685. *The Chemistry of Fungi. Part XLI.*[1] *The Structure of Rotiorin.*

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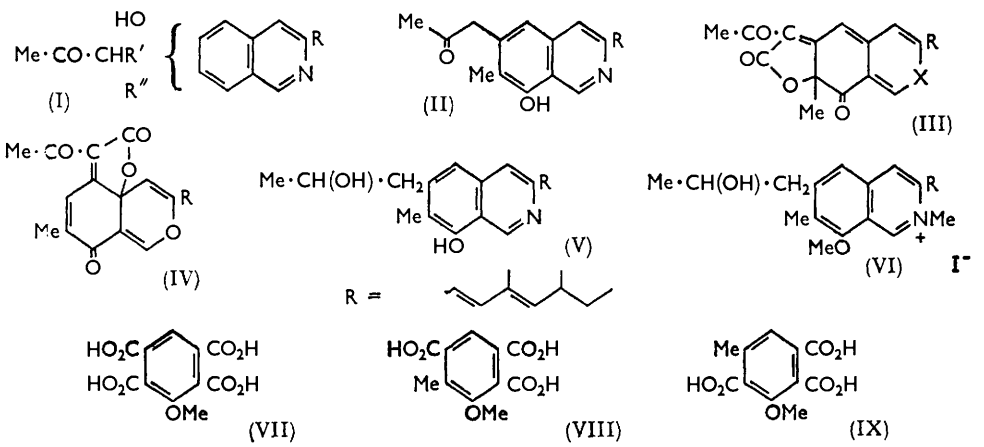
Rotiorin, previously [2] represented by the provisional structure (IV), has been defined as (III; X = O) by the assignment of the unequivocal structure (II) to the key degradation product, aporotioramine. Revised structures are also allocated to the various methylation products of aporotioramine and its derivatives.

The general structural features of rotiorin, a metabolite of *Penicillium sclerotiorum* van Beyma, have been previously described.[2] Thus, *inter alia*, on treatment with ammonia, rotiorin, $C_{23}H_{24}O_5$, furnishes the heterocyclic nitrogen derivative, rotioramine, $C_{23}H_{25}NO_4$. The latter compound is reductively aromatised to yield a substituted isoquincline, aporotioramine, $C_{22}H_{27}NO_2$, to which the partial structure (I; R′ = H, R″ = Me or *vice versa*) was allocated. The hydroxyl group of aporotioramine was assigned to the 6- or 8-position of the isoquinoline nucleus on the basis of the properties of the *O*- and *N*-methylated derivatives of aporotioramine, and the provisional structure (IV) was assigned to rotiorin.[2] However, the unequivocal definition of the structures of the cognate metabolites, sclerotiorin [3] (XII), rubropunctatin [4] (XIIIa), and monascorubrin [5] (XIIIb), together with the accumulation of additional evidence, has led us to revise the structure of rotiorin to (III; X = O).*



This paper describes the evidence which establishes structure (II) for aporotioramine and hence defines rotiorin and rotioramine as (III; X = O) and (III; X = NH), respectively.

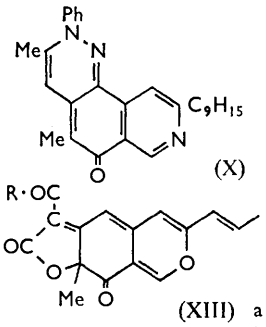
Methylation of aporotioraminol [2] (V), the sodium borohydride reduction product of aporotioramine, gives *NO*-dimethylaporotioraminol iodide (VI), which is oxidised by potassium permanganate in alkaline solution to the tetracarboxylic acid (VII), the tetramethyl ester of which is identical with an authentic specimen.[6] Aporotioramine is therefore an 8-hydroxyisoquinoline and on the basis of the partial structure (II) is substituted

* This conclusion, which was initially based principally on the chemical similarity of rotiorin to its congener, sclerotiorin [3] (XII), and to the related fungal metabolite, rubropunctatin [4] (XIII), has been previously reported.[4]

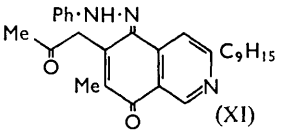
[1] Part XL, Holker, Ross, Staunton, and Whalley, *J.*, 1962, 4150.
[2] Jackman, Robertson, Travers, and Whalley, *J.*, 1958, 1825.
[3] Dean, Staunton, and Whalley, *J.*, 1959, 3004.
[4] Haws, Holker, Kelly, Powell, and Robertson, *J.*, 1959, 3598.
[5] Fielding, Haws, Holker, Powell, Robertson, Stanway, and Whalley, *Tetrahedron Letters*, 1960, **5**, 24.
[6] Roberts, *J.*, 1955, 2989.

in the 6- and 7-positions by an acetonyl residue and by a methyl group. Under milder conditions of oxidation the nuclear methyl group is retained, and a new tricarboxylic acid (VIII) was isolated as the trimethyl ester, $C_{14}H_{16}O_7$. The alternative structure (IX) for this acid is excluded because of the large difference between the melting point (88°) reported [7] for the trimethyl ester of acid (IX) and that observed (112°) for the corresponding derivative of the acid obtained from aporotioramine. The formation of acid (VIII) together with the previously reported evidence [2] for the partial structure (I) unequivocally defines aporotioramine as (II).

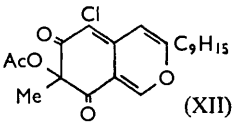
Collateral evidence was provided by the treatment of an alkaline solution of aporotioramine with benzenediazonium chloride. On the basis of the molecular formula, the insolubility in alkali, and the absence in the infrared spectrum of bands characteristic of an OH, NH, or isolated carbonyl group, the red crystalline product, $C_{28}H_{29}N_3O$, must have structure (X). This reaction has its parallel in the chemistry of rubropunctatin [4]



(X)

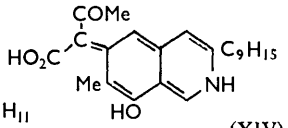


(XI)

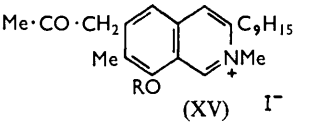


(XII)

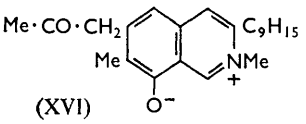
(XIII) a: R = C_5H_{11}
b: R = C_7H_{15}



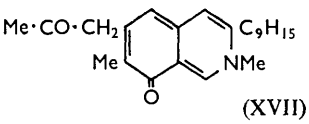
(XIV)



(XV) I^-



(XVI)



(XVII)

and presumably involves the tautomeric form (XI) of the initial coupling product which successively undergoes cyclisation and dehydration to produce the cinnoline (X). The formation of this cinnoline requires the presence of the acetonyl residue in the 6-position and this confirms the structure for the tricarboxylic acid (VIII) and hence the corresponding structure (II) for aporotioramine. In agreement with this, aporotioraminol (V) forms a normal, alkali-soluble, red dye, $C_{28}H_{23}NO_2$, on similar treatment with benzenediazonium chloride.

As required for an 8-hydroxyisoquinoline devoid of a substituent in the 5-position (or of a substituent which would be readily extruded from that position), aporotioramine furnishes a positive Gibbs test under the modified conditions described by King, King, and Manning.[8] Although the rapid fading of the blue colour produced by aporotioramine caused difficulty in the determination the absorption maximum was between 645 and 665 mμ, *i.e.*, within the requisite range.[8] The optical density of the solution at 655 mμ decreased linearly with time, a further characteristic [8] of a positive Gibbs test.

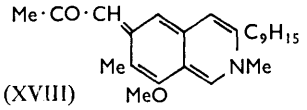
The assignment of the unequivocal structure (II) to aporotioramine together with the information previously reported [2] defines rotiorin as (III; X = O) and rotioramine as (III; X = NH). By analogy with the chemistry of rubropunctatin the first step in the aromatisation of rotioramine would be reduction to the intermediate (XIV), followed by tautomerisation and decarboxylation to produce aporotioramine (II). In agreement with this mechanism it has now been established that the conversion of rotioramine (III; X =

[7] Asahina and Fujikawa, *Ber.*, 1935, **68**, 1558.
[8] King, King, and Manning, *J.*, 1957, 563.

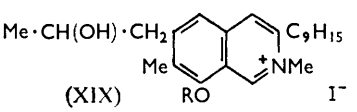
NH) into aporotioramine (II) is accompanied by the formation of one molecular proportion of carbon dioxide.

The orientation of the acetonyl group as in (II) provides a more rational interpretation than that suggested previousy [2] for the behaviour of the methylation products of aporotioramine. Thus, *N*-methylaporotioramine hydriodide [now formulated as (XV; R = H)] when treated with sodium hydrogen carbonate solution forms *N*-methylaporotioramine, the red colour of which is to be attributed [9] to the presence of a betaine system (XVI) or the equivalent quinonoid system (XVII).

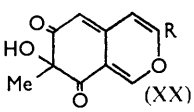
The bright red compound which is produced when *NO*-dimethylaporotioramine iodide (XV; R = Me) is treated with 2N-sodium hydroxide was previously believed to be the free base. However, the colour change from yellow to red suggests the formation of the anhydro-base (XVIII) rather than a true or pseudo-base (cf. Katritzky [10]). The reverse change [2] would be expected on acidification. The methoxyl group in (XVIII) is part of a vinylogous ester system with respect to the side-chain carbonyl group, and therefore might be expected to undergo the observed [2] hydrolysis in alkali to afford *N*-methylaporotioramine (XVI).



(XVIII)



(XIX)



(XX)

This explanation of the behaviour of these methylation products is confirmed by an examination of the corresponding methylation products of aporotioraminol (V). Aporotioraminol is methylated by methyl iodide in acetone to give the yellow *N*-methylaporotioraminol hydriodide (XIX; R = H) which furnishes the unstable, red compound, of type (XVI) or (XVII) when treated with sodium hydrogen carbonate solution. Hence this colour change is independent of the side-chain carbonyl group. In contrast, *NO*-dimethylaporotioraminol iodide (XIX; R = Me) remains yellow when treated with alkali and on this basis it may be concluded that an anhydro-base of type (XVIII) is not formed in the absence of the activating influence of the carbonyl group in the C_3 side-chain.

On the basis of structure (III; X = O) for rotiorin it has been proposed [3,4,11] that both rotiorin and sclerotriorin are biosynthesised from a common acetate derived precursor of the type (XX). Sclerotiorin would be formed by acetylation and chlorination, whereas esterification of the tertiary hydroxyl group by acetoacetic acid followed by aldol condensation and dehydration would give rotiorin. This scheme or an equivalent variant accords most satisfactorily with the biogenetic evidence.[12]

Experimental

Aporotioramine.—The following method of converting rotiorin into aporotioramine gives an improved yield. A solution of rotiorin (1 g.) in chloroform (100 ml.) was shaken with concentrated ammonia solution (5 ml.) and water (20 ml.). Five minutes later, an excess of 2N-hydrochloric acid was added, the chloroform layer separated, and zinc dust (1 g.) was introduced; then acetic acid (1 ml.) was carefully added to the vigorously agitated mixture. The purple colour of the chloroform solution was rapidly discharged and after 10 min. the chloroform solution was separated, filtered, washed successively with 2N-hydrochloric acid, 2N-sodium hydrogen carbonate, and water, and dried. Purification of the product remaining after removal of the solvent *in vacuo* gave aporotioramine [2] (0·6 g.) which formed cream-coloured needles, m. p. 186°, from methanol.

[9] Saxena, Stafford, and Stafford, *J.*, 1959, 1579.
[10] Katritzky, *J.*, 1955, 2586.
[11] Whalley, "Recent Developments in the Chemistry of Natural Phenolic Compounds," Chapter 2, ed. W. D. Ollis, Pergamon Press, London, 1961.
[12] Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576; Birch, Cassera, Fitton, Holker, Smith, Thompson, and Whalley, *J.*, 1962, 3583; Holker, Staunton, and Whalley, unpublished observations.

Aporotioraminol.—The following improved method of preparation has been used. A solution of potassium borohydride (1·1 g.) in water (15 ml.) was added to aporotioramine (1 g.) dissolved in alcohol (40 ml.) containing 2N-sodium hydroxide (3 ml.). After 2 hr. the mixture was poured into an excess of 2N-hydrochloric acid. Treatment of a methanolic solution of the yellow precipitate of aporotioraminol hydrochloride with an excess of 2N-sodium hydrogen carbonate furnished aporotioraminol [2] which separated from methanol in plates (0·75 g.), m. p. 240° (decomp.).

N-*Methylaporotioraminol Hydriodide.*—A solution of aporotioraminol (220 mg.) in acetone (50 ml.) containing methyl iodide (1 ml.) was refluxed during 4 hr. and concentrated to 10 ml. 24 Hr. later the crystalline precipitate was purified from ethyl acetate–methanol, yielding N-*methylaporotioraminol hydriodide* (180 mg.), yellow needles, m. p. 240° (decomp.) (Found: C, 57·3; H, 6·6; N, 2·6; I, 26·2. $C_{23}H_{32}INO_2$ requires C, 57·4; H, 6·7; N, 2·9; I, 26·4%).

NO-*Dimethylaporotioraminol Iodide.*—Aporotioraminol (1 g.) was dissolved in boiling acetone (500 ml.) containing methyl iodide (5 ml.). After 3 hr. the boiling solution was treated with potassium hydrogen carbonate (5 g.) and heated under reflux for a further 2 hr. NO-*Dimethylaporotioraminol iodide* separated from the filtered and concentrated solution and was crystallised from ethyl acetate–methanol in bright yellow needles (1 g.), m. p. 216° (decomp.) (Found: C, 56·1; H, 6·7; N, 2·6; OMe, 6·2. $C_{23}H_{31}INO{\cdot}OMe,H_2O$ requires C, 56·2; H, 7·0; N, 2·7; OMe, 6·0%).

Oxidation of NO-*Dimethylaporotioraminol Iodide.*—(*a*) A suspension of *NO*-dimethylaporotioraminol iodide (0·5 g.) in N-sodium hydroxide (50 ml.) containing potassium permanganate (1 g.) was shaken during 1 hr. Additional potassium permanganate (2 g.) was added to the mixture which was then maintained at 80° until it became colourless. The filtered solution was then acidified with 2N-sulphuric acid, heated to 80°, and treated with an excess of potassium permanganate. The cooled mixture was decolourised with sulphur dioxide and continuously extracted with ether (10 hr.). The colourless, acidic product remaining after removal of the ether was treated with an excess of ethereal diazomethane. The resultant ester was purified by chromatography on alumina from ether–light petroleum (b. p. 60—80°) and crystallised from light petroleum (b. p. 60—80°) to give *tetramethyl* 3-*methoxybenzene*-1,2,4,5-*tetracarboxylate* in needles (11 mg.), m. p. 106°, identical with a specimen prepared from 3-methoxybenzene-1,2,4,5-tetracarboxylate [6] (Found: C, 52·7; H, 4·8. $C_{15}H_{16}O_9$ requires C, 52·9; H, 4·7%).

(*b*) A stirred suspension of *NO*-dimethylaporotioraminol iodide (0·5 g.) in N-sodium hydroxide (250 ml.) was maintained at 80° and powdered potassium permanganate (2·9 g.) was added during 2 hr. The iodide rapidly dissolved and 3 hr. later more permanganate (0·1 g.) was added to the colourless solution and heating was continued. After 30 min. the residual oxidising agent was destroyed by addition of methanol. The filtered solution was then acidified, treated at 80° with an excess of potassium permanganate, and decolourised with sulphur dioxide. The product was isolated as described in (*a*) and after treatment with ethereal diazomethane gave *trimethyl* 3-*methoxy*-4-*methylbenzene*-1,2,5-*tricarboxylate* (20 mg.) in needles, m. p. 112° (Found: C, 56·7; H, 5·6; OMe, 42·0; *C*-Me, 3·5. $C_9H_3O(OMe)_4{\cdot}CH_3$ requires C, 56·8; H, 5·4; OMe, 41·9; 1*C*-Me, 5·1%).

Benzenediazonium Coupling Product of Aporotioramine.—A solution of benzenediazonium chloride (40 ml.) was prepared from aniline (1·28 g.) at 0°. This solution (2 ml.) was added to a stirred solution of aporotioramine (235 mg.) in 2N-sodium hydroxide (5 ml.) containing alcohol (5 ml.) and water (10 ml.). The resultant mixture was acidified with acetic acid, and the precipitate purified to yield 2,5-*dihydro*-8-(3,5-*dimethylpenta*-2,4-*dienyl*)-3,4-*dimethyl*-6-*oxopyrido*[3,4-*h*]*cinnoline* (X), in bright red needles (212 mg.), m. p. 270° (Found: C, 78·9; H, 7·2; N, 9·6. $C_{28}H_{29}N_3O$ requires C, 79·4; H, 6·9; N, 9·9%).

Benzeneazoporotioraminol.—Prepared similarly from aporotioraminol (230 mg.), *benzeneazoaporotioraminol* (150 mg.) separated from alcohol in orange needles, m. p. 196° (Found: C, 75·7; H, 7·5; N, 9·4. $C_{28}H_{33}N_3O_2$ requires C, 75·8; H, 7·5; N, 9·5%).

Estimation of the Carbon Dioxide Produced during the Aromatisation of Rotioramine.—When treated by the method previously described [2] rotioramine (0·52 g.) in acetic acid (50 ml.) gave rise to a precipitate of barium carbonate (254 mg., 0·92 mol. of CO_2).

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