804. *The Chemistry of Fungi. Part XL.*[1] *Further Evidence for the Structure of Sclerotiorin.*

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Treatment of methylated derivatives of aposclerotioramine and sclerotinol with potassium permanganate has given, in each case, a compound shown to be 6-chloro-3,5-dimethoxytrimellitic acid (V). Oxidation of di-*O*-acetylsclerotinol with chromic oxide, followed by saponification, gave an acid, shown to be 2-chloro-3,5-dihydroxy-4,6-dimethylphenylacetic acid (XI) by unequivocal synthesis of the dimethyl ether. These results, in conjunction with previous evidence, confirm the structures (IIIa) and (VI) previously assigned to aposclerotioramine and sclerotinol, respectively. Consequently, a firmer foundation is laid for structure (Ia) for sclerotiorin.

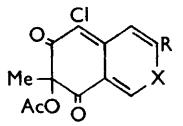
Although the structure (Ia), proposed for the mould metabolite sclerotiorin, is supported by much evidence,[2-6] no rigid proof of it has yet been offered. We now report further degradative and synthetic experiments, which in conjunction with previous evidence, permit the unique structural definition of a number of products derived from sclerotiorin.

The conversion of sclerotiorin into sclerotioramine by ammonia, and reductive aromatisation of this compound to aposclerotioramine, have been described.[3,5] The formulæ (Ia) for sclerotiorin and (IIa) for sclerotioramine depend to a large extent on structure (IIIa) for aposclerotioramine.[2,6] Confirmation of the latter structure has now been obtained. Conversion of aposclerotioramine into the dimethyl ether methosulphate [5] (IV) and oxidation of this with potassium permanganate gave 6-chloro-3,5-dimethoxytrimellitic acid (V). The proof of this structure (V) rests on the facts that, when heated, the acid (V) formed an anhydride still containing a free carboxyl group, and that when this anhydride was treated with boiling concentrated hydrochloric acid it formed 2-chloro-3,5-dihydroxybenzoic acid, characterised as the dimethyl ether. With boiling hydriodic acid under similar conditions, the anhydride formed 3,5-dihydroxybenzoic acid. The formation of acid (V) in the above degradation of the aposclerotioramine derivative (IV), and the production of pyridine-2,4,5-tricarboxylic acid by oxidation of sclerotioramine with nitric acid,[6] and of (+)-2-(3,5-dimethylheptyl)pyridine-4,5-dicarboxylic acid by oxidation of tetrahydrosclerotioramine (IIb) or its apo-derivative (IIIb) with potassium permanganate or hydrogen peroxide [6] clearly establish structure (IIIa) for aposclerotioramine.

Exhaustive hydrogenation [3] of sclerotiorin results in the expulsion of two carbon atoms and the formation of two compounds sclerotinol, $C_{19}H_{29}ClO_3$, and sclerotol, $C_{19}H_{27}ClO_3$, which were tentatively formulated as (VI) and (IX), respectively.[6] It has now been shown that, in keeping with structure (VI) for sclerotinol, its dimethyl ether [3] (VII) gives the

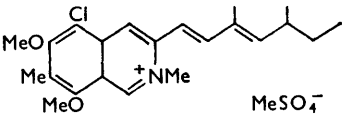
[1] Part XXXIX, Fielding, Holker, Jones, Powell, Richmond, Robertson, and Whalley, *J.*, 1961, 4579.
[2] Dean, Staunton, and Whalley, *J.*, 1959, 3004.
[3] Eade, Page, Robertson, Turner, and Whalley, *J.*, 1957, 4913.
[4] Graham, Page, Robertson, Travers, Turner, and Whalley, *J.*, 1957, 4924.
[5] Fielding, Graham, Robertson, Travers, and Whalley, *J.*, 1957, 4931.
[6] Fielding, Robertson, Travers, and Whalley, *J.*, 1958, 1814.

tricarboxylic acid (V) on oxidation with potassium permanganate. Complete confirmation of the structure (VI) for sclerotinol followed from oxidation of the di-*O*-acetyl derivative[3] (VIII) with chromic oxide to a diacetoxy-acid, $C_{13}H_{15}ClO_6$, which has been shown as follows to have structure (X). Saponification gave the parent dihydroxy-acid,

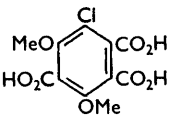


(I ; X = O)
(II ; X = NH)
(a : R = ·CH:CH·CMe:CH·CHMeEt)
(b : R = ·CH_2·CH_2·CHMe·CH_2·CHMeEt)

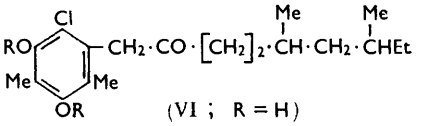
(III)



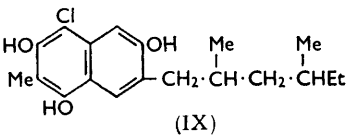
(IV)



(V)



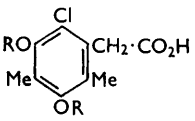
(VI ; R = H)
(VII ; R = Me)
(VIII ; R = Ac)



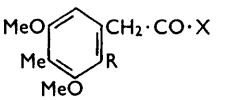
(IX)

which was converted into the dimethoxy-acid by reaction with dimethyl sulphate and potassium carbonate in acetone. The dimethoxy-acid has been shown to have structure (XII) by unequivocal synthesis.

The complete elucidation of the structures of aposclerotioramine (IIIa) and sclerotinol (VI), together with arguments previously presented,[2] provide overwhelming support for structure (IIa) for sclerotiorin.



(X ; R = Ac)
(XI ; R = H)
(XII ; R = Me)



(XIII ; R = H, X = OH)
(XIV ; R = H, X = NH_2)
(XV ; R = H, X = OMe)
(XVI ; R = CHO, X = OMe)
(XVII ; R = Me, X = OEt)
(XVIII ; R = Me, X = OH)

Synthesis of 2-Chloro-3,5-dimethoxy-4,6-dimethylphenylacetic acid (XII).—3,5-Dimethoxy-*p*-toluic aid was converted into the homologous phenylacetic acid (XIII) by the Arndt–Eistert procedure, Wolff rearrangement of the diazo-ketone being carried out with ammonia and silver oxide to give the amide (XIV), which was then hydrolysed to the acid (XIII). The methyl ester (XV) of this acid was converted into the 2-formyl derivative (XVI) by the Gattermann procedure or, in much higher yield, by using dichloromethyl methyl ether and aluminium chloride according to the directions of Rieche and his co-workers.[7] Reduction of the methyl ester (XVI) under the Clemmensen conditions in aqueous ethanol was accompanied by trans-esterification to give the ethyl ester (XVII), which was converted into the free acid (XVIII) on saponification. Chlorination of the ester (XVII) with sulphuryl chloride and aluminium chloride, which leads to nuclear rather than side-chain halogenation,[8] followed by saponification of the product, gave 2-chloro-3,5-dimethoxy-4,6-dimethylphenylacetic acid (XII) identical with the corresponding compound derived from sclerotinol.

[7] Rieche, Gross, and Höft, *Chem. Ber.*, 1960, **93**, 88.
[8] Silberrad, *J.*, 1921, **119**, 2029; 1922, **121**, 1015; 1925, **127**, 2677.

Experimental

Infrared absorption measurements were made on dispersions in mineral oil with a Perkin–Elmer model 21 instrument. Light petroleum used had b. p. 60—80°.

Oxidation of NOO-*Trimethylaposclerotioramine Methyl Sulphate* (IV).—A stirred suspension of this compound [5] (2 g.) in boiling N-sodium hydroxide (500 ml.) was treated with potassium permangante (11 g., in small portions) during 8 hr. and the mixture was boiled for a further 2 hr. The manganese dioxide was collected and washed with hot water, and the combined filtrate and washings were acidified with 2N-sulphuric acid and treated at 80° with an excess of potassium permanganate to destroy oxalic acid. After removal of the excess of oxidising agent with sulphur dioxide, the solution was concentrated to 400 ml. and continuously extracted with ether during 12 hr. After evaporation of the ether, the residue (1 g.) was dissolved in acetone (20 ml.) and the solution diluted with benzene (180 ml.); the *monohydrate* of 6-chloro-3,5-dimethoxytrimellitic acid (V) separated in needles (0·2 g.), which after repeated crystallisation from acetone–benzene, had m. p. 218° (decomp.), $\nu_{max.}$ 3185, 2862, 1700, 1592, and 1572 cm.$^{-1}$ [Found: C, 40·9; H, 3·3; OMe, 19·2. $C_9H_3ClO_6(OMe)_2,H_2O$ requires C, 40·9; H, 3·4; OMe, 19·2%]. The acetone–benzene mother-liquors were percolated through a column (20 × 1·5 cm.) of silica gel, and the eluate (200 ml.) evaporated, giving 6-*chloro*-3,5-*dimethoxytrimellitic acid anhydride*, needles (0·3 g.; from benzene), m. p. 197—198°, $\nu_{max.}$ 3030—2860, 1852, 1786, 1724, 1639, and 1587 cm.$^{-1}$ [Found: C, 46·5; H, 2·7; OMe, 21·5; Cl, 12·4. $C_9HClO_5(OMe)_2$ requires C, 46·2; H, 2·4; OMe, 21·9; Cl, 12·2%]. Further elution with acetone–benzene (1 : 9) (200 ml.) gave 6-*chloro*-3,5-*dimethoxytrimellitic acid* (V) which separated from acetone–benzene in prisms (0·2 g.), m. p. 200° (decomp.), $\nu_{max.}$ 2862, 1700, and 1653 cm.$^{-1}$ [Found: C, 43·4; H, 3·1; OMe, 21·6; Cl, 11·6. $C_9H_3ClO_6(OMe)_2$ requires C, 43·4; H, 3·0; OMe, 21·7; Cl, 11·5%]. Finally, elution with acetone–benzene (1 : 1) (100 ml.) gave needles (20 mg.), m. p. 218° (decomp.), of the monohydrate of 6-chloro-3,5-dimethoxytrimellitic acid.

Both 6-chloro-3,5-dimethoxytrimellitic acid and its monohydrate, when sublimed at 190°/0·5 mm., gave 6-chloro-3,5-dimethoxyanhydrotrimellitic acid, m. p. and mixed m. p. 197—198°.

Degradation of 6-*Chloro*-3,5-*dimethoxyanhydromellitic Acid.*—(*a*) *With hydrochloric acid.* A solution of the above acid anhydride (200 mg.) in concentrated hydrochloric acid (10 ml.) was heated under reflux for 15 hr. The product was isolated in ether (3 × 10 ml.) and crystallised from water as prisms (70 mg.), m. p. 252°, undepressed on admixture with 2-chloro-3,5-dihydroxybenzoic acid (m. p. 256—258°). This product (70 mg.) with dimethyl sulphate (200 mg.) and anhydrous potassium carbonate (1 g.) in boiling acetone (4 ml.) gave in 3 hr. a crude product which was saponified with boiling 2N-sodium hydroxide (10 ml.) containing methanol (1 ml.) for 1 hr. Acidification of the cooled mixture gave a precipitate of 2-chloro-3,5-dimethoxybenzoic acid, needles, m. p. 183° (from aqueous methanol) [Found: C, 49·7; H, 4·3; OMe, 28·9; Cl, 15·9. Calc. for $C_7H_3O_2Cl(OMe)_2$: C, 50·0; H, 4·2; OMe, 28·7; Cl, 16·2%]. This material had m. p., mixed m. p., and infrared spectrum identical with those of an authentic sample.

(*b*) *With hydriodic acid.* The acid anhydride (200 mg.) in acetic acid (4 ml.) and constant-boiling hydriodic acid (40 ml.) were heated under reflux until a test drop, on evaporation, gave material developing no colour in alcohol with a solution of ferric chloride. Removal of the solvent *in vacuo* and crystallisation of the residue from water gave needles (30 mg.) of 3,5-dihydroxybenzoic acid, m. p. and mixed m. p. 234—235° (decomp.).

Oxidation of Di-O-*methylsclerotinol* (VII).—This compound (1 g.) was oxidised with potassium permanganate (8 g.), as described for compound (IV). The product was isolated in ether by continuous extraction, treated with boiling benzene (40 ml.) for 4 hr., and adsorbed on a column (20 × 1·5 cm.) of silica gel from the solution in benzene. Elution with acetone–benzene (1 : 40) (100 ml.) gave 6-chloro-3,5-dimethoxytrimellitic acid anhydride, which separated from benzene in needles (30 mg.), m. p. and mixed m. p. 198°.

Oxidation of Di-O-*acetylsclerotinol* (VIII).—This compound (1 g.) in boiling acetic acid (100 ml.) was treated with chromium trioxide (4 g., in portions), and 15 min. later the excess of reagent was destroyed with methanol. The combined solutions from four experiments were evaporated *in vacuo*, and the residue in benzene was adsorbed on a column (20 × 1·5 cm.) of silica gel. Eluted with benzene–chloroform (4 : 1), 3,5-*diacetoxy*-2-*chloro*-4,6-*dimethylphenylacetic*

acid (X) separated from carbon tetrachloride in needles (0·1 g.), m. p. 192° (Found: C, 53·1; H, 4·6; *C*-Me, 16·1. $C_{14}H_{15}ClO_6$ requires C, 53·4; H, 4·8; 4*C*-Me, 19·6%).

When saponified with 2N-sodium hydroxide (3 drops) for 5 min. at room temperature, the diacetoxy-acid (X) (80 mg.) in methanol (5 ml.) gave *2-chloro-3,5-dihydroxy-4,6-dimethylphenylacetic acid* (XI), which was isolated in ether after acidification of the reaction mixture and dilution with water. Crystallised from benzene, the acid (XI) formed needles (50 mg.), m. p. 160° (Found: C, 47·8; H, 5·6; Cl, 13·8; *C*-Me, 12·7. $C_{10}H_{11}ClO_4,H_2O$ requires C, 48·4; H, 5·2; Cl, 14·1; 2*C*-Me, 12·1%).

Treatment of this acid (40 mg.) by the methyl sulphate–potassium carbonate–acetone method during 3 hr., followed by hydrolysis of the product with potassium hydroxide in aqueous methanol during 24 hr. at room temperature, gave *2-chloro-3,5-dimethoxy-4,6-dimethylphenylacetic acid* (XII) which separated from benzene–light petroleum in needles (30 mg.), m. p. 146° [Found: C, 55·6; H, 6·2. $C_{10}H_9O_4Cl(OMe)_2$ requires C, 55·8; H, 5·8%].

Synthesis of 2-Chloro-3,5-dimethoxy-4,6-dimethylphenylacetic Acid (XII).—*3,5-Dimethoxy-4-methylphenylacetic acid* (XIII). Prepared from 3,5-dimethoxy-*p*-toluic acid (5 g.) by use of thionyl chloride under reflux for 1 hr., the *acid chloride* was isolated by distillation at 112°/0·5 mm. and crystallised from light petroleum as needles (5·0 g.), m. p. 78—80°, $\nu_{max.}$ 1761 and 1745 cm.$^{-1}$ (Found: C, 55·7; H, 5·3; Cl, 16·3; OMe, 29·0. $C_{10}H_{11}ClO_3$ requires C, 56·0; H, 5·2; Cl, 16·5; OMe, 28·9%).

Dropwise addition of this chloride (5·0 g.) in ether (200 ml.) to a stirred solution of diazomethane in ether at 0° (from 20 g. of *N*-nitrosomethylurea), followed by storage overnight at 0° and removal of the ether at 30° *in vacuo*, gave a pale yellow solid (5·3 g.) which separated from light petroleum in yellow needles (5·1 g.) of the *diazo-ketone*, m. p. 128—130°, $\nu_{max.}$ 2128 cm.$^{-1}$ (Found: C, 60·1; H, 5·8; N, 11·2. $C_{11}H_{12}N_2O_3$ requires C, 60·0; H, 5·5; N, 12·7%).

A solution of the diazo-ketone (5 g.) in dioxan (100 ml.) was added during 20 min. to a stirred solution of silver nitrate (0·5 g.) in aqueous ammonia (*d* 0·880) (50 ml.) and water (50 ml.) at 70°. After 1 hr. more silver nitrate (0·2 g.) in ammonia (5 ml.) and water (5 ml.) was added and the mixture was kept at 70° for a further hour. After filtration and dilution with water (250 ml.), the cooled mixture was extracted with chloroform (3 × 100 ml.); the extract was washed with water (2 × 100 ml.), dried ($MgSO_4$), and evaporated, giving *3,5-dimethoxy-4-methylphenylacetamide* (XIV) which separated from water (charcoal) in needles (2·6 g.), m. p. 158—160°, $\nu_{max.}$ 3344, 3145, and 1647 cm.$^{-1}$ (Found: C, 63·0; H, 7·2; N, 7·0. $C_{11}H_{15}NO_3$ requires C, 63·1; H, 7·2; N, 6·7%).

Hydrolysis of this amide (2·6 g.) with potassium hydroxide (3 g.) in methanol (60 ml.) under reflux for 5 hr., followed by dilution with water (25 ml.), concentration (to 30 ml.), and acidification by hydrochloric acid, gave *3,5-dimethoxy-4-methylphenylacetic acid* (XIII) which separated from water in plates (2·1 g.), m. p. 129—130°, $\nu_{max.}$ 1698 cm.$^{-1}$ (Found: C, 62·8; H, 6·7; OMe, 29·5. $C_{11}H_{14}O_4$ requires C, 62·8; H, 6·7; OMe, 29·5%).

Prepared with diazomethane in ether and methanol, the *methyl ester* (XV) distilled at 119°/0·1 mm. and had m. p. 34°, $\nu_{max.}$ 1736 cm.$^{-1}$ (Found: C, 64·4; H, 7·4; OMe, 41·7. $C_{12}H_{16}O_4$ requires C, 64·3; H, 7·2; OMe, 41·5%).

Methyl 2-Formyl-3,5-dimethoxy-4-methylphenylacetate (XVI).—(i) A mixture of methyl 3,5-dimethoxy-4-methylphenylacetate (XV) (2·0 g.), zinc chloride (2·5 g.), and hydrogen cyanide (6 ml.) in ether (100 ml.) was saturated with hydrogen chloride at 0°. Next day the aldimine hydrochloride, which had separated as a gummy solid, was washed with ether (5 × 50 ml.) by decantation, and dissolved in water (25 ml.) at 0°. When the pH was adjusted to 6·0 with 2N-sodium hydrogen carbonate *methyl 2-formyl-3,5-dimethoxy-4-methylphenylacetate* (XVI) (100 mg.) separated; it crystallised from light petroleum in needles, m. p. 103—104°, $\nu_{max.}$ 1675 and 1742 cm.$^{-1}$ (Found: C, 61·7; H, 6·5. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·4%). Prepared in the usual way, the *2,4-dinitrophenylhydrazone* separated from ethyl acetate in orange needles, m. p. 219—220° (Found: C, 53·1; H, 4·9; N, 13·1. $C_{19}H_{20}N_4O_8$ requires C, 52·8; H, 4·7; N, 13·0%). Unchanged methyl 3,5-dimethoxy-4-methylphenylacetate (1·6 g.) was recovered from the supernatant ether and washings from the aldimine hydrochloride.

(ii) A mixture of methyl 3,5-dimethoxy-4-methylphenylacetate (XV) (1·6 g.) and aluminium chloride (3·0 g.) in methylene chloride (30 ml.) at 0° was stirred during dropwise addition of dichloromethyl methyl ether (2 ml.) at 0° during 10 min. When no more hydrogen chloride was evolved (15 min.) the mixture was poured on ice, and the organic layer was separated and washed successively with water (2 × 30 ml.), 2N-sodium hydrogen carbonate (2 × 30 ml.), and

water (30 ml.). After drying ($MgSO_4$), the methylene chloride was evaporated and the residue crystallised from light petroleum, giving the aldehyde-ester (XVI) in needles (1·7 g.), m. p. and mixed m. p. 103—104° (Found: C, 62·1; H, 6·4; OMe, 36·8. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·4; OMe, 36·9%).

Ethyl 3,5-*Dimethoxy*-2,4-*dimethylphenylacetate* (XVII).—The above aldehyde-ester (1·12 g.) in hot ethanol (10 ml.) was added, in small portions during 20 min., to a refluxing mixture of concentrated hydrochloric acid (15 ml.) and water (15 ml.) containing zinc amalgam (from 12 g. of zinc powder and 1·5 g. of mercuric chloride in 1·5 ml. of concentrated hydrochloric acid and 30 ml. of water). After a further hour's heating under reflux more concentrated hydrochloric acid (2 ml.) was added and the heating continued for ½ hr. After decantation from the excess of zinc amalgam, the solution was diluted with water (50 ml.), and the product isolated in ether (3 × 50 ml.). Purified by distillation at 122°/0·05 mm. *ethyl* 3,5-*dimethoxy*-2,4-*dimethylphenylacetate* (XVII) had m. p. 42—44°, $\nu_{max.}$ 1733 cm.$^{-1}$ [Found: C, 66·6; H, 7·9; OMe + OEt (calc. as OMe), 39·1. $C_{14}H_{20}O_4$ requires C, 66·6; H, 8·0; OMe, 20·6; OEt, 17·5%]. When saponified with potassium hydroxide (1·1 g.) in methanol (10 ml.) at room temperature overnight, this ester (0·25 g.) gave the *parent acid* (XVIII) which formed needles (0·20 g.), m. p. 108—109°, $\nu_{max.}$ 1721 cm.$^{-1}$ from light petroleum (Found: C, 64·2; H, 7·4; OMe, 27·4. $C_{12}H_{16}O_4$ requires C, 64·3; H, 7·2; 2OMe, 27·7%).

2-*Chloro*-3,5-*dimethoxy*-4,6-*dimethylphenylacetic Acid* (XII).—A stirred solution of ethyl 3,5-dimethoxy-2,4-dimethylphenylacetate (XVII) (0·32 g.) in carbon tetrachloride (20 ml.) at 0° was treated successively with aluminium chloride (0·15 g.) and sulphuryl chloride (0·18 g.) in carbon tetrachloride (2 ml.), the rates of addition being controlled so that the temperature did not rise above 5°. The mixture was then allowed to warm to room temperature and poured on ice (50 g.), and the organic layer was separated, washed with 2N-sodium hydrogen carbonate (2 × 20 ml.) and water (20 ml.), dried ($MgSO_4$), and evaporated. The crude product (0·3 g.) was saponified with potassium hydroxide (0·16 g.) in methanol (15 ml.) overnight at room temperature. This mixture was then diluted with water (20 ml.) and washed with ether (2 × 25 ml.), and the free acid was liberated with 2N-sulphuric acid and isolated in ether (3 × 20 ml.). Thus obtained, 2-chloro-3,5-dimethoxy-4,6-dimethylphenylacetic acid (XII) separated from benzene–light petroleum in needles (0·2 g.), m. p. 147—148°, $\nu_{max.}$ 1706 cm.$^{-1}$ (Found: C, 55·7; H, 5·9; Cl, 13·5. Calc. for $C_{12}H_{15}ClO_4$: C, 55·7; H, 5·9; Cl, 13·7%).

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