372. *The Chemistry of Fungi. Part XXXIV.* Rotiorin, a Metabolite of* Penicillium sclerotiorum *van Beyma.*

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The new pigment, rotiorin, $C_{23}H_{24}O_5$ or less probably $C_{22}H_{22}O_5$, from *P. sclerotiorum*, is similar in many respects to sclerotiorin. On alkaline degradation rotiorin furnishes 4 : 6-dimethylocta-2 : 4-dienoic acid, and with ammonia gives rotioramine, $C_{23}H_{25}O_4N$, which is readily aromatised to an extended *iso*quinolone and on oxidation yields 2-(3 : 5-dimethyl-*n*-heptyl)-pyridine-4 : 5-dicarboxylic acid.

The structure of rotiorin is discussed.

THE present paper describes preliminary studies of a new pigment, rotiorin, isolated [1,2] from the mycelium of *Penicillium sclerotiorum* van Beyma, the methods being similar to those employed for sclerotiorin.[2,3,4] Rotiorin, which is optically active and has the molecular formula $C_{23}H_{24}O_5$ or $C_{22}H_{22}O_5$, is devoid of methoxyl and hydroxyl groups, but, from the infrared absorption spectrum, appears to contain several carbonyl residues. From the results of numerous analyses of the compound and its derivatives the formula, $C_{23}H_{24}O_5$, appears the more likely and, although absolute distinction is not yet possible this will be used in the present communication. The pigment is not reduced by sulphur dioxide or sodium dithionite in aqueous dioxan and, although reaction occurred with hydroxylamine, semicarbazide, and phenylhydrazine, crystalline derivatives could not be isolated.

Rotiorin is extremely sensitive to alkali and on degradation with sodium hydroxide solution gave 4 : 6-dimethylocta-2 : 4-dienoic acid (I) and 2 : 4-dimethylhex-2-enal [2] (II); degradation with sodium hydrogen carbonate solution afforded, in addition to the dienoic acid, small amounts of acetaldehyde. Despite repeated attempts it has not been possible to isolate a crystalline product from the hydrogenation or the acetylation of rotiorin. On ozonolysis rotiorin gave (+)-α-methylbutyraldehyde; no other product could be isolated. Repeated attempts to obtain a ketone, analogous to pentanorsclerotiorone,[2] by oxidation with chromic acid or by ozonolysis were unsuccessful.

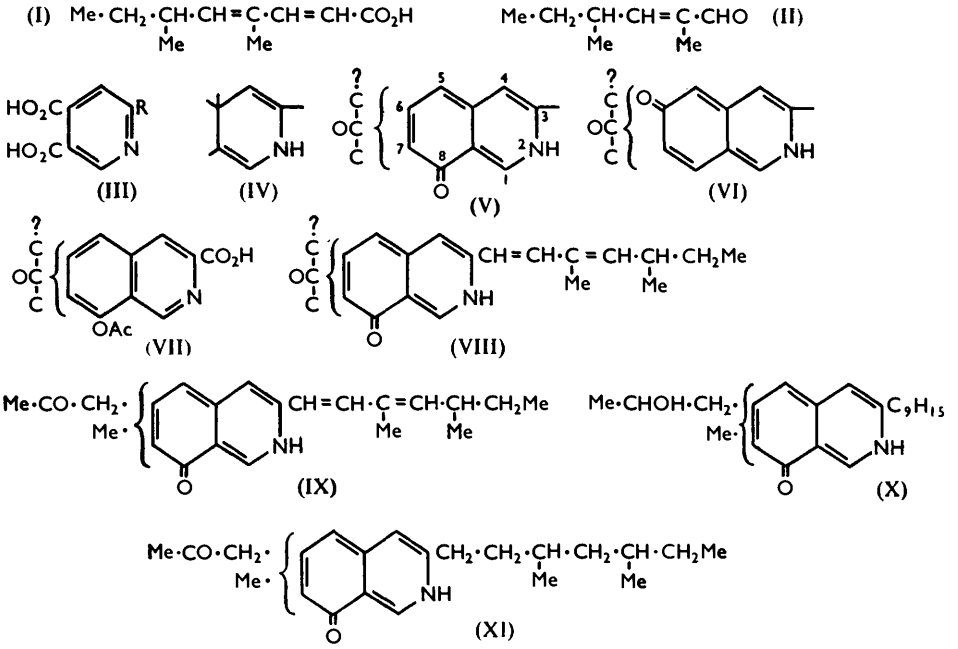
Like its congener, sclerotiorin, rotiorin reacts rapidly with ammonia to form rotioramine, $C_{23}H_{25}O_4N$, which on ozonolysis gives the aldehyde (II) but on degradation with alkali does not furnish the dienoic acid (I), acetaldehyde, or ammonia. Further, on oxidation with nitric acid, rotioramine, like sclerotioramine, furnishes berberonic acid (III; R = CO_2H), showing that the base contains the system (IV). With methylamine rotiorin yields *N*-methylrotioramine, $C_{24}H_{27}O_4N$, in low yield, which is also obtained in even smaller amounts by the *N*-methylation of rotioramine. Rotioramine is readily aromatised with zinc and alkali or with zinc and acetic acid (cf. the behaviour of sclerotioramine [4]) to give *apo*rotioramine, $C_{22}H_{27}O_2N$, which is readily soluble in aqueous sodium hydroxide solution, furnishes an unstable monohydrochloride and a monoacetyl derivative, $C_{24}H_{29}O_3N$, and exhibits the properties of an *iso*quinoline. From the infrared spectrum (1745 cm.$^{-1}$, aromatic acetate) together with the decrease in m. p. observed in passing from *apo*rotioramine to the acetate it is clear that this derivative is an *O*-acetate. The presence of an isolated carbonyl residue in *apo*rotioramine is shown by the infrared absorption at 1698 cm.$^{-1}$, in conjunction with the formation of a 2 : 4-dinitrophenylhydrazone (isolated as the hydrochloride), $C_{28}H_{31}O_5N_5$,HCl, $\lambda_{max.}$ 360 mμ (log ε, 4·43). Similarly, the hydrochloride of the mono-*O*-acetate exhibits aromatic acetate (1758 cm.$^{-1}$) and isolated carbonyl

* Part XXXIII, preceding paper.

[1] Powell, Robertson, and Whalley, *Chem. Soc. Special Publ.*, 1956, No. 5, p. 27.
[2] Eade, Page, Robertson, Turner, and Whalley, *J.*, 1957, 4913.
[3] Graham, Page, Robertson, Travers, Turner, and Whalley, *J.*, 1957, 4924.
[4] Fielding, Graham, Robertson, Travers, and Whalley, *J.*, 1957, 4931.

(1699 cm.$^{-1}$) absorption in the infrared spectrum. Volatile acids are not produced during the aromatisation of rotioramine to *apo*rotioramine.

Methylation of *apo*rotioramine with methyl sulphate and alkali furnishes *N*-methyl-*apo*rotioramine, which is devoid of hydroxyl and methoxyl groups, is rather unstable, and is identical with *N*-methyl*apo*rotioramine obtained from the aromatisation of *N*-methyl-rotioramine. The formation of this derivative, which is insoluble in alkali and exhibits infrared absorption at 1725 cm.$^{-1}$ (unconjugated carbonyl) and at 1623 cm.$^{-1}$ ($\alpha\beta\alpha'\beta'$-unsaturated carbonyl), in conjunction with the solubility of *apo*rotioramine in aqueous alkali and the oxidation of rotioramine to berberonic acid indicates the presence of an extended *iso*quinolone residue (V) or (VI) in *apo*rotioramine.



Ozonolysis of *O*-acetyl*apo*rotioramine gives *O*-acetyl*apo*rotaminic acid, $C_{16}H_{15}O_4N$, of type (VII), the positive ferrous sulphate reaction of which indicates that the carboxyl group and hence the original C_9H_{15} residue, is situated in the *ortho*-position to the nitrogen atom, as in sclerotioramine.[4] Consequently the structure for *apo*rotioramine may be expanded to (VIII). Since *O*-acetyl*apo*rotaminic acid contains three *C*-methyl residues and furnishes an almost quantitative yield of iodoform, the partial structure (VIII) for *apo*rotioramine may be further expanded to (IX) in which the molecular formula allows the isolated carbonyl group to be separated from the aromatic nucleus by only one carbon atom. Further, *apo*rotioramine does not readily yield an anhydro-compound devoid of the isolated carbonyl residue (*i.e.*, a 2-methylfuran derivative) and hence the quinolone-oxygen atom and the $CH_3{\cdot}CO{\cdot}CH_2{\cdot}$ residue are not likely to be in the *ortho*-position to each other.[5]

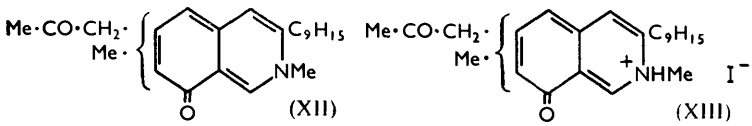
Ozonolysis of *O*-benzoyl*apo*rotioramine yields *O*-benzoyl*apo*rotaminic acid which, as expected, appears to contain two *C*-methyl residues.

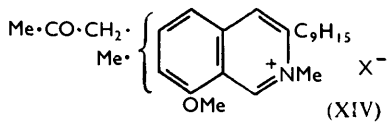
Reduction of the isolated carbonyl residue of *apo*rotioramine with potassium boro-hydride gives the secondary alcohol, *apo*rotioraminol (X) (infrared absorption at 3250 cm.$^{-1}$). On ozonolysis its di-*O*-acetate yields di-*O*-acetylrotaminolic acid which in agreement with previous considerations contains four *C*-methyl residues.

[5] Whalley and Lloyd, *J.*, 1956, 3213.

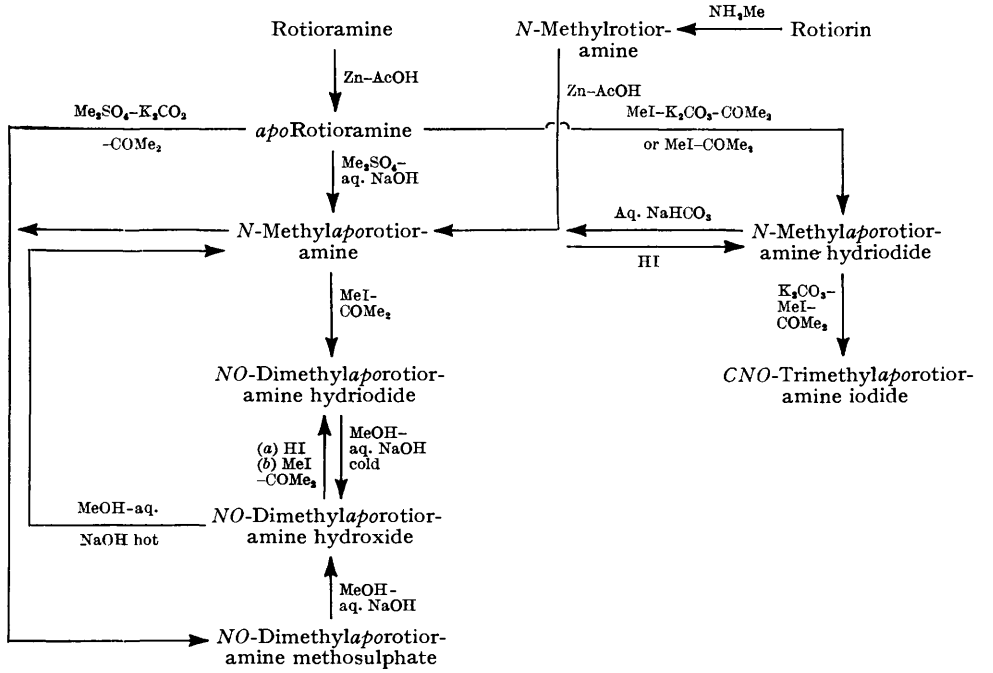
Hydrogenation of rotioramine to tetrahydrorotioramine has not been achieved, but reduction of *O*-acetyl*apo*rotioramine furnishes *O*-acetyltetrahydro*apo*rotioramine, characterised as the hydrochloride, $C_{24}H_{34}O_3NCl$, whilst *apo*rotioramine gives tetrahydro*apo*rotioramine $C_{23}H_{33}O_2N$ (XI) which is oxidised by potassium permanganate to the cinchomeronic acid [6] (III; R = $CH_2{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2Me$).

The action of methylating agents on *apo*rotioramine is complex but in agreement with the established behaviour of *iso*quinolones. Thus whilst methyl sulphate and alkali yield *N*-methyl*apo*rotioramine (XII), methyl iodide in acetone gives *N*-methyl*apo*rotioramine hydriodide (XIII) which readily yields the free base (XII) with sodium hydrogen carbonate.

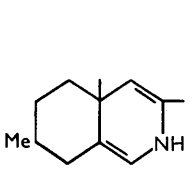




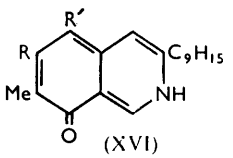
Methylation of either *apo*rotioramine or *N*-methyl*apo*rotioramine with methyl sulphate–potassium carbonate in acetone furnished *NO*-dimethyl*apo*rotioramine methosulphate (XIV; X = $MeSO_4$). With cold alkali this gave the unstable, insoluble base (XIV; X = OH) which was converted by hydriodic acid into the salt (XIV; X = I) and by warm alkali into *N*-methyl*apo*rotioramine. The same iodide (XIV; X = I) has been formed from *N*-methyl*apo*rotioramine by direct addition of methyl iodide to the quinonoid system. The action of methyl iodide–potassium carbonate–acetone on *apo*rotioramine, *N*-methyl-*apo*rotioramine hydriodide (XIII), or *NO*-dimethyl*apo*rotioramine iodide (XIV; X = I) furnished a product which is probably a *CNO*-trimethyl iodide. These results are summarised in the Chart.



The Structure of Rotiorin.—The experimental evidence indicates that *apo*rotioramine has the molecular formula, $C_{22}H_{27}O_2N$, and an extended *iso*quinolone structure containing one *C*-methyl and an acetonyl group as substituents. The isolation of 2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid from the potassium permanganate oxidation of tetrahydro*apo*rotioramine confirms the presence of an *iso*quinolone system which must be of type (V) or (VI) and is devoid of substituents in the 1- and the 4-position. Analogy with sclerotiorin [6] suggests that the nuclear *C*-methyl residue occupies the 7-position of the *iso*quinolone residue as in (XV). In addition, since *apo*rotioramine does not appear to have the *iso*quinolone-oxygem atom in the *ortho*-position to the acetonyl residue, as is indicated by our failure to prepare an anhydro-derivative (although this may be ascribed to the existence of the *iso*quinolone in the amide form), the formula for *apo*rotioramine may be expanded to (XVI; R = CH_2·COMe, R′ = H, or *vice versa*) although at this stage other equivalent formulations derived from the alternative structure (VI) for the *iso*quinolone moiety cannot be exluded; by analogy with sclerotiorin [6] (XVI; R = H, R′ = CH_2·COMe) appears the more probable.



(XV)



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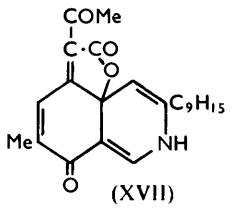

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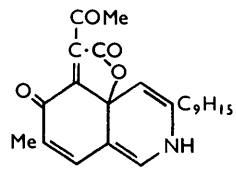
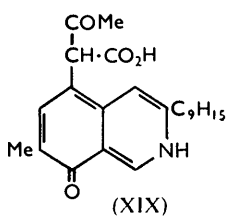
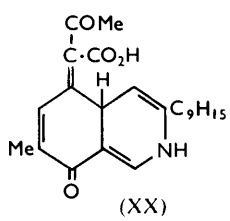


(XVIII)

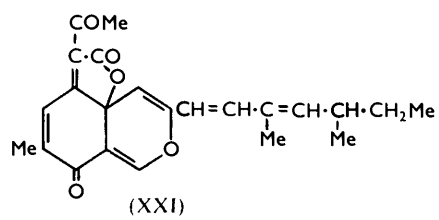
On the basis of the C_{23} formula for rotiorin the conversion of rotioramine into *apo*-rotioramine involves the addition of two hydrogen atoms and the elimination of two oxygen atoms and one carbon atom (which is not extruded as formic acid). These considerations and the foregoing experimental facts in conjunction with the arguments advanced during the derivation of a structure for the closely related sclerotiorin,[6] and the assumption that the aromatisation of rotioramine to *apo*rotioramine does not involve an intramolecular rearrangement, may be rationalised in terms of structure (XVII) for rotioramine, although obvious variants, *e.g.*, (XVIII), cannot be excluded. Reductive aromatisation of rotioramine then proceeds by way of (XIX) which may be formed by the mechanism envisaged for the aromatisation of sclerotioramine and its derivatives,[6] or perhaps more probably by direct hydrogenolysis of the diallylic lactone to (XX) (cf. the conversion



(XIX)



(XX)



(XXI)

of ψ-santonin into dihydro-ψ-santonin [7]), followed by a prototropic shift to (XIX). Spontaneous extrusion of carbon dioxide from the β-keto-acid (XIX) yields *apo*rotioramine. On the assumption that the conversion of rotiorin into rotioramine does not involve a rearrangement rotiorin is represented by (XXI) or an equivalent variant.

[6] Fielding, Robertson, Travers, and Whalley, preceding paper.
[7] Clemo and Cocker, *J.*, 1946, 30.

Experimental

Rotiorin.—Only comparatively small amounts of rotiorin were obtained when *Penicillium sclerotiorum* van Beyma was cultivated for the production of sclerotiorin on a Czapek–Dox type of medium. A somewhat improved yield resulted when the mould was grown on a Raulin–Thom medium containing glucose (6·0 g., all amounts per l.), ammonium tartrate (2·6 g.), tartaric acid (2·6 g.), ammonium phosphate (0·4 g.), potassium carbonate (0·4 g.), magnesium carbonate (0·26 g.), ammonium sulphate (0·17 g.), zinc sulphate (0·05 g.), and ferrous sulphate (0·05 g.) at 25° for 14—17 days, in penicillin flasks each containing 500 ml. of medium. The thick orange-red mycelial felts were collected, dried at room temperatue for 7 days, milled, and percolated with ether for 24 hr. Evaporation of this extract followed by purification from methanol furnished sclerotiorin, m. p. 206—207°. Subsequent exhaustive percolation of the mycelium with more ether gave rotiorin which was purified by repeated recrystallisation from alcohol, forming long red needles, m. p. 246° (decomp.), which sublimed unchanged at 190°/0·005 mm. and had a negative ferric reaction, $[\alpha]_D^{22}$ +5,080° (*c* 0·002 in $CHCl_3$), $\nu_{max.}$ 1745, 1660, 1640, 1620 cm.$^{-1}$, and $\lambda_{max.}$ 238, 242, 282, 312, and 493 mμ (log ε 5·71, 5·71, 5·65, and 5·91 respectively) (Found: C, 72·4, 72·0, 72·0; H, 6·3, 6·9, 6·4; O, 21·2, 21·4. Calc. for $C_{22}H_{22}O_5$: C, 72·1; H, 6·0; O, 21·9. Calc. for $C_{23}H_{24}O_5$: C, 72·6; H, 6·3; O, 21·1%). This pigment is sparingly soluble in the usual organic solvents and insoluble in 2N-aqueous sodium carbonate or hydroxide but readily dissolves in aqueous-alcoholic sodium hydroxide, giving a deep-red solution. The compound is not extracted from ethereal solution by aqueous sodium hydroxide. 1000 Flasks furnished *ca.* 8 kg. of dried mycelium from which sclerotiorin (300—350 g.) and rotiorin (100—150 g.) were extracted; the yield of rotiorin varied from one batch to another for no apparent reason.

Degradation of Rotiorin with Alkali.—(*a*) A mixture of rotiorin (1 g.) and 2N-aqueous sodium hydroxide (60 ml.) was slowly distilled during 2½ hr., in nitrogen, and the volume of the reaction mixture maintained by the addition of distilled water (200 ml.) as required. The pigment rapidly formed a red solution which later deposited a black solid, leaving a supernatant orange liquor which was decanted, acidified with 2N-sulphuric acid, filtered, and distilled, giving 4 : 6-dimethylocta-2 : 4-dienoic acid (10 mg.), m. p. and mixed m. p. 92°, $[\alpha]_D^{19}$ +67·6° (*c* 2 in EtOH), which was isolated from the distillate with ether (Found: C, 71·6; H, 9·6. Calc. for $C_{10}H_{16}O_2$: C, 71·4; H, 9·5%). With an excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate this distillate furnished the 2 : 4-dinitrophenylhydrazone of 2 : 4-dimethylhexa-2-enal which separated from alcohol in orange needles (60 mg.), m. p. and mixed m. p. 158°, identical with a specimen from sclerotiorin [2] (Found: C, 55·0; H, 6·1; N, 18·4. Calc. for $C_{14}H_{18}O_4N_4$: C, 54·9; H, 5·9; N, 18·3%). In another experiment this aldehyde was isolated as the semicarbazone from light petroleum (b. p. 60—80°) in plates, m. p. and mixed m. p. 148° (Found: C, 59·3; H, 9·2; N, 23·1. Calc. for $C_9H_{17}ON_3$: C, 59·0; H, 9·3; N, 22·9%).

(*b*) A mixture of rotiorin (1 g.) and N-aqueous sodium hydrogen carbonate (60 ml.) was distilled slowly for 2½ hr. with the addition of distilled water (200 ml.) to maintain the volume. Mixed with an excess of 2 : 4-dinitrophenylhydrazine sulphate solution, the distillate furnished the 2 : 4-dinitrophenylhydrazone of acetaldehyde which separated from alcohol in yellow plates (0·1 g.), m. p. and mixed m. p. 163°, having the requisite infrared absorption spectrum (Found: C, 43·5; H, 3·6; N, 24·6. Calc. for $C_8H_8O_4N_4$: C, 42·9; H, 3·6; N, 25·0%). Acidification of the cooled hydrolysate with 2N-sulphuric acid, followed by distillation of the filtered solution, gave 4 : 6-dimethylocta-2 : 4-dienoic acid (0·15 g.), m. p. and mixed m. p. 92°.

Ozonolysis of Rotiorin.—A stream of ozone and oxygen was passed through a solution of rotiorin (1 g.) in ethyl acetate (150 ml.) at 0° for 135 min. The originally red solution became pale yellow. After isolation the ozonide was decomposed with water (250 ml.) for 12 hr. and the clarified hydrolysate distilled, yielding (+)-α-methylbutyraldehyde which was isolated as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 133°.

Rotioramine.—(*a*) A mixture of aqueous ammonia (*d* 0·88; 25 ml.) and rotiorin (5 g.) was shaken gently for 15 min., and the resulting purple solution poured into an excess of 2N-hydrochloric acid at 0°. Purified from alcohol, the precipitate gave *rotioramine* in violet, rectangular, plates (3·5 g.), m. p. 261° (decomp.), $\nu_{max.}$ 1730, 1710, 1640, 1605, 1590, 3200, 3025 cm.$^{-1}$ (Found: C, 72·6, 72·6, 72·6, 72·7; H, 6·3, 6·5, 6·7, 6·7; N, 3·7, 3·7, 3·7. $C_{22}H_{23}O_4N$ requires C, 72·3; H, 6·3; N, 3·8. $C_{23}H_{25}O_4N$ requires C, 72·8; H, 6·6; N, 3·7%).

(*b*) A solution of rotiorin (1 g.) in ether (3 l.) was shaken for 1 min. with aqueous ammonia

(*d* 0·88; 5 ml.); the ammoniacal liquor was separated from the ethereal solution which was washed with water, followed by dilute hydrochloric acid, and evaporated to about 500 ml. On being kept this gave rotioramine (0·8 g.), m. p. and mixed m. p. 261° (decomp.). Rotioramine is insoluble in 2N-aqueous sodium hydroxide but readily dissolves in 2N-aqueous sodium hydroxide containing 1% of alcohol, to form a solution which remains clear on dilution with water.

N-*Methylrotioramine*.—A solution of rotiorin (0·3 g.) in ether (1 l.) was shaken with aqueous methylamine (from 2 ml. of base and 20 ml. of water) for 1 hr. and the aqueous layer acidified with dilute hydrochloric acid. The ethereal solution was separated, washed, and evaporated, leaving N-*methylrotioramine*, which crystallised from alcohol in prisms (30 mg.), m. p. 226° (decomp.) (Found: C, 74·0; H, 7·7. $C_{24}H_{27}O_4N$ requires C, 73·3; H, 6·9. $C_{23}H_{25}O_4N$ requires C, 72·8; H, 6·6%). Attempts to prepare this compound by the methylation of rotioramine under a variety of conditions were unsuccessful although occasionally traces of the *N*-methyl derivative were obtained.

Oxidation of Rotioramine.—Concentrated nitric acid (6 ml.) was diluted with water (1 ml.) and added to rotioramine (2 g.). Next day the resulting red solution was heated on the steam-bath for 21 hr.; more concentrated nitric acid (10 ml.) and water (1 ml.) were added after 7 and after 14 hr. The mixture was then diluted with water, evaporated to small volume, and again diluted and evaporated. This was repeated until all the nitric acid had been removed. The residual solution was then cooled, extracted with ether, treated with charcoal, and concentrated, to give berberonic acid, m. p. and mixed m. p. 238° (decomp.), having the requisite infrared absorption spectrum.

apo*Rotioramine*.—A mixture of rotioramine (2 g.), zinc dust (2 g.), and acetic acid (20 ml.) was heated under reflux in nitrogen for 15 min. and the orange solution cooled, diluted with ether (50 ml.), and filtered into a saturated solution of sodium hydrogen carbonate (500 ml.). The liquor was extracted with ether, and the combined extracts were washed, dried, and evaporated to *ca.* 20 ml. apo*Rotioramine* then separated in cream-coloured needles (1·2 g.) and on purification from alcohol had m. p. 186°, a negative ferric reaction in alcohol, and $[\alpha]_D^{22}$ +57° (*c* 0·2 in $CHCl_3$) [Found: C, 78·0, 78·0, 78·6, 78·6; H, 8·0, 8·0, 8·1, 8·0; N, 3·9, 4·0, 4·0%; *M* (Rast), 304. $C_{22}H_{27}O_2N$ requires C, 78·3; H, 8·0; N, 4·2%; *M*, 337. $C_{21}H_{25}O_2N$ requires C, 78·0; H, 7·8; N, 4·3%; *M*, 323]. *apo*Rotioramine is unchanged on sublimation *in vacuo* and readily dissolves in 2N-aqueous sodium hydroxide from which it is precipitated unchanged by carbon dioxide. The solution in aqueous methanol containing hydrochloric acid deposits bright yellow needles of an unstable hydrochloride, m. p. 230°, which on crystallisation from aqueous alcohol regenerates *apo*rotioramine. Formed with alcoholic–acidic 2 : 4-dinitrophenylhydrazine, the 2 : 4-*dinitrophenylhydrazone* of *apo*rotioramine hydrochloride separated from alcohol in yellow needles, m. p. 252° (decomp.) (Found: C, 60·8; H, 5·9; N, 12·1. $C_{28}H_{31}O_5N_5$,HCl requires C, 60·7; H, 5·6; N, 12·6. $C_{27}H_{29}O_5N_5$,HCl requires C, 60·1; H, 5·6; N, 13·0%). Pyridine–acetic anhydride at room temperature for 12 hr. gave a quantitative yield of O-*acetyl*apo*rotioramine*, plates, m. p. 86° [from light petroleum (b. p. 40—60°)] (Found: C, 75·7, 75·3; H, 7·6, 7·8; N, 3·5, 3·7. $C_{24}H_{29}O_3N$ requires C, 76·0; H, 7·7; N, 3·7. $C_{23}H_{27}O_3N$ requires C, 75·6; H, 7·5; N, 3·8%). This acetate is readily deacetylated with 2N-aqueous sodium hydroxide to the parent base. Addition of 2N-hydrochloric acid to this acetate in alcohol furnished the *hydrochloride* in green needles, m. p. 166° (Found: C, 66·6, 66·3; H, 7·5, 7·4; N, 3·3. $C_{23}H_{27}O_3N$,HCl,H_2O requires C, 65·9; H, 7·1; N, 3·3. $C_{24}H_{29}O_3N$,HCl,H_2O requires C, 66·5; H, 7·4; N, 3·2%. Found in specimen dried at 80°: C, 68·3; H, 7·0. $C_{24}H_{29}O_3N$,HCl requires C, 69·4; H, 7·2%). With pyridine–benzoyl chloride, *apo*rotioramine (1·5 g.) furnished O-*benzoyl*apo*rotioramine*, pale yellow needles (1·0 g.), m. p. 148° (from methanol) (Found: C, 79·1; H, 6·8; N, 2·5. $C_{29}H_{31}O_3N$ requires C, 79·0; H, 7·0; N, 3·1%).

Reduction of apo*Rotioramine*.—Potassium borohydride (0·5 g.) in water (10 ml.) was added during 1 hr. to *apo*rotioramine (0·5 g.) suspended in alcohol (15 ml.) and distilled water (5 ml.). The base rapidly dissolved and was then gradually replaced by a white precipitate. Next day the mixture was added to an excess of dilute hydrochloric acid, and the resulting green unstable hydrochloride isolated and decomposed with 2N-aqueous sodium hydrogen carbonate to give apo*rotioraminol* which separated from alcohol in plates (0·4 g.), m. p. 240° (Found: C, 77·6; H, 8·8. $C_{22}H_{29}O_2N$ requires C, 77·9; H, 8·6. $C_{21}H_{27}O_2N$ requires C, 77·5; H, 8·4%). The *hydrochloride* separated from aqueous alcohol in pale yellow needles, m. p. 224° (Found:

C, 70·1; H, 8·3. $C_{22}H_{29}O_2N$,HCl requires C, 70·3; H, 8·0%). Prepared from *apo*rotioraminol (1 g.) by pyridine–acetic anhydride di-*O*-acetyl*apo*rotioraminol (1 g.) was obtained as an oil which furnished quantitatively a *hydrochloride* in yellow needles, m. p. 105° (from ethanol) (Found: C, 67·8; H, 7·6. $C_{26}H_{33}O_4N$,HCl requires C, 67·9; H, 7·4. $C_{25}H_{31}O_4N$,HCl requires C, 67·4; H, 7·2%).

Di-O-*acetyl*apo*rotaminolic Acid.*—A mixture of oxygen and ozone was passed through a solution of di-*O*-acetyl*apo*rotioraminol (0·5 g.) in ethyl acetate (50 ml.) for 20 min. Removal of the solvent furnished the ozonide as a colourless gum which was decomposed with water (10 ml.) during 12 hr. Purification of the resultant product from ethanol gave *di*-O-*acetyl*apo*rotaminolic acid* in needles (0·1 g.), m. p. 196° (Found: C, 62·3; H, 5·7; *C*-Me, 16·4. $C_{18}H_{19}O_6N$ requires C, 63·6; H, 5·6; 4*C*-Me, 17·4. $C_{17}H_{17}O_6N$ requires C, 61·6; H, 5·2; 4*C*-Me, 18·1%). This acid has a negative reaction with ferric chloride but exhibits a bright orange colour with ferrous sulphate in alcohol.

Ozonolysis of O-*Acetyl*apo*rotioramine.*—A slow stream of ozone and oxygen was passed through a solution of *O*-acetyl*apo*rotioramine (1 g.) in ethyl acetate (25 ml.) at room temperature for 40 min. Removal of the solvent in a vacuum followed by decomposition of the ozonide with water (20 ml.) for 12 hr. furnished a pale yellow solid which was purified from methanol, giving O-*acetyl*apo*rotaminic acid* in needles (0·25 g.), m. p. 240° (decomp.), with a negative ferric reaction and absorption at 1754 (aromatic acetate) and at 1724 $cm.^{-1}$ (isolated carbonyl) (Found: C, 63·0, 63·4, 63·4; H, 5·1, 4·9, 5·2; N, 4·2, 4·2. $C_{15}H_{13}O_5N$ requires C, 62·7; H, 4·6; N, 4·9. $C_{16}H_{15}O_5N$ requires C, 63·8; H, 5·0; N, 4·7%). This acid, which is insoluble in 2N-aqueous sodium carbonate but readily soluble in cold 2N-aqueous sodium hydroxide, gives a bright orange colour with ferrous sulphate and is unchanged by acetylating reagents. When a suspension of *O*-acetyl*apo*rotaminic acid (0·1 g.) in alcohol (50 ml.) was saturated with hydrogen chloride and heated under reflux for 3 hr., evaporation of the clear yellow solution gave a yellow solid which was purified by ethanol, to yield apo*rotaminic acid hydrochloride* (0·05 g.) in yellow needles, m. p. 265° (Found: C, 56·9; H, 4·7. $C_{14}H_{13}O_4N$,HCl requires C, 56·8; H, 4·7%).

Ozonolysis of O-*Benzoyl*apo*rotioramine.*—A stream of ozonised oxygen was passed through a solution of *O*-benzoyl*apo*rotioramine (0·75 g.) in ethyl acetate (20 ml.) at room temperature, until the solution was colourless (*ca.* 45 min.). Removal of the solvent under reduced pressure followed by decomposition of the ozonide with water (10 ml.) during 12 hr. furnished a solid which was purified from methanol, to yield O-*benzoyl*apo*rotaminic acid* in prisms (0·08 g.), m. p. 280—285° (decomp.) (Found: C, 66·5; H, 5·0; *C*-Me, 7·3. $C_{21}H_{17}O_5N$ requires C, 69·4; H, 4·7. $C_{21}H_{17}O_5N,H_2O$ requires C, 66·1; H, 5·0; 2*C*-Me, 7·8%).

Hydrogenation of O-*Acetyl*apo*rotioramine and of* apo*Rotioramine.*—A solution of this acetate (1 g.) in alcohol (50 ml.) containing palladium–charcoal (from 1 g. of charcoal and 0·15 g. of palladium chloride) was shaken in hydrogen until 2·5 mol. had been absorbed. Evaporation of the filtered solution gave a glass but treatment in alcohol with 2N-hydrochloric acid furnished O-*acetyltetrahydro*apo*rotioramine hydrochloride* which separated from acetone in almost colourless needles (0·6 g.), m. p. 130° (Found: C, 68·6; H, 7·9. $C_{24}H_{33}O_3N$,HCl requires C, 68·7; H, 8·1. $C_{23}H_{31}O_5N$,HCl requires C, 68·1; H, 8·0%).

When a solution of *apo*rotioramine (0·5 g.) in ethyl acetate (100 ml.) containing the same catalyst was shaken in hydrogen the absorption of *ca.* 2½ mol. of gas occurred in 2½ hr., giving a colourless solution which contained an amorphous product. This was converted into *tetrahydro*apo*rotioramine hydrochloride* which separated from ethyl acetate in yellow plates (0·3 g.), m. p. 150° (Found: C, 70·1, 69·8; H, 8·3, 8·5; N, 3·4. $C_{22}H_{31}O_2N$,HCl requires C, 70·0; H, 8·5; N, 3·7. $C_{21}H_{29}O_2N$,HCl requires C, 69·5; H, 8·2; N, 3·9%).

Oxidation of O-*Acetyltetrahydro*apo*rotioramine Hydrochloride.*—A suspension of this hydrochloride (1 g.) in 2N-aqueous sodium hydroxide (20 ml.) was warmed until complete dissolution occurred (5 min.). A solution of potassium permanganate (3 g.) in water (75 ml.) was added gradually, initially at room temperature and then finally at 70° to complete the oxidation. The warm mixture was filtered, the cooled, acidified filtrate extracted with ether (2 × 150 ml.), and the extract dried and evaporated, to yield a product which on purification from aqueous acetone gave 2-(3 : 5-dimethyl-*n*-heptyl)pyridine-4 : 5-dicarboxylic acid in needles (0·04 g.), m. p. and mixed m. p. 195°.

Methylation of apo*Rotioramine.*—(*a*) A solution of the compound (1 g.) in acetone (30 ml.) containing methyl iodide (3 ml.) was heated under reflux for 4 hr., and the crystalline precipitate

was collected and purified from methanol, giving the *hydriodide* of *N*-methyl*apo*rotioramine in yellow needles (0·8 g.), m. p. 252° (decomp.) (Found: C, 57·2, 57·6, 57·2; H, 6·1, 6·2, 6·3; N, 2·8; OMe, 0. $C_{23}H_{29}O_2N$,HI requires C, 57·4; H, 6·3; N, 2·9. $C_{22}H_{27}O_2N$,HI requires C, 56·8; H, 5·8; N, 3·0%). Trituration of this salt (1 g.) with aqueous sodium hydrogen carbonate gave N-*methyl*apo*rotioramine* which was purified from benzene, forming unstable red needles (0·5 g.), m. p. 196° (decomp.) (Found: C, 74·5, 73·7; H, 8·4, 8·2; N, 3·6; NMe, 5·4; OMe, 0. $C_{23}H_{29}O_2N,H_2O$ requires C, 74·8; H, 8·4; N, 3·9. $C_{22}H_{27}O_2N,H_2O$ requires C, 74·4; H, 8·2%). The same product, m. p. and mixed m. p. 196°, was obtained in poor yield by treating *apo*rotioramine with methyl sulphate and aqueous sodium hydroxide at room temperature or *N*-methylrotioramine with zinc and alkali under the conditions employed for the preparation of *apo*rotioramine.

(*b*) When a solution of *N*-methyl*apo*rotioramine (0·6 g.) and methyl iodide (3 ml.) in acetone (15 ml.) was heated under reflux for 1 hr., purification of the crystalline precipitate gave NO-*dimethyl*apo*rotioramine iodide* which separated from methanol in yellow needles (0·4 g.), m. p. 216°, containing ionisable iodine (Found: C, 58·1; H, 6·8; N, 2·8; OMe, 6·5; NMe, 5·4. $C_{24}H_{32}O_2NI$ requires C, 58·4; H, 6·5; N, 2·8; OMe, 6·3; NMe, 5·9. $C_{23}H_{30}O_2NI$ requires C, 57·6; H, 6·3; N, 3·0; OMe, 6·5; NMe, 6·1%). Treatment of this salt (1 g.) in methanol with excess of 2N-aqueous sodium hydroxide gave an orange precipitate of NO-*dimethyl*apo*rotioramine hydroxide* which crystallised from methanol in rather unstable, hygroscopic orange needles (0·5 g.), m. p. 180° (decomp.) (Found: N, 4·2; OMe, 7·9; NMe, 5·2. $C_{24}H_{33}O_3N$ requires N, 3·7; OMe, 8·1; NMe, 7·6%). Addition of hydriodic acid to a solution of this base in acetone regenerated quantitatively the parent iodide, m. p. and mixed m. p. 216° (decomp.), which was also obtained when the free base was refluxed in acetone solution with methyl iodide. When a solution of *NO*-dimethyl*apo*rotioramine hydroxide in methanol at 60° was treated with 2N-aqueous sodium hydroxide *N*-methyl*apo*rotioramine was formed in low yield.

Methylation of either *apo*rotioramine (0·5 g.) or *N*-methyl*apo*rotioramine (0·5 g.) with methyl sulphate–potassium carbonate in boiling acetone gave the methosulphate of *O*-dimethyl-*apo*rotioramine which separated from ethyl acetate in yellow needles (0·2 g.), m. p. 172°. The addition of 2N-aqueous sodium hydroxide to a solution of this methosulphate in methanol furnished *NO*-dimethyl*apo*rotioramine hydroxide in orange needles, m. p. and mixed m. p. 180° (decomp.).

(*c*) After *N*-methyl*apo*rotioramine hydriodide (1 g.) or *NO*-dimethyl*apo*rotioramine iodide (1 g.) had been heated with potassium carbonate (2 g.) and methyl iodide (2 ml.) in boiling acetone (100 ml.) for 6 hr., purification of the product from methanol gave a *substance* in yellow prisms (0·8 g.), m. p. 202° (decomp.), containing ionisable halogen (Found: C, 58·7, 59·1; H, 6·8, 6·8; N, 2·8, 2·6; OMe, 6·5, 7·3; NMe, 7·2; I, 25·5. $C_{25}H_{34}O_2NI$ requires C, 59·2; H, 6·7; N, 2·8; OMe, 6·1; NMe, 5·7; I, 25·0. $C_{24}H_{32}O_2NI$ requires C, 58·5; H, 6·5; N, 2·8; OMe, 6·3; NMe, 5·9; I, 25·7%). The mixed m. p. with *NO*-dimethyl*apo*rotioramine iodide was *ca.* 186° (decomp.).

NO-Dimethyl*apo*rotioramine iodide was recovered unchanged when treated with methyl sulphate–potassium carbonate in boiling acetone for 6 hr.

The ultraviolet absorption spectra were determined in 95% alcohol with a Unicam S.P. 500 Spectrophotometer whilst the infrared spectral data were obtained in Nujol by using a Perkin-Elmer Model 21 double-beam spectrophotometer. The analyses were by Mr. A. S. Inglis, M.Sc., and his associates of this Department.

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