its place the dihydroxynaphtha-*p*-quinone, tetrahydrosclerotoquinone, $C_{19}H_{22}O_2(OH)_2$ (XXXVII) is formed. Reductive acetylation of this quinone gives tetra-*O*-acetyltetrahydrosclerotoquinol (XXXIX). A comparison of the ultraviolet absorption spectra of tetrahydro- and di-*O*-acetyltetrahydro-sclerotoquinone and tetra-*O*-acetyltetrahydrosclerotoquinol with those of 2-hydroxyl-1,4-naphthaquinone, 1,4-naphthaquinone and naphthalene respectively, clearly established that tetrahydrosclerotoquinone is a naphthalene derivative, and a 1:4 and not a 1:2-quinone.

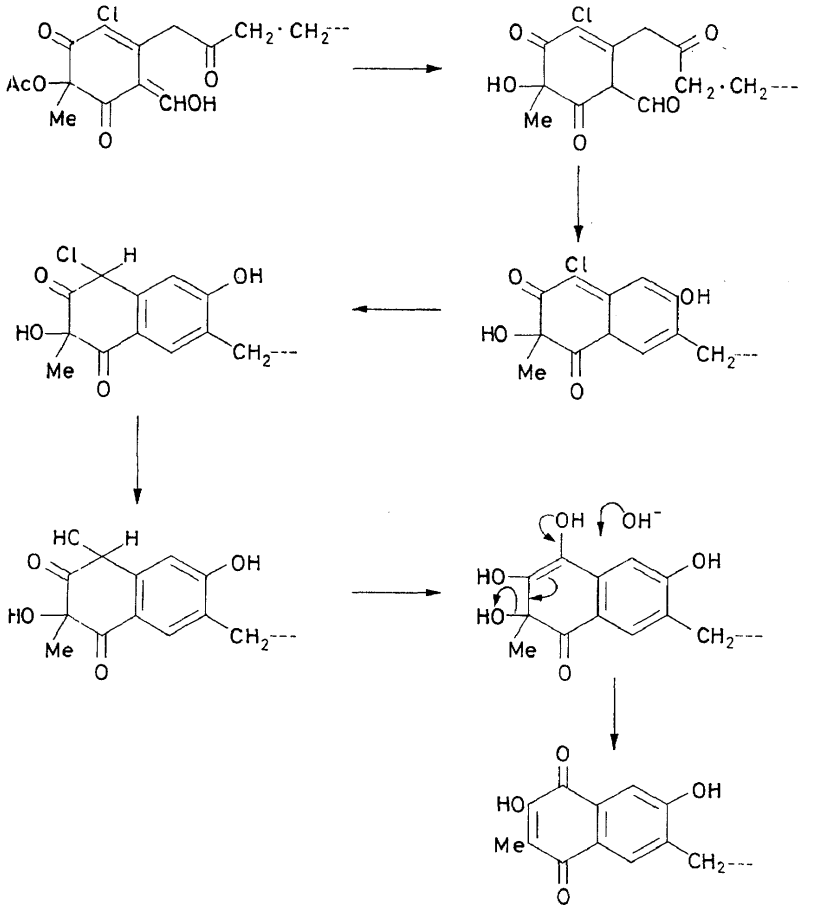
In agreement with this, tetrahydro-di-*O*-methylsclerotoquinone (XXXVIII) was oxidized to (+)-4-(2,4-dimethyl-n-hexyl)-5-methoxyphthalic acid (XL), which furnished an anhydride and upon demethylation yielded the corresponding hydroxy acid, devoid of a ferric reaction.

Similarly, alkali degradation of dihydrosclerotiorin (IV) furnishes the analogous dihydrosclerotoquinone (XLI). Ozonolysis of tetra-*O*-acetyldihydrosclerotoquinol followed by deacetylation and aerial oxidation of the product gave the quinone (XLII), which on sublimation formed the coumaro-quinone (XLIII). The acetonyl *p*-quinone (XLII) was also obtained[2], as expected, by alkali degradation of dihydropentanorsclerotiorone (V).

The genesis of the naphthaquinones of type (XXXVII) is readily appreciated in terms of the sequence[3] outlined opposite.

The formation of these quinones provided strong circumstantial evidence that in *e.g.* tetrahydrosclerotiorin, the chlorine atom is situated as in (XLIV) with respect to the potential hydroxyl groups. Consequently, aposclerotioramine has the orientation (XXVI): rational formulae for the various cognate derivatives follow.

Independent confirmation[14] for the structure (XXVI) was provided



from the investigations concerning sclerotinol (p. 567) and by the oxidation with potassium permanganate of the di-*O*-methylether methosulphate (XLVII), of aposclerotioramine to 6-chloro-3,5-dimethoxytrimellitic acid (XV).

The derivation of the structure for sclerotioramine (and hence for sclerotiorin) now only required the insertion into aposclerotioramine of the acetyl residue eliminated as acetic acid. This requirement leads inevitably[3] to the structure (XVII) for sclerotioramine and hence to (I) for sclerotiorin.

Although sclerotiorin is rapidly destroyed by alkali, selective deacetylation of *e.g.* sclerotioramine (XVII) to desacetylsclerotioramine (XLV) can be achieved. Acetylation of this alcohol regenerates sclerotioramine. In accord with these conclusions oxidation[3] of tetrahydro-*N*-methylaposclerotioramine (XLVI) with lead tetraacetate gave a high yield of a substance having the general properties of tetrahydro-*N*-methylsclerotioramine (XXI): indeed the materials were spectroscopically identical[3]. Although the stereochemical complexity of the side-chain and the lack of stereospecificity at the point of

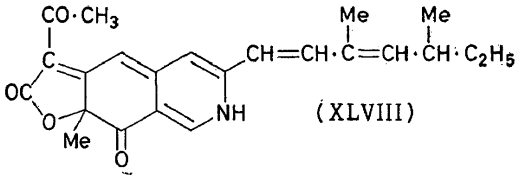
acetoxylation prevented complete identification the nature of the reaction provided compelling support for the structure (I) of sclerotiorin.

A laevorotatory sclerotiorin, $[\alpha]_D$ −482° has recently been isolated[17] from *P. hirayamae* Udagawa. Since this metabolite yields the (+)-dienoic acid (XI) upon hydrolysis, (−) sclerotiorin is 7-epi-(+) sclerotiorin[18].

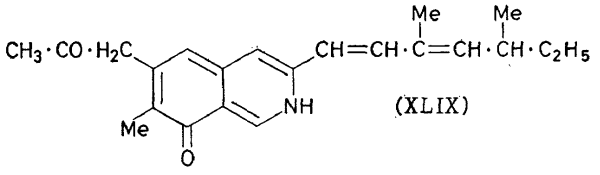
ROTIORIN

The pigment, rotiorin (VI), was obtained[4] from the mycelium of *P. sclerotiorum* van Beyma, as a co-metabolite of sclerotiorin.

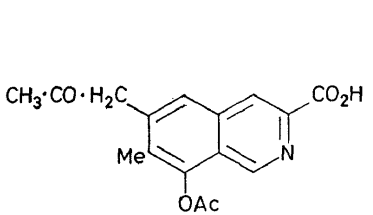
Rotiorin is extremely sensitive to the action of alkali which degrades it to



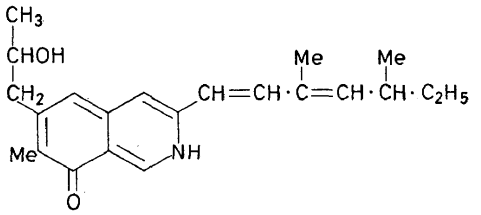
(XLVIII)



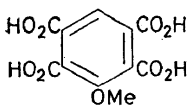
(XLIX)



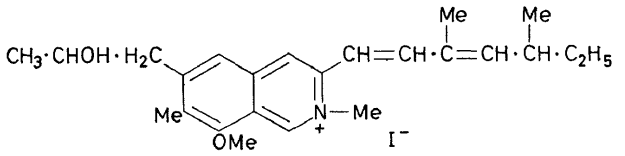
(L)



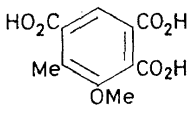
(LI)



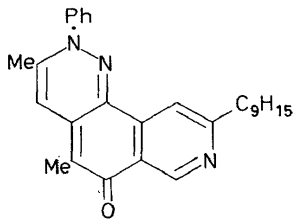
(LIII)



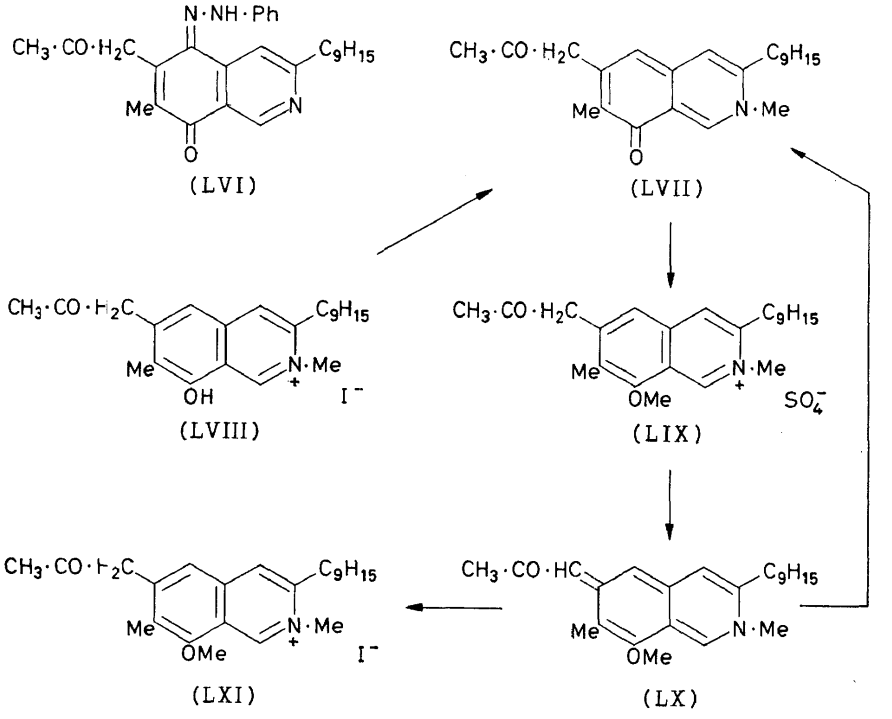
(LII)



(LIV)



(LV)



4,6-dimethylocta-2,4-dienoic acid (XI) together with 2,4-dimethylhexa-2-enal (XII). Extensive investigation of the hydrogenation of rotiorin failed to produce an homogeneous product.

Like sclerotiorin, rotiorin reacts rapidly with ammonia. The product, rotioramine, $C_{23}H_{25}NO_4$, (XLVIII), furnishes 2,4-dimethylhexa-2-enal (XII) on ozonolysis but does not yield ammonia or the dienoic acid (XI) when degraded with alkali. Oxidation of rotioramine with nitric acid furnished berberonic acid (XXXIV) (*cf.* sclerotioramine). Rotioramine is aromatized by the action of zinc and alkali or zinc and acetic acid (*cf.* sclerotioramine) to the amphoteric aporotioramine, $C_{22}H_{27}NO_2$ (XLIX) with the extrusion of the lactonic carbonyl group as a molecular proportion of carbon dioxide. The general properties of aporotioramine clearly indicated[4] the presence of an isoquinolone system with the quinolone oxygen located at C–6 or C–8. The presence of the acetonyl residue was shown by the isolated carbonyl absorption at 1698 cm^{-1} and the formation of iodoform. Oxidation of tetrahydroaporotioramine (prepared by hydrogenation of aporotioramine) yielded (+)-2-(3,5-dimethyl-n-heptyl)pyridine-4,5-dicarboxylic acid (XXXV) (*cf.* sclerotiorin).

Ozonolysis of *O*-acetylaporotioramine gave *O*-acetylaporotaminic acid, $C_{16}H_{15}NO_5$ (L), the red ferrous sulphate reaction of which showed that the carboxyl residue, and hence the unsaturated C_9 side-chain, is situated in the *ortho*-position to the nitrogen, as in sclerotioramine. Further, since aporotioramine did not yield an anhydro-compound (*i.e.* a 2-methyl furan) the acetonyl residue is not *ortho*- to the quinolone oxygen function. Unequivocal

definition of the orientation of aporotioramine (and hence of rotiorin) was obtained as follows[5].

Methylation of aporotioraminol (LI), the sodium borohydride reduction product of aporotioramine, gave *NO*-dimethylaporotioraminol iodide (LII), which was oxidized by potassium permanganate to anisole-2,3,5,6-tetracarboxylic acid (LIII). Aporotioramine is thus an 8-hydroxyisoquinoline. Under milder conditions of oxidation (LII) gave the tribasic acid (LIV). Aporotioramine was thus unequivocally defined as (XLIX). In agreement with this structure, aporotioramine gave a positive Gibbs test and coupled with benzene diazonium chloride to furnish the cinnoline, $C_{28}H_{29}N_3O$, (LV). This reaction has its parallel in the chemistry of rubropunctatin (*q.v.*) and presumably involves the tautomeric form (LVI) of the initial coupling product which successively undergoes cyclization and dehydration to yield (LV). In agreement with this, aporotioraminol (LI) formed a normal coupling product, $C_{28}H_{33}N_3O_2$.

In keeping with its structure as an isoquinolone, aporotioramine yields an extensive series of methylation products. Thus whilst the action of methylsulphate and alkali gives *N*-methylaporotioramine (LVII), methyl-iodide in acetone forms *N*-methylaporotioramine hydriodide (LVIII), which is readily converted into the base (LVII). Methylation of either aporotioramine or of *N*-methylaporotioramine with methylsulphate–potassium carbonate in acetone gives *NO*-dimethylaporotioramine methosulphate (LIX). With cold alkali this forms the red, anhydro base (LX), which is converted into *NO*-dimethylaporotioramine iodide (LXI) by addition of hydriodic acid. The addition of methyl iodide to the quinonoid *N*-methylaporotioramine yields the same salt (LXI). In accordance with its formulation as a vinylogous ester the methoxyl group in (LX) was hydrolysed by warm alkali to *N*-methylaporotioramine.

RUBROPUNCTATIN, MONASCORUBRIN AND MONASCIN

In 1895 Went showed[19] that the fungus *Monascus purpureus* was responsible for the colour of "Red-Rice" ("Ang Khak"), a preparation used extensively in Asia as a colouring matter for food stuffs and alcoholic beverages, *e.g.* the Taiwan Wine, Hong-Ru[26]. The orange coloured monascorubrin (VIII) together with the yellow pigment, monascin† (IX) were first isolated from *M. purpureus* Wentii by Nishikawa[20]. Subsequently, Karrer and Saloman[21] obtained monascin and reported a preliminary investigation of the metabolite[21, 22].

More recently[9] our systematic survey of metabolites of the *Monascus* genus resulted in the isolation of monascin (IX) and rubropunctatin (VII) from *M. rubropunctatus* Sato, monascin and "monascorubrin" from *M. purpureus* Wentii (*cf.* Nishikawa[20]) and monascin from *M. rubriginosus* Sato.

Our investigations have established the constitutions of these pigments and their affinity with sclerotiorin and rotiorin.

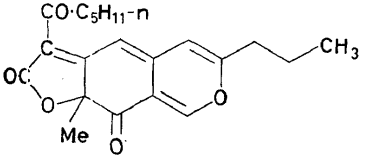
† The Japanese workers[23] prefer the name monascoflavin for this pigment on the grounds that the term monascin has been used for a yellow pigment isolated[24] from *M. paxii* Lingelsheim. This duplication of names appears unjustified, since *M. paxii* is no longer available and since monascoflavin might well be identical with monascin.

RUBROPUNCTATIN

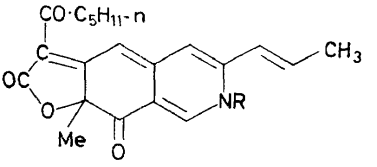
Rubropunctatin[6] (VII) differs from rotiorin (VI) only in the nature of the two pendant alkyl residues, and its structural definition closely follows that of rotiorin.

Thus ozonolysis of rubropunctatin yields acetaldehyde (from the propenyl residue) whilst oxidation with alkaline hydrogen peroxide forms n-hexanoic acid. Although difficult to control (*cf.* rotiorin) hydrogenation gives, in low yield, dihydrorubropunctatin (LXII) which, in accord with its structure does not furnish acetaldehyde on ozonolysis.

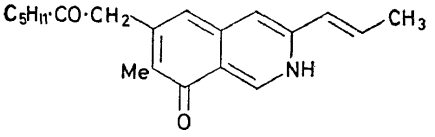
Rubropunctatin forms rubropunctatamine, $C_{21}H_{23}NO_4$ (LXIII) with ammonia, and *N*-methylrubropunctatamine (LXIV) with methylamine. Reductive aromatization of rubropunctatamine yields carbon dioxide together with the amphoteric isoquinolone, aporubropunctatamine,



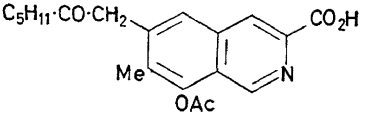
(LXII)



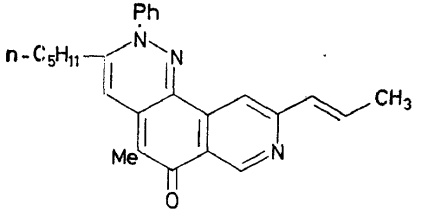
(LXIII; R=H)
(LXIV; R=Me)



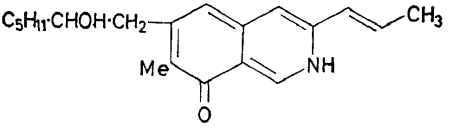
(LXV)



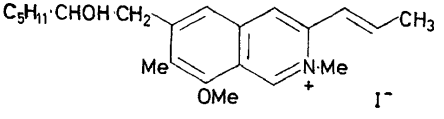
(LXVI)



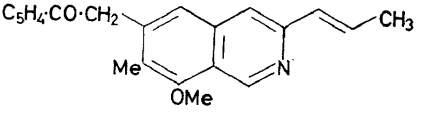
(LXVII)



(LXVIII)



(LXIX)



(LXX)

$C_{20}H_{25}NO_2$ (LXV), which forms an *O*-acetate and *O*-methyl ether (*cf.* aporotioramine), and contains three carbon methyl residues and an isolated carbonyl group.

As in the case of aporotioramine the isoquinoline nature of aporubropunctatamine was established, *inter alia*, by, (*a*) ozonolysis of *O*-acetylaporubropunctatamine to acetaldehyde and the bisnor-acid, $C_{20}H_{23}NO_5$ (LXVI), the red ferrous sulphate reaction of which established the position of the propenyl residue as *ortho*- to the heterocyclic nitrogen; (*b*) oxidation of rubropunctatamine with nitric acid to berberonic acid (XXXIV); and (*c*) the general spectral properties of *O*-acetyldihydro-aporubropunctatamine.

In aporubropunctatamine the 3-propenyl isoquinolone residue accounts for twelve of the twenty carbon atoms: the n-pentyl residue disposes of another five; of the remaining three one is in the isolated keto group and the second is in a carbon methyl residue: hence aporubropunctatamine must have either an acetonyl substituent together with a n-pentyl residue or a methyl group and a β-oxo-n-heptyl moiety. Since aporubropunctatamine does not give an iodoform reaction the former possibility is excluded. The definition of the orientation of aporubropunctatamine as (LXV) closely follows that of the analogous aporotioramine. Thus, *e.g.* aporubropunctatamine coupled with benzene diazonium chloride to yield the cinnoline (LXVII), whilst aporubropunctataminol (LXVIII) furnished a normal coupling product. In addition[7], oxidation of the methiodide (LXIX) of *O*-methylaporubropunctataminol gave anisole-2,3,5,6-tetracarboxylic acid (LIII).

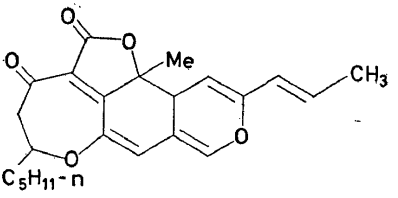
The structure (VII) for rubropunctatin thus follows.

MONASCORUBIN

During work upon monascin (*q.v.*) isolated from the mycelium of *M. purpureus* Wentii we obtained minor amounts of a second pigment, which underwent a series of reactions parallel to those of rubropunctatin and which was apparently identical[7] with "monascorubrin" first isolated by Nishikawa[20]. Our "monascorubin" proved to be an inseparable mixture of rubropunctatin (VII), as the minor component, together with monascorubrin (VIII). In the sequel trivial names enclosed in quotes refer to mixtures.

The properties of monascorubin closely simulate[7] those of rubropunctatin. Thus, *e.g.* reaction with ammonia yields "monascorubramine" which is reduced by zinc and acetic acid to "apomonascorubramine" together with one molecular equivalent of carbon dioxide. All derivatives of "monascorubrin" have properties similar to the analogous derivatives of rubropunctatin, the only consistent difference between the two series being that the derivatives of "monascorubrin" had a slightly higher carbon and hydrogen content than the corresponding compounds from rubropunctatin. The situation was resolved by a mass spectrometric examination of *O*-methylaporubropunctatamine (LXX) and the corresponding "*O*-methylapomonascorubramine". This showed that whereas the former compound was essentially one component of M.W. 325, in agreement with formula (LXX), "*O*-methylapomonascorubramine" was a mixture of two major

components of molecular weights, 325 and 353 in the ratio of 1:4. In gas chromatography the component of M.W. 325 had the same retention time as *O*-methylaporubropunctatamine (LXX). Thus it seemed that the material of M.W. 353 probably differed from (LXX) only by two additional methylene groups. The position of these methylene groups was established by oxidation of "dihydromonascorubramine", followed by isolation of the monobasic aliphatic acids. Separation of these acids on paper gave spots corresponding to n-hexanoic acid (also identified as the *p*-bromophenacyl derivative) and n-octanoic acid. Consequently, our "monascorubrin" is a mixture of rubropunctatin (VII) and monascorubin which is represented by the structure (VIII)[7].



(LXXI)

The independent work of Nakanishi and his co-workers[25] upon monascorubrin seems to have been performed with material substantially uncontaminated by rubropunctatin. These workers originally assigned structure (LXXI) to monascorubrin, but after further investigation[26] withdrew (LXXI) in favour of our structure (VIII).

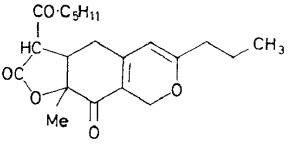
MONASCIN

In a preliminary examination of this yellow, optically active pigment Karrer *et al.*[21, 22] allocated the provisional formula, $C_{20}H_{24}O_5$, to monascin and showed that, *inter alia*, it furnished n-hexanoic and acetic acids on oxidation and, although resistant to acetylation or benzoylation, contained one active hydrogen (Zerewitinoff). Our analytical results[8] upon monascin and its numerous derivatives, together with a mass spectral examination of tetrahydromonascin (LXXIII), established the molecular formula as $C_{21}H_{26}O_5$. Thus we quickly adopted the provisional structure (IX) for monascin since: (*a*) monascin and rubropunctatin are co-metabolites of *M. rubropunctatus* Sato, (*b*) both furnish n-hexanoic acid on oxidation and acetaldehyde upon ozonolysis, and (*c*) the molecular formula of monascin is equivalent to that of a tetrahydrorubropunctatin. In accord with formula (IX) monascin, as distinct from its congeners, does not react with ammonia. The structure (IX) for monascin has been substantiated as follows.

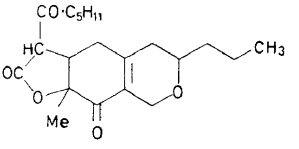
Hydrogenation of monascin forms successively dihydro- (LXXII), tetrahydro- (LXXIII) and hexahydro- (LXXIV) derivatives, which do not yield acetaldehyde on ozonolysis. The ultraviolet absorption spectra are compatible with these structures for monascin and its hydro- derivatives. Thus, in agreement with the presence of a fully substituted $\alpha\beta$-unsaturated ketone system in tetrahydromonascin (LXXIII), the spectrum has λ_{max} 244 and 320 mμ (log ϵ 4·01 and 1·93). Further, the nuclear magnetic resonance

spectrum of (LXXIII) is devoid of a vinylic hydrogen peak. Empirical calculations for the linear conjugated system in dihydromonascin (LXXII) gives a value for λ_{max} of 374 mμ. Since the principal high intensity absorption band occurs at λ_{max} 365 mμ (log ϵ 4·18) the agreement is acceptable, particularly in view of the limited data available for assessing the bathochromic contribution of the ethereal oxygen atom.

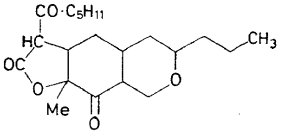
Reduction of tetrahydromonascin with zinc and acetic acid yields principally tetrahydroapomonascin (LXXV), together with carbon dioxide in a molecular proportion to the amount of tetrahydroapomonascin formed.



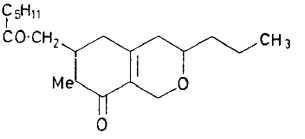
(LXXII)



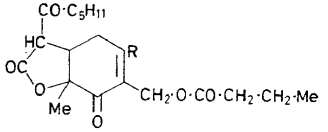
(LXXIII)



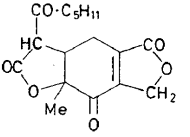
(LXXIV)



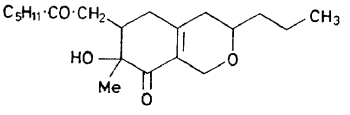
(LXXV)



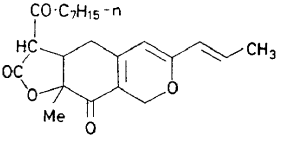
(LXXVI; R = CHO)
(LXXVII; R = CO_2H)



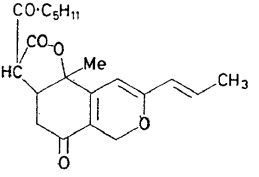
(LXXVIII)



(LXXIX)



(LXXX)



(LXXXI)

Unlike monascin and its hydrogenated derivatives, tetrahydroapomonascin is devoid of active hydrogen (negative Zerewitinoff), and is insoluble in sodium hydroxide solution. This is in agreement with the acidity of these compounds being associated with the tertiary hydrogen at C* in (IX), whilst the absence of corresponding enolic properties is probably ascribable to the *trans*-fusion of the γ-lactone ring junction†, in which case the generation of an enol would be energetically unfavourable.

Whilst carbon dioxide is produced by the reduction of monascin with zinc and acetic acid (although no other recognizable product could be isolated), hexahydromonascin (LXXIV) is unchanged under these conditions, from which it may be concluded that the double bond of the $\alpha\beta$-unsaturated ketone group in monascin and its tetrahydro derivative is necessary for reduction under mild conditions (*cf.* similar observations[27] in the steroid series).

The infrared spectra of monascin and its derivatives support the proposed structures. Thus, the spectrum of tetrahydroapomonascin (LXXV) has bands at 1709 (side chain ketone carbonyl), and at 1656 and 1629 cm^{-1} ($\alpha\beta$-unsaturated ketone). Hexahydromonascin (LXXIV) has bands at 1786 (γ-lactone) and 1739 (cyclohexanone carbonyl), 1718 cm^{-1} (side chain carbonyl residue). The high wavenumbers of the γ-lactone and cyclohexanone residues are in agreement with their relationship in the α-ketol lactone residue. Similar shifts are apparent in the spectrum of tetrahydromonascin (LXXIII), which has bands at 1786 (γ-lactone), 1718 (side-chain carbonyl), 1695 ($\alpha\beta$-unsaturated ketone), and 1623 cm^{-1} (double bond stretch in $\alpha\beta$-unsaturated ketone). The carbonyl stretching regions of monascin (IX) and of dihydromonascin (LXXII) are similar, both having bands at 1785 (γ-lactone), 1715 (side-chain carbonyl), and 1669 and 1628 cm^{-1} ($\alpha\beta$-unsaturated carbonyl group).

Unequivocal chemical evidence of a vinyl ether system in dihydromonascin (LXXII) has been obtained. Thus, oxidation of (LXXII) with sodium dichromate gave the formyl-butyrate ester (LXXVI) and the corresponding carboxylic acid (LXXVII). Hydrolysis of (LXXVII) furnished n-butyric acid and the dilactone (LXXVIII), which exhibited the requisite spectral and chemical characteristics.

The position of the angular methyl group in monascin is in agreement with the structures of its congeners and with the presence in the nuclear magnetic resonance spectra of monascin, dihydro- and tetrahydromonascin, of a strong, sharp singlet at τ 8·50. This singlet is absent in tetrahydroapomonascin. Supporting chemical evidence although not unequivocal is available for the position of the nuclear C–Me residue. Thus, treatment of tetrahydromonascin with barium hydroxide gave a non-acidic gum which exhibited bands at 3448, 1709 and 1667 cm^{-1} in its infrared spectrum. The absence of absorption at 1770 cm^{-1} suggests that this product was predominantly the α-ketol (LXXIX). In agreement with this view, oxidation with lead tetra-acetate produced an acidic gum which, unlike tetrahydromonascin, gave a positive iodoform reaction. Hence the acidic oxidation product probably contained a CH_3CO—C residue in agreement with the location of the angular methyl residue in formula (IX) for monascin.

† The n.m.r. data of Nakanishi *et al.*[26] support this stereochemical assignment.

In an independent examination of monascin Nakanishi *et al.*[23] deduced the formula (IX) or (LXXXI) for monascin, with a marked preference for the latter structure. However, in our view the formation of iodoform from tetrahydromonascin and of tetrahydroapomonascin, under very mild reductive conditions are not readily explicable in terms of structure (LXXXI).

Although monascin is apparently a tetrahydro- derivative of rubropunctatin, the interconversion of monascin and rubropunctatin has yet to be achieved.

In view of the co-occurrence[8, 9] in *M. purpureus* of monascin and "monascorubrin" and since the latter substance is a mixture[7] of rubropunctatin (VII) and the higher homologue, monascorubrin (VIII) it seemed possible that monascin too might be a similar mixture of compounds (IX) and (LXXX). Although equivocal, a mass spectrometric examination of samples of tetrahydromonascin prepared from monascin elaborated by *M. purpureus* and *M. rubropunctatus* indicated only small amounts, if any, of the homologue (LXXX) in our specimens. Samples of monascin produced by both fungi were therefore oxidized and the volatile acids examined. These consisted predominantly of n-hexanoic acid together with traces of an acid which had the R_f values of n-octanoic acid, arising presumably from trace quantities of (LXXX).

It will be apparent that our structural determinations in this investigation were substantially completed before the advent of nuclear magnetic resonance to the organic chemist: only in the concluding stages of our work on monascin was this technique available to us. Consequently these investigations constitute one of the last major structural problems to be resolved essentially by "classical" methods. It is thus interesting to speculate how much of the fascinating chemistry of these chameleon-like (both literally and metaphorically!) compounds would have remained undisclosed in the presence of nuclear magnetic resonance.

BIOSYNTHESIS

Incorporation studies with sodium [1-^{14}C]-acetate and [1-^{14}C]-formate have shown [28, 29] that sclerotiorin, rotiorin, rubropunctatin and monascin are derived by the well-established poly β-ketide pathway. In each compound two chains must be present: the extra one increases in complexity from acetyl in sclerotiorin to acetoacetyl which has undergone ring closure (in rotiorin) and finally to the n-hexanoylacetyl moiety with similar ring closure as in monascin and rubropunctatin[6, 30].

We have recently obtained additional information concerning the biosynthesis of sclerotiorin and rotiorin by *P. sclerotiorum* van Beyma from incorporation experiments with diethyl [1-^{14}C]- and [2-^{14}C]-malonate and sodium (1-^{14}C)-butyrate[31].

The labelled metabolites were degraded by established processes. The carbon dioxide evolved during the formation of aporotioramine was isolated as barium carbonate. In the studies with diethyl [1-^{14}C]-malonate and sodium (1-^{14}C)-butyrate comparison of the activities of the terminal and adjacent two-carbon units in the main chain was very important. Thus, both metabolites were ozonized to give 2-methylbutyric acid which was

degraded to 1-methylpropylamine, [isolated as *N*-(1-methylpropyl)-2,4-dinitroaniline], and carbon dioxide (isolated as barium carbonate). These two products contained the radioactivity of C_2 and C_4 respectively, [*e.g.* in (I) and (VI)], being derived from the terminal and first chain-building units.

The distributions of labels in sclerotiorin and rotiorin derived from diethyl [1-^{14}C]- and [2-^{14}C]-malonate and sodium [1-^{14}C]-butyrate are summarized in columns (1) and (2) of *Tables 1–3* respectively, and show that sclerotiorin derived from both [1-^{14}C]- and [2-^{14}C]-malonate is labelled in the terminal unit of the poly-β-ketide chains. However, comparison of the relative molar activities of the *N*-(1-methylpropyl)-2,4-dinitroaniline and the barium carbonate from [1-^{14}C]-malonate (*Table 1*) shows that the terminal unit of the chain is less radioactive than the second unit. We interpret

Table 1. Distribution of label from diethyl [1-^{14}C]-malonate in sclerotiorin and rotiorin
Diethyl [1-^{14}C]-malonate (100 μC) gave sclerotiorin (4·5 μC) and rotiorin (1·8 μC)

Compound	(1) *Counts per h*	(2) *R.M.A.*	(3) *Calc. R.M.A. for distributions in Figure 1*
Sclerotiorin, (I)	6,950	146	—
Sclerotioramine, (XVII)	6,810	143	146
Di-*O*-acetylaposclerotioramine, (XXIX)	6,025	139	142
4,6-Dimethylocta-2,4-dienoic acid, (XI)	6,531	65·3	64·4
Barium carbonate from Schmidt reaction	19,096	19·1	19·4
N-(1-Methylpropyl)-2,4-dinitroaniline	612	6·12	6·16
Rotiorin, (VI)	4,050	93·4	—
Rotioramine, (XLVIII)	4,050	93·4	93·4
Aporotioramine, (XLIX)	4,140	91·1	89·9
Barium carbonate from reductive aromatization	3,560	3·56	3·55
Barium carbonate from Schmidt reaction	11,000	11·0	11·8
N-(1-Methylpropyl)-2,4-dinitroaniline	420	4·20	3·55

Table 2. Distribution of label from diethyl [2-^{14}C]-malonate in sclerotiorin and rotiorin
Diethyl [2-^{14}C]-malonate (100 μC) gave sclerotiorin (8·4 μC) and rotiorin (3·7 μC)

Compound	(1) *Counts per h*	(2) *R.M.A.*	(3) *Calc. R.M.A. for distributions in Figure 1*
Sclerotiorin, (I)	12,200	256	—
Sclerotioramine, (XVII)	12,200	256	256
Di-*O*-acetylaposclerotioramine, (XXIX)	10,400	239	247
4,6-Dimethylocta-2,4-dienoic acid, (XI)	11,300	113	111
Barium carbonate from Schmidt reaction	890	0·89	0·00
N-(1-methylpropyl)-2,4-dinitroaniline	4,305	43·1	42·9
Rotiorin, (VI)	20,600	474	—
Rotioramine, (XLVIII)	20,500	472	474
Aporotioramine, (XLIX)	21,300	468	459
Barium carbonate from reductive aromatization	2,700	2·70	0·00
Barium carbonate from Schmidt reaction	2,400	2·40	0·00
N-(1-methylpropyl)-2,4-dinitroaniline	7,600	76·0	76·1

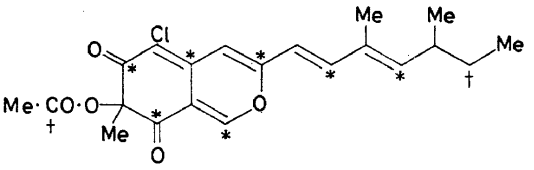
Table 3. Distribution of label from sodium [1-^{14}C]-butyrate in sclerotiorin and rotiorin
Sodium [1-^{14} C]-butyrate (500 μC) gave sclerotiorin (11·4 μC) and rotiorin (5·8 μC)

Compound	(1) *Counts per h*	(2) *R.M.A.*	(3) *Calc. R.M.A. for distributions in Figure 3*
Sclerotiorin, (I)	22,200	466	—
Sclerotioramine, (XVII)	22,300	468	466
Di-*O*-acetylaposclerotioramine, (XXIX)	18,100	416	414
4,6-Dimethylocta-2,4-dienoic acid, (XI)	21,100	210	207
Barium carbonate from Schmidt reaction	50,200	50·2	51·8
N-(1-methylpropyl)-2,4-dinitroaniline	5,080	50·8	51·8
Rotiorin, (VI)	10,400	239	—
Rotioramine, (XLVIII)	10,400	239	239
Aporotioramine, (XLIX)	9,100	200	201
Barium carbonate from reductive aromatization	38,200	38·2	38·2
Barium carbonate from Schmidt reaction	21,800	21·8	22·2
N-(1-methylpropyl)-2,4-dinitroaniline	2,240	22·4	22·2

this to mean that the biosynthesis of the main chain in sclerotiorin proceeds by the acetate + malonate pathway, but that some of the malonate is decarboxylated to acetate which is then incorporated into the terminal unit. On the assumption that the radioactivity of the terminal unit is a *direct* measure of this decarboxylation and further, that the main chain is derived by uniform incorporation of malonate into the chain-building units, then the percentage distribution of label may be calculated for sclerotiorin. This information is summarized for [1-^{14}C]- and [2-^{14}C]-malonate in *Figures 1a* and *2a* respectively. Comparison of columns (2) and (3) in *Tables 1* and *2* shows that this distribution agrees with the experimental findings.

Perhaps the most important observation in the experiments with rotiorin is that the acetoacetate equivalent which forms the β-oxolactone system does not appear to be derived from acetate + malonate. Thus, in rotiorin derived from [1-^{14}C]-malonate (*Table 1*), the relative molar activity of the terminal unit of the main chain is very similar to that of the second unit of the acetoacetate equivalent (determined as barium carbonate from reductive aromatization of rotioramine). Since the former unit comes directly from acetate (labelled by decarboxylation of malonate), it is highly likely that the latter unit also arises in the same way. The alternative explanation, namely that the acetoacetate is biosynthesized from acetate + malonate at a different period in time to that of the main chain, and therefore, labelled to a different extent, can be discounted, since in this case it would be expected that in [1-^{14}C]-acetate derived material there would be a similar difference in activity between the two chains and this is not the case. Hence the acetoacetate unit in rotiorin arises directly from two acetate units by a different pathway to that of acetate + malonate operating to the main chain. Calculated on the basis that the acetoacetate equivalent comes from acetate without participation of malonate, the distribution of label in rotiorin derived from [1-^{14}C]- and [2-^{14}C]-malonate is shown in *Figures 1b* and *2b* respectively. Comparison of columns (2) and (3) in *Tables 1* and *2* shows the agreement with experimental results.

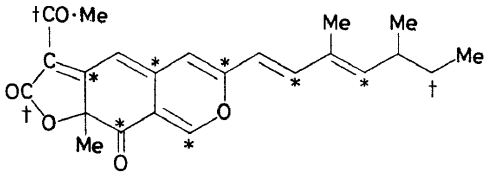
(*a*) Sclerotiorin



Carbons labelled † have 4·2% of the total activity

Carbons labelled * have 13·1% of the total activity

(*b*) Rotiorin

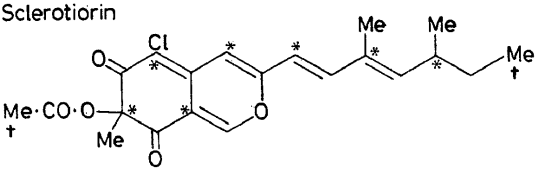


Carbons labelled † have 3·8% of the total activity

Carbons labelled * have 12·7% of the total activity

Figure 1. Calculated distribution of label in sclerotiorin and rotiorin derived from diethyl [1-^{14}C]-malonate

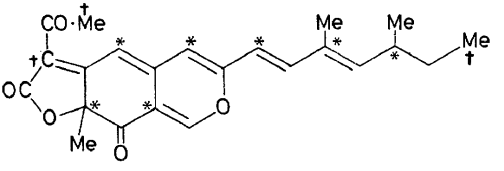
(*a*) Sclerotiorin



Carbons labelled † have 3·4% of the total activity

Carbons labelled * have 13·3% of the total activity

(*b*) Rotiorin



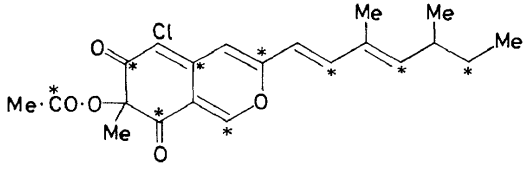
Carbons labelled † have 3·1% of the total activity

Carbons labelled * have 13·0% of the total activity

Figure 2. Calculated distribution of label in sclerotiorin and rotiorin derived from diethyl [2-^{14}C]-malonate

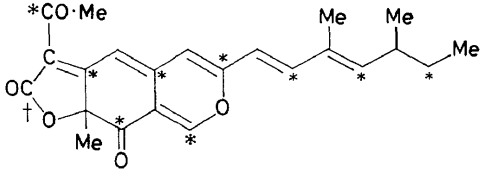
Experiments incorporating sodium [1-^{14}C]-butyrate into sclerotiorin (*Table 3*) show a distribution of label identical with that obtained by incorporation of sodium [1-^{14}C]-acetate. Hence the butyrate must be degraded to acetate before incorporation. Further, the equal distribution of label in the first two units of the main chain precludes the possibility that a four-carbon unit from butyrate forms a terminal unit in the chain. The experimental results (*Table 3*) clearly show that the main chain of rotiorin formed from [1-^{14}C]-butyrate is derived as in sclerotiorin. However, the carboxyl carbon atom of the β-oxolactone system in rotiorin has a much higher activity than any other carbon atom in the molecule. This provides strong evidence for the view that the butyrate is oxidized to acetoacetate which is then incorporated as an intact unit. Calculation of the distribution of label on this basis is shown in *Figure 3b* and is in agreement with the

(*a*) Sclerotiorin



Carbons labelled * have 11·1% (1/9) of the total activity

(*b*) Rotiorin



Carbons labelled * have 9·3% of the total activity

Carbons labelled † have 16% of the total activity

Figure 3. Calculated distribution of label in sclerotiorin and rotiorin derived from sodium [1-^{14}C]-butyrate

experimental results in *Table 3*. These experiments thus indicate that two pathways of biosynthesis operate in rotiorin: the main chain is derived by the acetate + malonate pathway and the β-oxolactone system is derived directly from two acetate units.

In conclusion I wish to thank the Irish National Committee for Chemistry for the invitation to address this Meeting. It is a pleasure to express my gratitude to Professor Alexander Robertson, F.R.S., for introducing me to this fascinating problem. I am

indebted to Dr J. S. E. Holker and to the many research men whose skill and enthusiasm were ultimately responsible for the successful outcome of these investigations. Dr L. M. Jackman kindly determined the nuclear magnetic resonance spectra and Dr J. B. Matthews and Mr A. Quayle of the Shell Refining Co. Ltd., were generously responsible for the mass spectral determinations.

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