722. *The Chemistry of Fungi. Part XXXVII.*[1] *The Structure of Rubropunctatin.*

By E. J. Haws, J. S. E. Holker, A. Kelly, A. D. G. Powell, and Alexander Robertson.

A new pigment, $C_{21}H_{22}O_5$, rubropunctatin has been isolated from *Monascus rubropunctatus* Sâto. With aqueous ammonia this gives a nitrogen analogue, rubropunctatamine, $C_{21}H_{23}O_4N$, which, on reduction with zinc and acetic acid is degraded to an 8-hydroxyisoquinoline derivative, aporubropunctatamine, $C_{20}H_{25}O_2N$, and one mol. of carbon dioxide. From degradative studies on the parent compound and these derivatives, aporubropunctatamine is shown to have structure (XII), whilst structures (XIXa) and (XVIII) are proposed for rubropunctatin and rubropunctatamine respectively.

Exhaustive hydrogenation of rubropunctatin gives hexahydroaporubropunctatin, $C_{20}H_{30}O_3$, for which structure (XXIa) is proposed.

The probable biogenesis of rubropunctatin is discussed and possible structural implications for the related compound rotiorin are tentatively suggested.

During an investigation into the metabolic products of the *Monascus* genus in this Department, a new red pigment, rubropunctatin was isolated from the mycelium of *M. rubropunctatus* Sâto. In a preliminary account of this work [2] a close relation was pointed out between the properties of the pigments sclerotiorin,[1,3,4,5,6] rotiorin,[7] monascin,[8] monascorubrin,[9] and rubropunctatin.

The present paper describes the structural investigations on rubropunctatin which have been carried out during the last five years.

Rubropunctatin has the molecular formula, $C_{21}H_{22}O_5$, although the formula, $C_{22}H_{24}O_5$, cannot be excluded by analysis alone and the latter was originally suggested.[2] This compound, which has a very high negative specific rotation, contains no hydroxyl (Zerewitinoff) and no methoxyl group. The ultraviolet [$\lambda_{max.}$ 218, 246, 290, and 460 mμ (log ε 4·17, 4·09, 3·98, and 4·30)] and infrared spectra [$\nu_{max.}$ 1757, 1724, 1656, 1636, and 1577 cm.$^{-1}$ (mull)] together indicated the presence of a highly conjugated chromophore containing carbonyl functions. The pigment was extracted from ethereal solution by dilute aqueous sodium hydroxide and was precipitated unchanged on immediate treatment of the alkaline solution with mineral acid. This, in conjunction with the absence of active hydrogen, suggested that the compound contains a potentially acidic function. Prolonged treatment with sodium hydroxide decomposed the pigment to a complex unresolved mixture of acidic products. The isolation of hexanoic acid by degradation of rubropunctatin with alkali [2] has not been substantiated. The pigment was not reduced by alkaline sodium dithionite in alcohol or by sulphur dioxide, and so does not contain a quinone system. Although there was an obvious reaction with 2,4-dinitrophenylhydrazine hydrochloride, a crystalline derivative could not be isolated.

Hydrogenation of rubropunctatin was extremely complex and will be discussed in detail below, but under very mild conditions in the presence of palladium on barium sulphate a dihydro-derivative was isolated. However, the conditions for this reaction appeared to be critical and difficultly reproducible.

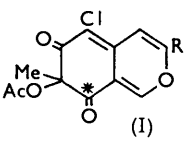
[1] Part XXXVI, Dean, Staunton, and Whalley, *J.*, 1959, 3004.
[2] Powell, Robertson, and Whalley, *Chem. Soc. Special Publ.*, 1957, No. 5, p. 27.
[3] Eade, Page, Robertson, Turner, and Whalley, *J.*, 1957, 4913.
[4] Graham, Page, Robertson, Travers, Turner, and Whalley, *J.*, 1957, 4924.
[5] Fielding, Graham, Robertson, Travers, and Whalley, *J.*, 1957, 4931.
[6] Fielding, Robertson, Travers, and Whalley, *J.*, 1958, 1814.
[7] Jackmann, Robertson, Travers, and Whalley, *J.*, 1958, 1825.
[8] Salvman and Karrer, *Helv. Chim. Acta*, 1932, **15**, 18; Geiger and Karrer, *Helv. Chim. Acta*, 1941, **24**, 289.
[9] Nishikawa, *J. Agric. Chem. Soc. Japan*, 1932, **8**, 1007.

On ozonolysis, rubropunctatin gave acetaldehyde which was isolated as the 2,4-dinitrophenylhydrazone. Although no other pure product could be isolated from this reaction, the result indicated the presence of an ethylidene group in the pigment. Oxidation of rubropunctatin with alkaline hydrogen peroxide or chromic oxide gave hexanoic acid, characterised as the *p*-bromophenacyl ester and as the piperazine salt, showing that the pigment contains an n-pentyl side-chain. Thus, of the three *C*-methyl groups shown to be present in the compound by Kuhn–Roth estimation, two are in ethylidene and n-pentyl side-chains.

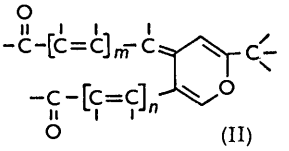
With dilute aqueous ammonia at room temperature rubropunctatin rapidly formed the violet compound, rubropunctatamine, $C_{21}H_{23}O_4N$. Dihydrorubropunctatamine was prepared by reaction of dihydrorubropunctatin with ammonia, and by hydrogenation of rubropunctatamine. With methylamine under similar conditions rubropunctatin gave *N*-methylrubropunctatamine. These nitrogen analogues are feebly basic, forming yellow hydrochlorides in concentrated hydrochloric acid, whence the parent bases are regenerated by dilution with water. Alcoholic suspensions of rubropunctatamine and its dihydro-derivative dissolved immediately on addition of 2N-sodium hydroxide but the *N*-methyl derivative was insoluble, showing that only the two former compounds are acidic. The extremely mild conditions under which the nitrogen analogues are formed by apparent replacement of O by NH or NMe and the properties of these compounds are analogous to the formation and properties of the corresponding derivatives of sclerotiorin [1,3] (Ia). A further similarity was the oxidation of rubropunctatamine by nitric acid to pyridine-2,4,5-tricarboxylic acid, which had been isolated on similar oxidation of sclerotioramine [6] and rotioramine.[7] It therefore seemed likely that rubropunctatin contained the same

(a) R = ·CH:CH·C(Me):CH·CHMeEt

(b) R = ·CH$_2$·CH$_2$·CH(Me)·CH$_2$·CHMeEt



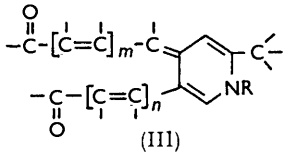
(I)



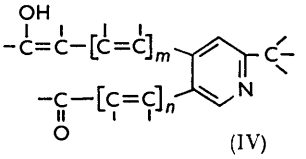
(II)

(a) R = H

(b) R = Me



(III)



(IV)

chromophoric system as sclerotiorin, or a closely related system of type (II), in which the replaceable oxygen function is independently conjugated with two carbonyl functions. On this basis rubropunctatamine would contain a chromophoric system of type (IIIa) and *N*-methylrubropunctatamine would contain system (IIIb). Accordingly the solubility of rubropunctatin and rubropunctatamine in alkali can be attributed respectively to a vinylogous lactone system and to an enolic hydroxyl group generated by amide-imine tautomerism of type (IIIa) $\rightleftharpoons$ (IV).

Rubropunctatamine with alkaline potassium permanganate and with ozone gave hexanoic acid and acetaldehyde respectively, establishing that the pentyl and the ethylidene side-chains of rubropunctatin are present in rubropunctatamine. Ozonolysis of dihydrorubropunctatamine did not produce acetaldehyde, so it is the double bond of the ethylidene group that is hydrogenated.

Reduction of rubropunctatamine with zinc and acetic acid gave one mol. of carbon dioxide and a colourless optically inactive compound, $C_{20}H_{25}O_2N$, aporubropunctatamine. This formed a dihydro-derivative, identical with the product obtained by reduction of dihydrorubropunctatamine with zinc and acetic acid. Similar reduction of *N*-methylrubropunctatamine gave the red compound *N*-methylaporubropunctatamine, $C_{21}H_{27}O_2N$. Although, as pointed out previously, it was impossible to differentiate between C_{21} and

C_{22} formulations for rubropunctatin and its nitrogen analogue on the basis of analytical determinations alone, elemental analyses for aporubropunctatamine and many derivatives establish the C_{20} formulation for this compound, and since in its formation from rubropunctatamine only one carbon atom appears to be extruded as carbon dioxide, the C_{21} rather than C_{22} formulation is preferred for the latter compound and hence for rubropunctatin.

Aporubropunctatamine is soluble in alkali, has an infrared band at 3120 cm.$^{-1}$, and gives an alkali-insoluble monoacetate: it thus contains a phenolic hydroxyl group. The infrared spectrum of the acetate was devoid of absorption in the 3 μ region and had a strong band at 1757 cm.$^{-1}$ due to a phenolic acetate group. Similarly, aporubropunctatamine with methyl iodide and potassium carbonate in acetone gave an *O*-methyl derivative in low yield, *O*-methylation rather than *N*-methylation being assumed on the basis of (*a*) the great similarity between the ultraviolet spectra of this derivative and the *O*-acetate,

FIG. 1. FIG. 2.

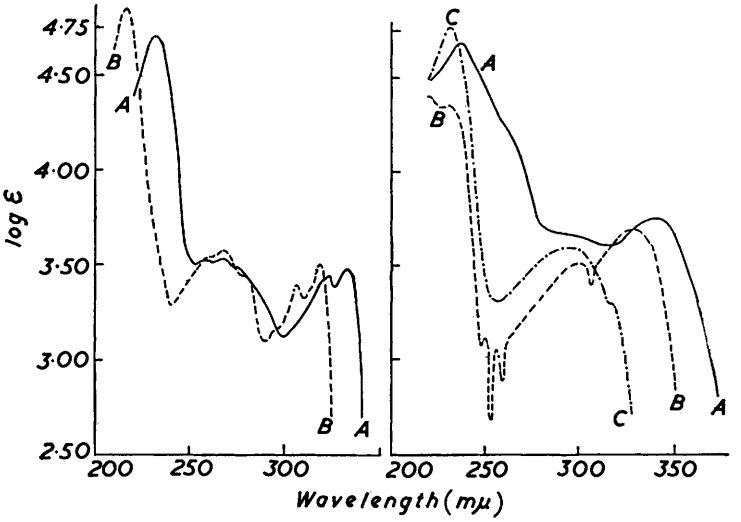


FIG. 1. *Absorption spectra of* (*A*) O-*acetyldihydroaporubropunctatamine, and* (*B*) *isoquinoline in* 95% *alcohol.*

FIG. 2. *Absorption spectra of* (*A*) *dihydroaporubropunctatamine and* (*B*) 8-*hydroxy-, and* (*C*) 6-*hydroxy-isoquinoline in* 95% *alcohol.*

(*b*) the quantitative yield of methyl iodide in the Zeisel determination, and (*c*) non-identity with *N*-methylaporubropunctatamine. The second oxygen function in aporubropunctatamine is ketonic, as *O*-methylaporubropunctatamine formed an oxime and reduction of aporubropunctatamine and its dihydro-derivative with potassium borohydride gave monohydric alcohols which formed diacetates showing infrared bands at 1735 (alcoholic acetate) and 1773 cm.$^{-1}$ (phenolic acetate). Borohydride reduction of *O*-methylaporubropunctatamine similarly gave a monohydric alcohol which formed a monoacetate showing an infrared band at 1735 cm.$^{-1}$. The ketonic carbonyl group in aporubropunctatamine appeared to be isolated from the main chromophore since the compound showed an infrared band at 1704 cm.$^{-1}$ and the ultraviolet spectrum of the borohydride reduction product from aporubropunctatamine showed no shift of absorption bands when compared with the spectrum of the parent ketone.

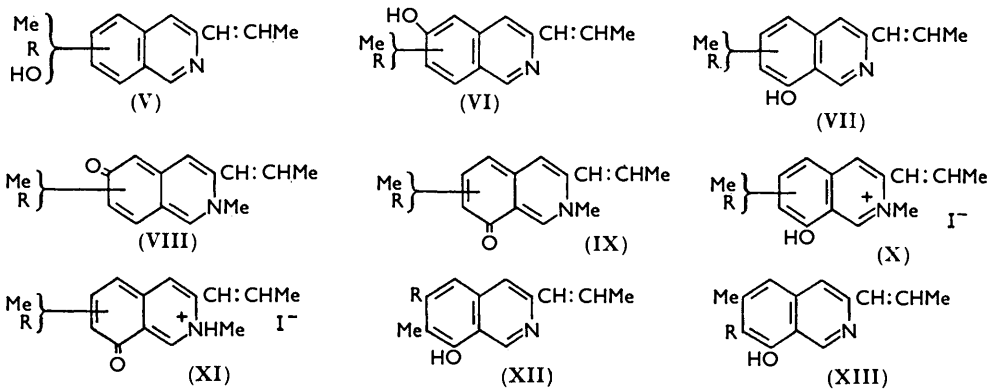
The presence of a propenyl group in aporubropunctatamine was demonstrated by ozonolysis of the *O*-acetyl derivative, acetaldehyde and the bisnor-acid $C_{20}H_{23}O_5N$ being produced. This propenyl group appeared to be conjugated with the main chromophoric system since the ultraviolet absorption bands of dihydroaporubropunctatamine and its

acetate were at 20 mμ lower wavelength than the corresponding bands of aporubropunctatamine and its acetate respectively.

The ultraviolet absorption spectra of *O*-acetyldihydroaporubropunctatamine and the diacetate of its borohydride reduction product were identical and similar to the spectrum of isoquinoline in intensity and relative position of the absorption bands (Fig. 1) but, as in the case of aposclerotioramine [5] and aporotioramine,[7] were at wavelengths longer by about 20 mμ. Since acetoxyl groups do not influence the ultraviolet absorption of aromatic systems [10] and the conjugation of the propenyl side-chain has been removed in the dihydro-derivatives, these spectra provide reasonable evidence regarding the isoquinoline nature of the nucleus in the apo-compounds. On the assumption that no skeletal rearrangement occurs in the formation of the apo-compounds from rubropunctatamine, the production of pyridine-2,4,5-tricarboxylic acid by oxidation of the latter compound supports this hypothesis and, further, indicates that the isoquinoline nucleus has a substituent in the 3-position. This substituent is the propenyl group since the ozonolysis acid from *O*-acetylaporubropunctatamine gave an intense red colour in alcohol with ferrous sulphate, a reaction which is characteristic of a carboxyl group in the *ortho*-position to an aromatic nitrogen atom.[11]

The nature of the remaining substituent groups in the isoquinoline skeleton of the apo-compounds can now be completely defined. The 3-propenylisoquinoline moiety accounts for twelve of the twenty carbon atoms in aporubropunctatamine, and as the pentyl side-chain would hardly be affected in the formation of the apo-compound from rubropunctatamine, this accounts for a further five. Of the remaining three carbon atoms, one is present in an isolated keto-group and a second must be in a methyl group to account for the observed *C*-methyl content of the apo-compounds. On this basis, aporubropunctatamine must contain either an acetonyl group together with a pentyl side-chain, or a methyl group together with a β-oxoheptyl group. The former possibility was excluded since aporubropunctatamine gave a negative iodoform test. Hence, this compound has the partial structure (V), in which the substituent groups are attached directly to the benzene ring of the isoquinoline skeleton.

The orientation of these substituents was established as follows. Aporubropunctatamine gave a methiodide, $C_{21}H_{27}O_2NI$, which generated *N*-methylaporubropunctatamine on treatment with sodium carbonate. This reaction, which contrasts with the formation



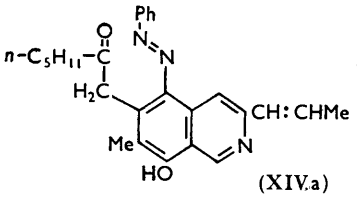
R = *n*-C_5H_{11}·CO·CH_2–

of an *O*-methyl ether described above, has parallels in the aposclerotioramine [5] and aporotioramine [7] series, and indicated that the phenolic hydroxyl group of aporubropunctatamine must be in a position which would permit amide-enol tautomerism, *i.e.*, the group

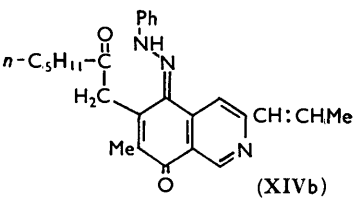
[10] Cooke, Macbeth, and Winzor, *J.*, 1939, 878.
[11] Ley, Schwarte, and Münnich, *Ber.*, 1924, **57**, 349.

must be at the 6- or 8-position of the isoquinoline nucleus, thus leading to the partial structures (VI) or (VII) for aporubropunctatamine, and (VIII) or (IX) for its *N*-methyl derivative. The methiodide could thus be regarded as (X) or a tautomeric *N*-methyl hydriodide (XI) (or equivalent structures derived from 6-hydroxyisoquinoline). A study of the ultraviolet spectrum of dihydroaporubropunctatamine (which is devoid of a side-chain conjugated with the nucleus) suggested an 8- in preference to a 6-hydroxyisoquinoline nucleus. Thus, Fig. 2 shows that the absorption bands in this spectrum are much more similar in intensity and relative position to the corresponding bands of 8- than to those of 6-hydroxyisoquinoline, except that the bands in the spectrum of the apo-compound are at about 20 mμ longer wavelengths than in 8-hydroxyisoquinoline owing to the bathochromic effects of the substituents. Further, aporubropunctatamine gave a blue colour ($\lambda_{max.}$ 650 mμ) with 2,6-dichlorobenzoquinone chlorimide under the conditions of King, King, and Manning,[12] indicating the presence of an unsubstituted position *para* to the phenolic hydroxyl group, *i.e.*, an 8-hydroxyisoquinoline group unsubstituted in the 5-position. Thus, aporubropunctatamine appeared to have structure (XII) or (XIII). Finally, this compound did not form an anhydro-compound when heated and therefore appeared not to contain a phenolic hydroxyl group in the *ortho*-position to the β-oxoheptyl side-chain. Thus, structure (XII) is preferred to structure (XIII) for aporubropunctatamine.

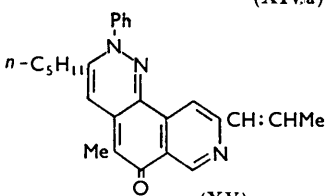
Confirmation was provided as follows. The potassium borohydride reduction product



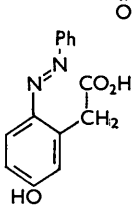
(XIV.a)



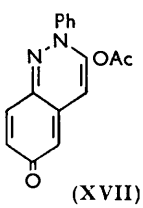
(XIVb)



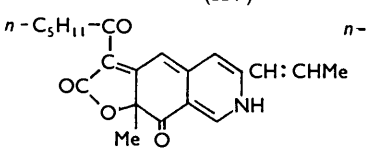
(XV)



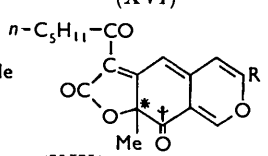
(XVI)



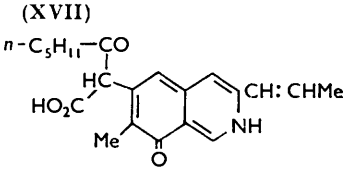
(XVII)



(XVIII)



(XIX)
(a) R = CH: CHMe
(b) R = $CH_2 \cdot CH_2Me$



(XX)

from aporubropunctatamine with benzenediazonium chloride gave an azo-dye $C_{26}H_{31}O_2N_3$ which gave an intensely coloured sodium salt with dilute sodium hydroxide. It is well known [13] that compounds with an azo-group in the *ortho*-position to a phenolic hydroxyl group, *e.g.*, 1-azo-2-naphthol, are insoluble in dilute alkali owing to hydrogen-bonding. Thus, with the apo-derivative, coupling has occurred in the *para*-position to the phenolic hydroxyl group, supporting the assumption that the *apo*-compounds contain an unsubstituted position *para* to the phenolic hydroxyl group.

Aporubropunctatamine with benzenediazonium chloride gave an alkali-insoluble red

[12] King, King, and Manning, *J.*, 1957, 563.
[13] Saunders, " The Aromatic Diazo Compounds," Arnold and Co., London, 1949, p. 198.

product, $C_{26}H_{27}ON_3$, containing a molecule of water less than the expected structure (XIVa). The infrared spectrum of this compound showed no absorption which could be attributed to either hydroxyl or isolated keto-groups and it was therefore assumed that the initially formed coupling product (XIVa ⇌ XIVb) underwent spontaneous intramolecular condensation to give the cinnoline derivative (XV). Although this type of cyclisation does not appear to have been reported previously, compounds of type (XVI) are cyclised [14] by acetic anhydride and sulphuric acid to cinnoline derivatives (XVII). The difference in ease of cyclisation of the ketone (XIV) and the carboxylic acid (XVI) is probably due to the greater reactivity of the keto-group towards nucleophiles. The cyclisation is being investigated further. If the cinnoline structure is correct, it confirms structure (XII) for aporubropunctatamine.

Provided that no molecular rearrangement occurs in the reductive aromatisation of rubropunctatamine to aporubropunctatamine and a mol. of carbon dioxide, the derivation of a complete structure for rubropunctatamine requires formal replacement of two hydrogen atoms by carbon dioxide. The structure derived for rubropunctatamine must explain the ease of aromatisation and contain a chromophore of type (II). Structure (XVIII) for rubropunctatamine and hence (XIXa) for rubropunctatin most adequately fulfil these conditions. The γ-lactone ring is theoretically derived from a γ-hydroxy-acid in which the alcoholic hydroxyl group is flanked by both a double bond and a carbonyl group. By analogy with the well-known reductive fission of α-ketol esters and allylic esters, similar fission of rubropunctatamine would be expected to occur readily with formation of an intermediate β-keto-acid (XX), which on decarboxylation and prototropic shift would afford aporubropunctatamine (XII). Further, the asymmetric carbon atom marked * in structure (XIXa) explains the optical activity of rubropunctatin. Although the intense colour of rubropunctatamine prevented determination of optical activity, it seems unlikely that the mild conditions used in the formation of this nitrogen analogue would destroy or racemise the asymmetric centre. Consequently it is assumed that rubropunctatamine contains an asymmetric centre which is destroyed in the formation of the apo-compounds. This hypothesis is fully accommodated in structures (XVIII) and (XII).

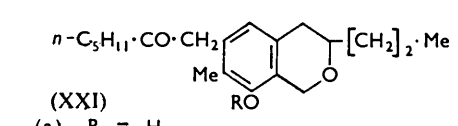
The infrared spectra of rubropunctatin and rubropunctatamine seem to be compatible with structures (XIXa) and (XVIII) respectively. On conversion of rubropunctatin into rubropunctatamine the principal absorption bands in the carbonyl stretching region all undergo bathochromic shifts. This is qualitatively analogous to the shift of carbonyl frequency on conversion of an ester into an amide, and indicates that all three carbonyl functions of rubropunctatin are conjugated with the ethereal oxygen atom, in agreement with structure (XIXa). Despite the difficulty of detailed spectral analysis in highly conjugated systems, the 1757 cm.$^{-1}$ band in the spectrum of rubropunctatin can be tentatively ascribed to the αβ-unsaturated γ-lactone, the 1656 cm.$^{-1}$ band to a highly conjugated ketonic carbonyl group, and the bands at 1636 and 1575 cm.$^{-1}$ to conjugated double bonds. The absorption at 1724 cm.$^{-1}$ in the spectrum of rubropunctatin seems anomalous at first sight. However, a similar band at 1715 cm.$^{-1}$ (in $CHCl_3$) in the spectrum of sclerotiorin has been ascribed to the carbonyl group marked * in formula (Ia), the abnormally short wavelength being attributed to strain in the region of this group.[1] Similar arguments can be applied to structure (XIXa) for rubropunctatin and indicate that the band at 1724 cm.$^{-1}$ is due to the carbonyl group marked †.

The interpretation of ultraviolet spectra in cross-conjugated systems as complex as that in rubropunctatin is extremely difficult. Even when the influence of the propenyl system is removed in the dihydro-derivative (XIXb), cross-conjugation still exists between the γ-lactone and the hexanoyl group. In the absence of detailed correlation it is interesting that the ultraviolet spectra show the chromophoric system of dihydrorubropunctatin

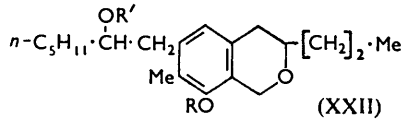
[14] Kornfeld, *J. Amer. Chem. Soc.*, 1948, **70**, 1373.

(XIXb) [$\lambda_{max.}$ 218 and 400 mμ (log ε 4·22 and 4·40)] to be more extensively conjugated than that of tetrahydrosclerotiorin (Ib) [$\lambda_{max.}$ 225 and 343 mμ (log ε 4·13 and 4·30)], in agreement with the proposed formulæ.

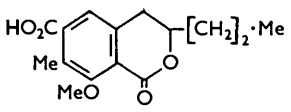
Exhaustive hydrogenation of rubropunctatin involved uptake of 5—6 mols. of hydrogen and was accompanied by remarkable fluorescent colour changes. Great difficulty was experienced in the isolation of a pure compound but, under controlled conditions, 10% of a compound $C_{20}H_{30}O_3$ was isolated which is though to be hexahydroaporubropunctatin (XXIa) for the following reasons. (1) The infrared spectrum showed bands at **3663** (phenolic OH), **1718** (isolated C=O), and **1626** cm.$^{-1}$ (aromatic). (2) The compound gave monoacetate (XXIb) and a monomethyl ether (XXIc). (3) Hexahydroaporubropunctatin, like aporubropunctatamine, gave a blue colour with Gibb's reagent, indicating an unsubstituted position *para* to the phenolic hydroxyl group. (4) The presence of a keto-group was established by the formation of an oxime from hexahydro-*O*-methylaporubropunctatin and by reduction of the phenol with potassium borohydride to the monohydric alcohol (XXIIa) which formed a diacetate (XXIIb). (5) Hexahydro-*O*-methylaporubropunctatin with potassium permanganate gave a carboxylic acid, $C_{15}H_{18}O_5$. Although insufficient of the acid has been obtained for characterisation, it probably has structure (XXIII), arising by oxidation of the hexanoyl side-chain and the benzyl ether system. A direct analogy for the oxidation of a similar benzyl ether system to a lactone is conversion of methyl dihydro-*O*-dimethylcitrinin (XXIV) into methyl dihydro-*O*-dimethylcitrinone (XXV) by chromic oxide or potassium permanganate.[15] Further, the infrared spectrum of the acid (XXIII) shows bands at 2646 and 1698 cm.$^{-1}$ (aryl acid) and 1718 cm.$^{-1}$ (δ-lactone of aryl acid).



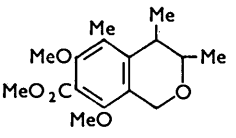
(XXI)
(a) R = H
(b) R = Ac
(c) R = Me



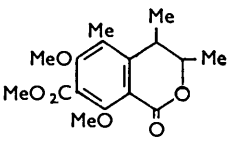
(XXII)
(a) R = R′ = H
(b) R = R′ = Ac



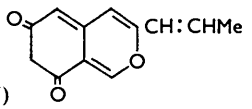
(XXIII)



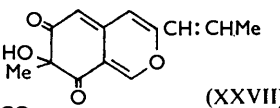
(XXIV)



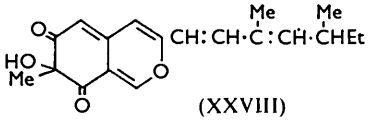
(XXV)



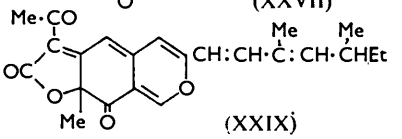
(XXVI)



(XXVII)



(XXVIII)



(XXIX)

At first sight it appears that rubropunctatin (XIXa) must be derived biogenetically from an unusual branched sequence of acetate units. However, an intermediate of type (XXVI) is probably first formed from a linear sequence of six acetate units, and this, on methylation from the C_1 pool and hydroxylation, would give an intermediate (XXVII). Acylation of the tertiary hydroxyl group with 3-oxo-octanoic acid, derived from a linear sequence of four acetate units, followed by aldol condensation and dehydration, would give rubropunctatin. This pattern of biogenesis is similar to that postulated for sclerotiorin

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

on the basis of labelled acetate studies,[16] where an intermediate of type (XXVIII) is apparently formed from a linear sequence of eight acetate units. Further, since rotiorin [7] is a co-pigment of sclerotiorin in *Penicillium sclerotiorum* van Beyma, it seems likely that it has structure (XXIX), derived from an intermediate of type (XXVIII) by acetoacetylation of the tertiary hydroxyl group and subsequent aldol condensation and dehydration. We have been informed by Dr. W. B. Whalley and his associates of this Department that structure (XXIX) for rotiorin is in better agreement with recent experimental findings than the structure recently suggested.[7]

Experimental

Unless otherwise stated, ultraviolet absorption spectra were measured for 95% alcohol solutions with a Unicam S.P. 500 spectrophotometer, infrared spectra for mineral oil dispersions with a Perkin–Elmer Model 21 instrument, and optical rotations for chloroform solutions.

Rubropunctatin and Monascin from Monascus rubropunctatus *Sâto.*—A culture of this organism, obtained from the Centraal-bureau voor Schimmelcultures, Baarn, Holland, was grown on Czapek–Dox solution (prepared according to Raistrick and Rintoul [17]) at 30° for 14—20 days until pigmentation was complete. The red mycelial mats were dried in air at room temperature, milled, and percolated successively with light petroleum (b. p. 60—80°) and ether. The former extract was concentrated and the crude pigment which separated was resolved by fractional crystallisation from ether into *rubropunctatin* (XIXa), orange needles, m. p. 156·5—157° (decomp.) (from ether or alcohol), $[\alpha]_D$ −3481 (*c* 1·07), $\lambda_{max.}$ 218, 246, 290, and 460 mμ (log ε 4·17, 4·09, 3·98, and 4·30), $\nu_{max.}$ 1757s, 1724s, 1656s, 1636s, and 1577s cm.$^{-1}$ [Found: C, 71·1, 71·2, 71·6; H, 6·3, 6·3, 6·5; *C*-Me, 11·1%; OMe, 0·0; active H, 0·0; *M* (Menzies–Wright), 369. $C_{21}H_{22}O_5$ requires C, 71·2; H, 6·3; 3*C*-Me, 12·7%; *M*, 354·4], and monascin, yellow plates, m. p. 142—144° (decomp.) (from ether), $[\alpha]_D$ +544° (*c* 1·27) [Found: C, 70·6, 70·1; H, 7·5, 7·5%; active H, 0·0; *M* (*X*-ray), 348. Calc. for $C_{21}H_{26}O_5$: C, 70·4; H, 7·3%; *M*, 358]. On concentration, the ether extract from the mycelium gave a further amount of rubropunctatin, uncontaminated with monascin. The yields of pigments from the mycelium were variable, 440 penicillin flasks, each containing 500 ml. of culture medium, producing up to 26 g. of rubropunctatin and up to 4 g. of monascin.

Rubropunctatin was soluble in the usual organic solvents but only sparingly soluble in light petroleum and carbon tetrachloride. Although insoluble in cold 2N-sodium hydroxide, the compound was extracted from solution in ether by 2N-sodium hydroxide to give an intensely violet solution, from which it was recovered unchanged by immediate acidification. Solutions of the pigment in alcohol were not decolorised by sodium dithionite and sodium hydroxide or by sulphur dioxide.

Rubropunctatin was extensively degraded by treatment with saturated aqueous barium hydroxide at 100° for 5 hr. or 5% sodium hydroxide in alcohol under reflux for 1 hr. Acidification and distillation in steam of the resultant hydrolysates gave mixtures of volatile acidic products which have not been separated.

Dihydrorubropunctatin (XIXb).—Hydrogenation of rubropunctatin (0·5 g.) suspended in ether (100 ml.) with hydrogen (approx. 2 mols. absorbed) at atmospheric pressure and 5% palladium–barium sulphate (500 mg.) was complete in 2 hr. The filtered solution was diluted with light petroleum (b. p. 40—60°) (15 ml.) and concentrated until crystallisation commenced. *Dihydrorubropunctatin* (292 mg.) was obtained as yellow needles, m. p. 118—119° [after repeated recrystallisation from light petroleum (b. p. 40—60°)], $[\alpha]_D$ −2474° (*c* 1·03), $\lambda_{max.}$ in cyclohexane 218 and 400 mμ (log ε 4·22 and 4·40) (Found: C, 70·9; H, 6·7. $C_{21}H_{24}O_5$ requires C, 70·8; H, 6·8%).

Ozonolysis of Rubropunctatin.—A stream of ozone and oxygen was led into a solution of rubropunctatin (1 g.) in acetic acid (50 ml.) until absorption was complete (*ca.* 2·5 hr.). The orange solution was poured into water (1 l.) containing zinc dust (3 g.), and 12 hr. later the insoluble brown amorphous residue and excess of zinc were collected. Acetaldehyde was removed from the aqueous filtrate by steam and characterised as the 2,4-dinitrophenylhydrazone derivative (308 mg., 51%), yellow blades (from alcohol), m. p. and mixed m. p. 164—166° (Found: C, 42·6; H, 3·6; N, 24·9. Calc. for $C_8H_8O_4N_4$: C, 42·9; H, 3·6; N, 25·0%).

[16] Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576.
[17] Raistrick and Rintoul, *Phil. Trans.*, 1931, *B*, **220**, 2.

Oxidation of Rubropunctatin.—(a) *With alkaline hydrogen peroxide.* Rubropunctatin (1 g.) in N-alcoholic sodium hydroxide (30 ml.) containing water (1·2 ml.) and 30% hydrogen peroxide (8 ml.) was kept at room temperature for 5 days during which the colour changed from deep purple to bright yellow (24 hr.) and then faded (4 days). The mixture was diluted with water (60 ml.) and acidified with hydrochloric acid, and excess of hydrogen peroxide was removed with sulphur dioxide. After isolation by continuous extraction with ether, the products were partially separated by distillation in steam, giving an intractable non-volatile residue (357 mg.) and a volatile oil (213 mg.) (isolated from the distillate with ether) from which hexanoic acid was characterised as the *p*-bromophenacyl ester, plates (from aqueous alcohol), m. p. and mixed m. p. 71—72° (Found: C, 53·9; H, 5·6. Calc. for $C_{14}H_{17}O_3Br$: C, 53·7; H, 5·5%), and as the piperazine salt, needles (from dioxan), m. p. and mixed m. p. 109° (Found: C, 59·9; H, 10·4; N, 8·6. Calc. for $C_{16}H_{34}O_4N_2$: C, 60·3; H, 10·8; N, 8·8%).

(b) *With chromic acid.* To a solution of rubropunctatin (500 mg.) in acetic acid (10 ml.) was added portionwise during 24 hr. a N-solution of chromic acid in acetic acid (50 ml.). After treatment with a few drops of methanol, the solvent was removed *in vacuo*, and the residue was shaken with concentrated hydrochloric acid (1 ml.) in water (100 ml.). Hexanoic acid (58 mg.) was separated in steam, isolated in ether, and characterised as the piperazine salt, needles (from dioxan), m. p. and mixed m. p. 109—110° (Found: C, 60·5; H, 10·7%). The residue from the steam-distillation was separated into intractable neutral (364 mg.) and acidic (100 mg.) fractions.

Rubropunctatamine (XVIII).—A solution of rubropunctatin (1 g.) in ether (350 ml.) was shaken for 1 min. with aqueous ammonia (*d* 0·88) (6 ml.) in water (250 ml.), the colour of the ether solution changing from orange to purple. After addition of concentrated hydrochloric acid (8 ml.), the ether layer was separated, the aqueous layer was further extracted with ether, and the combined ether extracts were concentrated (to *ca.* 240 ml.) and cooled. The dark purple crystals which separated were collected and recrystallised from a small volume of alcohol, giving *rubropunctatamine* in purple needles (660 mg.), m. p. 217—218° (decomp.), $\lambda_{max.}$ 252, 305, 382 (sh), 426, and 542 mμ (log ε 4·16, 4·43, 3·93, 4·17, and 4·24), $\nu_{max.}$ 3067m, 1715s, and 1706s (doublet), 1657w, 1626s, and 1550(s + b) cm.$^{-1}$ (Found: C, 71·3; H, 6·5; N, 4·0. $C_{21}H_{23}O_4N$ requires C, 71·4; H, 6·6; N, 4·0%). Rubropunctatamine was soluble in concentrated hydrochloric acid to a yellow solution, and was precipitated unchanged by water. The pigment, when suspended in alcohol, dissolved immediately on addition of 2N-sodium hydroxide, and was precipitated unchanged on acidification.

Dihydrorubropunctatamine.—(*a*) Hydrogenation of rubropunctatamine (1·0 g.) in alcohol (150 ml.) at atmospheric pressure with palladium–charcoal (from 50 mg. of palladium chloride and 500 mg. of charcoal) was allowed to proceed until 1·2 mols. of gas had been absorbed. On isolation, *dihydrorubropunctatamine* separated from alcohol in red needles (700 mg.), m. p. 212—213° (decomp.), $\lambda_{max.}$ 229, 243, 270, 418, and 503 mμ (log ε 4·15, 4·16, 4·19, 4·22, and 4·29), $\nu_{max.}$ 3070(m + b), 1715 and 1701s (doublet), and 1637s cm.$^{-1}$ (Found: C, 71·2; H, 7·3; N, 3·7. $C_{21}H_{25}O_4N$ requires C, 71·0; H, 7·1; N, 3·9%).

(*b*) Dihydrorubropunctatin (504 mg.) was dissolved in aqueous ammonia (*d* 0·88; 15 ml.), and after ½ hr. at room temperature the solution was diluted with water (200 ml.) and acidified with hydrochloric acid. The resultant red precipitate was collected and crystallised from benzene–light petroleum (b. p. 80—100°), giving dihydropunctatamine in red needles (414 mg.), m. p. and mixed m. p. 212—213° (decomp.) (Found: C, 70·6; H, 7·2; N, 4·1%).

A suspension of this compound in alcohol dissolved immediately on addition of a drop of 2N-sodium hydroxide.

N-*Methylrubropunctatamine.*—Treatment of rubropunctatin (2 g.) in ether (750 ml.) with 25% aqueous methylamine (25 ml.) in water (500 ml.), as described for the preparation of rubropunctatamine, gave N-*methylrubropunctatamine* which separated from alcohol in almost black needles (1·05 g.), m. p. 209—210° (decomp.), $\nu_{max.}$ 1715s, and 1631s cm.$^{-1}$ (Found: C, 72·2; H, 7·0; N, 3·9. $C_{22}H_{25}O_4N$ requires C, 71·9; H, 6·9; N, 3·8%). A suspension of this compound in alcohol did not dissolve on addition of 2N-sodium hydroxide.

Ozonolysis of Rubropunctatamine.—A stream of ozone and oxygen was led into a solution of rubropunctatamine (500 mg.) in ethyl acetate (250 ml.) at room temperature until the original purple colour was discharged (1·5 hr.). During evaporation of the solvent *in vacuo* at room temperature the purple colour was restored. The residue was treated with water (25 ml.), and 12 hr. later the aqueous layer was decanted from the amorphous purple residue. Acetaldehyde

was isolated from the aqueous phase by distillation in steam, and characterised as the 2,4-dinitrophenylhydrazone, yellow needles (from alcohol), m. p. and mixed m. p. 164·5—166°.

Ozonolysis of dihydrorubropunctatamine under similar conditions gave a similar amorphous purple residue but no acetaldehyde.

Oxidation of Rubropunctatamine.—(a) *With nitric acid.* Rubropunctatamine (1 g.) was warmed at 100° with nitric acid (*d* 1·52; 6 ml.) and water (1 ml.) for 18 hr. with additions of more nitric acid (4·5 ml. and 7 ml.) after 5 hr. and 8 hr. The residue was evaporated with water (15 ml.) and subsequently dissolved in water (15 ml.), washed with ether, and boiled with a small amount of charcoal. After filtration, concentration to 4 ml. gave pale yellow prisms which recrystallised from hot water giving pyridine-2,4,5-tricarboxylic acid in prisms (340 mg.), m. p. and mixed m. p. 242—243° (decomp.) (after drying), having an infrared spectrum identical with that of an authentic sample.

(b) *With potassium permanganate.* Rubropunctatamine (1 g.), suspended in 3% aqueous sodium hydroxide (30 ml.) at room temperature, was treated with portions (5 ml.) of 5% aqueous potassium permanganate, reduction of each portion being complete before addition of the next. After addition of 70 ml. the mixture was warmed to 90° and further quantities of the reagent added until oxidation was complete (210 ml. in total). Hexanoic acid was separated by distillation in steam, isolated in ether, and characterised as the *p*-bromophenacyl ester (245 mg.), m. p. and mixed m. p. 69·5—71°.

Aporubropunctatamine (XII).—Successive portions of zinc dust (*ca.* 5 × 750 mg.) were added to rubropunctatamine (1·0 g.) in alcohol (100 ml.) and acetic acid (10 ml.) at 30—40° until the colour of the solution changed from purple to yellow-orange (*ca.* 5 min.). After filtration, the solution was poured into 2N-sodium hydrogen carbonate (300 ml.), and the resultant amorphous orange precipitate was collected and crystallised from alcohol (75 ml.) to give colourless needles (640 mg.) of *aporubropunctatamine*, m. p. 197—198° (red melt), unchanged on sublimation at 190°/0·5 mm., $[\alpha]_D$ ±0° (*c* 6·9 in pyridine), $\lambda_{max.}$ 261, 306, and 356 mμ (log ε 4·56, 3·85, and 3·69), $\nu_{max.}$ 3120m (phenolic OH), 1709s (isolated C=O), 1656w, 1626m, 1586m, and 1567s cm.$^{-1}$ (Found: C, 77·1; H, 8·0; N, 4·9; *C*-Me, 13·3. $C_{20}H_{25}O_2N$ requires C, 77·1; H, 8·1; N, 4·5; 3*C*-Me, 14·5%). In a second experiment under conditions designed to exclude carbon dioxide, carbon dioxide produced in the reduction was estimated by conversion into barium carbonate (Found: 561 mg. of $BaCO_3$ from 1·000 g. of rubropunctatamine. $1CO_2$ requires 558 mg. of $BaCO_3$). Aporubropunctatamine had the following properties: (*a*) A suspension in alcohol dissolved immediately on addition of 2N-sodium hydroxide, (*b*) addition of dilute hydrochloric acid gave a yellow solution of the hydrochloride, (*c*) a solution in methanol did not give iodoform, and (*d*) Gibb's reagent in pyridine gave an instantaneous bright blue colour. Prepared with acetic anhydride and pyridine, O-*acetylaporubropunctatamine* formed needles [from light petroleum (b. p. 60—80°)], m. p. 114—115°, $\lambda_{max.}$ 252, 290, 299, and 342 mμ (log ε 4·71, 4·16, 4·10, and 3·46) with shoulders at 280 and 353 mμ (log ε 4·07 and 3·39), $\nu_{max.}$ 1757s (phenolic OAc), 1704s (isolated ketonic C=O), 1639w, 1597w, 1572w, and 1205s (phenolic OAc) cm.$^{-1}$ (Found: C, 74·9; H, 7·6; N, 4·0. $C_{22}H_{27}O_3N$ requires C, 74·8; H, 7·7; N, 4·0%). This compound, in alcoholic suspension, did not dissolve in 2N-sodium hydroxide, but was soluble in dilute hydrochloric acid.

O-*Methylaporubropunctatamine.*—Aporubropunctatamine (500 mg.) in acetone (35 ml.) was heated under reflux for 2 hr. with methyl iodide (0·5 ml.) and potassium carbonate (800 mg.), more methyl iodide (0·25 ml.) being added after 1 hr. After cooling of the mixture, the potassium carbonate was collected and washed with hot acetone (3 × 10 ml.), and the combined filtrates were concentrated (to 5 ml.), diluted with water (15 ml.), and heated to boiling; the clear solution was then allowed to cool. The solid which separated was collected, dried, and extracted with hot light petroleum (b. p. 40—60°). On cooling, the extract deposited O-*methylaporubropunctatamine* (230 mg.) which on recrystallisation from light petroleum (b. p. 40—60°) gave needles, m. p. 80—81°, $\lambda_{max.}$ 253, 280, 290, 300, and 341 mμ (log ε 4·77, 4·09 4·09, 4·18, and 3·55), $\nu_{max.}$ 1704s (ketonic C=O), 1660w, 1631w, 1595w, and 1572m cm.$^{-1}$ (Found: C, 77·4; H, 8·4; N, 4·3; OMe, 8·8. $C_{21}H_{27}O_2N$ requires C, 77·5; H, 8·4; N, 4·3; OMe, 9·5%). Prepared with hydroxylamine hydrochloride and pyridine the *oxime* formed needles (from ether), m. p. 122—124° (Found: N, 7·9. $C_{21}H_{28}O_2N_2$ requires N, 8·2%).

N-*Methylaporubropunctatamine.*—(*a*) Aporubropunctatamine (1 g.) and methyl iodide (1 ml.) in acetone (75 ml.) were heated under reflux for 4 hr., and the resultant solution concentrated to 20 ml. *Aporubropunctatamine methiodide* (1·2 g.) separated as yellow prisms,

m. p. 218—219°, not raised by subsequent recrystallisation from ethanol and ether, $\lambda_{max.}$ 264, 330, and 401 mμ (log ε 4·19, 3·27, and 3·44), $\nu_{max.}$ 3195m (phenolic OH), 1706s (isolated ketonic C=O), 1626s, 1563m, and 1508w cm.$^{-1}$ (Found: C, 55·4; H, 6·5; N, 2·8; I, 28·1. $C_{21}H_{28}O_2NI$ requires C, 55·6; H, 6·2; N, 3·1; I, 28·0%). This compound (500 mg.) in methanol (10 ml.) with 2N-sodium carbonate (10 ml.) and water (30 ml.) gave a red precipitate of N-*methylaporubropunctatamine* which separated from ethyl acetate and light petroleum (b. p. 60—80°) in orange needles (330 mg.), m. p. 172—174°, $\lambda_{max.}$ 241, 370, and 481 mμ (log ε 4·51, 3·78, and 3·85) with an inflexion at 275 mμ (log ε 4·32), $\nu_{max.}$ 1701s (isolated ketonic C=O), 1623s, and 1563m cm.$^{-1}$ (Found: C, 76·9; H, 8·4; N, 4·3. $C_{21}H_{27}O_2N$ requires C, 77·5; H, 8·4; N, 4·3%).

(*b*) Reduction of *N*-methylrubropunctatamine (1 g.) with zinc and acetic acid, according to the method for the preparation of aporubropunctatamine, gave *N*-methylaporubropunctatamine in orange needles (500 mg.) [from benzene and light petroleum (b. p. 60—80°)], m. p. and mixed m. p. 173·5—174° (Found: C, 77·4; H, 8·4; N, 4·7%).

Dihydroaporubropunctatamine.—(*a*) Hydrogenation of aporubropunctatamine (1·0 g.) in alcohol (150 ml.) with 10% palladium–charcoal (500 mg.) at atmospheric pressure was terminated when *ca.* 1·2 mols. had been absorbed. *Dihydroaporubropunctatamine* formed needles (660 mg.) (from alcohol), m. p. 118—119°, $\lambda_{max.}$ 238, and 343 mμ (log ε 4·69, and 3·69), $\nu_{max.}$ 3145m (phenolic OH), 1708s (ketonic C=O), 1639m, 1599w, and 1560s cm.$^{-1}$ (Found: C, 76·8; H, 8·8; N, 4·5. $C_{20}H_{27}O_2N$ requires C, 76·6; H, 8·7; N, 4·5%).

(*b*) Reduction of dihydrorubropunctatamine (0·5 g.) with zinc and acetic acid by the usual method gave dihydroaporubropunctatamine (0·3 g.), m. p. and mixed m. p. 118—119°, having an infrared spectrum identical with the compound prepared as in (*a*). Prepared with acetic anhydride and pyridine O-*acetyldihydroaporubropunctatamine* formed needles [from light petroleum (b. p. 60—80°)], m. p. 82—83°, $\lambda_{max.}$ 232, 268, 321, and 334 mμ (log ε 4·79, 3·60, 3·48, and 3·54), $\nu_{max.}$ 1754s (phenolic OAc), 1708s (isolated ketonic C=O), 1642w, 1600w, 1568w, and 1225s (phenolic OAc) cm.$^{-1}$ (Found: C, 74·0; H, 8·1; N, 4·1. $C_{22}H_{29}O_3N$ requires C, 74·3; H, 8·2; N, 3·9%).

Reduction of Aporubropunctatamine, its Dihydro-derivative, and O-Methyl Derivative with Potassium Borohydride.—To a suspension of aporubropunctatamine (250 mg.) in methanol (10 ml.) and water (2 ml.) was added 2N-sodium hydroxide until a homogeneous solution was obtained. Potassium borohydride (250 mg.) in water (5 ml.) was then added, and the mixture kept at 35—40° for 6 hr., then kept at room temperature for 18 hr. Acidification with acetic acid and crystallisation of the precipitate from methanol gave prisms (180 mg.) of the *dialcohol*, m. p. 207—208°, $\lambda_{max.}$ 259, 308, and 352 mμ (log ε 4·68, 3·82, and 3·63), $\nu_{max.}$ 3300m (alcoholic OH), 3106m (phenolic OH), 1626s, 1585m, and 1560s cm.$^{-1}$ (Found: C, 76·1; H, 8·8; N, 4·4. $C_{20}H_{27}O_2N$ requires C, 76·6; H, 8·7; N, 4·5%). With acetic anhydride and pyridine, the reduction product formed a *di*-O-*acetate*, needles (from aqueous alcohol), m. p. 91—92°, $\lambda_{max.}$ 252, 290, 300, 343, and 354 mμ (log ε 4·72, 4·11, 4·08, 3·47, and 3·42), $\nu_{max.}$ (in CCl_4) 1773s and 1190s (phenolic OAc), 1733s and 1220s (alcoholic OAc), and 1626m cm.$^{-1}$ (Found: C, 72·3; H, 7·8; N, 3·8. $C_{24}H_{31}O_4N$ requires C, 72·5; H, 7·9; N, 3·5%).

Similar treatment of dihydroaporubropunctatamine (1·3 g.) with potassium borohydride gave the *dihydro-derivative* of the above reduction product, rhombs (910 mg.) (from aqueous alcohol), m. p. 164—165°, $\lambda_{max.}$ 241 and 343 mμ (log ε 4·69, and 3·66), $\nu_{max.}$ 3289m (alcoholic OH), 3125m (phenolic OH), 1628s, 1592m, and 1562s cm.$^{-1}$ (Found: C, 75·9; H, 9·4; N, 4·6. $C_{20}H_{29}O_2N$ requires C, 76·1; H, 9·3; N, 4·4%). The *di*-O-*acetate* formed needles (from aqueous alcohol), m. p. 97—99°, $\lambda_{max.}$ 231, 269, 321, and 333 mμ (log ε 4·93, 3·62, 3·54, and 3·60), $\nu_{max.}$ (in CCl_4) 1773s and 1190s (phenolic OAc), 1730s and 1220m (alcoholic OAc), and 1623m cm.$^{-1}$ (Found: C, 72·6; H, 8·5; N, 3·6; Ac, 22·0. $C_{24}H_{33}O_4N$ requires C, 72·2; H, 8·3; N, 3·5; Ac, 21·5%).

Prepared by methylation of the above reduction product from aporubropunctatamine with methyl iodide and potassium carbonate in acetone, or by reduction of *O*-methylaporubropunctatamine with sodium borohydride, the *methoxy-alcohol* separated from aqueous methanol in needles, m. p. 94—95°, $\lambda_{max.}$ 254, 292, 304, and 342 mμ (log ε 4·73, 4·00, 3·93, and 3·52) with an inflection at 354 mμ (log ε 3·47), $\nu_{max.}$ 3289m (alcoholic OH), 1650w, 1623s, 1580m, and 1653s cm.$^{-1}$ (Found: C, 76·7; H, 8·7; N, 4·3; OMe, 9·2. $C_{21}H_{29}O_2N$ requires C, 77·0; H, 8·9; N, 4·3; OMe, 9·5%). The O-*acetate* formed needles (from aqueous methanol), m. p. 79—80°, $\lambda_{max.}$ 253, 292, 303, 343, and 353 mμ (log ε 4·74, 4·07, 4·01, 3·52, and 3·47), $\nu_{max.}$ (in CCl_4) 1733s and 1233s (alcoholic OAc), 1647w, 1618m, 1575m, and 1558 cm.$^{-1}$ (Found: C, 74·7; H, 8·8; N, 3·8. $C_{23}H_{31}O_3N$ requires C, 74·8; H, 8·5; N, 3·8%).

Ozonolysis of O-*Acetylaporubropunctatamine*.—This compound (1·0 g.) in ethyl acetate (100 ml.) at room temperature was treated with ozone and oxygen until the yellow colour initially formed in the solution had faded (*ca.* 20 min.). After evaporation of the solvent *in vacuo*, the residue was treated with water (20 ml.), and 12 hr. later the brown precipitate was collected and crystallised from methanol, giving the *bisnor-acid* in needles (220 mg.), m. p. 244°, $\nu_{max.}$ 2500(w + b) (CO_2H), 1750s and 1196s (phenolic OAc), and 1700s (CO_2H and isolated ketonic C=O) cm.$^{-1}$ (Found, after thorough drying: C, 67·0; H, 6·7; N, 3·9; O, 22·3. $C_{20}H_{23}O_5N$ requires C, 67·2; H, 6·5; N, 3·9; O, 22·4%). Prepared with diazomethane the *methyl ester* formed needles, m. p. 145—146° (from methanol), $\nu_{max.}$ 3370w (solvate water), 1767s (phenolic OAc), 1715s (Me ester and ketonic C=O), 1629w, and 1567w cm.$^{-1}$ (Found: C, 66·1; H, 6·7; N, 3·7. $C_{21}H_{25}O_5N,0{\cdot}5H_2O$ requires C, 66·3; H, 6·9; N, 3·7%). The aqueous filtrate left after separation of the bisnor-acid was distilled in steam; acetaldehyde was isolated from the distillate as the 2,4-dinitrophenylhydrazone, yellow needles (0·17 g. 33%) (from alcohol), m. p. and mixed m. p. 166—166·5°.

Azo-dye from the Borohydride Reduction Product of Aporubropunctatamine.—A solution (40 ml.) of benzenediazonium chloride was prepared in the usual way at 0° from aniline (1·28 g.). Part (2 ml.) of this solution was added dropwise at 0° to a stirred solution of the borohydride reduction product from aporubropunctatamine (218 mg.) in N-sodium hydroxide (10 ml.) and methanol (10 ml.). The resultant deep red solution was neutralised with 20% acetic acid, and the red precipitate collected and crystallised from alcohol, giving orange needles (150 mg.) of the *azo-dye*, m. p. 194° (decomp.), $\lambda_{max.}$ 251, 409, and 486 mμ (log ε 4·44, 4·03, and 4·16), $\nu_{max.}$ 3310m (alcoholic OH), 3086sh (phenolic OH), 1645w, 1608m, and 1548s cm.$^{-1}$ (Found, after thorough drying: C, 74·6; H, 7·7; N, 10·1. $C_{26}H_{31}O_2N_3$ requires C, 74·8; H, 7·5; N, 10·1%). This compound, when suspended in a small amount of methanol or ether, dissolved immediately on addition of dilute sodium hydroxide to give an intense red colour. The original orange colour was restored by acidification.

The Cinnoline Derivative (XV).—Part (2 ml.) of a benzenediazonium chloride solution [from aniline (1·28 g.) in a total volume of 40 ml.] was added dropwise at 0° to aporubropunctatamine (216·5 mg.) in N-sodium hydroxide (10 ml.) and methanol (15 ml.). Coagulation of the red precipitate, which separated immediately, was effected by neutralisation with 20% acetic acid, and the product was collected and crystallised from alcohol, giving the *cinnoline derivative* (XV) in vermillion red needles (223 mg.) m. p. 233—234°, $\lambda_{max.}$ 249, 280, 325, 408, and 443 mμ (log ε 4·49, 4·32, 4·03, 4·29, and 4·10), $\nu_{max.}$ 1645w, 1618w, 1592s, 1582s, 1567s, and 1536m cm.$^{-1}$ (Found, after thorough drying: C, 78·6; H, 7·0; N, 10·8. $C_{26}H_{27}ON_3$ requires C, 78·6; H, 6·9; N, 10·6%). This compound, when suspended in a small amount of methanol or ether, did not dissolve or change colour on addition of dilute sodium hydroxide.

Hexahydroaporubropunctatin (XXIa).—Hydrogenation of rubropunctatin (500 mg.) in acetic acid (100 ml.) at atmospheric pressure with 10% palladium–carbon (500 mg.) was complete after the apparent absorption of 5—6 mols. A solution of the product (a pale yellow oil) in ether was successively extracted with saturated sodium hydrogen carbonate and 2N-sodium hydroxide solutions, and the products were recovered by acidification of the extracts and separation in ether. The " acidic " (trace) and " phenolic " (70 mg.) extracts were intractable and were discarded. The " neutral " material (360 mg.) was adsorbed on a column of silica gel (4″ × ½″) from solution in benzene–light petroleum (b. p. 60—80°) (1 : 1) (10 ml.). Successive elution with the same solvent mixture (2 × 40 ml.) and benzene (2 × 40 ml.) gave *hexahydroaporubropunctatin* (XXIa) which separated from light petroleum in needles (60 mg.), m. p. 76—77°, $\lambda_{max.}$ 282 mμ (log ε 3·36), $\nu_{max.}$ 3663m (phenolic OH), 1718s (isolated ketonic C=O), and 1626w cm.$^{-1}$ (Found: C, 75·7, 75·6; H, 9·3, 9·5; *C*-Me, 12·3. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5; 3*C*-Me, 14·2%). Further elution with more polar solvents gave only intractable material. With acetic anhydride and pyridine the phenol (XXIa) gave an *acetate* (XXIb) which separated from aqueous alcohol in needles, m. p. 71·5—72·5°, $\lambda_{max.}$ 266 mμ (log ε 2·55), $\nu_{max.}$ (in CCl_4) 1761s and 1193s (phenolic OAc), 1706s (isolated ketonic C=O), and 1616w cm.$^{-1}$ (Found: C, 73·3; H, 9·0. $C_{22}H_{32}O_4$ requires C, 73·3; H, 9·0%). With dimethyl sulphate and potassium carbonate in acetone the phenol (XXIa) gave a *methyl ether* (XXIc), needles (from aqueous alcohol), m. p. 49—50°, $\lambda_{max.}$ 272 mμ (log ε 2·62) (Found: C, 75·9; H, 9·8; OMe, 9·1. $C_{21}H_{32}O_3$ requires C, 75·9; H, 9·7; OMe, 9·3%). With hydroxylamine hydrochloride and pyridine the methyl ether gave an *oxime*, needles (from aqueous alcohol), m. p. 80—81° (Found: C, 72·6; H, 9·7; N, 4·2. $C_{21}H_{33}O_3N$ requires C, 72·6; H, 9·6; N, 4·1%).

Reduction of Hexahydroaporubropunctatin with Potassium Borohydride.—Hexahydroaporubropunctatin (100 mg.) in methanol (10 ml.) was treated with potassium borohydride (50 mg.) in water (5 ml.) for 16 hr. at room temperature. After acidification with 2N-hydrochloric acid (10 ml.) and water (50 ml.) the precipitate was collected and crystallised from aqueous alcohol, giving the *dialcohol* (XXIIa) in plates (70 mg.), m. p. 99—100°, $\lambda_{max.}$ 274, and 282 mμ (log ε 2·98, and 3·00), $\nu_{max.}$ 3379m and 1587w cm.$^{-1}$ (Found: C, 74·7; H, 10·5. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%). Prepared with acetic anhydride and pyridine, the *diacetate* (XXIIb) separated from aqueous alcohol in needles, m. p. 62—63°, $\lambda_{max.}$ 268mμ (log ε 2·56), $\nu_{max.}$ 1773s and 1196s (phenolic OAc), 1736s and 1238s (alcoholic OAc), 1626w, and 1575w cm.$^{-1}$ (Found: C, 71·8; H, 9·4. $C_{24}H_{36}O_5$ requires C, 71·3; H, 9·0%).

Oxidation of Hexahydro-O-methylaporubropunctatin.—A solution of this compound (250 mg.) in acetone (25 ml.) was heated under reflux during the addition of potassium permanganate (1·2 g.), in small portions, during 14 hr. After distillation of acetone from the mixture, the residue was treated with water (25 ml.) and sulphur dioxide. The product was isolated in ether and extracted with saturated sodium hydrogen carbonate solution. Acidification of this extract with 2N-hydrochloric acid gave a white precipitate which was collected and crystallised from water, giving the *acid* (XXIII) in needles (25 mg.), m. p. 151—152°, $\nu_{max.}$ 2646m and 1698s (aryl CO_2H), 1718s (δ-lactone of aryl acid), 1600w, and 1563w cm.$^{-1}$ (Found: C, 64·4; H, 6·7; OMe, 11·1. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5; OMe, 11·1%).

The authors are indebted to Drs. F. M. Dean, W. B. Whalley, and Mr. J. Staunton of this Department for helpful discussions and for information about unpublished work on rotiorin. One of them (E. J. H.) thanks the Department of Scientific and Industrial Research for a Maintenance Grant. The microanalyses were performed by Mr. A. S. Inglis, M.Sc., and his associates of this Department.

UNIVERSITY OF LIVERPOOL. [*Received, June 4th,* 1959.]