NOTES

Metabolites of pathogenic fungi. VII. On the structure and stereochemistry of xanthomegnin, vioxanthin, and viopurpurin, pigments from *Trichophyton violaceum*[1,2]

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Recently, we proposed structure **1** for xanthomegnin (1) and tentatively assigned structures **2** and **3** for vioxanthin and viopurpurin respectively (2). We should now like to present evidence which confirms the structure of vioxanthin (**2**), adds weight to the structure proposed for viopurpurin, and which defines the stereochemistry of the lactone ring in **1** and **3**.

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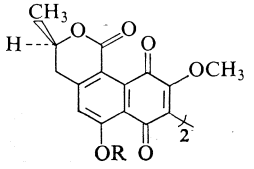
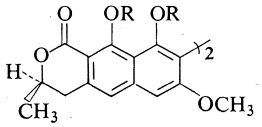
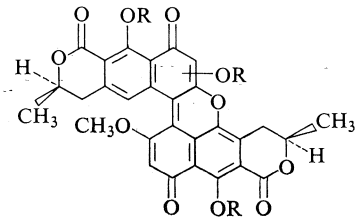
Lithium aluminium hydride reduction of **4**, an oxidation product of **1** (1), followed by ozonolysis and oxidation with silver oxide (3), gave β-hydroxybutyric acid, $[\alpha]_D -22.8°$. R-β-hydroxybutyric acid has $[\alpha]_D -24°$ (4). The configuration of the asymmetric center in **4**, and therefore in the lactone ring of xanthomegnin, is R.



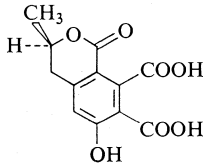
1, Xanthomegnin, R = H
1*a*, R = Ac



2, Vioxanthin, R = H
2*a*, R = CH_3
2*b*, R = Ac



3, Viopurpurin, R = H
3*a*, R = CH_3
3*b*, R = Ac



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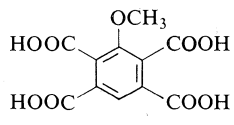

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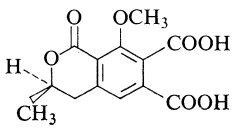

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[2]For part VI in this series, see ref. 9.

[3]Abstracted from part of the Ph.D. Thesis of A. S. Ng, McGill University, 1968.

Oxidation of trimethylviopurpurin (**3***a*) with alkaline hydrogen peroxide gave lactone **6**, identical in all respects with the known lactone obtained by oxidation of floccosin (5). The optical rotatory dispersion (o.r.d.) curves of **4** and **6** are

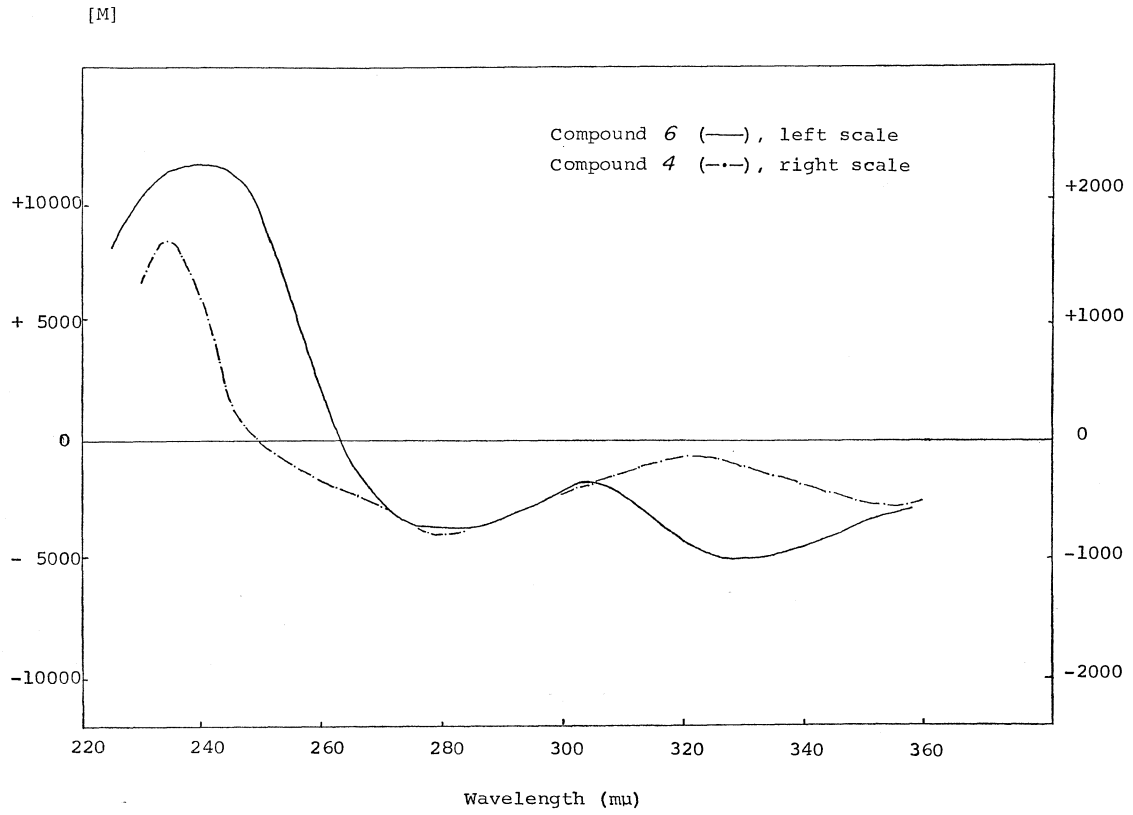


FIG. 1. The optical rotatory dispersion curves of **4** and **6**.

very similar (Fig. 1). It may therefore be concluded that the lactone rings in **4** and **6**, and therefore in **1** and **3**, have the same absolute configuration. This is not unreasonable, especially since both pigments were obtained from the same organism. No degradation product of **2** containing the intact lactone ring could be obtained. It is, however, quite probable that it too has the same absolute stereochemistry.

In our original paper on xanthomegnin (1), we had proposed the S-configuration for the lactone ring, based on an apparently unwarranted extension of the Hudson–Klyne rule (6). It is conceivable that the o.r.d. curve of the acid (or sodium salt) of **1** (see Fig. 3) is sufficiently different from **1** to simulate an apparent reversal of the ΔM_D, (which is taken to reflect the absolute stereochemistry at the asymmetric center) upon opening of the lactone ring.

Vioxanthin has been assigned structure **2**, based mainly on spectroscopic data (2). The linear arrangement of the three rings has now been confirmed by oxidation of **2***a* with alkaline permanganate to the known anisole-2,3,5,6-tetracarboxylic acid[4] and the dimeric nature has been corroborated by mass spectrometry (molecular ion of **2***a*: *m/e* 602).

Biogenetic considerations had governed the assignment of the positions of the dimer linkage and of the methoxy groups in vioxanthin (2). This assignment has now been confirmed by o.r.d. of vioxanthin (**2**) and its tetramethyl ether **2***a* and tetraacetate **2***b* (Fig. 2), which shows a very large Cotton effect (M_D = 480 000) associated with the hindered rotation at the dimer linkage. This large effect cannot be associated with the dissymmetry of the lactone ring (see Fig. 1), and excludes the alternative arrangement in which the

[4]We should like to thank Dr. J. S. E. Holker, Department of Organic Chemistry, University of Liverpool, England, for the infrared spectra of an authentic sample.

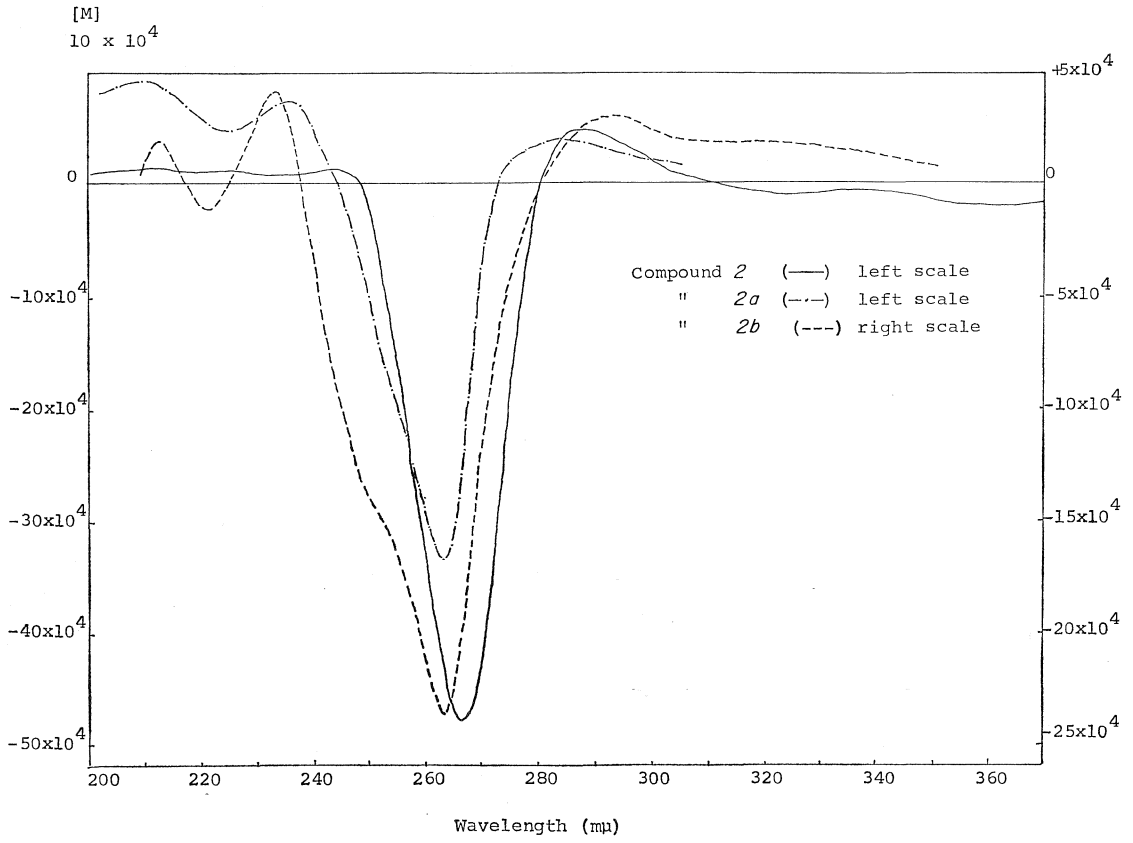
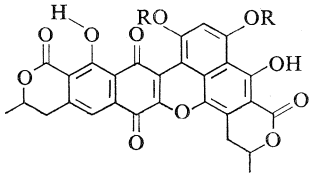


FIG. 2. The optical rotatory dispersion curves of **2**, **2***a*, and **2***b*.

positions of the methoxy group and the dimer linkage are switched, and in which no optical activity due to hindered rotation around the dimer-linkage should be observed.

Not enough model compounds of types **1** and **2** have been described to permit the assignment of the absolute stereochemistry of the binaphthyl linkage in xanthomegnin and vioxanthin (7).

An alternative structure for viopurpurin, which cannot be excluded at the present time, is represented by **7**.



7, R = H,CH_3

Experimental

β-*Hydroxybutyric Acid from Xanthomegnin (1)*

Xanthomegnin (500 mg) was dissolved in 50 ml 1% aqueous potassium hydroxide. To the alkaline solution, 10 ml 50% hydrogen peroxide was dropped in slowly with stirring and the solution was stirred for 5 h. The solution was then acidified with concentrated hydrochloric acid to pH 4, and extracted with ether in a liquid–liquid extractor for 48 h.

The oxidation product **4** was then dried *in vacuo*, and dissolved in 50 ml anhydrous tetrahydrofuran. To the tetrahydrofuran solution, 1 g of lithium aluminium hydride was added cautiously, and the mixture stirred for 24 h. The excess reagent was decomposed with 1.6 ml 10% sodium hydroxide and 2 ml of distilled water. After the decomposition was completed, the solution was filtered, the filtrate was taken to dryness, and an oil was obtained. It showed one major spot on thin-layer chromatography (t.l.c.). The crude reduced product was dissolved in 40 ml of chloroform and ozonized at room temperature for 2 h. After the solvent was removed, 20 ml of distilled water and 500 mg of silver oxide were added to the ozonide. The mixture was warmed on a water bath for 20 min at 70°, the oxide was filtered off, and the filtrate acidified

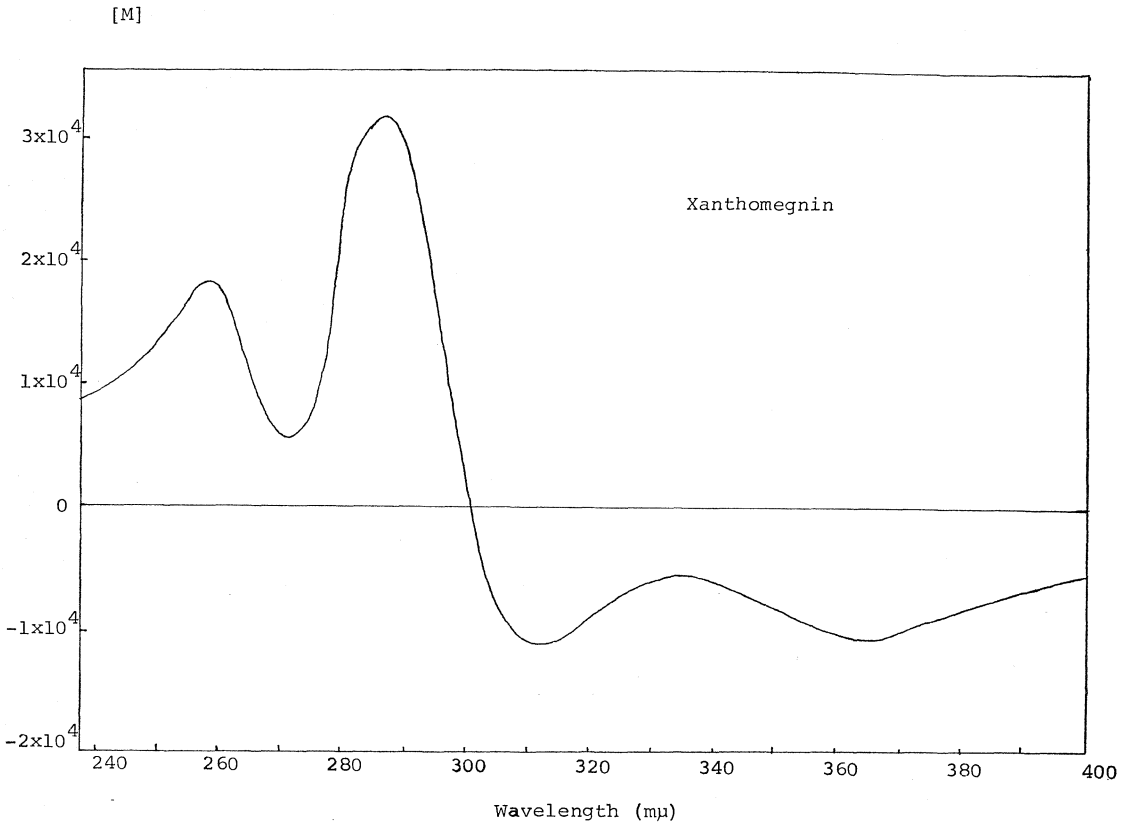


FIG. 3. The optical rotatory dispersion curve of the acid (or sodium salt) of **1**.

with concentrated hydrochloric acid. After removal of silver chloride, the filtrate was extracted with ether; β-hydroxybutyric acid was obtained and identified by t.l.c. The chromatogram was sprayed with methyl orange indicator after developing in acetone. A red spot having the same R_f value as authentic β-hydroxybutyric acid was observed. The acid was further identified by comparison of its retention time with that of an authentic sample on vapor-pressure chromatography (v.p.c.). Optical rotation of the acid was $[\alpha]_D - 22.8°$ ($CHCl_3$).

Tetramethyl Vioxanthin (2a)

To an acetone (20 ml) solution of vioxanthin (50 mg), were added 5 ml of dimethyl sulfate and 2 g of anhydrous potassium carbonate. The mixture was refluxed with stirring for 5 h, cooled, and the solid removed. Acetone was removed by evaporation *in vacuo*. The excess dimethyl sulfate was hydrolyzed with 5% aqueous sodium hydroxide solution. The alkaline solution was acidified with concentrated hydrochloric acid and extracted with chloroform. The chloroform solution was washed with distilled water until free from acid. The pale-colored tetramethyl vioxanthin obtained from the chloroform solution was purified by using t.l.c. (silicagel plates; benzene: hexane, 3:7); 40 mg of pure tetramethyl vioxanthin, m.p. 170–171°, was obtained as a powder and could not be crystallized.

λ_{max} (EtOH) 350 mμ (ε 5380), 320 mμ (ε 8840), 307 mμ ε 9250), 264 mμ (ε 53 000).

Anal. Calcd. for $C_{34}H_{34}O_{10}$ (mol. wt., 602): C, 67.76; H, 5.46; O, 26.60. Found[5] (mol. wt. (osmometric), 583): C, 66.82; H, 5.41; O, 27.69.

Mass spectrum: parent peak at *m/e* 602. The nuclear magnetic resonance (n.m.r.) spectrum differed from that of **2** mainly by the absence of the hydroxyl hydrogens and the presence of methoxy signals at 3.55 and 3.9 p.p.m.

Oxidation of Tetramethyl Vioxanthin

Tetramethyl vioxanthin (20 mg) was suspended in 20 ml of 5% aqueous sodium hydroxide solution. About 20 ml of 5% aqueous potassium permanganate was added portionwise and the solution was heated to reflux for 18 h. Manganese dioxide was removed and the filtrate acidified with dilute sulfuric acid. Sodium bisulfite was added to destroy the remaining manganese dioxide. The acidic solution was then extracted with ether in a liquid–liquid extractor for 48 h. The material obtained (2 mg) from the ether extract showed a spot corresponding to authentic anisole-2,3,5,6-tetracarboxylic acid (8) on t.l.c. Its infrared (i.r.) spectrum was also identical to that of the authentic sample. Crystallization was not possible because of the scarcity of the sample.

[5]Not enough sample to repeat the analysis.

Anisole-2,3,5,6-tetracarboxylic Acid Methyl Ester

The crude anisole-2,3,5,6-tetracarboxylic acid (2 mg) was dissolved in 1 ml of methanol. The solution was methylated with ethereal diazomethane for 30 min. The methylated product was dissolved in ether and injected into a DC 710 v.p.c. column (Model F & M 700, temperature 200 °C). Three peaks appeared with retention times at 0.95, 1.45, and 3.75 min. The retention time of an authentic sample of anisole-2,3,5,6-tetracarboxylic acid methyl ester (8) was 3.75 min.

Trimethyl Viopurpurin (3a)

Viopurpurin triacetate (**3***b*) (40 mg) was dissolved in absolute methanol. A methanol solution of sodium methoxide was added with stirring. The orange colored solution turned blue slowly. After stirring for 2 h, the solvent was removed and methyl iodide added to destroy the excess sodium methoxide. The resulting blue solid was suspended in 20 ml of spectrograde acetone, and 2 g of anhydrous potassium carbonate and 5 ml of dimethyl sulfate were added to the acetone solution. The mixture was refluxed for 8 h. After cooling, the solid was removed. The solvent was taken off by evaporating *in vacuo*. The excess dimethyl sulfate was hydrolyzed by stirring with 5% aqueous sodium hydroxide for 3 h. After the hydrolysis was complete, the alkaline solution was acidified with concentrated hydrochloric acid and extracted with chloroform; 30 mg of red crystalline trimethyl viopurpurin were obtained.[6] Recrystallization from methanol gave blood-red crystals, m.p. 173–174 °C.

λ_{max} (EtOH) 355 mμ (ε 1220), 278 mμ (ε 24 600), 270 mμ (ε 24 000), 218 mμ (ε 14 000); λ_{max} ($CHCl_3$) 455 mμ (ε 1030).

The mass spectrum showed a molecular ion peak at *m/e* 586 (calcd: 586). The n.m.r. of **3***a* showed the following peaks: δ 1.58 (6H, d), 3.10 (4H, d), 4.70 p.p.m. (2H, multiplet), corresponding to $CH_3CH(O)CH_2$—Ar; 7.8 (1H, s), 7.88 p.p.m. (1H, s) corresponding to two aromatic hydrogens; 4.11 (6H, s), 4.15 (3H, s), 4.2 p.p.m. (3H, s) corresponding to four methoxy groups.

[6]The material was used for degradation work. No analysis was obtained.

Alkaline Hydrogen Peroxide Oxidation of Trimethyl Viopurpurin (3a)

Trimethyl viopurpurin (20 mg) was dissolved in 5 ml of methanol and 5 ml of 20% sodium hydroxide was added. To the solution, 10 ml of 50% hydrogen peroxide was added slowly with stirring. Stirring was continued for 5 h, when the intense orange color faded to pale amber. The solution was then acidified with concentrated hydrochloric acid and extracted with ether in a liquid–liquid extractor for 24 h. The ether solution was evaporated to dryness: 3 mg of lactone **6** were obtained which on recrystallization from ether had a m.p. 192–194°. The m.p. was not depressed upon admixture of an authentic sample, and the i.r. spectrum and R_f value (t.l.c.) were identical with those of an authentic sample (5).

Acknowledgments

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1. G. Just, W. Day, and F. Blank. Can. J. Chem. **41**, 74 (1963).
2. F. Blank, A. S. Ng, and G. Just. Can. J. Chem. **44**, 2873 (1966).
3. K. J. Van der Merwe, P. S. Steyn, and D. L. Fourie. J. Chem. Soc. 7083 (1965).
4. R. S. Cahn and C. K. Ingold. J. Chem. Soc. 612 (1951); R. S. Cahn, C. K. Ingold, and V. Prelog. Experientia, **12**, 81 (1956).
5. J. Tudor. Ph.D. Thesis. McGill University, Montreal, Quebec. 1966.
6. C. S. Hudson. J. Am. Chem. Soc. **32**, 338 (1910). W. Klyne. Chem. Ind. London, 1198 (1954).
7. K. Mislow, M. A. W. Glass, R. E. O'Brien, P. Rutkin, D. H. Steinberg, J. Weiss, and C. Djerassi. J. Am. Chem. Soc. **84**, 1455 (1962).
8. J. C. Roberts. J. Chem. Soc. 2989 (1955).
9. C. Pace-Asciak, G. Just, and F. Blank. Can. J. Microbiol. **14**, 90 (1968).

Some derivatives of 1-benzazepine

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A new rearrangement of a substituted 5*H*-1-benzazepine-5-one is described. A new route to certain dimethyl-1-benzazepines has been developed and linked up with a known synthesis. Several new derivatives of 1-benzazepine are reported.

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A number of 1-benzazepines based on the ketolactam **1**, (R = R′ = Me) have been described (1) and screened for pharmacological activity (2). Continuing our studies of this heterocyclic system, although we were unable to prepare analogues **1**, (RR′ = $(CH_2)_3$) and **1**, (R = Cl, R′ = Me) based on 5-acetylaminoindane and 3-methyl-4-chloroacetanilide respectively, we