

## 1,5,8-Trihydroxy-6-methoxy-3-methylantraquinone from *Laurera purpurina* (Nyl.) Zahlbr.\*

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A red pigment, 1,5,8-trihydroxy-6-methoxy-3-methylantraquinone, (I) has been isolated from *Laurera purpurina* (Nyl.) Zahlbr. The structure has been verified by a synthesis from parietin using Elbs persulfate oxidation.

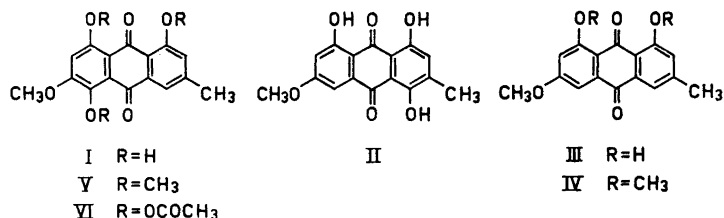
The crustaceous dark red coloured lichen *Laurera purpurina* which grows in the tropics, belongs to the order *Pyrenocarpeae* of which few members have been studied chemically. The present investigation was performed using material collected from the bark of palms growing at the Ivory Coast.

The lichen material on extraction with hexane afforded a crude pigment mixture which was analysed by TLC on polyamide. It gave yellow, orange and red spots, which gave characteristic colour reactions on spraying with a solution of magnesium acetate. One of the main components was isolated by preparative chromatography on silicagel plates and by chromatography on a polyamide column. Crystallisation from benzene and sublimation *in vacuo* gave red needles of the pigment, m.p. 250–251°. Elemental analysis, methoxyl group determination and the mass spectrum indicated the composition  $C_{15}H_9O_5(OCH_3)$ . Prolonged methylation with methyl methanesulphonate in acetone gave a yellow methyl derivative,  $C_{15}H_6O_2(OCH_3)_4$ , and acetylation afforded a triacetate,  $C_{15}H_6O_2(OCH_3)(OCOCH_3)_3$ . These results suggest the presence of a hydroxyantraquinone structure.

The IR-spectrum of the pigment exhibits a broad band around  $2900\text{ cm}^{-1}$  due to chelated hydroxyl groups and a single band in the carbonyl region at  $1600\text{ cm}^{-1}$  (KBr disc), characteristic of a 1,4,5-trihydroxyantraquinone structure.<sup>2</sup> Comparison of the UV-spectra, especially in the region 400–500 nm, of the pigment and its demethylation product,  $C_{15}H_{10}O_6$ , with published spectra of tri- and tetrahydroxyantraquinones<sup>3-5</sup> supports the presence of an antraquinone ring with three  $\alpha$ -hydroxyl groups in the pigment. The

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methoxyl group must be in a  $\beta$ -position since the IR-spectrum of the demethylation product retains a single carbonyl band at  $1604\text{ cm}^{-1}$  and shows a hydroxyl band at  $3300\text{ cm}^{-1}$  (KBr disc) ascribed to an unchelated hydroxyl group. In contrast to the parent pigment, the demethylation product is soluble in a sodium carbonate solution.

These results can be accounted for by either of the structures I or II. Other alternatives are less likely for biogenetic reasons only. Formulae I and II both represent hydroxylated derivatives of the wide spread lichen compound parietin (3-*O*-methylemodin) (III). Like most antraquinones isolated from lichens parietin contains an oxygen arrangement which apart from the extra oxygen atom in the 10-position is consistent with biosynthesis *via* the acetate-malonate route.<sup>6</sup> Formula II, however, represents an *Aspergillus* pigment, erythroglaucin,<sup>7</sup> m.p.  $205\text{--}206^\circ$ , and can be excluded.

The structure I for the pigment has been verified by synthesis. Parietin was subjected to Elbs oxidation with potassium peroxodisulphate<sup>8</sup> in a water — pyridine solution. A compound, chromatographically indistinguishable from the *Laurera* pigment, was isolated in low yield by preparative TLC. The identity was confirmed by mixed melting point determination and by comparison of IR- and mass spectra. The reaction mixture gave a second spot on TLC with the same  $R_F$ -value and colour reactions as an authentic sample of erythroglaucin. In addition, spots due to higher oxidation products were observed.

The *Laurera* pigment was further characterised by the NMR-spectrum of the trimethyl ether V and the triacetate VI. Chemical shifts are given in Table 1, where corresponding values for tri-*O*-methylemodin are included. The chemical shifts of the methyl groups are consistent with their  $\beta$ -position. The aromatic protons of position 7 of the trimethyl ether V and the triacetate VI respectively, give the expected singlet signals whereas the corre-

Table 1. Proton chemical shifts ( $\delta$  ppm) in  $\text{CDCl}_3$ -solutions.

	3-CH <sub>3</sub>	6-OCH <sub>3</sub>	2-H	4-H	5-H	7-H
IV	2.43(s)(3H)	3.92; 3.98(9H)	7.15(br)	7.70(br)	7.35(d) <sup>a</sup>	6.80(d) <sup>a</sup>
V	2.43(s)(3H)	3.97(s)(9H)	7.05(br)	7.55(br)	—	6.80(s)
VI	2.44(br)(12H) <sup>b</sup>	3.95(s)(3H)	7.17(br)	7.90(br)	—	6.95(s)

<sup>a</sup>  $J=2$  cps.

<sup>b</sup> Coincides with acetyl protons.

sponding proton in tri-*O*-methylemodin appears as a doublet ( $J=2$  cps). These signals fall well within the region reported for the corresponding protons of 1,3-dimethoxy antraquinones.<sup>9</sup> The protons at positions 2 and 4, flanking a methyl group, give broadened signals.

1,5,8-Trihydroxy-6-methoxy-3-methylantraquinone has been synthesised from 5-bromo-tri-*O*-methylemodin by Tanaka and Kaneko.<sup>10</sup> Synthesis from emodine by oxidative alkali fusion and subsequent partial methylation has more recently been reported by Steglich, Lösel and Reininger.<sup>11</sup> These latter authors isolated a pigment, xanthorin, formulated as I and evidently identical with the *Laurera* pigment, from *Xanthoria elegans* (Link) Th. Fr. It occurs in small amounts only (0.01 % of dry lichen), accompanied by parietin (1 %). The content of the pigment I in *Laurera purpurina* was estimated to be at least 1 % but parietin could not be detected in the extract. A minor component, however, gave a spot on TLC which in all respects was identical with a spot of 1,5,6,8-tetrahydroxy-3-methyl-antraquinone, obtained by demethylation of I.

To avoid confusion the name earlier proposed by us for the pigment I, lauropurpon,<sup>1</sup> has not been used.

## EXPERIMENTAL

Melting points were determined on a Kofler micro hot stage and are corrected. UV-spectra were recorded in 99.5 % ethanol on a Beckman DK 2 spectrophotometer. IR spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer. NMR spectra were measured in  $\text{CDCl}_3$  solution with a Varian A-60 spectrophotometer at 60 Mc/s. Tetramethyl silane was used as internal standard and chemical shifts are given as  $\delta$  TMS.

Parietin, m.p. 205–206°, was isolated from *Xanthoria parietina* L.Fr. Methylation with dimethyl sulphate and potassium carbonate in acetone gave tri-*O*-methylemodin, m.p. 228–229°, (IV).

*Chromatographic investigations.* The isolation procedures were followed by TLC on polyamide (Merck) with methanol-water (4:1) (three consecutive developments) as solvent. The spots were observed in visible and UV-light before and after spraying with a solution of magnesium acetate (0.5 % in methanol) and subsequent heating (100° for 10 min). Three main spots ( $R_F$  0.05; 0.4; 0.55) and some minor spots ( $R_F$  0; 0.08; 0.65; 0.75) were observed. The spot  $R_F$  0.05 refers to the pigment (I).

*Extraction.* The lichen material (75 g bark containing 10–20 % of lichen thalli) was extracted with hexane for 35 h in a Soxhlet apparatus. The extract deposited a product A which was collected (0.95 g). The mother liquors gave an oily residue (0.2 g).

*Isolation of pigment (I).* a) The product A (250 mg) dissolved in chloroform (12 ml) was subjected to preparative TLC on plates (20×20 cm) with 1 mm layer of Silicagel G (Merck), prewashed with methanol – ether (4:1) and reactivated. Toluene – ethyl formate – formic acid (5:4:1) was used as solvent. The appropriate zones ( $R_F$  ca. 0.7) were combined and extracted with chloroform in a Soxhlet apparatus. Evaporation gave the pigment (60 mg) which was crystallised from benzene – ethanol (2:1) and sublimed (220°, 10 mm) to give red needles (15 mg), m.p. 250–251°, undepressed on admixture with a sample of 1,5,8-trihydroxy-6-methoxy-3-methylantraquinone m.p. 253–254°, synthesised as described below (Found: C 64.1; H 4.05;  $\text{OCH}_3$  10.2; mass spectrum  $M^+ = 300$  m.u. (base peak).  $\text{C}_{18}\text{H}_9\text{O}_5(\text{OCH}_3)$  (300.28) requires C 64.0; H 4.03;  $\text{OCH}_3$  10.3).  $\lambda_{\text{max}}$  EtOH 235 nm ( $\log \epsilon$  4.40), 258 nm (4.55), 306 nm (4.10), 462.5 nm (4.05), 485 nm (4.16), 489.5 nm (4.25), 511 nm (4.06), 525 nm (4.06).

b) Polyamide (Machery and Nagel) (2.5 g) was treated with a solution of product A (430 mg) in chloroform and the solvent was removed *in vacuo*. The amide was packed

on the top of a column (3.7 × 20 cm) made from prewashed (methanol) polyamide (37 g). The column was eluted consecutively with methanol, chloroform and acetic acid. The methanol eluate (300 mg) gave two main spots ( $R_F$  0.4 and 0.55) on TLC as described above and was not investigated further. The chloroform eluate gave the desired pigment (145 mg,  $R_F$  0.05) which was crystallised from benzene to give red needles (65 mg), m.p. 250–251°.

The acetic acid eluate (15 mg) gave a red-violet spot (methanol–water 4:1,  $R_F$  0; pyridine–acetone 4:1,  $R_F$  0.08). This fraction was chromatographically indistinguishable from the demethylation product of the pigment (I) described below.

*Demethylation of pigment (I).* A mixture of pigment (50 mg), hydrobromic acid ( $d=1.50$ , 1.5 ml) and acetic acid (10 ml) was refluxed for 24 h. Water (10 ml) was added and the crystalline product was filtered off. Crystallisation from acetic acid and sublimation (160°, 0.001 mm) gave the demethylation product as dark red-violet needles, m.p. 280–290° (decomp.) (Found: C 62.7; H 3.42; mass spectrum  $M^+=286$  m.u. (base peak).  $C_{15}H_{10}O_6$  (286.25) requires C 62.9; H 3.52).  $\lambda_{max}$  EtOH 236.5 (log  $\epsilon$  4.50), 259 (4.33), 305 (4.04), 494 (4.16), 516.5 (4.03), 529 (4.05), 568 (3.31).  $\nu_{max}$ (KBr) 3280, 2900 (broad), 1604  $cm^{-1}$ .

*Methylation of pigment (I).* Methyl methanesulphonate (1.7 g) and potassium carbonate (2 g) were added to a solution of the pigment (75 mg) in dry acetone (40 ml) and the mixture was refluxed for 95 h. Water was added, the excess methane sulphonate was destroyed and the product was isolated by extraction with ethyl ether. Crystallisation from toluene and sublimation (160°, 0.4 mm) gave the permethyl derivative (V) as yellow needles (75 mg), m.p. 189–190°. Tanaka and Kaneko<sup>10</sup> report m.p. 185–186°. (Found: C 65.4; H 5.33;  $OCH_3$  37.0; mass spectrum  $M^+=342$  m.u. (base peak).  $C_{15}H_6O_2(OCH_3)_4$  (342.34) requires C 66.7; H 5.30;  $OCH_3$  36.3.  $\nu_{max}$ (KBr) 1656  $cm^{-1}$ .

*Acetylation of pigment (I).* Perchloric acid (0.05 ml) was added to a solution of the pigment (22 mg) in pyridine (5 ml) and acetic anhydride (1.5 ml). After 4 days the reaction mixture was poured into water. The product was filtered off and crystallised from ethanol to give the acetate (VI) as yellow needles (23 mg), m.p. 245–247°. (Found: C 61.75; H 4.28; mass spectrum  $M^+=426$  m.u.  $C_{15}H_6O_2(OCOCH_3)_2(OCH_3)$  (426.35) requires C 62.0; H 4.28).  $\nu_{max}$ (KBr) 1770, 1678, 1660  $cm^{-1}$ .

*Elbs persulfate oxidation of parietin.* Parietin (1.75 g, 6.15 mmoles) and sodium hydroxide (1.25 g, 31 mmoles) were dissolved in a mixture of water (20 ml) and pyridine (10 ml). Potassium persulfate (1.65 g, 6.15 mmoles) in water (20 ml) was added in portions of 2 ml over 48 h. After another 48 h water (100 ml) was added and the solution was neutralised to pH 7 with hydrochloric acid. Most of the unreacted parietin (1.4 g) was extracted from the solution with chloroform (2 × 150 ml). The aqueous layer was acidified with conc. hydrochloric acid (10 ml) and heated for 2 h on a boiling water bath. The precipitate was collected and subjected to preparative TLC as described above. Each plate was developed twice with toluene–ethyl acetate (3:1) and then once with toluene–ethyl acetate–formic acid (15:5:1), the plates being dried at 50° between each stage. The appropriate zones were collected and the pigment (23 mg) was isolated by extraction with chloroform in a Soxhlet apparatus. Crystallisation from benzene and sublimation (175°, 0.1 mm) gave 1,5,8-trihydroxy-6-methoxy-3-methylantraquinone (I) as needles, m.p. 253–254°. Mass spectrum  $M^+=300$  (base peak).

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