986. *The Chemistry of Fungi. Part XXVIII.* Sclerotiorin and its Hydrogenation Products.*

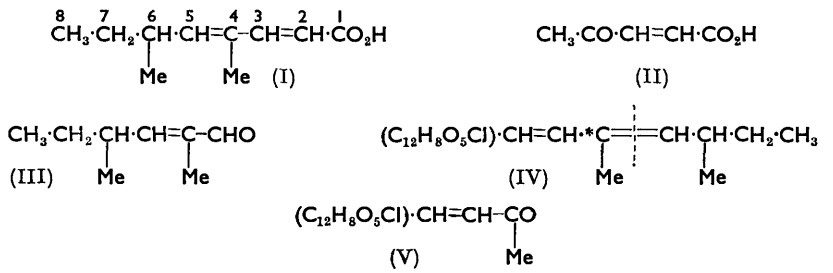
By R. A. Eade, H. Page, Alexander Robertson, K. Turner, and W. B. Whalley.

Degradation of the yellow, chlorine-containing pigment sclerotiorin, $C_{21}H_{23}O_5Cl$, from *Penicillium sclerotiorum* van Beyma, with alkali gives hydrochloric acid, formic acid, and 4 : 6-dimethylocta-*trans*-2 : 4-dienoic acid (I). With ammonia and primary amines sclerotiorin reacts to give amino-compounds of the general formula $C_{21}H_{23}O_4NCl{\cdot}R$; the nature of this reaction is discussed.

Hydrogenation of sclerotiorin gives tetrahydrosclerotiorin, $C_{21}H_{27}O_5Cl$, by the saturation of the diene side chain, whilst reduction with hydriodic acid forms dihydrosclerotiorin, $C_{21}H_{25}O_5Cl$, by the saturation of the double bond in the side chain adjacent to the sclerotiorin nucleus. Prolonged hydrogenation of sclerotiorin or dihydro- or tetrahydro-sclerotiorin yields two phenols, sclerotinol, $C_{19}H_{29}O_3Cl$, and sclerotol, $C_{19}H_{27}O_3Cl$, the constitutions of which are discussed.

From *P. sclerotiorum* van Beyma a second analogous colouring matter, rotiorin, has been isolated.

First isolated from the mycelium of *Penicillium sclerotiorum* van Beyma by Reilly and Curtin,[1,2] who proposed the empirical formula $C_{20}H_{19}O_5Cl$ or $C_{20}H_{21}O_5Cl$, the yellow chlorine-containing metabolite sclerotiorin was examined by Watanabe[3] who also obtained the compound from *P. multicolor* Grigorieva Manoilova and Poradielova and suggested the formula, $C_{21}H_{23}O_5Cl$. Since methylation and acetylation experiments were unsuccessful this author concluded that the pigment was devoid of a hydroxyl group and, from its failure to liberate iodine from acidified potassium iodide solution and its inertness towards sodium hydrogen sulphite, inferred that it did not contain a quinonoid system. On oxidation with chromic oxide sclerotiorin gave a yellow compound, $C_{16}H_{13}O_6Cl$, together with α-methylbutyric acid, whilst degradation with alkali furnished formic acid and a crystalline, optically active, unsaturated acid, $C_{10}H_{16}O_2$, which was reduced catalytically to the optically active saturated acid, $C_{10}H_{20}O_2$, characterised as the *p*-bromophenacyl derivative. From these results and the application of Woodward's rules[4] to its ultraviolet absorption spectrum, Watanabe[3] suggested that the acid, $C_{10}H_{16}O_2$, was 4 : 6-dimethylocta-2 : 4-dienoic acid (I).



About the same time Birkinshaw[5] also reported the isolation of sclerotiorin from the same organisms and described preliminary experiments on the metabolite for which he

* Part XXVII, *J.*, 1957, 3497.

[1] Reilly and Curtin, *Biochem. J.*, 1940, **34**, 1419.
[2] *Idem, ibid.*, 1943, **37**, 36.
[3] Watanabe, *J. Pharm. Soc. Japan*, 1952, **72**, 807.
[4] Woodward, *J. Amer. Chem. Soc.*, 1941, **63**, 1123; 1942, **64**, 72, 76.
[5] Birkinshaw, *Biochem. J.*, 1952, **52**, 283.

proposed the empirical formula, $C_{21}H_{23}O_5Cl$, but considered that the alternative formula, $C_{21}H_{25}O_5Cl$, was not entirely excluded. Birkinshaw confirmed the formation of the acid, $C_{10}H_{16}O_2$, established the relative position of the two double bonds by the preparation of a maleic anhydride adduct, and concluded that it had the constitution (I) since ozonolysis furnished (+)-α-methylbutyraldehyde along with β-acetylacrylic acid (II). The presence of an aldehydic or ketonic carbonyl group in sclerotiorin was inferred from the production of a dark red derivative, $C_{27}H_{27(29)}O_8N_4Cl$ with 2 : 4-dinitrophenylhydrazine, a red derivative, $C_{21}H_{23(25)}O_4N_2Cl$, with hydroxylamine, and a red product, $C_{21}H_{24(26)}O_4NCl$, with aqueous ammonia.

In an independent investigation * initiated in these laboratories in 1950, sclerotiorin was isolated from *P. sclerotiorum* and *P. multicolor*, and from its analytical results together with those of numerous derivatives and degradation products the metabolite clearly appeared to have the empirical formula $C_{21}H_{23}O_5Cl$; along with sclerotiorin from the mycelium of *P. sclerotiorum* there was obtained a second pigment, rotiorin, $C_{23(22)}H_{24(22)}O_5$, the chemistry of which will be described in a subsequent memoir. In preliminary work it was found that sclerotiorin is optically active, and from its infrared absorption spectrum and its failure to be acetylated or methylated under the usual conditions, appeared to be devoid of hydroxyl groups, a view which was substantiated by the negative Zerewitinoff estimation. With diazomethane it furnished an intractable gum and with methyl iodide or methyl sulphate and potassium carbonate intractable brown solids; the metabolite is unstable even in the mild alkaline conditions of the acetone–potassium carbonate methylation. Though the behaviour of sclerotiorin with aqueous-alcoholic sodium hydroxide is characteristic of a lactone clear evidence supporting the presence of this system is absent owing, in the main, to the complex behaviour of the compound; in attempts to detect a lactone system application of the standard methylation procedure [6] resulted either in unchanged sclerotiorin or in complete degradation.

Although sclerotiorin is moderately stable towards cold or hot dilute mineral acids and is unchanged by hydrogen chloride in ethyl acetate it is very sensitive to alkali (cf. Watanabe [3] and Birkinshaw [5]). Degradation with aqueous sodium hydrogen carbonate or preferably with dilute aqueous sodium hydroxide (cf. Watanabe [3]) readily gives hydrochloric acid, formic acid, the conjugated dienoic acid, $C_{10}H_{16}O_2$, which is clearly 4 : 6-dimethylocta-2 : 4-dienoic acid (I), and 2 : 4-dimethylhex-2-enaldehyde (III). The ultraviolet absorption spectrum of the dienoic acid indicates the conjugation of the diene system and of this system with the carboxyl group,[8,9] whilst infrared absorption at 988 cm.$^{-1}$ (m) indicates the *trans*-arrangement of the αβ-disubstituted double bond (cf. Crombie *et al.*[10]). Hydrogenation of the acid yields (+)-4 : 6-dimethyl-*n*-octanoic acid, the constitution of which has been confirmed by synthesis.[10] The physical properties in conjunction with the production of (+)-α-methylbutyraldehyde (cf. Birkinshaw [5]) and of pyruvaldehyde together with an aldehyde, $C_8H_{14}O$ (III), by ozonolysis served to

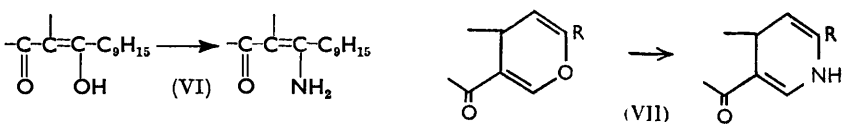
* Simultaneously with the initiation of the work on sclerotiorin and rotiorin (H. Page, Ph.D. thesis, Liverpool, 1953), studies on the pigments, rubropunctatin, monascorubrin, and monascin produced by members of the genus *Monascus* were resumed in collaboration with Dr. A. D. G. Powell (late of this Department); earlier work on this group, chiefly on monascin, had been carried out in collaboration with Dr. Richmond in 1937—1939 whose results (Ph.D. thesis, Liverpool, 1940) covered the ground described by Geiger and Karrer (*Helv. Chim. Acta*, 1941, **24**, 289). As indicated elsewhere,[7] sclerotiorin and rotiorin are members of a closely related group of fungal pigments which have been shown to include rubropunctatin, monascorubrin, and (possibly) monascin (Dr. Powell, Ph.D. thesis, Liverpool, 1954).

In an earlier communication [7] the generic name azaphilone was applied to this group of substances. Although this has certain advantages it is now clear that the general application must remain in abeyance until the nature of the compounds has been more clearly defined. A.R.

[6] Canter and Robertson, *J.*, 1931, 1875.
[7] Powell, Robertson, and Whalley, *Chem. Soc. Special Publ.*, 1957, No. 5, p. 27.
[8] Morton, "The Application of Absorption Spectra to the Study of Vitamins, Hormones and Enzymes," Hilger, London, 2nd edn., 1942, p. 19.
[9] Crossley and Hilditch, *J.*, 1949, 3353.
[10] Crombie, Manzoor-i-Khuda, and Smith, *J.*, 1957. 479.

establish the detailed structure of the dienoic acid. The structure of 2 : 4-dimethylhex-2-enaldehyde (III) is in agreement with the bright red colour of the 2 : 4-dinitrophenylhydrazone, which is indicative of αβ-unsaturation, whilst the ultraviolet absorption spectrum of the semicarbazone [$\lambda_{max.}$ 262 mμ (ε 32,400)] lies within the range [$\lambda_{max.}$ 267 ± 7·5 mμ (ε 10,000—35,000)] characteristic of the C:C·CH:N·NH·CO·NH_2 grouping.[11] Ozonolysis of sclerotiorin furnished the aldehyde (III) whilst scission of the side chain in sclerotiorin with ozone, as indicated at the broken line in the partial formula (IV), gave rise to (+)-α-methylbutyraldehyde and a ketone, pentanorsclerotiorone $C_{16}H_{13}O_6Cl$, which contains the sclerotiorin nucleus intact and appears to be identical with the product obtained in low yield by Watanabe [3] on oxidation of sclerotiorin with chromic oxide. Formulation of this oxidation product as the methyl ketone (V) is in accordance with, *inter alia*, the structure of the side chain of sclerotiorin, the failure of the product to form a dimedone derivative, and the ready production of iodoform whereas sclerotiorin does not undergo the haloform reaction. In agreement with structure (V) the conjugation of the newly introduced carbonyl group is indicated by the appearance of an additional band in the infrared absorption spectrum at 1698 $cm.^{-1}$ (m). Further, the optical activity of this ketone, from which the asymmetric carbon atom present in the side chain has been removed, indicates that the sclerotiorin kernel possesses at least one source of optical activity.

Despite the orange colour, sclerotiorin does not exhibit the usual quinonoid reactions (cf. Watanabe [3]), and is, for example, inert towards sulphur dioxide and bisulphites whilst catalytic reduction initially saturates the side chain with the formation of a tetrahydro-derivative in which the nucleus remains intact (see below). Although reductive acetylation of sclerotiorin led to a deep-seated degradation with the formation of an extremely difficultly separable, complex mixture, the deepening of colour on dissolution in alcoholic alkali indicates a partial or pseudo-quinonoid system. Attempts to confirm this by the formation of an anilinoquinone on treatment with aniline resulted in the production of a red derivative, *N*-phenylsclerotioramine, $C_{27}H_{28}O_4NCl$, in which one oxygen atom of sclerotiorin has been replaced by the PhN residue and which did not have the expected properties of an anilinoquinone. Similarly, with methylamine sclerotiorin readily furnished *N*-methylsclerotioramine, $C_{22}H_{26}O_4NCl$, and with hydrazine a deep purple *N*-aminosclerotioramine, $C_{21}H_{24}O_4NCl$ (cf. Birkinshaw [5]). The close similarity between the infrared absorption spectra of sclerotiorin and sclerotioramine indicates that the reaction with primary amines is probably not accompanied by a deep-seated change; the spectra may be regarded as compatible with the change $-O- \longrightarrow -NH-$. Further, the production of 2 : 4-dimethylhex-2-enaldehyde by ozonolysis of sclerotioramine shows that the aliphatic side chain is not concerned in the formation of sclerotioramine. From their general properties it appears that these substances are of the enamine type and may well be produced from a β-diketo-, β-aldehydo-, or keto-ester system (VI) or its cyclic equivalent (VII). Since the degradation of sclerotioramine, which occurs with both acid



and alkali, does not give ammonia, formic acid, or 4 : 6-dimethylocta-2 : 4-dienoic acid (I) it seems highly probable that the nitrogen atom of the amino-compounds is contained in a heterocyclic system involving the acid residue (I) and the formyl moiety as in (VII). The formation of the amino-compounds from ammonia and primary amines only and the failure of secondary or tertiary amines to react support this concept which implies the necessity of the group $-NH_2$ in the reacting amine. In agreement with a structure of the

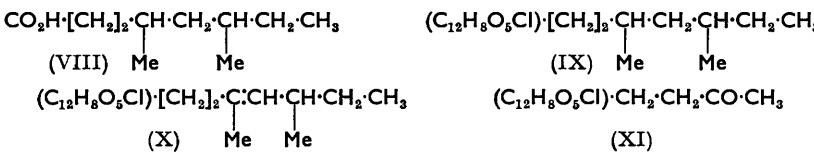
[11] Evans and Gillam, *J.*, 1943, 565.

kind (VII) the infrared spectrum of sclerotioramine exhibits absorption in the :NH stretching region at 3205 (m) and 3058 cm.$^{-1}$ (s), whilst in *N*-methylsclerotioramine absorption is absent in this region, thus showing that the nitrogen atom is tertiary.

In contrast to the other analogous products, *N*-phenylsclerotioramine is formed by way of a comparatively stable, intermediate, yellow adduct, $C_{27}H_{30}O_5NCl$, which is more readily soluble in aqueous solutions than the parent compound or *N*-phenylsclerotioramine, does not exhibit hydroxyl group absorption in the infrared spectrum, and is converted into the phenylamino-compound by hydrogen ions but much more readily by hydroxyl ions. This behaviour in conjunction with the intensification of colour observed in the formation of the amino-compounds supports the concept that the products are enamines where the intermediate adduct of aniline and sclerotiorin is of a salt-like nature (cf. Cromwell [12]).

Sclerotioramine is also a natural product since it has been isolated, together with sclerotiorin, from the mycelium of *P. multicolor* grown under special conditions.

Tetrahydrosclerotiorin, $C_{21}H_{27}O_5Cl$, which is readily formed by catalytic hydrogenation, along with a mixture of intractable degradation products, gave, on oxidation with chromic oxide, 4 : 6-dimethyl-*n*-octanoic acid (VIII), thereby showing that the hydrogenation of sclerotiorin to tetrahydrosclerotiorin is confined to the saturation of the side chain. Since this reaction involves the production of a new asymmetric centre at the C-atom* in formula (IV), tetrahydrosclerotiorin (IX) is a mixture of two diastereoisomerides, m. p. 142—144°, of which one only was originally obtained pure. Subsequently, by a prolonged fractional crystallisation this mixture has been resolved to give α-tetrahydro-, m. p. 118°, and β-tetrahydro-sclerotiorin, m. p. 159°. For present degradation experiments the mixture, m. p. 142—144°, was employed but it seems likely from the separation results that the β-diastereoisomeride greatly predominates in the mixture. For the same reason all tetrahydrosclerotiorin derivatives and degradation products retaining the hydrogenated side-chain intact may be expected to be mixtures of two diastereoisomerides; this applies, *e.g.*, to sclerotinol and sclerotol described below. Though Watanabe [3] reported the preparation of a substance, m. p. 130—132°, in low yield, believed to be hexahydrosclerotiorin no evidence for the existence of this derivative has been obtained in the present work, and it appears likely that the product was impure tetrahydrosclerotiorin.



Tetrahydrosclerotiorin shows infrared absorption at 1748 (s), 1727 (m), 1647 (vs), 1590 (m), and 1536 (m) cm.$^{-1}$. It has been found that all the derivatives of sclerotiorin which retain the sclerotiorin nucleus have a peak close to 773 cm.$^{-1}$ (s—m) (unpublished work in this laboratory) which may be tentatively ascribed to aliphatic chlorine absorption.[13] Thus any absorption peak arising in these derivatives at or close to double this frequency, *i.e.*, 1536 cm.$^{-1}$, may be provisionally attributed to the first overtone of the chlorine vibration and not to double-bond vibration. Hence if the peak at 1536 cm.$^{-1}$ exhibited by tetrahydrosclerotiorin is assigned to this overtone stretching frequency there remain four peaks attributable to double bonds. The peaks at 1748 and 1727 cm.$^{-1}$ may, with reasonable certainty, be assigned to the C=O stretching and, since sclerotiorin does not appear to contain an aromatic residue, the peaks at 1590 and 1647 cm.$^{-1}$ may be ascribed to a double bond conjugated with a carbonyl group.[14]

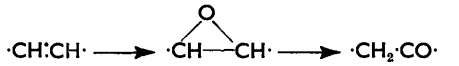
Reduction of sclerotiorin under carefully controlled conditions with hydriodic acid gave

[12] Cromwell, *Chem. Rev.*, 1946, **38**, 83.
[13] Bellamy, " Infra-Red Spectra of Complex Molecules," Methuen, London, 1954, p. 271.
[14] Ref. 13, p. 117.

dihydrosclerotiorin, whose partial structure (X) was readily established by ozonolysis to (+)-α-methylbutyraldehyde and the methyl ketone, pentanordihydrosclerotiorone, $C_{16}H_{15}O_6Cl$ (XI).

On prolonged catalytic hydrogenation with a palladium–charcoal catalyst in acetic acid sclerotiorin, or dihydro- or tetrahydro-sclerotiorin, undergoes a complex change with the elimination of two carbon atoms and the formation of two optically active phenolic products, sclerotinol, $C_{19}H_{29}O_3Cl$, and sclerotol, $C_{19}H_{27}O_3Cl$, in comparatively poor yields; reduction in alcohol or acetic acid with a platinum catalyst gave only sclerotinol, in low yield, accompanied by much intractable gum. Of these two, the main product is sclerotinol, a dihydric phenol forming a diacetate, a dimethyl ether, and a di-*p*-nitrobenzoate. The third oxygen atom in sclerotinol is present in a carbonyl group which readily forms derivatives and, from the ultraviolet absorption spectrum and the infrared absorption at 1712 cm.$^{-1}$ (s), together with the pale yellow colour of the 2 : 4-dinitrophenylhydrazone, is clearly an aliphatic unconjugated carbonyl residue. Further, the resistance of di-*O*-acetyl- and di-*O*-methyl-sclerotinol to oxidation, in conjunction with the infrared spectral data, indicates that this carbonyl group is ketonic and not aldehydic. Since it has a negative ferric reaction in alcohol and cannot be oxidised to a quinone, sclerotinol appears to be a resorcinol and not a catechol or quinol derivative. Moreover, because the saturated alkyl residue equivalent to the acid (VIII) is present, sclerotinol can contain only one benzenoid ring. Because this phenol does not couple with benzenediazonium chloride or give a fluorescein reaction with phthalic acid or anhydride it is probable that the benzenoid system is substituted in the *o*- and *p*-position to the hydroxyl groups although steric effects on the coupling reaction cannot be excluded entirely; it seems likely that the aromatic system is fully substituted. The molecular rotation indicates that the optical activity of sclerotinol is associated only with the asymmetric carbon atom of the alkyl residue and, although experimental proof has not yet been possible, the compound may well be, for the reasons which apply to tetrahydrosclerotiorin, a mixture of two diastereoisomerides; this applies also to sclerotol. The chlorine atom of sclerotinol cannot be removed by catalytic hydrogenation with Raney nickel or palladium–barium carbonate or by the action of Raney nickel in boiling alcohol. Di-*O*-methylsclerotinol is unaffected by the same reagents, by boiling alcoholic alkali with or without Raney nickel, or by alcoholic silver acetate. These observations indicate that the halogen in this phenol is aromatic and not aliphatic.

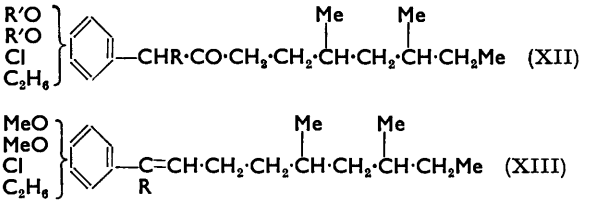
On reduction with lithium aluminium hydride sclerotinol gave a secondary alcohol. Similarly formed from the di-*O*-methyl ether, the secondary alcohol $C_{19}H_{29}OCl(OMe)_2$, which exhibited strong infrared hydroxylic absorption at 3676 cm.$^{-1}$ in place of the carbonyl absorption of the parent ether, gave on dehydration the ethenoid derivative $C_{19}H_{27}Cl(OMe)_2$, the light absorption of which indicates that the double bond is conjugated with the aromatic nucleus. Attempts to hydroxylate the olefin with osmium tetroxide were unsuccessful and, unexpectedly, ozonolysis regenerated the parent ketone in high yield, a reaction which may proceed thus:

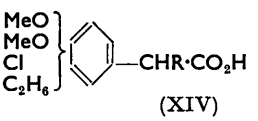


in accordance with the conversion of amyrin acetate into 3β-acetoxy-13*a*-ursan-12-one [15] and similar to the oxidation of, *e.g.*, 2-methylbut-2-ene to 3-methylbutan-2-one [16] with chromic acid. Interaction of di-*O*-methylsclerotinol and methylmagnesium iodide gave a tertiary alcohol, $C_{22}H_{37}O_3Cl$, which exhibited infrared hydroxyl absorption at 3670 cm.$^{-1}$ (s) but not carbonyl absorption. Phenylmagnesium bromide afforded an analogous tertiary alcohol, but neither alcohol could be satisfactorily dehydrated or oxidised.

[15] Allan, Spring, and Stevenson, *J.*, 1955, 3072.
[16] Hickinbottom, Peters, and Wood, *J.*, 1955, 1360.

Since the carbonyl group of sclerotinol, which is not conjugated with the nucleus, in all probability appears as the carboxyl of the dienoic acid (I) it seems likely that sclerotinol and its dimethyl ether contain the systems (XII; R′ = H) and (XII; R′ = Me) respectively and hence the olefin may be formulated as (XIII). If a benzyl ketone residue is





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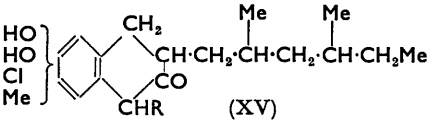

(XV)

present it seems likely that this does not carry a phenolic hydroxyl in the *o*-position because attempts to convert sclerotinol into a benzofuran derivative by standard methods were unsuccessful. It is not clear whether R is hydrogen or a methyl residue. In attempts to decide this, the oxime of di-*O*-methylsclerotinol was subjected to the Beckmann reaction, giving a product, $C_{21}H_{34}O_3NCl$, with the expected absorption at 1642, 1553, and 3279 cm.$^{-1}$, but as it could not be hydrolysed under fairly drastic conditions or reduced with lithium aluminium hydride this substance may not be the expected amide. That the carbonyl system in sclerotinol is not contained in a cyclic system follows from the fact that, although it has not so far been possible to oxidise di-*O*-methylsclerotinol satisfactorily, the corresponding diacetate gave a small yield of a monobasic acid (XIV), having the properties of a phenylacetic acid. The isolation of a phenylacetic acid and not a benzoic acid is in keeping with the observed resistance of highly hindered phenylacetic acids to further oxidation.[17]

Sclerotol, which closely resembles sclerotinol and has almost identical ultraviolet and infrared absorption spectra, contains two *meta*-phenolic hydroxyl groups, and forms a dimethyl ether $C_{19}H_{25}OCl(OMe)_2$. As in sclerotinol the carbonyl group is not conjugated with the aromatic system. Sclerotol, however, possesses two hydrogen atoms less than sclerotinol and may have a structure of β-tetralone type (XV).

Experimental

Sclerotiorin.—This was obtained from *Penicillium sclerotiorum* van Beyma and *P. multicolor* Grigorieva-Manoilova and Poradielova grown on a modified Czapek–Dox medium as described by Curtin and Reilly.[1,2] Extraction of the dried milled mycelium (3 kg.) from *P. sclerotiorum* van Beyma with light petroleum (b. p. 60—80°) furnished the crude compound which was purified from methanol, giving the metabolite (200—250 g.) in orange-yellow needles, m. p. 206°, unchanged on sublimation at 170°/0·01 mm., giving a negative ferric and iodoform reaction (cf. Watanabe [3] and Birkinshaw [5]), $[\alpha]_D^{20}$ +493° (*c* 0·298 in EtOH), $\lambda_{max.}$ 224, 287, 365, 449 mμ (log ε 4·06, 4·11, 4·50, and 4·11 respectively) (Found: C, 64·4, 64·5, 64·3, 64·2; H, 6·1, 5·7, 6·1, 5·9; Cl, 8·7, 9·1. Calc. for $C_{21}H_{23}O_5Cl$: C, 64·5; H, 5·9; Cl, 9·1%). Sclerotiorin is readily soluble in alcohol, methanol, ethyl acetate, or benzene, sparingly soluble in light petroleum (b. p. 60—80°) or ether. Though insoluble in cold 2N-aqueous sodium hydroxide, the compound dissolves very slowly on prolonged contact with this reagent but is not extracted from an ethereal solution by it. It readily dissolves in aqueous-alcoholic 2N-sodium hydroxide, forming a deep purple-red solution from which it is not precipitated on dilution with water but is recovered unchanged upon immediate acidification.

Percolation, with ether, of the residues left from the light petroleum extract, followed by concentration of the liquor, gave a semicrystalline solid which was purified from methanol, to

[17] Lloyd and Whalley, *J.*, 1956, 3209.

furnish *rotiorin* in red needles (*ca.* 8 g.), m. p. 246° (decomp.) (Found: C, 72·6, 72·4, 72·4, 72·7; H, 6·8, 6·2, 6·3, 6·5%; OMe, 0; *M*, 366. $C_{23}H_{24}O_5$ requires C, 72·6; H, 6·4%; *M*, 380).

From the dried, powdered, mycelium (3·5 kg.) of *P. multicolor*, which did not seem to contain rotiorin, sclerotiorin (600—700 g.) was extracted directly with ether. On one occasion *P. multicolor* G.-M.P. was grown on the normal modified Czapek–Dox medium for five weeks at 20—30° and on extraction the dried mycelium gave sclerotiorin contaminated with an orange-red impurity which was concentrated by removing the greater part of the sclerotiorin with hot light petroleum (b. p. 60—80°). The red residue was then purified by chromatography on neutralised aluminium oxide from benzene. Sclerotiorin was rapidly eluted by benzene, followed, more slowly, by a red band, which furnished sclerotioramine in red needles, m. p. and mixed m. p. 235° (decomp.), having an infrared spectrum identical with that of a specimen prepared from sclerotiorin.

60—80% of the starting material was recovered when sclerotiorin was treated with boiling acetic anhydride–sodium acetate, acetic anhydride–sulphuric acid at 100° (5 min.), or acetic anhydride–pyridine at 100° (1 hr.).

Degradation of Sclerotiorin with Alkali.—(*a*) Sclerotiorin (2 g.) was heated with boiling 5% aqueous potassium hydroxide (100 ml.) for 5 min., the dark brown filtered solution was acidified with 2N-sulphuric acid, and a solution of the brown amorphous precipitate in ether was washed with 2N-aqueous sodium hydrogen carbonate and then 2N-aqueous sodium hydroxide. Acidification of the former extract gave 4 : 6-dimethylocta-2 : 4-dienoic acid which separated from light petroleum (b. p. 60—80°) in prisms (0·8 g.), m. p. 92°, $[\alpha]_D^{20}$ +64·4° (*c* 2·12 in EtOH), $\lambda_{max.}$ 261 mμ (log ε 4·433) (Watanabe [3] records $[\alpha]_D^{23}$ +68·7° in EtOH) (Found: C, 71·5; H, 9·4%; equiv., 170. Calc. for $C_{10}H_{16}O_2$: C, 71·4; H, 9·6%; equiv., 168). Hydrogenation of this acid (2 g.) in alcohol (50 ml.) with platinic oxide (0·1 g.) was complete in 30 min., giving the 4 : 6-dimethyl-*n*-octanoic acid, b. p. 98°/1 mm., $[\alpha]_D^{20}$ +19·4° (*c* 2·38 in EtOH), $[M]_D^{20}$ +2336° (Found: C, 69·9; H, 11·6. Calc. for $C_{10}H_{20}O_2$: C, 69·7; H, 11·7%). Acidification of the aqueous sodium hydroxide extract furnished an intractable solid.

The combined filtrates from five degradation experiments were distilled with steam until the distillate was neutral. This distillate, which contained chloride ions, was neutralised with 0·1N-aqueous sodium hydroxide and evaporated to dryness, leaving the colourless mixed sodium salts (1·7 g.), a portion of which, on treatment with *o*-phenylenediamine, gave benziminazole (from formic acid), m. p. and mixed m. p. 169°; this furnished a picrate, m. p. and mixed m. p. 225°. These compounds depressed the m. p. of 2-methylbenziminazole and its picrate respectively.

The same results were obtained when the degradation was carried out with zinc dust and 5% aqueous potassium hydroxide.

(*b*) A mixture of sclerotiorin (5 g.) and 10% aqueous sodium hydroxide (200 ml.) was slowly distilled, with the addition of sufficient distilled water to maintain the volume, until a test portion of the distillate no longer gave a precipitate with aqueous 2 : 4-dinitrophenylhydrazine sulphate. Treatment of the distillate with an excess of this reagent precipitated 2 : 4-*dimethylhex-2-enaldehyde* 2 : 4-*dinitrophenylhydrazone* (0·3 g.) which separated from methanol in red needles, m. p. 159° (Found: C, 55·3; H, 6·2; N, 18·1. $C_{14}H_{18}O_4N_4$ requires C, 54·9; H, 5·9; N, 18·3%). The presence of other 2 : 4-dinitrophenylhydrazones in the crude product could not be detected and acidification of the alkaline hydrolysate furnished 4 : 6-dimethylocta-2 : 4-dienoic acid (1 g.).

Repetition of this experiment, but with addition of a saturated solution of sodium acetate (4·5 g.) and semicarbazide hydrochloride (1 g.) to the concentrated distillate (30 ml.), gave 2 : 4-*dimethylhex-2-enaldehyde semicarbazone* which separated from light petroleum (40—60°) in needles, m. p. 149°, $[\alpha]_D^{30}$ +72° (*c* 0·83 in EtOH), $\lambda_{max.}$ 262 mμ (log ε 4·51) (Found: C, 59·3; H, 9·2; N, 23·1. $C_9H_{17}ON_3$ requires C, 59·0; H, 9·4; N, 22·9%).

(*c*) Degradation of sclerotiorin (1 g.) with boiling 2N-aqueous sodium hydrogen carbonate (40 ml.) for 3 hr. as in method (*b*) furnished a distillate (50 ml.) from which 2 : 4-dimethylhex-2-enaldehyde was isolated as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 159°. Other aldehydic or ketonic substances were not detected.

Ozonolysis of 4 : 6-*Dimethylocta-2* : 4-*dienoic Acid.*—A mixture of ozone and oxygen was passed through a solution of this acid (0·9 g.) in ethyl acetate (90 ml.) at room temperature for 30 min. and, after the removal of the solvent in a vacuum, the gummy residue was treated with an excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. One hour later the mixture of

semicrystalline products (1·8 g.) was collected, triturated with 2N-aqueous sodium hydrogen carbonate, washed with hot alcohol, and purified from dioxan, giving pyruvaldehyde bis-2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 300° (decomp.) (Found: N, 25·6. Calc. for $C_{15}H_{12}O_8N_8$: N, 25·9%). Concentration of the alcoholic washings gave 2 : 4-dimethylhex-2-enaldehyde 2 : 4-dinitrophenylhydrazone (0·2 g.), m. p. and mixed m. p. 159°.

When the time of ozonolysis was extended to 2 hr. the product consisted of the bis-2 : 4-dinitrophenylhydrazone of pyruvaldehyde together with (+)-α-methylbutyraldehyde 2 : 4-dinitrophenylhydrazone which was isolated from the alcoholic washings of the crude product as plates (0·2 g.), m. p. 122—124°. Purified by chromatography from benzene–light petroleum (b. p. 60—80°) (1 : 1) on aluminium oxide this had m. p. 132°, $[\alpha]_D^{20}$ +29·8° (*c* 1·37 in ethyl acetate) (Badin and Pacsu [18] give m. p. 132—133°, $[\alpha]_D^{20}$ +31·2° in acetone) (Found: C, 49·9; H, 5·3; N, 20·7. Calc. for $C_{11}H_{14}O_4N_4$: C, 49·6; H, 5·3; N, 21·0%). The infrared absorption was identical with that of the 2 : 4-dinitrophenylhydrazone of (±)-α-methylbutyraldehyde.

Ozonolysis of Sclerotiorin.—A stream of ozone and oxygen was passed into a solution of sclerotiorin (0·5 g.) in ethyl acetate (50 ml.) at room temperature for 1 hr. and the residue left on removal of the solvent in a vacuum was treated with water (250 ml.) and barium carbonate (2 g.). 12 Hr. later the aqueous solution was decanted from an orange solid and distilled into an excess of 2 : 4-dinitrophenylhydrazine sulphate, giving a precipitate which on purification by chromatography furnished 2 : 4-dimethylhex-2-enaldehyde 2 : 4-dinitrophenylhydrazone (50 mg.), m. p. and mixed m. p. 159°.

Ozonolysis of sclerotiorin (2 g.), in ethyl acetate (100 ml.), at room temperature for 1·75 hr. with subsequent decomposition of the ozonide with water (100 ml.) for 12 hr. and distillation of the aqueous solution gave the 2 : 4-dihydrophenylhydrazone of (+)-α-methylbutyraldehyde (0·4 g.) in yellow plates, m. p. and mixed m. p. 132°.

The dried orange solid obtained from the ozonide was purified from alcohol, giving a ketone, pentanorsclerotiorone, in stout, orange needles (0·5—0·8 g.), m. p. 234° (decomp.) with darkening from *ca.* 222°, $[\alpha]_D^{20}$ +381° (*c* 0·91 in $CHCl_3$), $\lambda_{max.}$ 243, 281, 311, 355, and 426 mμ (log ε 4·04, 4·25, 4·08, 4·23, and 3·84 respectively), identical with the product formed in much lower yield by the oxidation of sclerotiorin with chromium trioxide [Watanabe [3] gives m. p. 233—235° (decomp.)] (Found: C, 57·2; H, 4·1; Cl, 10·2. Calc. for $C_{16}H_{13}O_6Cl$: C, 57·1; H, 3·9; Cl, 10·6%). This ketone, which readily furnished iodoform under standard conditions, is sparingly soluble in alcohol, benzene, or acetic acid, insoluble in ether or light petroleum (b. p. 60—80°), and readily soluble in cold 2N-aqueous sodium hydroxide. The alcoholic solution, which has a negative ferric reaction, becomes an intense purple-red on the addition of 2N-aqueous sodium hydroxide or sodium carbonate.

Reductive Acetylation of Sclerotiorin (*with* N. B. GRAHAM).—Zinc dust (6 g.) was added gradually to a solution of sclerotiorin (10 g.) and sodium acetate (5 g.) in boiling acetic anhydride (75 ml.) and after the vigorous reaction had subsided the mixture was heated under reflux for 15 min. The colourless solution was poured into water (250 ml.) and, on purification from alcohol, the precipitate gave a product in pale yellow needles (6 g.), m. p. 207—216°, which appeared to be a mixture [Found: C, 65·3, 65·2; H, 6·9, 6·5; Cl, 9·8%; *M* (Rast), 637, 647, 778; *M* (Menzies–Wright), 784, 908]. This product was insoluble in 2N-aqueous sodium hydroxide, had a negative ferric reaction in alcohol, and did not react with ammonia or alcoholic 2 : 4-dinitrophenylhydrazine sulphate. On repeated purification from alcohol this mixture (2 g.) ultimately furnished a fraction (0·4 g.), m. p. 220—222°, which appeared to be a substantially homogeneous substance (Found: C, 65·6; H, 6·1; Cl, 9·8%).

Interaction of Sclerotiorin with Ammonia and Primary Amines.—Sclerotiorin (4 g.) rapidly dissolved in aqueous ammonia (30 ml.; *d* 0·88) at room temperature, and 20 min. later the deep red solution was diluted with water (50 ml.), filtered, and poured into concentrated hydrochloric acid (35 ml.) and water (150 ml.) at 0°. Purification of the well-washed red precipitate from aqueous alcohol gave sclerotioramine in red needles (3·7 g.), m. p. 234° (decomp.) (Birkinshaw [5] gives m. p. 228—229°), $\lambda_{max.}$ 230, 345, and 504 mμ (log ε, 4·14, 4·60, and 3·83) (Found: C, 64·6, 64·7; H, 6·2, 6·0; N, 3·5, 3·5; Cl, 9·6. Calc. for $C_{21}H_{24}O_4NCl$: C, 64·6; H, 6·2; N, 3·6; Cl, 9·1%). Sclerotioramine, which readily forms a red solution in 2N-aqueous sodium carbonate or sodium hydroxide, is insoluble in 2N-aqueous sodium hydrogen carbonate, has a negative ferric reaction in alcohol, and forms an unstable yellow hydrochloride on treatment with hydrochloric acid.

[18] Badin and Pacsu, *J. Amer. Chem. Soc.*, 1945, **67**, 1353.

On addition of hydrazine hydrate (0·5 g.) to a solution of sclerotiorin (1 g.) in methanol (10 ml.), followed by dilution of the resulting red solution immediately with water (100 ml.), a red semicrystalline solid separated which on purification from aqueous methanol furnished N-*aminosclerotioramine* in purple needles (0·8 g.), m. p. 232° (decomp.) (Found: C, 62·6; H, 6·4; N, 6·4; Cl, 8·7. $C_{21}H_{25}O_4N_2Cl$ requires C, 62·4; H, 6·2; N, 6·9; Cl, 8·8%).

Addition of 33% aqueous methylamine (2 ml.) to a suspension of sclerotiorin (1 g.) in methanol (10 ml.) gave a purple red solution which on immediate dilution with water precipitated N-*methylsclerotioramine*, forming purple needles (0·9 g.), m. p. 212° (decomp.), from aqueous methanol (Found: C, 65·2; H, 6·4; N, 3·3; *N*-Me, 6·2; OMe, 0. $C_{22}H_{26}O_4NCl$ requires C, 65·5; H, 6·5; N, 3·5; *N*-Me, 7·2%).

A solution of sclerotiorin (1 g.) in alcohol (150 ml.), containing aniline (0·2 ml.), was kept at 50° for 1 hr. and diluted with water. Crystallised from dilute alcohol, the resultant precipitate gave the *aniline adduct* in yellow plates (1 g.), m. p. 169° (Found: C, 67·0, 67·0; H, 6·4, 6·1; N, 3·0, 2·7; Cl, 7·5. $C_{27}H_{30}O_5NCl$ requires C, 67·0; H, 6·2; N, 2·9; Cl, 7·3%). Addition of 2N-sulphuric acid (2 drops) or 2N-aqueous sodium hydroxide (2 drops) to a suspension of this adduct (1 g.) in alcohol (25 ml.) produced an immediate deep red solution, which was kept at room temperature for 0·5 hr. and diluted with water. Purified from aqueous methanol, the precipitate gave N-*phenylsclerotioramine* in red needles (0·9 g.), m. p. 189°, insoluble in aqueous or alcoholic sodium hydroxide solution (Found: C, 69·9; H, 6·0; N, 3·0; Cl, 7·6. $C_{27}H_{28}O_4NCl$ requires C, 69·6; H, 6·1; N, 3·0; Cl, 7·6%). The same derivative, m. p. and mixed m. p. 189°, was formed by interaction of sclerotiorin (1 g.) and aniline (0·5 g.) in alcohol (10 ml.) at room temperature for 48 hr.

Ozonolysis of Sclerotioramine.—A stream of ozone and oxygen was passed into a suspension of sclerotioramine (1 g.) in ethyl acetate (100 ml.) at room temperature for 25 min., the resulting red solution was evaporated in a vacuum, and the residual red glass treated with water (150 ml.). 12 Hr. later the intractable, red resin was removed and the solution distilled into aqueous 2 : 4-dinitrophenylhydrazine sulphate, giving 2 : 4-dimethylhex-2-enaldehyde 2 : 4-dinitrophenylhydrazone (0·1 g.), m. p. and mixed m. p. 159°.

Tetrahydrosclerotiorin.—A solution of sclerotiorin (5 g.) in acetic acid (100 ml.) containing a palladium catalyst (from 0·25 g. of palladium chloride and 1 g. of charcoal) was shaken in hydrogen until the absorption of gas was about 880 ml. (*ca.* 3 mol.); the orange solution gradually changed to pale yellow. Evaporation of the filtered solution in a vacuum left an oil which crystallised from methanol, giving tetrahydrosclerotiorin (mixed isomers) in pale yellow needles (3·25 g.), m. p. 141—144°, insoluble in cold 2N-aqueous sodium hydroxide, having $[\alpha]_D^{21}$ +215° (*c* 0·1 in EtOH) and $\lambda_{max.}$ 225, 343 mμ (log ε 4·13, 4·30 respectively) (Found: C, 64·2, 64·1, 64·1, 63·9; H, 7·0, 6·8, 6·9, 6·6; Cl, 9·9. Calc. for $C_{21}H_{27}O_5Cl$: C, 63·9; H, 6·9; Cl, 9·0%). Addition of 2N-aqueous sodium hydroxide to an alcoholic solution of this compound gave a deep orange solution which remained clear on dilution with water; on immediate acidification unchanged tetrahydrosclerotiorin was then precipitated.

By prolonged fractional crystallisation from alcohol, tetrahydrosclerotiorin, m. p. 143—144°, was resolved into β-*tetrahydrosclerotiorin*, m. p. 159°, $\lambda_{max.}$ 225, 343 mμ (log ε 4·15, 4·34), $[\alpha]_D^{21}$ +230° (*c* 0·11 in EtOH) (Found: C, 63·8; H, 6·6; Cl, 7·9%), together with α-*tetrahydrosclerotiorin*, m. p. 112°, $\lambda_{max.}$ 225, 343 mμ (log ε 4·15, 4·34), $[\alpha]_D^{21}$ +206° (*c* 0·15 in EtOH) (Found: C, 63·9; H, 7·0%). A mixture of α- and β-tetrahydrosclerotiorin had m. p. *ca.* 135°.

Concentration of the mother-liquors from the purification of crude tetrahydrosclerotiorin gave a yellow oil.

Hydrogenation of sclerotiorin (5 g.) in alcohol (100 ml.) gave tetrahydrosclerotiorin (3·1 g.) along with an oil.

Oxidation of Tetrahydrosclerotiorin.—A solution of chromic oxide (1·7 g.) in acetic acid (50 ml.) was added in 24 hr. to tetrahydrosclerotiorin (1 g.) in acetic acid (40 ml.) and, after removal of the solvent in a vacuum, the acidic fraction was isolated from an ethereal solution of the oxidation product with aqueous sodium hydrogen carbonate and purified by distillation at 115°/1 mm. Thus obtained, 4 : 6-dimethyl-*n*-octanoic acid was converted into the *p*-bromophenacyl ester which separated from aqueous methanol in plates, m. p. 31—34°, undepressed on admixture with the ester of the authentic acid prepared by the hydrogenation of 4 : 6-dimethylocta-2 : 4-dienoic acid; the infrared absorption spectra of the two specimens were identical.

Dihydrosclerotiorin (with N. B. GRAHAM).—When sclerotiorin (5 g.) was added quickly to a

stirred mixture of acetic anhydride (15 ml.) and hydriodic acid (15 ml.; *d* 1·7) at 80°, iodine was liberated immediately. 5 Min. later the mixture was poured into an excess of concentrated aqueous sodium sulphite, and next day the solid was collected and purified from methanol, giving *dihydrosclerotiorin* in pale yellow needles (0·5—1·0 g.), m. p. 192° (decomp.), $[\alpha]_D^{21}$ +184°, with a negative ferric reaction (Found: C, 64·1, 64·1; H, 6·6, 6·7; Cl, 8·9, 8·9; I, 0. $C_{21}H_{25}O_5Cl$ requires C, 64·2; H, 6·4; Cl, 9·0%). The conditions for this reaction are critical, particularly the temperature which must be within the limits 65—80°. Dihydrosclerotiorin, which sublimes unchanged, is readily soluble in alcohol and insoluble in cold 2N-aqueous sodium hydroxide. Addition of 2N-aqueous sodium hydroxide to the compound dissolved in methanol gives an orange solution which remains clear on dilution with water; on immediate acidification unchanged dihydrosclerotiorin is precipitated.

Larger quantities of dihydrosclerotiorin were prepared by the following somewhat less tedious procedure. Sclerotiorin (25 g.) was added rapidly to a stirred mixture of hydriodic acid (50 ml.; *d* 1·7) and acetic acid (from 75 ml. of anhydride) at 75°, and 4 min. later the solution was poured into concentrated aqueous sodium hydrogen sulphite (500 ml.) at 0°. Collected immediately and purified from methanol, the solid gave dihydrosclerotiorin (2·5 g.), m. p. and mixed m. p. 192°. Repeated attempts under a variety of conditions failed to improve the yield of this product.

After treatment with hydrobromic acid under similar conditions sclerotiorin was recovered unchanged.

Ozonolysis of Dihydrosclerotiorin.—A stream of ozone and oxygen was passed through a solution of dihydrosclerotiorin (0·6 g.) in ethyl acetate (150 ml.) at room temperature for 40 min. and the solvent removed in a vacuum. Water (15 ml.) was added to the residual gum and next day the resulting solid was purified from alcohol, giving *dihydropentanorsclerotiorone* in yellow needles, m. p. 196—198°; mixed with dihydrosclerotiorin it had m. p. *ca.* 162° (Found: C, 56·6, 56·6, 56·4; H, 4·7, 4·7, 4·8; Cl, 11·1. $C_{16}H_{15}O_6Cl$ requires C, 56·8; H, 4·4; Cl, 10·5%). Distilled into 2 : 4-dinitrophenylhydrazine sulphate solution, the aqueous hydrolysate gave a precipitate of the 2 : 4-dinitrophenylhydrazone of (+)-α-methylbutyraldehyde in golden plates, m. p. and mixed m. p. 128—129°.

Exhaustive Hydrogenation of Sclerotiorin.—A solution of sclerotiorin (5 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 (*ca.* 5 hr.); about 6 mol. (1700 ml.) were absorbed. Removal of the solvent left a colourless oil which crystallised from light petroleum (b. p. 60—80°), giving *sclerotinol* in needles (1·8 g.), m. p. 86°, with a negative ferric reaction, $[\alpha]_D^{20}$ +3·26° (*c* 2·36 in $CHCl_3$), $[M]_D^{20}$ +1108°, $\lambda_{max.}$ 299 mμ (log ε 3·34) (Found: C, 66·9, 67·0, 66·9; H, 8·3, 8·1, 8·4; Cl, 10·5, 10·5. $C_{19}H_{29}O_3Cl$ requires C, 66·9; H, 8·6; Cl, 10·4%). This compound, which is readily soluble in the usual organic solvents except light petroleum, readily dissolves unchanged in cold 2N-aqueous sodium hydroxide.

From the concentrated mother-liquors left from purification of sclerotinol, *sclerotol* was obtained which separated from light petroleum (b. p. 60—80°) in needles (0·3 g.), m. p. 147°, with a negative ferric reaction, $[\alpha]_D^{18}$ +5·2° (*c* 2·27 in $CHCl_3$), $[M]_D^{19}$ +1757°, $\lambda_{max.}$ 287 mμ (log ε 3·37) (Found: C, 67·2, 67·1; H, 8·1, 7·8; Cl, 10·5, 10·6. $C_{19}H_{27}O_3Cl$ requires C, 67·3; H, 8·0; Cl, 10·5%). The yield of sclerotol, which was always small, was very variable and occasionally nil.

When tetrahydrosclerotiorin (5 g.) in acetic acid (100 ml.) was hydrogenated under the same conditions until the absorption of hydrogen (1120 ml., 4 mol.) ceased, sclerotinol (2·5 g.) and sclerotol (0·35 g.) were obtained. Similarly, dihydrosclerotiorin (1 g.) in acetic acid (100 ml.) gave sclerotinol, m. p. and mixed m. p. 86° (256 ml., 4·2 mol. of hydrogen were absorbed).

Prolonged hydrogenation of sclerotiorin or tetrahydrosclerotiorin in alcohol or ethyl acetate resulted in the uptake of the requisite quantities of hydrogen, but the products were oils which did not yield crystalline material before or after chromatography from light petroleum on silica or neutralised aluminium oxide.

When a platinum catalyst was employed the hydrogenation of sclerotiorin (1 g.) or of tetrahydrosclerotiorin (1 g.) in alcohol (25 ml.) or acetic acid (25 ml.) proceeded comparatively rapidly (*ca.* 1 hr.), giving sclerotinol (25 mg.), m. p. 85—86°, as the only isolable product; this was separated with difficulty from the accompanying gum by chromatography from light petroleum (b. p. 60—80°) on silica gel, followed by crystallisation from light petroleum (b. p. 60—80°).

Derivatives of Sclerotinol.—By the standard method, sclerotinol gave (0·65 g.) the *di-p-nitrobenzoate*, needles (1·2 g.) (from alcohol), m. p. 152—153° (Found: C, 62·3, 61·9; H, 5·7, 5·5; N, 4·2, 4·1. $C_{33}H_{35}O_9N_2Cl$ requires C, 62·0; H, 5·5; N, 4·4%). The 2 : 4-*dinitrophenylhydrazone* of this di-*p*-nitrobenzoate crystallised from aqueous alcohol in yellow needles, m. p. 203° (Found: C, 57·1; H, 5·0; N, 9·9; Cl, 4·3. $C_{39}H_{39}O_{12}N_6Cl$ requires C, 57·2; H, 4·8; N, 10·3; Cl, 4·3%). Sclerotinol, by the sodium acetate or pyridine method, furnished the *diacetate* (in high yield), which separated from light petroleum (b. p. 60—80°) in needles, m. p. 98° (Found: C, 64·8; H, 7·9; Cl, 8·6. $C_{23}H_{33}O_5Cl$ requires C, 65·0; H, 7·8; Cl, 8·4%). Deacetylation of this diacetate (0·2 g.) in alcohol (10 ml.) with 10% aqueous potassium hydroxide (5 ml.) for 3 hr. regenerated sclerotinol (0·15 g.), m. p. and mixed m. p. 86°, after purification. The 2 : 4-*dinitrophenylhydrazone* of this acetate separated from aqueous alcohol in pale yellow needles, m. p. 123° (Found: C, 57·6; H, 6·2; N, 9·3; Cl, 5·9. $C_{29}H_{37}O_8N_4Cl$ requires C, 57·5; H, 6·0; N, 9·2; Cl, 5·7%).

By the methyl sulphate–potassium carbonate method for 2 hr. sclerotinol (0·5 g.) gave the *dimethyl ether* (0·4 g.), b. p. 120°/0·01 mm., $\lambda_{max.}$ 274 mμ (log ε 2·86) [Found: C, 68·6; H, 9·3; Cl, 9·6; OMe, 16·8. $C_{19}H_{27}OCl(OMe)_2$ requires C, 68·4; H, 9·0; Cl, 9·6; OMe, 16·8%]. Methyl sulphate and aqueous alkali afforded a poor yield of the same product. Demethylation of di-*O*-methylsclerotinol (0·4 g.) with a boiling mixture of hydriodic acid (8 ml.; *d* 1·7) and acetic acid (from 6 ml. of acetic anhydride) for 30 min. regenerated sclerotinol (0·1 g.), m. p. and mixed m. p. 86°. The *oxime* of di-*O*-methylsclerotinol was obtained as an oil which was purified by distillation at 140—145°/0·01 mm. and then slowly crystallised, having m. p. *ca.* 25° [Found: C 65·9; H, 8·8; Cl, 9·1; N, 4·0; OMe, 16·2. $C_{19}H_{28}ONCl(OMe)_2$ requires C, 65·7; H, 8·9; Cl, 9·2; N, 3·7; OMe, 16·3%].

Derivatives of Sclerotol.—Prepared from sclerotol (0·3 g.) by pyridine–acetic anhydride on the steam-bath for ½ hr., the *diacetate* separated from light petroleum (b. p. 60—80°) in needles (0·3 g.), m. p. 106° (Found: C, 65·5; H, 7·6; Cl, 8·4. $C_{23}H_{31}O_5Cl$ requires C, 65·3; H, 7·4; Cl, 8·4%). Mixed with the acetate of sclerotinol it had m. p. *ca.* 90°. By the methyl sulphate–potassium carbonate method, sclerotol (0·5 g.) gave the *dimethyl ether* (0·3 g.), b. p. 125—130°/0·01 mm. [Found: C, 68·6; H, 8·5; Cl, 9·7; OMe, 16·8. $C_{19}H_{25}OCl(OMe)_2$ requires C, 68·7; H, 8·5; Cl, 9·7; OMe, 16·9%]. The *di-p-nitrobenzoate* of sclerotol separated from aqueous alcohol in needles, m. p. 106° (Found: C, 62·2; H, 5·6; N, 4·3; Cl, 5·5. $C_{33}H_{33}O_9N_2Cl$ requires C, 62·2; H, 5·2; N, 4·4; Cl, 5·6%), and gave a 2 : 4-*dinitrophenylhydrazone*, yellow needles, m. p. 122° (from aqueous alcohol) (Found: C, 57·2; H, 4·8; N, 10·5; Cl, 4·4. $C_{39}H_{37}O_{12}N_6Cl$ requires C, 57·3; H, 4·6; N, 10·3; Cl, 4·3%).

Reduction of Sclerotinol and of Di-O-methylsclerotinol.—A solution of the phenol (0·5 g.) in ether (10 ml.) was added slowly to lithium aluminium hydride (2 g.) in ether (100 ml.), and the mixture then heated under reflux for 2 hr. On isolation in the usual manner the *product* separated from light petroleum (b. p. 60—80°) in needles, m. p. 79°, $\lambda_{max.}$ 287 mμ (log ε 3·27) (Found: C, 66·4; H, 9·0; Cl, 10·1; O, 14·2. $C_{19}H_{31}O_3Cl$ requires C, 66·5; H, 9·1; Cl, 10·3; O, 14·0%). A mixture of this alcohol and sclerotinol had m. p. *ca.* 70°. The infrared absorption spectrum showed the absence of carbonyl groups.

A solution of di-*O*-methylsclerotinol (0·6 g.) in ether (15 ml.) was added slowly to lithium aluminium hydride (3 g.) in ether (100 ml.), and the mixture heated under reflux for 2 hr. The resulting *alcohol* was an oil (0·5 g.), b. p. 120—130°/0·01 mm., the infrared spectrum of which exhibited hydroxyl but not carbonyl absorption; it had $\lambda_{max.}$ 279 mμ (log ε 2·90) [Found: C, 68·1; H, 9·8; Cl, 9·6; OMe, 16·5. $C_{19}H_{29}OCl(OMe)_2$ requires C, 68·0; H, 9·5; Cl, 9·6; OMe, 16·7%].

Dehydration of this alcohol (0·5 g.) with sodium hydrogen sulphate (2 g.) at 180° for 2 hr. gave the dehydration product (0·3 g.), b. p. 125°/0·01 mm., which did not show infrared hydroxyl absorption and had $\lambda_{max.}$ 279 mμ (log ε 3·01) [Found: C, 71·4; H, 9·4; Cl, 10·1; OMe, 17·8. $C_{19}H_{27}Cl(OMe)_2$ requires C, 71·4; H, 9·4; Cl, 10·1; OMe, 17·6%]. Ozonolysis of this compound in ethyl acetate or chloroform, followed by distillation of the product, gave an almost quantitative yield of di-*O*-methylsclerotinol, identified by the infrared absorption spectrum (Found: C, 68·7; H, 9·2%).

Beckmann Rearrangement of Di-O-methylsclerotinol Oxime.—A solution of the oxime (0·4 g.) in ether (15 ml.) was treated with thionyl chloride (1 drop), the solvent evaporated, water added, and the product isolated with ether and purified from light petroleum (b. p. 60—80°), giving a *product* in needles (0·35 g.), m. p. 125° [Found: C, 65·6; H, 8·9; N, 3·4; Cl, 9·2; OMe,

16·3. $C_{19}H_{28}ONCl(OMe)_2$ requires C, 65·7; H, 8·9; N, 3·7; Cl, 9·2; OMe, 16·3%]; the infrared absorption spectrum has a band at 1642 cm.$^{-1}$ (amide carbonyl [19]) which is not present in the spectrum of the oxime. This product was not hydrolysed by boiling 6N-alcoholic potassium hydroxide for 3 hr., boiling 35% sulphuric acid for 10 hr., or concentrated sulphuric acid at room temperature. It was unaffected by aqueous potassium permanganate solution in acetone at room temperature, by sodium in alcohol, or by lithium aluminium hydride in ether for 6 hr.

Action of Grignard Reagents upon Di-O-methylsclerotinol.—A solution of di-*O*-methylsclerotinol (0·6 g.) in ether (15 ml.) was added to an excess of phenylmagnesium bromide in ether (50 ml.), and the mixture was boiled for 1 hr., decomposed with ice and 2N-sulphuric acid, and extracted with ether. The residue left on evaporation of the extract was distilled at 140°/0·01 mm., giving the *phenyl derivative* (0·6 g.) which slowly crystallised in needles, m. p. *ca.* 20° [Found: C, 72·4; H, 8·8; Cl, 8·2; OMe, 13·7. $C_{25}H_{33}OCl(OMe)_2$ requires C, 72·6; H, 8·7; Cl, 8·0; OMe, 13·9%].

Similarly interaction of di-*O*-methylsclerotinol (0·3 g.) and excess of methylmagnesium iodide furnished the *methyl derivative* (0·2 g.), b. p. 130°/0·01 mm. [Found: C, 69·9; H, 9·2; Cl, 8·9; OMe, 15·6. $C_{20}H_{31}OCl(OMe)_2$ requires C, 68·6; H, 9·7; Cl, 9·2; OMe, 16·1%]. The absorption at 3670 cm.$^{-1}$ indicates the presence of a tertiary hydroxyl group.

The ultraviolet absorption spectra were determined with a Unicam S.P.• 500 spectrophotometer in 95% alcohol, and the infrared spectra in Nujol with a Grubb-Parsons double-beam spectrophotometer. The analyses were by Mr. A. S. Inglis, M.Sc., and his associates of this Department.

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[19] Ref. 13, p. 176.