Pigments of *Gnomonia erythrostoma*. Part II.[1] Epierythrostominol and Epideoxyerythrostominol

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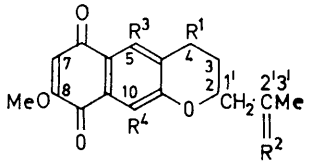
Epierythrostominol and epideoxyerythrostominol have been isolated from cultures of *Gnomonia erythrostoma* and shown to be 3,4-dihydro-4,5,10-trihydroxy-2-(2-hydroxypropyl)-8-methoxy-2*H*-naphtho[2,3-*b*]pyran-6,9-quinone (3) and the side-chain 2-epimer (5) of deoxyerythrostominol (2), respectively.

DURING repetition of the purification of the pigments from deep cultures of *Gnomonia erythrostoma*[1] a more polar red pigment, which has been named epierythrostominol, was isolated. Epierythrostominol was shown by mass spectrometry to have the formula $C_{17}H_{18}O_8$ and had a u.v. spectrum very similar to that of erythrostominone (1),[1] but unlike the latter, its i.r. spectrum showed that it was not a ketone. The n.m.r. spectrum of epierythrostominol, which revealed that it was not a methyl ketone and showed a multiplet at τ 5·72, suggested that the pigment contained a side-chain 2-hydroxy-group as in deoxyerythrostominol (2).[1] Hence epierythrostominol was assigned structure (3), and this has been confirmed by relating it to both erythrostominone and deoxyerythrostominol. In the course of this work epierythrostominol and deoxyerythrostominol were found to be epimeric at the side-chain 2-position (C-2′).

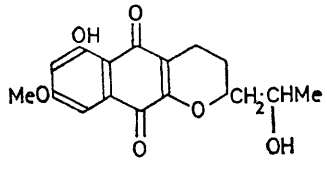
Reduction of erythrostominone with sodium borohydride (*cf.* ref. 1), and preparative layer chromatography (p.l.c.) (development $\times 12$) of the product, gave two closely separated major red bands. Recovery of material from the slower-running band gave an alcohol identical with epierythrostominol, thus confirming the structure of the latter. The faster-running band afforded its 2′-epimer, erythrostominol (4). The n.m.r. spectra of the epimers differed slightly, their i.r. spectra (in $CHBr_3$) showed small differences between 1050 and 1100 cm^{-1}, and their mass spectra were virtually identical.

Hydrogenation of epierythrostominol (*cf.* ref. 1) did not, as expected, yield the natural pigment deoxyerythrostominol (2),[1] but gave its previously unknown 2′-epimer, epideoxyerythrostominol (5). However, hydrogenation of erythrostominol, followed by p.l.c., gave a quinone identical with deoxyerythrostominol, together with a small amount of an orange product which consisted mainly of the monohydroxyquinone (8) but contained some of its isomers (6) and/or (7) (*cf.* ref. 1). The 2′-epimers (2) and (5) differed in m.p. and slightly in R_F value, but their i.r. spectra were almost identical except in the region 1050—1100 cm^{-1}, and their mass spectra differed only in the relative intensities of some ions.

Following the preparation of epideoxyerythrostominol, re-examination of natural deoxyerythrostominol[1] by p.l.c. showed that it contained a small proportion of its 2′-epimer (5).



	R^1	R^2	R^3	R^4	
(1)	OH	O	OH	OH	
(2)	H	H,OH	OH	OH	
(3)	OH	H,OH	OH	OH	
(4)	OH	H,OH	OH	OH	[2′-epimer of (3)]
(5)	H	H,OH	OH	OH	[2′-epimer of (2)]
(6)	H	H,OH	OH	H	
(7)	H	H,OH	H	OH	



(8)

EXPERIMENTAL

Optical rotations were measured with an NPL Automatic Polarimeter 143D with acetone as solvent. I.r. spectra were obtained for solutions in $CHBr_3$ with a Perkin Elmer 157G spectrometer. Details of chromatographic materials and conditions used for determination of m.p.s and u.v., n.m.r., and mass spectra are reported in Part I.[1]

Isolation of Epierythrostominol and Epideoxyerythrostominol.—A dark red solid (1·42 g) extracted from *Gnomonia erythrostoma* broth[1] was chromatographed on Kieselgel G (25 × 4·5 cm). Elution with chloroform–methanol–formic acid (97 : 1 : 2) afforded deoxyerythrostominone (103 mg),

[1] B. E. Cross, M. N. Edinberry, and W. B. Turner, *J.C.S. Perkin I*, 1972, 380.

followed by fractions containing deoxyerythrostominol (120 mg), erythrostominone (746 mg), and a gum which crystallised from acetone–light petroleum as red microcrystals (52 mg) of *epierythrostominol* (3), m.p. variable, but after melting it crystallised on cooling and all samples remelted at 187—191°, $[\alpha]_D^{22}$ +256° (*c* 0·1) (Found: C, 58·35; H, 5·1%; *m/e* 350·0996. $C_{17}H_{18}O_8$ requires, C, 58·3; H, 5·2%; *M*, 350·1002), $\nu_{max.}$ 3570 and 1605 cm^{-1}; $\lambda_{max.}$ 231, 277, 317, 482sh, 514, and 551 nm (ε 32,200, 8050, 7860, 6970, 8440, and 5400); τ 8·67 (3H, d, *J* 5·3 Hz, 3′-H_3), 7·97 (4H, m, 3-H_2 and 1′-H_2), 6·05 (3H, s, 8-OMe), 5·72 (2H, m, 2-H and 2′-H), 5·04 (1H, m, 4-H), 3·58 (1H, s, 7-H), −2·63 (1H, s, 5- or 10-H), and −3·21 (1H, s, 5- or 10-OH).

The fractions containing deoxyerythrostominol were purified by p.l.c. Development (×11) with chloroform–formic acid (199 : 1) partially separated two bands. Recovery of material from the trailing band followed by crystallisation from acetone gave red needles (4 mg), m.p. 178—183°, identical (t.l.c. and i.r. spectrum) with the epideoxyerythrostominol prepared later. Attempts to isolate deoxyerythrostominol, free from epideoxyerythrostominol, from the major band proved unsuccessful. The presence of small quantities of epideoxyerythrostominol in samples of deoxyerythrostominol isolated from fermentations probably accounts for the discrepancy in m.p.s between these samples (m.p. 139—141°) (*cf.* ref. 1) and the specimen (m.p. 147—150°) prepared later from erythrostominol.

Reduction of Erythrostominone with Sodium Borohydride.—Erythrostominone (275 mg) in methanol (110 ml) was added during 15 min to an ice-cold stirred solution of sodium borohydride (330 mg) in methanol (120 ml). The red colour of the solution immediately changed to yellow. Stirring was continued for 5 min at 0° and for a further 5 min at room temperature. The solution was diluted with water (60 ml), glacial acetic acid (7 ml) was added, and the mixture was left for 30 min. Concentration *in vacuo* followed by recovery in ethyl acetate gave a gum (268 mg) which was purified by p.l.c. Development (×12) with chloroform–methanol–formic acid (48 : 1 : 1) separated two close-running red bands. Material from the faster-running band, recovered in acetone, gave a gum which crystallised from acetone–light petroleum to give red plates (83 mg) of *erythrostominol* (4), m.p. 180—182°, $[\alpha]_D^{26}$ +237° (*c* 0·1) (Found: *m/e* 350·1000. $C_{17}H_{18}O_8$ requires, *M*, 350·1002), $\nu_{max.}$ 3570 and 1605 cm^{-1}; τ 8·70 (3H, d, *J* 5·5 Hz, 3′-H_3), 8·02 (4H, m, 3-H_2 and 1′-H_2), 6·05 (3H, s, 8-OMe), 5·78 (1H, m, 2- or 2′-H), 5·52 (1H, m, 2- or 2′-H), 5·02 (1H, m, 4-H), 3·68 (1H, s, 7-H), −2·58 (1H, s, 5- or 10-OH), and −3·23 (1H, s, 5- or 10-OH).

Recovery of material from the second band in acetone afforded a gum which crystallised from acetone–light petroleum as red microcrystals (59 mg), identical (t.l.c., $[\alpha]_D$, i.r. and mass spectra) with epierythrostominol.

Hydrogenation of Epierythrostominol.—Hydrogenation of epierythrostominol (35 mg) in glacial acetic acid (15 ml) over 10% palladised charcoal (14 mg) for 1·5 h led to the uptake of 1·1 mol. equiv. of hydrogen. Recovery of the product gave a gum (33 mg) which was purified by p.l.c. Development (×2) with chloroform–methanol–formic acid (48 : 1 : 1) gave two red bands. Recovery of the front-running band in chloroform gave a gum which crystallised from acetone as red needles (5 mg) of *epideoxyerythrostominol* (5), m.p. 178—182°, $[\alpha]_D^{22}$ +334° (*c* 1·0) (Found: *m/e* 334·1049. $C_{17}H_{18}O_7$ requires *M*, 334·1052), $\nu_{max.}$ 3560 and 1603 cm^{-1}; τ 8·70 (3H, d, *J* 6·2 Hz, 3′-H_3), 8·17 (4H, m, 3-H_2 and 1′-H_2), 7·31 (2H, m, 4-H_2), 6·06 (3H, s, 8-OMe), 5·68 (2H, m, 2-H and 2′-H), 3·59 (1H, s, 7-H), −2·73 (1H, s, 5- or 10-H), and −3·27 (1H, s, 5- or 10-H). The second band contained starting material (8·6 mg).

Hydrogenation of Erythrostominol.—Erythrostominol (43 mg) in glacial acetic acid (15 ml) was hydrogenated over 10% palladised charcoal (20 mg) for 25 min; 1·3 mol. equiv. of hydrogen was absorbed and the solution became colourless. Filtration followed by evaporation *in vacuo* gave a gum which on p.l.c. gave three major bands after development (×10) with chloroform–methanol–formic acid (97 : 1 : 2). Recovery of material from the orange leading band in acetone gave a solid which crystallised from ethanol as orange needles (5 mg), m.p. 125—139° (*cf.* ref. 1), of a mixture of the isomeric dihydroxyquinones (8) and (6) and/or (7) which failed to separate on further chromatography and crystallisation (Found: *m/e* 318. Calc. for $C_{17}H_{18}O_6$: *M*, 318), $\nu_{max.}$ 3550 (OH), 1680 (C=O of quinone), 1632 (H-bonded C=O of quinone), and 1609 (C=C) cm^{-1}. Its n.m.r. spectrum showed that the quinone (8) was the major component. Similarly, recovery of material from the second band and crystallisation from acetone–light petroleum gave red needles (4 mg), m.p. 147—150°, $[\alpha]_D^{24}$ +289° (*c* 0·1), identical (t.l.c., i.r. and mass spectra) with deoxyerythrostominol. The third band contained starting material (10 mg).

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