371. *The Chemistry of Fungi. Part XXXIII.* The Oxidation of Sclerotioramine and the Structure of Sclerotiorin.*

By H. C. Fielding, Alexander Robertson, R. B. Travers, and W. B. Whalley.

Ozonolysis of *O*-acetylsclerotioramine gives sclerotaminic acid, $C_{13}H_{10}O_6NCl$, which exhibits the properties of a substituted pyridonecarboxylic acid and on reductive aromatisation yields acetic acid together with *apo*sclerotaminic acid (Part XXX [1]). Oxidation of sclerotioramine, and of *apo*sclerotioramine with nitric acid, forms berberonic acid whilst the action of alkaline hydrogen peroxide on tetrahydrosclerotioramine produces (+)-2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid.

The structure of sclerotiorin is discussed.

The structure of *apo*sclerotioramine, formed by the reductive aromatisation of sclerotioramine, was discussed in Part XXX [1] and the present communication deals with the oxidation of the parent amine and its derivatives. In the decomposition of the ozonide from *O*-acetylsclerotioramine the alkyl side-chain of the base is removed as 2 : 4-dimethylhexa-2-enal (I; see Chart), the acetoxy-residue is hydrolysed, and there is formed sclerotaminic acid, $C_{13}H_{10}O_6NCl$, which contains a *C*-methyl group, retains the kernel of sclerotioramine and exhibits the pyridone-like properties [1] of its precursor. Thus, on methylation with diazomethane the acid gave methyl *N*-methylsclerotaminate, $C_{13}H_8O_5Cl(NMe)\cdot OMe$, and with methyl iodide–potassium carbonate formed methyl *O*-methylsclerotaminate, $C_{13}H_8O_4NCl(OMe)_2$ and with methyl sulphate–potassium carbonate a mixture of methyl *O*-methyl- and methyl *N*-methyl-sclerotaminate.

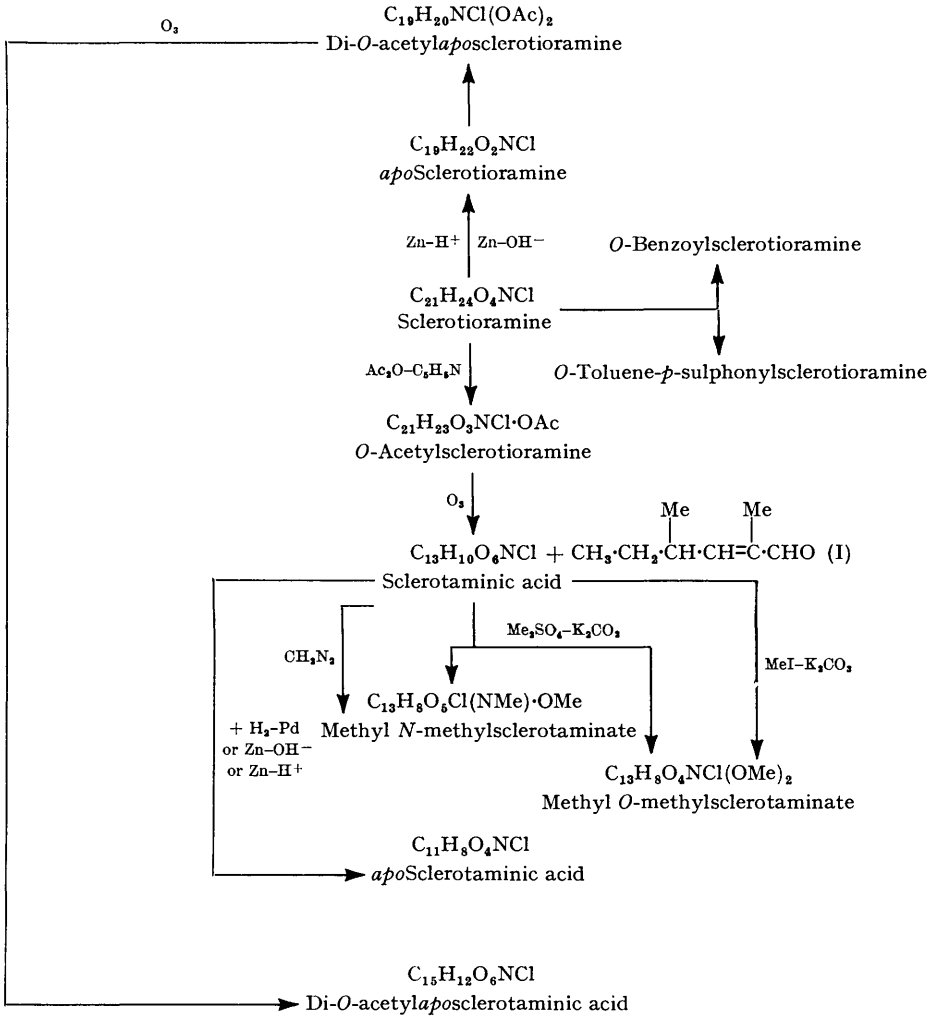
As with the parent sclerotioramine, reductive aromatisation of sclerotaminic acid is effected with hydrogen and a palladium or platinum catalyst in alcohol or acetic acid or with zinc and alkali or with zinc and acid, and is accompanied by simultaneous elimination of two carbon atoms as acetic acid, giving rise to *apo*sclerotaminic acid which is identical with the acid obtained by the ozonolysis of di-*O*-acetyl*apo*sclerotioramine followed by deacetylation of the product; [1] sclerotaminic acid and its derivatives show considerable resistance to oxidation with ozone. The formation of *apo*sclerotaminic acid by the alternative routes proves that the C_9 alkyl side-chain does not participate in any of the reactions involved. The interaction of sclerotioramine with benzoyl chloride and toluene-*p*-sulphonyl chloride affords derivatives which, by analogy with the acetate of the base,[1] are formulated as the *O*-benzoate and the *O*-toluene-*p*-sulphonate. On ozonolysis these derivatives give rise to the corresponding *O*-benzoyl-, $C_{20}H_{14}O_7NCl$, and *O*-toluene-*p*-sulphonyl-sclerotaminic acid, $C_{20}H_{16}O_8NClS$, respectively. The foregoing inter-relations are summarised in the Chart.

On oxidation with nitric acid sclerotioramine and its tetrahydro-derivative gave rise to berberonic acid (pyridine-2 : 4 : 5-tricarboxylic acid) (II), and oxidation of tetrahydro*apo*-sclerotioramine with potassium permanganate or alkaline hydrogen peroxide furnished an optically active, dicarboxylic acid, $C_{16}H_{23}O_4N$, which is also formed by the oxidation of tetrahydrosclerotioramine with the same reagents and is characterised as the dimethyl ester. This acid, which has a negative ferrous sulphate reaction in alcohol and thus does not have a carboxyl group in *ortho*-position to the nitrogen atom, gives berberonic acid on oxidation with nitric acid; and, since it exhibits a fluorescein reaction in the usual manner, it is a substituted cinchomeronic acid containing the C_9 saturated alkyl side-chain of tetrahydrosclerotioramine and tetrahydro*apo*sclerotioramine which on oxidation provides the 2-carboxyl group of berberonic acid. In agreement with its general properties and

* Part XXXII, preceding paper.

[1] Fielding, Graham, Robertson, Travers, and Whalley, *J.*, 1957, 4931.

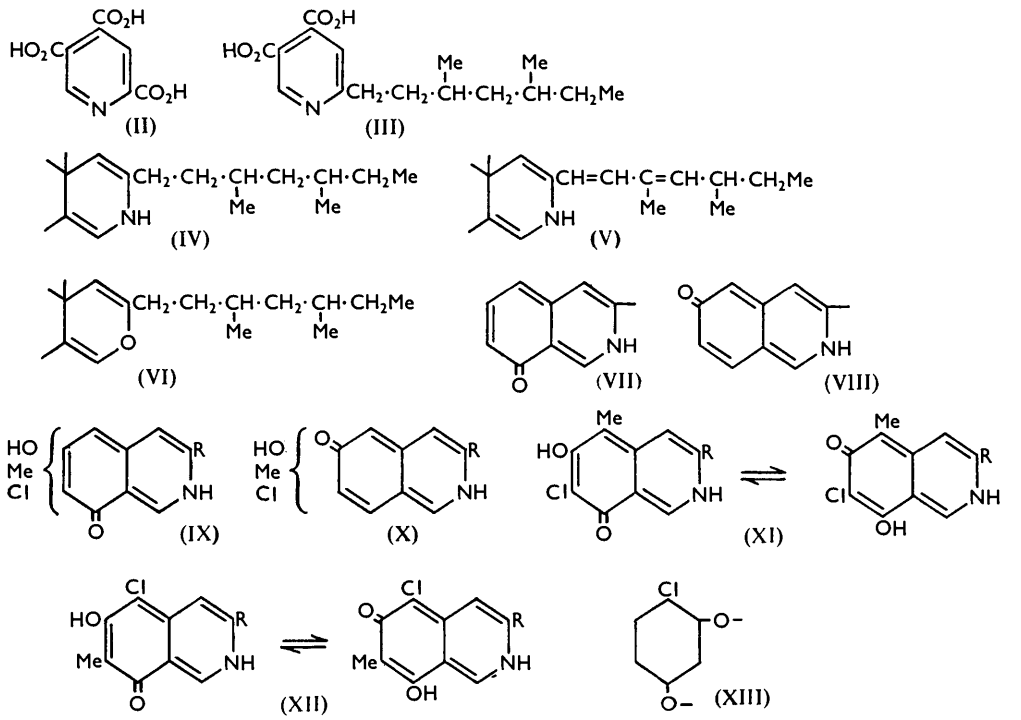
molecular rotation this substituted cinchomeronic acid is clearly (+)-2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid (III), and from the comparatively mild conditions employed in its production it is reasonably certain that the residue (IV) obtains in tetrahydrosclerotioramine and hence the system (V) is present in sclerotioramine, a view



compatible with the formation of the amino-compounds from a progenitor of the type (VI) on the assumption that this conversion does not involve a rearrangement (cf. Part XXVIII [3]). Further, the formation of the acid (III) supports conclusively the *iso*quinoline structures proposed in Part XXX [1] for *apo*sclerotaminic acid and its derivatives and, moreover, this heterocyclic kernel cannot be an α-*iso*quinolone but must be an *iso*quinolone of the type (VII) or (VIII), containing a chlorine atom, a hydroxyl group, and a *C*-methyl residue which does not arise in the aromatisation process since, from the Kuhn–Roth estimation, sclerotaminic acid also contains a *C*-methyl group. Hence the formula for *apo*sclerotaminic acid and for *apo*sclerotioramine may be expanded to (IX; R = CO_2H) and

[2] Graham, Page, Robertson, Travers, Turner, and Whalley, *J.*, 1957, 4924.
[3] Eade, Page, Robertson, Turner, and Whalley, *J.*, 1957, 4913.

(IX; R = C_9H_{15}) or (X; R = CO_2H) and (X; R = C_9H_{15}) respectively. Since *apo*-sclerotaminic acid and tetrahydro*apo*sclerotioramine do not give a ferric reaction and are not readily oxidised it is reasonably certain that the hydroxyl group and the quinolone-oxygen atom are in the *meta*-position to each other and thus the compounds are resorcinol derivatives and not catechol or quinol derivatives; consequently *apo*sclerotaminic acid and *apo*sclerotioramine are respectively represented by (XI or XII; R = CO_2H) and

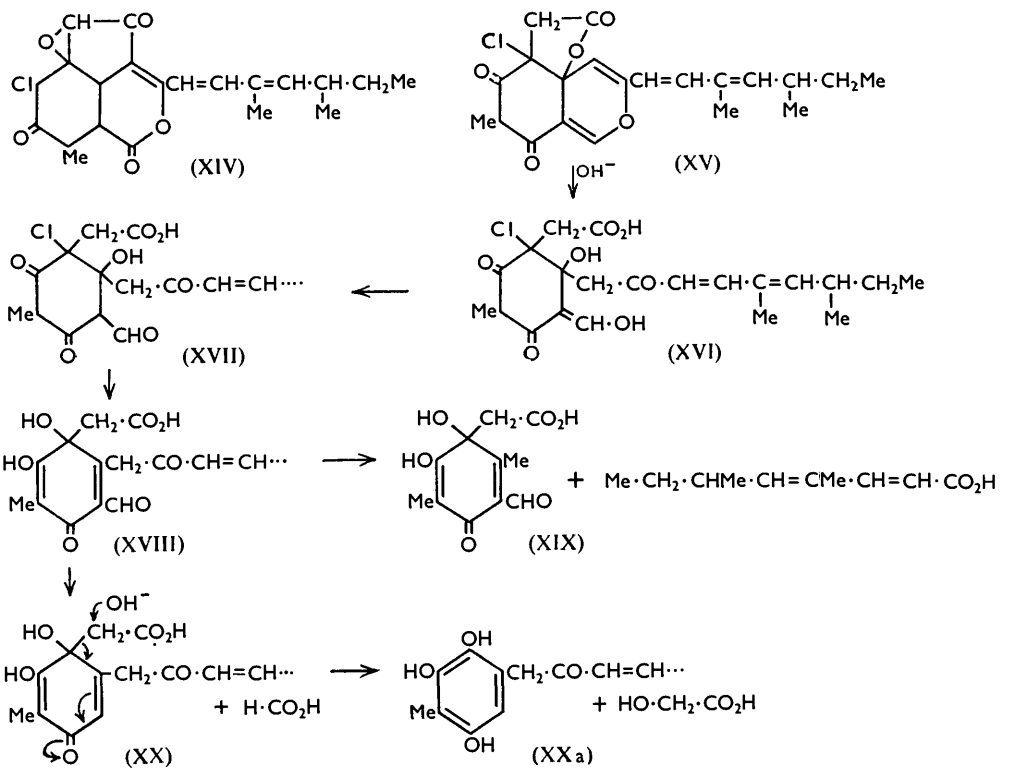


(XI or XII; R = C_9H_{15}). The formation of tetrahydrosclerotoquinone from tetrahydrosclerotiorin (Part XXIX [2]) suggests that in tetrahydrosclerotiorin the chlorine atom is situated as in (XIII) relatively to the potential hydroxyl groups and it follows that *apo*-sclerotaminic acid has the orientation (XII; R = CO_2H) or a tautomeric modification of it, with *apo*sclerotioramine as (XII; R = C_9H_{15}); rational formulations for the various *O*- and *N*-methyl derivatives [1] follow from the structures of the parent compounds.

The Structure of Sclerotiorin

In view of the exceptionally mild conditions under which this metabolite reacts with ammonia to form the base sclerotioramine [3] it seems reasonable to assume that the replacement of the heterocyclic oxygen atom is not accompanied by an intramolecular rearrangement, a view which is compatible with the infrared data. Further, the ready reductive aromatisation of sclerotioramine and its derivatives to yield the respective *apo*-compounds, together with approximately one molecular proportion of acetic acid, under a wide variety of conditions, affords substantial evidence that a molecular rearrangement does not occur during the aromatisation. On the basis of these assumptions in conjunction with the structures deduced earlier [1-3] for the degradation products of sclerotiorin, the derivation of a complete formulation for sclerotioramine and hence of the parent metabolite implies the insertion into the *apo*sclerotioramine system of a residue $C_2H_2O_2$, in which the ·C·C·O· is intact to account for the production of acetic acid during aromatisation. In this

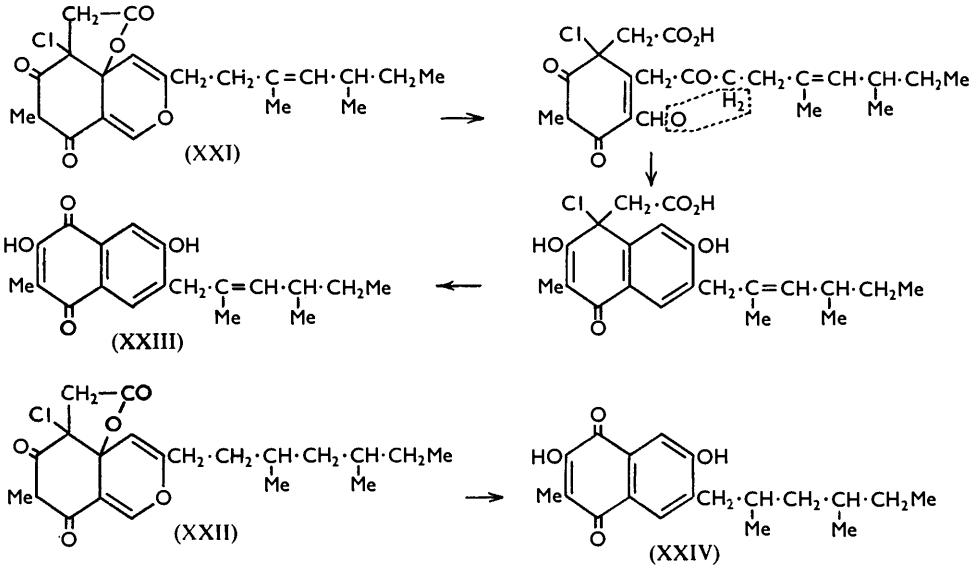
connection it may be noted that the isolation of the oxidation products, the C_9-alkylcinchomeronic acid and berberonic acid described in the present work, excludes the type of structure (XIV) proposed provisionally.[4] On the basis of the experimental facts now available there are several ways in which the $C_2H_2O_2$ residue may be inserted (by reversing the aromatisation process) into the *apo*sclerotioramine system so as to comply with the requisite empirical formula. Of the structures which may thus be derived for sclerotioramine and hence for sclerotiorin, (XV) appears at present to be the most acceptable for the metabolite. On the basis of (XV) the degradation of sclerotiorin with alkali would be expected to proceed by way of (XVI), (XVII), XVIII), (XIX), and (XX) (although not necessarily in this sequence) with the ultimate production of 4 : 6-dimethylocta-2 : 4-dienoic acid along with formic acid. Similarly, by the series of reactions previously suggested [2] di- and tetra-hydrosclerotiorin (XXI) and (XXII) could give rise to di- and tetra-hydrosclerotoquinone (XXIII) and (XXIV) respectively together with formic acid accompanied



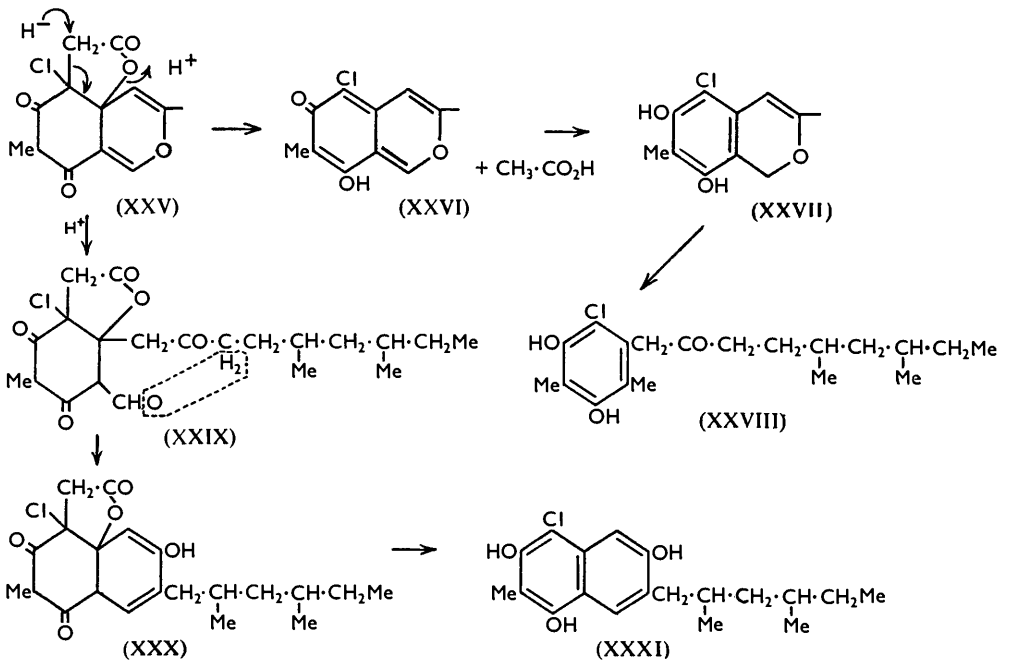
by the extrusion of the C_2 fragment as glycollic acid as in (XX). The alternative genesis of these quinones from an intermediate of, *e.g.*, type (XVIII) with the elimination of the C_2 residue by a retroaldol condensation would furnish acetic acid. The repeated failure to isolate acetic acid in the alkaline degradation of sclerotiorin (and of di- and tetrahydrosclerotiorin) may be due to the predominance of the aromatisation sequence of type (XX) ⟶ (XXa) in which the C_2 fragment is eliminated as glycollic acid, a view in keeping with the failure of β-hydroxy-acids to undergo the retroaldol condensation readily, and with the similar aromatisation of *O*-methylhomofuscin,[5] geodin,[6] and erdin.[6] In connection with the alkaline degradation of sclerotiorin and its derivatives it must be

[4] Powell, Robertson, and Whalley, *Chem. Soc. Special Publ.*, 1956, No. 5, p. 27.
[5] Barton and Hendrickson, *J.*, 1956, 1028.
[6] Calam, Clutterbuck, Oxford, and Raistrick, *Biochem. J.*, 1947, **41**, 458.

noted that the yields of characterisable products are invariably poor and that much intractable resin is produced.



In the prolonged hydrogenation of sclerotiorin (XV) or of tetrahydrosclerotiorin (XXII) the reaction probably proceeds by concerted attack of H^+ and H^- as indicated in formula (XXV) to yield acetic acid together with the pyrono-hemiquinone (XXVI) which

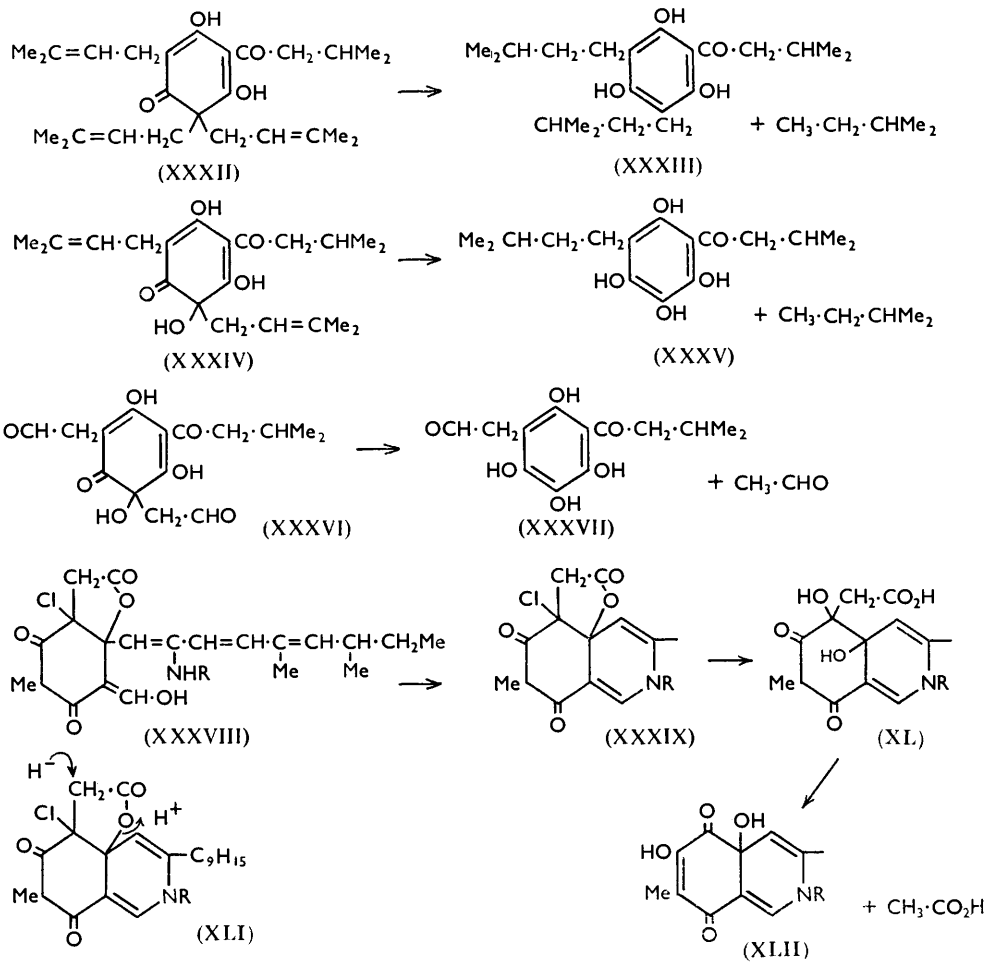


then gives the dihydro-derivative (XXVII) (cf. the hydrogenation of, *e.g.*, citrinin [7]) and ultimately sclerotinol [1] (XXVIII) by fission of the vinyl ether system in (XXVII). The

[7] Brown, Robertson, Whalley, and Cartwright, *J.*, 1949, 867.

acid-catalysed fission of tetrahydrosclerotiorin to an intermediate type (XXIX) and its subsequent cyclisation to (XXX) would account for the production of small quantities of sclerotol (XXXI) in addition to the major product, sclerotinol, when acetic acid is employed as the solvent for exhaustive hydrogenation.

This aromatisation of sclerotiorin with simultaneous extrusion of acetic acid has a close parallel in the behaviour of numerous morphine derivatives with acetic anhydride, *e.g.*, thebaine is thus aromatised to acetylthebaol with the extrusion of the *N*-methylethylamine residue as 2-acetoxyethyl-*N*-acetylmethylamine. This and the closely associated reactions in the morphine series have been interpreted by Stork [8] as proceeding



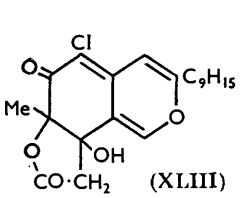
by synchronous attack of acetate anions and acetyl cations. Even closer analogies to the sclerotiorin series are provided by the reductive aromatisation of various natural and synthetic *gem*-substituted derivatives of phloroglucinol: thus aromatisation of lupulone (XXXII) by hydrogenation with a palladium catalyst at room temperature proceeds readily with extrusion of *iso*pentane [9,10] and the formation of the phloroglucinol (XXXIII), whilst humulone (XXXIV) furnishes the tetrahydric phenol (XXXV) and *iso*pentane.[9,10]

[8] Stork, in Manske and Holmes, " The Alkaloids," Academic Press, New York, 1952, p. 175.
[9] Riedl, *Chem. Ber.*, 1952, **85**, 692.
[10] Wieland, *Ber.*, 1925, **58**, 102, 2012.

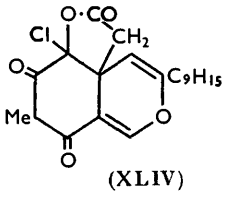
On the other hand reduction of lupulone (XXXII) with a platinum catalyst saturates the double bonds in the *iso*pentene side-chains and aromatisation does not occur in the absence of activation provided by the allyl system. The activation provided by the carbonyl of the $\cdot CH_2 \cdot CO \cdot$ system in schlerotiorin is not readily suppressed by hydrogenation and thus the extrusion of acetic acid occurs as readily with a platinum as with a palladium catalyst. Further, the allylic-lactone character of sclerotiorin will facilitate hydrogenolysis. Further analogies to the behaviour of sclerotiorin are provided in the aromatisation of (XXXVI) to (XXXVII) by zinc and very dilute acid [11] and of 4-dichloromethyl-4-methyl*cyclo*hexa-2 : 5-dienone with zinc and acetic acid to *p*-cresol and methylene chloride.[12] The aromatisation of lupulone and related compounds has recently been interpreted by Riedl and Nickl [13] in terms of the synchronous attack of H^+ and H^- as suggested in the present work to explain the behaviour of sclerotiorin.

The interaction of sclerotiorin with ammonia or primary amines seems likely to proceed by way of an open-chain form (XXXVIII) of the formyl ketone system in sclerotiorin, and to be similar to the ready formation of 4-methoxycarbonyl-7-methyl-1-*iso*quinolone from 4-methoxycarbonyl-7-methyl*iso*coumarin or the hydrated open-chain acid derived from it.[14] It is clear that degradation of the resulting bases (XXXIX) with acid or alkali would not give rise to ammonia, formic acid, or 4 : 6-dimethylocta-2 : 4-dienoic acid but to a small quantity of acetic acid by a retroaldol condensation by way of (XL) to a product of type (XLII) which would be susceptible to further degradation to the intractable products characteristic of the prolonged action of acid or alkali on the parent base. The reductive aromatisation of sclerotioramine to *apo*sclerotioramine with the simultaneous extrusion of the C_2 fragment as acetic acid may be interpreted in the same manner as the first stage of the exhaustive hydrogenation of tetrahydrosclerotiorin, *i.e.*, by way of (XLI) to give (XII; $R = C_9H_{15}$).

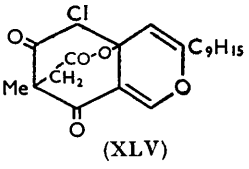
On the basis of formula (XV), sclerotiorin would be expected to exhibit reactions associated with the α-chloro-ketone system, *e.g.*, halogen exchange with sodium iodide in acetone. The metabolite, however, is moderately stable to prolonged heating with sodium



(XLIII)



(XLIV)



(XLV)

iodide in acetone. Steric considerations could be a factor in the resistance of the chlorine to replacement by iodine; *e.g.*, whilst phenacyl chloride is very rapidly converted into phenacyl iodide, α-chloro*iso*butyrophenone is extremely inert towards sodium iodide in acetone.[15] The infrared spectral data of tetrahydrosclerotiorin and its derivatives have previously [1] been tentatively interpreted by the assignment of the highest carbonyl frequency to an α-chloro-ketone, but on the present views this band must now be allocated to the γ-lactone and hence the carbonyl group of the α-chloro-ketone appears as the band of an isolated carbonyl group (1705—1709 $cm.^{-1}$). Investigations concerning the spectra of α-halogeno-ketones [16,17] have demonstrated that isolated-carbonyl frequency is observed if the chromophores are approximately at right angles.

The structure (XV) now suggested for sclerotiorin may be considered as arising

[11] Howard and Tatchall, *J.*, 1954, 2400.
[12] Auwers and Keil, *Ber.*, 1902, **35**, 4207.
[13] Riedl and Nickl, *Chem. Ber.*, 1956, **89**, 1838.
[14] Ungnade, Nightingale, and French, *J. Org. Chem.*, 1945, **10**, 533.
[15] Reeve, McCaffery, and Kaiser, *J. Amer. Chem. Soc.*, 1954, **76**, 2281.
[16] Jones, Ramsey, Herling, and Dobriner, *ibid.*, 1952, **74**, 2828.
[17] Bellamy, Thomas, and Williams, *J.*, 1956, 3704.

biogenetically from the addition of the elements of acetic acid to a fundamental quinone-like compound (XXVI), the reactivity of which would follow from the well-established behaviour of quinones in forming 2 : 3-adducts. On this basis alternative structures, *e.g.*, (XLIII), (XLIV), or (XLV), may be derived but as these are in one way or another incompatible with the present experimental findings they need not be further considered at present.

Experimental

Sclerotaminic Acid.—A stream of ozone and oxygen was passed into a solution of *O*-acetylsclerotioramine (2 g.) in ethyl acetate (20 ml.) at room temperature until the deep red solution became pale yellow (*ca.* 2 hr.). Decomposition of the ozonide with water (50 ml.) for 12 hr. yielded a red solid which on purification from aqueous methanol furnished *sclerotaminic acid* in scarlet needles or plates (0·8 g.), m. p. 201° (Found: C, 47·4; H, 3·6; N, 4·3; Cl, 11·2; *C*-Me, 5·4. $C_{13}H_{10}O_6NCl,H_2O$ requires C, 47·4; H, 3·8; N, 4·3; Cl, 10·8; *C*-Me, 5·0%). This acid is readily soluble in 2N-aqueous sodium hydrogen carbonate with effervescence, and gives an intense purple colour with ferrous sulphate in alcohol and a violet colour with copper sulphate.

Distillation of the aqueous liquors left on isolation of the solid gave (+)-α-methylbutyraldehyde, isolated and characterised as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 128°. When the time of ozonolysis was reduced to 1 hr. 2 : 4-dimethylhexa-2-enaldehyde was isolated and characterised as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 158°.

Treatment of sclerotaminic acid (0·5 g.) in methanol (10 ml.) with an excess of diazomethane for 15 min. gave *methyl* N-*methylsclerotaminate* which separated from aqueous methanol in brick-red needles (0·2 g.), m. p. 76° (Found: C, 52·6; H, 4·1; N, 4·2; OMe, 8·2. $C_{13}H_8O_5ClNMe{\cdot}OMe$ requires C, 53·0; H, 4·1; N, 4·1; OMe, 9·1%). This ester, which is insoluble in 2N-aqueous sodium hydrogen carbonate and has a negative ferrous sulphate reaction in alcohol, was recovered unchanged after treatment with methyl sulphate– or methyl iodide–potassium carbonate in boiling acetone for 2 hr. Methylation of sclerotaminic acid (0·5 g.) by methyl iodide–potassium carbonate in boiling acetone (25 ml.) for 1½ hr. gave *methyl* O-*methylsclerotaminate* which formed brick-red needles (0·25 g.), m. p. 108°, from aqueous methanol, with a negative ferrous sulphate reaction in alcohol [Found: C, 53·2; H, 4·0; N, 4·0; OMe, 17·0. $C_{13}H_8O_4NCl(OMe)_2$ requires C, 53·0; H, 4·1; N, 4·1; OMe, 18·3%]. This compound is insoluble in 2N-aqueous sodium carbonate and was recovered unchanged after having been heated with methyl sulphate–potassium carbonate in boiling acetone.

Methylation of sclerotaminic acid (0·5 g.) with methyl sulphate–potassium carbonate in boiling acetone (25 ml.) for 4 hr. gave a mixed product, m. p. 95—100°, which by chromatography from ether–benzene (2 : 1) on neutralised aluminium oxide followed by elution with the same solvent was resolved into methyl *O*-methylsclerotaminate (0·2 g.), m. p. and mixed m. p. 108°, and methyl *N*-methylsclerotaminate (0·05 g.), m. p. and mixed m. p. 76°.

A stream of ozone and oxygen was passed into a solution of sclerotaminic acid (1 g.) in ethyl acetate (50 ml.) at room temperature for 3 hr. and the solvent evaporated, leaving a colourless glass which was decomposed with water (50 ml.). Next day the red solid was purified from aqueous methanol, yielding unchanged starting material (0·6 g.), m. p. and mixed m. p. 200°. The residual aqueous liquors did not give a precipitate with aqueous 2 : 4-dinitrophenylhydrazine sulphate. Ozonolysis for much longer periods gave the same result. Under similar conditions methyl *N*-methylsclerotaminate was not attacked by ozone.

Di-O-acetylaposclerotaminic Acid.—(*a*) A solution of sclerotaminic acid (1 g.) in acetic acid (100 ml.) containing palladium–charcoal (from 1 g. of charcoal and 0·25 g. of palladium chloride) was shaken in hydrogen until absorption ceased (about 1 mol.) and the initially red solution became yellow. Evaporation of the filtered solution followed by purification of the residue from methanol gave *aposclerotaminic acid* in pale yellow needles (0·45 g.), m. p. >300° (Found: C, 48·4; H, 4·5; N, 5·1; *C*-Me, 4·5. $C_{11}H_8O_4NCl,H_2O$ requires C, 48·6; H, 3·7; N, 5·2; 1*C*-Me, 5·5%). On acetylation this compound gave di-*O*-acetyl*apo*sclerotaminic acid,[1] m. p. and mixed m. p. 233° (Found: C, 53·3; H, 3·5; N, 4·0. Calc. for $C_{15}H_{12}O_6NCl$: C, 53·4; H, 3·6; N, 4·2%), which with methanolic diazomethane furnished methyl di-*O*-acetyl*apo*sclerotaminate in needles, m. p. 184°, identical (mixed m. p. and infrared absorption spectrum) with a specimen obtained from di-*O*-acetyl*apo*sclerotioramine [1] (Found: C, 54·5; H, 4·2; N, 3·9. Calc. for

$C_{16}H_{14}O_6NCl$: C, 54·6; H, 4·0; N, 4·0%). When this hydrogenation was effected in alcohol (100 ml.) the yield of the same product was (0·6 g.).

(*b*) A solution of sclerotaminic acid (2 g.) in 2N-aqueous sodium hydroxide (20 ml.) was shaken with zinc dust (2 g.) until the deep red solution became yellow (*ca.* 15 min.). The mixture was filtered into an excess of 2N-sulphuric acid at 0° and the precipitate purified from methanol or dioxan, giving *apo*sclerotaminic acid in pale yellow needles (1·2 g.), m. p. >300° (Found: C, 48·7; H, 3·8; N, 5·0. Calc. for $C_{11}H_8O_4NCl,H_2O$: C, 48·6; H, 3·7; N, 5·2%). The di-*O*-acetate had m. p. and mixed m. p. 235° (Found: C, 53·3; H, 3·7; N, 4·2. Calc. for $C_{15}H_{12}O_6NCl$: C, 53·4; H, 3·6; N, 4·2%). Sclerotaminic acid (2 g.), which had been distilled with water under reduced pressure to remove traces of volatile acidic material, until the distillate was neutral, and which had a negative acetyl value, was degraded with zinc and alkali, and the acidic aqueous liquors remaining after the isolation of the crude *apo*sclerotaminic acid were distilled (with the addition of water as required) until all the volatile acid was removed. After being neutralised (phenolphthalein) with aqueous sodium hydroxide the distillate was evaporated and the sodium salts treated with *o*-phenylenediamine in the usual manner, yielding 2-methylbenziminazole, m. p. and mixed m. p. 176°.

(*c*) A solution of sclerotaminic acid (0·5 g.) in alcohol (50 ml.) containing platinic oxide (50 mg.) was shaken in hydrogen for 2 hr. Absorption of gas then ceased and the pale yellow solution deposited crystals. The warmed solution was filtered and the solvent evaporated, giving *apo*sclerotaminic acid which separated from alcohol in green-yellow needles (0·3 g.), m. p. >320°, and was identified by conversion into the diacetate, m. p. and mixed m. p. 235°.

Methylation of apo*Sclerotaminic Acid.*—Methylation of this acid (0·5 g.), suspended in ether, with diazomethane furnished *methyl* NO-*dimethyl*apo*sclerotaminate* which separated from dioxan in needles, m. p. 130°, insoluble in cold 2N-aqueous sodium hydrogen carbonate [Found: C, 56·4; H, 4·3; N, 5·0. $C_{11}H_5O_2Cl(NMe)(OMe)_2$ requires C, 56·9; H, 4·8; N, 4·7%].

Methylation of methyl *apo*sclerotaminate (0·6 g.) by methyl iodide–potassium carbonate in boiling acetone (30 ml.) for 3 hr. gave methyl di-*O*-methyl*apo*sclerotaminate which separated from aqueous acetone in needles (0·4 g.), m. p. 168°, identical (m. p., mixed m. p., and infrared spectrum) with the compound from di-*O*-acetyl*apo*sclerotioramine (Part XXX [1]) [Found: C, 57·0; H, 4·9; N, 5·3; OMe, 30·7. Calc. for $C_{11}H_5ONCl(OMe)_3$: C, 56·9; H, 4·8; N, 4·7; OMe, 31·5%]. The residue left on evaporation of the mother-liquors from the purification of methyl di-*O*-methyl*apo*sclerotaminate was chromatographed from benzene on activated aluminium oxide. Elution of a red band with the same solvent yielded an amorphous product. Subsequently a yellow band was eluted with methanol which provided a yellow solid and on crystallisation from the same solvent this gave methyl *NO*-dimethyl*apo*sclerotaminate in yellow needles, m. p. and mixed m. p. 224° [Found: C, 56·8; H, 4·5; N, 4·7; OMe, 21·5. Calc. for $C_{12}H_8O_2NCl(OMe)_2$: C, 56·9; H, 4·8; N, 4·7; OMe, 21·0%].

The same products were obtained in like proportion by methylation of *apo*sclerotaminic acid with methyl iodide– or methyl sulphate–potassium carbonate.

Hydrolysis of the di-*O*-methyl ester (1 g.) with boiling 2N-aqueous sodium hydroxide (25 ml.) for 1 hr. furnished *di*-O-*methyl*apo*sclerotaminic acid*, forming needles (0·7 g.), m. p. 227°, from methanol [Found: C, 55·5; H, 4·6; N, 4·7; OMe, 20·2; Cl, 12·0. $C_{11}H_6O_2NCl(OMe)_2$ requires C, 55·4; H, 4·3; N, 4·9; OMe, 22·0; Cl, 12·6%] which regenerated the ester with methyl iodide–potassium carbonate. Hydrolysis of the ester (0·2 g.) with boiling concentrated hydrochloric acid (15 ml.) for 3 hr. also gave di-*O*-methyl*apo*sclerotaminic acid (0·15 g.), m. p. and mixed m. p. 227°.

On being boiled with hydrobromic acid (2 ml.; *d* 1·49) for 4 hr. methyl di-*O*-methyl*apo*sclerotaminate (0·2 g.) gave *apo*sclerotaminic acid, m. p. >300°, identified by conversion into the di-*O*-acetate, m. p. and mixed m. p. 235°.

Chlorination of Methyl apo*Sclerotaminate.*—A solution of this ester (0·2 g.) in phosphorus oxychloride (5 ml.) was heated at 130° for 1 hr., and the cooled mixture poured on ice and neutralised with sodium hydrogen carbonate. Purification of the precipitate from dioxan gave a *chloro-derivative* (0·09 g.) of the ester, m. p. 175° in needles (Found: C, 49·5; H, 3·5; Cl, 19·8. $C_{12}H_9O_3NCl_2$ requires C, 50·4; H, 3·2; Cl, 24·8%).

O-*Toluene*-p-*sulphonate of Sclerotioramine.*—A solution of toluene-*p*-sulphonyl chloride (2 g.) in pyridine (10 ml.) was added to sclerotioramine (2 g.), dissolved in pyridine (20 ml.) at 0°, and after having been kept at 0° for 1 hr. and then at room temperature for 4 hr. the solution was again cooled to 0° and more toluene-*p*-sulphonyl chloride (2 g.) added; 24 hr. later the red

mixture was poured on ice, the product isolated with ether, and the ethereal solution washed successively with ice-cold 2N-aqueous sodium hydroxide, water, 2N-hydrochloric acid, and water. Evaporation of the dried solution left a syrup which crystallised from methanol, giving the O-*toluene*-p-*sulphonate* of sclerotioramine in yellow needles (2·1 g.), m. p. 154° (Found: C, 61·5; H, 5·6; N, 2·6; Cl, 7·2. $C_{28}H_{30}O_6NClS$ requires C, 61·7; H, 5·5; N, 2·6; Cl, 6·4%). On being warmed with concentrated ammonia or hydrochloric acid this ester slowly regenerated sclerotioramine.

A stream of ozone and oxygen was passed through a solution of the foregoing ester (0·2 g.) in ethyl acetate (25 ml.) for 15 min., and on evaporation of the solvent in a vacuum the residual ozonide was decomposed with water (25 ml.) for 12 hr., giving a colourless solid which on purification from methanol furnished the O-*toluene*-p-*sulphonate* of sclerotaminic acid in needles (0·08 g.), m. p. 174° (decomp.), soluble in cold 2N-aqueous sodium hydrogen carbonate and having a negative ferric chloride and ferrous sulphate reaction in alcohol (Found: C, 54·8; H, 3·3; N, 3·3; Cl, 9·1. $C_{20}H_{16}O_8NClS$ requires C, 51·6; H, 3·5; N, 3·0; Cl, 7·7%). Formed with diazomethane, the *methyl ester* separated from methanol in yellow needles, m. p. 146° (Found: C, 52·6; H, 3·7; Cl, 8·1. $C_{21}H_{18}O_8NClS$ requires C, 52·6; H, 3·8; Cl, 7·4%).

The aqueous liquors from the ozonolysis furnished (+)-α-methylbutyraldehyde, characterised as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 128°.

O-*Benzoylsclerotioramine*.—Sclerotioramine (2 g.), dissolved in pyridine (25 ml.), was treated at 0° with a solution of benzoyl chloride (1 ml.) in pyridine (5 ml.) and 3 hr. later the mixture was poured on ice and extracted with ether. The ethereal extract was successively washed with 2N-aqueous sodium hydroxide, water, 2N-hydrochloric acid, and water, dried, and evaporated, leaving a residue which on crystallisation from methanol gave O-*benzoylsclerotioramine* in yellow needles (1·5 g.), m. p. 186° (Found: C, 68·3; H, 5·7; N, 2·8. $C_{28}H_{28}O_5NCl$ requires C, 68·0; H, 5·7; N, 2·8%). Ozonolysis of this benzoate (0·5 g.) in ethyl acetate (25 ml.) at room temperature for ½ hr. followed by decomposition of the ozonide in the usual manner furnished O-*benzoylsclerotaminic acid* in needles (0·1 g.), m. p. 152° from methanol (Found: C, 57·4; H, 3·2; N, 3·6. $C_{20}H_{14}O_7NCl$ requires C, 57·8; H, 3·4; N, 3·4%).

Oxidation of Sclerotioramine.—(*a*) Concentrated nitric acid (*d* 1·52; 20 ml.) and water (1·5 ml.) were added (with cooling) to sclerotioramine (5 g.) and next day the mixture was heated on the steam-bath for 26 hr. with addition of more nitric acid (15 ml.) and water (1 ml.) after 6 hr. and 14 hr. respectively. The pale yellow solution was evaporated on the steam-bath to remove traces of nitric acid, and the residue treated with water (50 ml.) and again evaporated. A solution of this solid in water (50 ml.) was extracted with ether, treated with charcoal at 100°, filtered, concentrated, and kept. Berberonic acid (pyridine-2 : 4 : 5-tricarboxylic acid) then slowly separated in needles (1·2 g.), m. p. and mixed m. p. 245° (decomp.) (after drying) with rapid heating, having an infrared absorption spectrum identical with that of an authentic specimen prepared by the oxidation of berberine sulphate with nitric acid (Found: C, 45·4; H, 2·6; N, 6·7; O, 44·7; Cl, 0. Calc. for $C_8H_5O_6N$: C, 45·5; H, 2·4; N, 6·6; O, 45·5%).

Oxidation of tetrahydrosclerotioramine (1 g.) with nitric acid under similar conditions furnished berberonic acid (0·1 g.), m. p. and mixed m. p. 245° (decomp.), and having the requisite infrared absorption spectrum.

(*b*) A solution of potassium permanganate (1·5 g.) in water (30 ml.) was added gradually to sclerotioramine (2 g.) dissolved in 0·5N-aqueous sodium hydroxide (100 ml.) at 10°. The solution was cleared with sulphur dioxide, filtered, and extracted with ether to furnish (+)-α-methylbutyraldehyde, identified as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 132°. A solution of potassium permanganate (4 g.) in water (80 ml.) was added gradually to the residual aqueous solution at 80° and, when colourless, the filtered solution was acidified with hydrochloric acid and evaporated to dryness in a vacuum and a solution of the residual solid in water (30 ml.) extracted with ether; then the aqueous solution was evaporated to dryness and the residue extracted with hot alcohol (2 × 10 ml.). Evaporation of the alcohol furnished a sticky solid which was purified from hot water, to give berberonic acid (0·1 g.), m. p. and mixed m. p. 245° (decomp.), further identified by the infrared spectrum (Found: C, 46·2; H, 2·6; N, 6·6; O, 45·3; Cl, 0. Calc. for $C_8H_5O_6N$: C, 45·5; H, 2·4; N, 6·6; O, 45·5%).

The oxidation of *apo*sclerotaminic acid (1·5 g.) with potassium permanganate (3·5 g.) by the method used for the oxidation of sclerotioramine gave berberonic acid (90 mg.), m. p. and mixed m. p. 245° (decomp.).

*Oxidation of Tetrahydro*apo*sclerotioramine.*—(*a*) A solution of potassium permanganate (1·5 g.) in water (30 ml.) was added gradually in 30 min. to tetrahydro*apo*sclerotioramine (1 g.) in 0·5N-aqueous sodium hydroxide (20 ml.) and the reaction completed by warming the mixture to 70°. The cooled mixture was filtered, acidified with 2N-hydrochloric acid, and extracted with ether, and the extract washed with 2N-aqueous sodium hydrogen carbonate. The ether solution then contained a negligible quantity of product but acidification of the bicarbonate extract yielded a precipitate of 2-(3 : 5-*dimethyl*-n-*heptyl*)*pyridine*-4 : 5-*dicarboxylic acid* which on purification from benzene–alcohol or aqueous acetone formed needles (0·2 g.), m. p. 194°, readily soluble in 2N-aqueous sodium hydrogen carbonate, having a negative ferric chloride and ferrous sulphate reaction in alcohol but giving an intense fluorescein reaction (Found: C, 65·6; H, 7·7; N, 4·9; O, 19·6; Cl, 0. $C_{16}H_{23}O_4N$ requires C, 65·5; H, 7·9; N, 4·8; O, 21·0%), $[\alpha]_D^{20}$ +12° (*c* 10·18 in EtOH), $[M]_D^{20}$ +3396°.

(*b*) Hydrogen peroxide (2 ml. of 100-vol.) at 0° was added to a solution of tetrahydro*apo*-sclerotioramine (0·6 g.) in N-aqueous sodium hydroxide (10 ml.), and the mixture was kept at this temperature for 18 hr., then gently warmed until effervescence began, cooled, acidified with 2N-hydrochloric acid, and extracted with ether. Evaporation of the dried extract furnished 2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid (0·13 g.), m. p. and mixed m. p. 194° after purification, which had an infrared absorption spectrum identical with that of the specimen prepared as by (*a*) (Found: C, 65·0; H, 7·8; N, 4·7%).

Oxidation of Tetrahydrosclerotioramine.—Prepared at 0°, a mixture of hydrogen peroxide (3 ml. of 100-vol.) and tetrahydrosclerotioramine (1 g.) in 2N-aqueous sodium hydroxide (20 ml.) was kept at 0° for 20 hr. with the addition of more peroxide (3 ml. of 100-vol.) after 2 hr., acidified, and extracted with ether. Evaporation of the dried extract left 2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid, forming needles (0·2 g.), m. p. and mixed m. p. 194°, from aqueous acetone (Found: C, 65·8; H, 7·8; N, 5·1. Calc. for $C_{16}H_{23}O_4N$: C, 65·6; H, 7·9; N, 4·8%). Esterification of this acid (0·8 g.) with ethereal diazomethane (20 ml.) at 0° furnished *dimethyl* 2-(3 : 5-*dimethyl*-n-*heptyl*)*pyridine*-4 : 5-*dicarboxylate* as a pale yellow oil (0·5 g.), b. p. 170°/0·75 mm., insoluble in 2N-aqueous sodium hydrogen carbonate [Found: C, 67·2; H, 8·5; N, 4·6; OMe, 18·3. $C_{16}H_{21}O_2N(OMe)_2$ requires C, 67·3; H, 8·5; N, 4·4; OMe, 19·3%].

Oxidation of 2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid (0·5 g.) with nitric acid by the method described previously gave berberonic acid (0·05 g.), m. p. and mixed m. p. 245° (decomp.), having the requisite infrared absorption spectrum.

Degradation of Sclerotiorin with Acid.—A suspension of sclerotiorin (10 g.) in 10N-sulphuric acid (250 ml.) was heated under reflux for 32 hr. by which time most of the sclerotiorin had dissolved. The cooled, diluted mixture was filtered and distilled with the addition of distilled water to maintain the volume of reaction liquid. The acid distillate was neutralised (phenolphthalein) with N-aqueous sodium hydroxide and evaporated, and the sodium salts (2 g.) were converted into methylbenziminazole (150 mg.), m. p. and mixed m. p. 174°. The mixed m. p. with benziminazole was *ca.* 148°.

Exhaustive Hydrogenation of Sclerotiorin in Alcohol.—A solution of sclerotiorin (5 g.) in sodium-treated alcohol (250 ml.) containing platinic oxide (50 mg.) was shaken in hydrogen until approximately 6 mols. of hydrogen had been absorbed (*ca.* 3 hr.). The colourless filtrate was saturated with ammonia, then the alcohol was removed under reduced pressure and the residue extracted with hot distilled water (20 ml.). Evaporation of the aqueous solution gave a colourless, crystalline residue (3 g.) which by interaction with *o*-phenylenediamine in the usual manner gave 2-methylbenziminazole (0·1 g.), m. p. and mixed m. p. 174° (mixed m. p. with benziminazole, *ca.* 154°). A control experiment under the same conditions gave no inorganic salts.

The ultraviolet absorption spectra were measured in 95% alcohol with a Unicam S.P. 500 Spectrophotometer, and the infrared spectral data were obtained in Nujol on a Grubb-Parsons S.3 double-beam spectrophotometer. The analyses were performed by Mr. A. S. Inglis, M.Sc., and his associates of this Department.

UNIVERSITY OF LIVERPOOL.

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