

Fungus Pigments

XXI.* Peniophorinin, a Further Pigment Produced by
Peniophora sanguinea Bres.

JARL GRIPENBERG

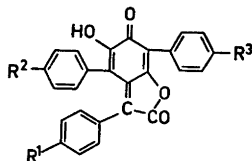
Department of Chemistry, Helsinki University of Technology, Otaniemi, Finland

The isolation of peniophorinin is described. Its chemical and spectral properties indicate that it is either 9-hydroxy-1-(*p*-hydroxyphenyl)-4-phenyl-7*H*-benzofuro[5,4-*c*][2]benzopyran-2,5-dione (4) or 9-hydroxy-4-(*p*-hydroxyphenyl)-1-phenyl-7*H*-benzofuro[5,4-*c*][2]benzopyran-2,5-dione (5), most likely the former.

The isolation of four pigments, A (5-*O*-methylxylerythrin),^{1,2} B (xylerythrin (1)),^{1,2} C (peniophorin (2) or (3)),^{1,3} and D,¹ from wood attacked by the fungus *Peniophora sanguinea* Bres. has been described in previous papers. Further chromatography of the mother liquors from which pigments B and C separated by crystallisation gave two more pigments, designated B₁ and B₂, which had *R_F* values between those of B and C in TLC analysis.

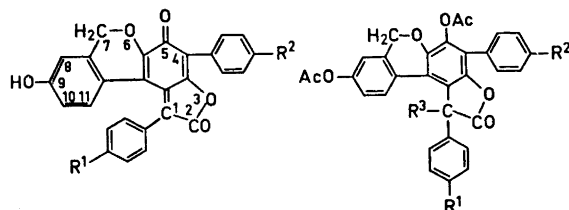
This paper deals with the pigment B₂, now termed peniophorinin, for which the structure 4 or 5 is proposed. The evidence is as follows.

Peniophorinin has the composition C₂₇H₁₆O₆ and thus contains one carbon atom more than peniophorin, and one carbon atom and one oxygen atom more than xylerythrin. The presence of two hydroxyl groups is shown by its con-



- 1 R¹=OH; R²=R³=H
 2 R¹=R²=OH; R³=H
 3 R¹=R³=OH; R²=H

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|---|--|----|--|
| 4 | R ¹ =OH; R ² =H | 10 | R ¹ =R ³ =OAc; R ² =H |
| 5 | R ² =OH; R ¹ =H | 11 | R ² =R ³ =OAc; R ¹ =H |
| 6 | R ¹ =OCH ₃ ; R ² =H | 12 | R ¹ =OAc; R ² =R ³ =H |
| 7 | R ² =OCH ₃ ; R ¹ =H | 13 | R ² =OAc; R ¹ =R ³ =H |
| 8 | R ¹ =OAc; R ² =H | | |
| 9 | R ² =OAc; R ¹ =H | | |

version to a dimethyl ether (6 or 7) and a diacetate (8 or 9). On further treatment of the diacetate with acetic anhydride, a colourless tetra-acetate (10 or 11) is formed. Reductive acetylation gives a dihydrotriacetate (12 or 13). These last two reactions are analogous to the reactions of xylyerythrin and peniophorin and serve to establish the presence of a quinone methide group also in peniophorin. There is also a lactone group in peniophorin as evidenced by an absorption band at 1760 cm^{-1} . This band is at 1750 cm^{-1} in the spectrum of xylyerythrin² and at 1775 cm^{-1} in the spectrum of peniophorin.³

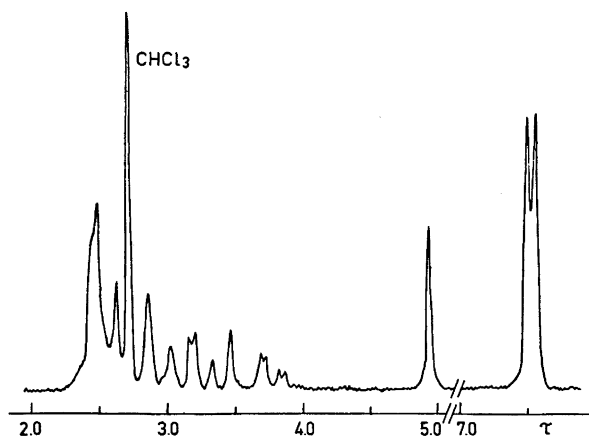
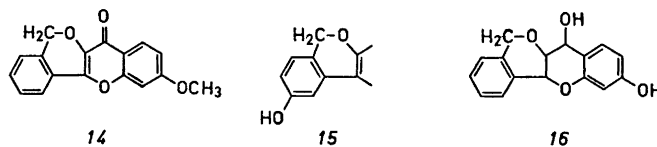


Fig. 1. NMR spectrum of peniophorin acetate.

The NMR spectrum of peniophorin acetate (Fig. 1) has the typical A_2B_2 -pattern of a *para*-substituted benzene ring with two two-proton doublets at τ 2.56 (partly hidden) and τ 2.94 and a coupling constant of 8.5 Hz. A broad singlet at τ 2.50 corresponding to five protons indicates a monosubstituted benzene ring.

The most important part, however, of the NMR spectrum is that due to the 1*H*-2-benzopyran system. A singlet (2H) at τ 4.96 is ascribed to the two benzylic protons. This value is in very good agreement with the values found for the analogous system in, for instance, **14**.⁴ The values of τ for six derivatives containing this system vary between 4.74 and 5.00.⁴⁻⁷



A reversed order of the methylene group and the oxygen atom in the cyclic ether would probably not give a very different value of τ , but this arrangement is considered extremely unlikely from a biogenetic point of view.

In the aromatic region, there is the typical pattern of protons in the 1, 2, and 4 positions, namely one-proton signals at τ 3.78 (dd; $J = 8.5$ and 2 Hz), 3.41 (d; $J = 8.5$ Hz) and 3.20 (d; $J = 2$ Hz). In addition to the arrangement given in **4** or **5**, the arrangement in part structure **15** would also agree with these coupling constants. A decision in favour of **4** or **5** can, however, be made by a consideration of the chemical shifts. It is noteworthy that the two protons, the signals of which show *ortho*-coupling, are very strongly shielded. Inspection of models shows that the plane of the aromatic ring attached to C-1 has to be nearly perpendicular to the plane of the main ring system. The two protons at positions 10 and 11 in **4** or **5** are then in the shielding cone of the aromatic ring,⁸ whereas only the *meta*-coupling proton would be shielded in **15**.

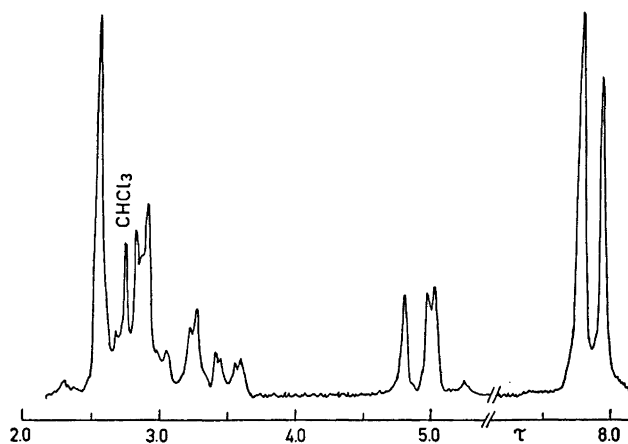


Fig. 2. NMR spectrum of peniophorinin leucoacetate.

The signals due to the cyclic benzyl ether group in the leucoacetate (12 or 13) (Fig. 2) form an AB system with $J = 14$ Hz. Drewes and Roux⁹ report a coupling constant of 16 Hz for some derivatives of mopanol (16). The reason for the difference between the acetate and the leucoacetate lies, of course, in the fact that C-1 is tetrahedral in the leucoacetate, and hence the benzylic protons are magnetically nonequivalent. The tetrahedral nature of this carbon atom also lessens the shielding effect of the benzene ring attached to it. The signal from the H-atom at C-11 is shifted downfield approximately 0.6 ppm, so that it is obscured by the signals from the *para*-substituted benzene ring and chloroform. The signal from the H-atom at C-10 is shifted downfield 0.27 ppm. The NMR spectrum of the leucoacetate contains, of course, also a singlet at τ 4.80 due to the new proton at C-1. The corresponding signal is found at τ 5.12² and τ 5.17³ in the spectra of the leucoacetates of xylytrythrin and peniophorin, respectively.

Whereas the structure of the 1*H*-2-benzopyran moiety of peniophorin thus appears firmly established, no decision between alternatives 4 and 5 can be made. Two possible structures, 2 and 3, may likewise be proposed for peniophorin.³ If peniophorin is 2 and peniophorin 4, peniophorin would be a dehydrogenation product of 5-*O*-methylpeniophorin. This last compound has not been found among the pigments produced by *P. sanguinea*, but the corresponding derivative of xylytrythrin has been isolated.² Weiss *et al.*^{5,6} and Matsuura and Matsushima⁴ have shown that a dehydrogenation very similar to the one referred to above can be effected in the flavone series by irradiation. Thus, for instance, 3,7-dimethoxyflavone is converted to 14.⁴ It was therefore very tempting to try to correlate peniophorin with peniophorin in this way. As monomethylation of peniophorin is difficult to achieve, peniophorin trimethyl ether³ was irradiated in the hope of obtaining peniophorin dimethyl ether. The irradiation was carried out with Pyrex-filtered light from both a mercury and a tungsten lamp. Several different solvents were tried. TLC-analyses showed that the starting material was relatively rapidly consumed, and that a very complex mixture of products resulted. None of the products appeared to be the desired peniophorin dimethyl ether. In a separate experiment it was furthermore found that peniophorin dimethyl ether is unstable to radiation. A search was therefore made for a common product among the irradiation products of peniophorin trimethyl ether and peniophorin dimethyl ether, which would have established the desired connection between these two substances. None of the major spots in thin-layer chromatograms of the two irradiated mixtures appeared to be identical, however. Although no relationship between peniophorin and peniophorin could thus be established, it is biogenetically very plausible that such a relationship exists. We therefore tentatively propose that peniophorin is 9-hydroxy-1-(*p*-hydroxyphenyl)-4-phenyl-7*H*-benzofuro[5,4-*c*][2] benzopyran-2,5-dione (4).

Consequently, structure 2 is considered the more likely structure of peniophorin.³

EXPERIMENTAL

The spectra were recorded with the following instruments: UV spectra (in dioxan) on a Beckman DK-2; IR spectra (KBr discs) on Beckman IR-5 and PE 125; NMR spectra on a Varian A 60 provided with a Varian C 1024 Time Averaging Computer; and mass spectra on a PE 270 B. Analyses were done by Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach, West Germany.

Isolation of peniophorinin. Various mother liquors obtained in the isolation of xylerythrin² and peniophorin³ were pooled, evaporated to dryness under vacuum and dissolved in chloroform. The thin-layer chromatogram of this solution had two spots in addition to those of xylerythrin and peniophorin. One, moving slightly behind the spot of xylerythrin, was designated B₁ and the second, which moved just ahead of the spot of peniophorin, B₂. The substances giving these spots were separated by column chromatography on silica gel impregnated with potassium dihydrogen phosphate (prepared by mixing 100 g of Merck's Kieselgel, 0.05–0.2 mm, with 50 ml of 0.5 M KH₂PO₄ and reactivating the mixture at 110° overnight). Elution was carried out with chloroform. The dark red effluent was collected in fractions of about 50 ml. These were analysed by TLC, and the fractions containing mainly pigment B₂ were pooled. Most of the chloroform was distilled off, whereupon peniophorinin separated as dark brown crystals. It decomposes at 305–315° without any sharp melting point. (Found: C 73.92; H 3.40. C₂₇H₂₆O₈ (436.4) requires C 74.31; H 3.70.) *m/e* 438, 436, 409. IR maxima: 3470, 1760, 1620, 1510, 1420, 1285, 1260, 1215, 1160, 1135, 827, 795, 730, 695 cm⁻¹. UV spectrum: λ_{max} 278 (4.40), 292 inf. (4.36), 380 inf. (3.97), 447 (4.25); λ_{min} 253 (4.13), 342 (3.74) nm (log ε).

Peniophorinin dimethyl ether. The methylation was carried out in the usual way² with dimethyl sulphate in acetone containing potassium carbonate. The ether melted at 209–210°. (Found: C 74.82; H 4.23; OCH₃ 13.20. C₂₉H₂₆O₈ (464.5) requires C 74.99; H 4.34; 2 OCH₃ 13.35.) *m/e* 466, 464, 437, 408. IR maxima: 1785, 1650, 1605, 1185, 1165, 792, 732 cm⁻¹. UV spectrum: λ_{max} 277 (4.46), 288 inf. (4.44), 443 (4.33); λ_{min} 253 (4.20), 343 (3.79) nm (log ε).

Acetylation of peniophorinin. Peniophorinin was acetylated with acetic anhydride containing a small amount of pyridine. After the mixture had stood 2 h, the acetic anhydride was decomposed by careful addition of water. The orange precipitate of peniophorinin diacetate (8) was recrystallised from chloroform-ethanol. M.p. 220–222°. (Found: C 71.06; H 3.79; C₃₁H₂₀O₈ (520.5) requires C 71.53; H 3.87.) *m/e* 520, 478, 450, 436, 422, 409, 393, 380, 363, 351. IR maxima: 1785, 1650, 1605, 1205, 1185, 1165, 792, 732 cm⁻¹. UV spectrum: λ_{max} 271 (4.39), 288 inf. (4.36), 370 inf. (4.16), 407 (4.28); λ_{min} 247 (4.21), 327 (3.84) nm (log ε). NMR spectrum (CDCl₃; 200 x): τ 2.50 (5H, br s), 2.56 (2H, d; *J* = 8.5 Hz), 2.94 (2H, d; *J* = 8.5 Hz), 3.20 (1H, d; *J* = 2 Hz), 3.41 (1H, d; *J* = 8.5 Hz), 3.78 (1H, dd; *J* = 8.5 and 2 Hz), 4.96 (2H, s), 7.70 (3H, s), 7.75 (3H, s).

When the acetylation mixture was allowed to stand for a longer time, the initial dark orange colour gradually faded. After one week, the solution was only faintly yellow. Decomposition of the acetic anhydride by careful addition of water caused precipitation of the tetraacetate 10 as silky needles with m.p. 247–249°. (Found: C 66.55; H 4.74. C₃₅H₂₆O₁₁ (622.6) requires C 67.52; H 4.21.) *m/e* 622, 580, 563, 538, 522, 480, 463, 438, 421, 409, 393. IR maxima: 1795, 1762, 1600, 1582, 1490, 1358, 1180, 1118, 1078, 1060, 1025, 1010, 900, 685 cm⁻¹. UV spectrum: λ_{max} 283 (4.22), 329 (4.15); λ_{min} 266 (4.10), 301 (3.92) nm (log ε).

Peniophorinin leucoacetate (12). This compound was prepared by reductive acetylation of peniophorinin as the corresponding derivative of xylerythrin.² Recrystallisation of the leucoacetate from chloroform-ethanol gave colourless crystals, m.p. 230–231°. (Found: C 69.80; H 4.03; C₃₃H₂₄O₉ (564.5) requires C 70.21; H 4.29.) *m/e* 564, 522, 505, 480, 463, 451, 438, 421, 409. IR maxima: 1820, 1765, 1600, 1500, 1370, 1200, 1053, 912, 890, 820, 693 cm⁻¹. UV spectrum: λ_{max} 282 (4.25), 323 (4.22); λ_{min} 260 (3.88), 297 (3.98) nm (log ε). NMR spectrum: (CDCl₃; 11x); τ 2.55 (5H, s), 2.8–3.9 (5H, m), 3.23 (1H, d; *J* = 2 Hz), 3.49 (1H, dd; *J* = 9 and 2 Hz), 4.80 (1H, s), 4.99 (2H, AB-quartet; *J* = 14 Hz).

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