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Fungus Pigments

XX.* On the Structure of Peniophorin, One of the Pigments Produced by *Peniophora sanguinea* Bres.

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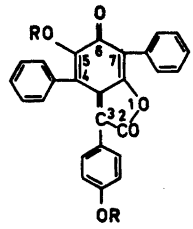
It is shown that peniophorin is an analogue of xylerythrin produced by the same fungus. Its chemical and spectral properties indicate that it is either 5-hydroxy-3,4-di(*p*-hydroxyphenyl)-7-phenylbenzofuran-2,6-dione (*3*) or 5-hydroxy-3,7-di(*p*-hydroxyphenyl)-4-phenylbenzofuran-2,6-dione (*4*), but more likely the former. A third isomer, 5-hydroxy-4,7-di(*p*-hydroxyphenyl)-3-phenylbenzofuran-2,6-dione(*5*), was synthesised and found to differ from peniophorin.

A few years ago, the isolation of four pigments from wood attacked by the fungus *Peniophora sanguinea* Bres. was reported.[1] The pigments were provisionally designated A, B, C, and D in order of decreasing R_F value in TLC.

Pigments A and B (xylerythrin) have the structure *1* and *2*, respectively.[1-3]



1 R = CH_3

2 R = H

This paper deals with the structure of pigment C, for which the name peniophorin is now proposed.

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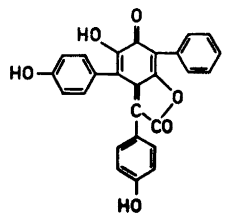
Peniophorin has the composition $C_{26}H_{16}O_6$, and thus differs from xylerythrin in having one oxygen atom more. It gives a trimethyl ether ($C_{29}H_{22}O_6$) and a triacetate ($C_{32}H_{22}O_9$). Upon prolonged treatment with acetic anhydride, the latter adds one molecule of acetic anhydride to form a colourless pentaacetate ($C_{36}H_{28}O_{12}$). Reductive acetylation of peniophorin gives a leucoacetate, which is a dihydrotetraacetate ($C_{34}H_{26}O_{10}$). All these reactions parallel closely the reactions of xylerythrin when allowance is made for one additional hydroxyl group. Further support for the close connection between xylerythrin and peniophorin is provided by the UV spectra of the acetate, the colourless acetate and the leucoacetate of peniophorin and the spectra of the corresponding derivatives of xylerythrin, as shown in Table 1.

Table 1. UV absorption spectra of derivatives of xylerythrin and peniophorin in ethanol.

	λ_{max} nm (log ε)	λ_{min} nm (log ε)
Xylerythrin acetate	242(4.31), 366(4.14), 400infl. (4.09)	221(4.24), 312(3.84)
Peniophorin acetate	247(4.39), 370(4.18), 400infl. (4.10)	222(4.30), 315(3.88)
Xylerythrin leucoacetate	220infl. (4.64), 250infl. (4.30), 290infl. (3.75)	
Peniophorin leucoacetate	220infl. (4.66), 250infl. (4.37), 290infl. (3.84)	
Xylerythrin "colourless acetate"	225(4.54), 292(3.64)	283(3.60)
Peniophorin "colourless acetate"	225infl. (4.36), 292(3.69)	284(3.66)

It can thus be safely assumed that peniophorin has the same basic skeleton as xylerythrin and can be formulated as a trihydroxy derivative of 3,4,7-triphenylbenzofuran-2,6-dione.

Oxidation of peniophorin trimethyl ether gives benzoic acid and anisic acid as the only aromatic acids. This fact shows that one of the hydroxyl groups has to be in position 5. Two of the aromatic rings have a hydroxyl group in the *para*-position, and the third is unsubstituted. This substitution pattern of the aromatic rings finds strong support in the NMR spectrum of the pentaacetyl derivative referred to above. This has, in the aromatic region,



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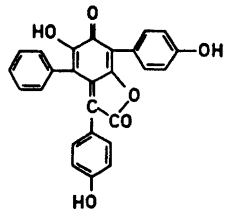

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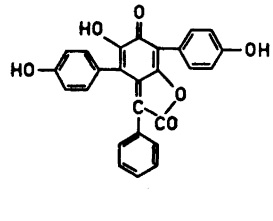
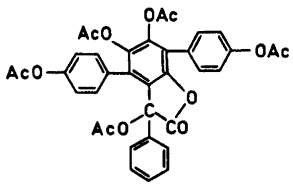


5

two separate A_2B_2-quartets, each corresponding to four protons and a slightly broadened singlet corresponding to five protons.

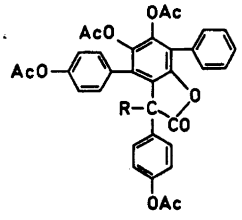
Peniophorin has thus one of the structures *3*, *4*, and *5*. Structure *5* should be amenable to synthesis by the route followed in the synthesis of xylerythrin.[3] Condensation of 2,5-diacetoxy-1,6-di(*p*-acetoxyphenyl)1,4-benzoquinone (atromentin tetraacetate) with phenylacetic acid in acetic anhydride in the presence of sodium acetate gave as the main product, in addition to atromentin leucoacetate, a colourless pentaacetate, which has to be *6*.



6

A small amount of the orange triacetate of *5* was also obtained. Acid hydrolysis of *6* gave *5*, which as well as the acetates, differed definitely from peniophorin and its corresponding derivatives, as judged by their different spectral and chromatographic properties. Structure *5* is thus eliminated as a possible structure for peniophorin. No definite decision can be made at the moment between the two remaining alternatives *3* and *4*.

For reasons outlined in the following paper,[4] structure *3* is to be preferred from a biogenetic point of view, and this structure is therefore tentatively suggested for peniophorin. On the basis of this conclusion, the leucoacetate and the colourless pentaacetate can be formulated as *7* and *8*, respectively, in accordance with the corresponding derivatives of xylerythrin.[3]



7 R = H

8 R = AcO

EXPERIMENTAL

The spectra were recorded with the following instruments: UV spectra (in ethanol, unless stated otherwise) on a Beckman DK-2, IR spectra (KBr discs) on Beckman IR-5 and PE 125, NMR spectra on a Varian A 60, and mass spectra on a PE 270 B. The analyses were done by Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach, West Germany.

Isolation of peniophorin. Extraction of the infected wood and chromatography of the extract was carried out as described before.[3] When all the xylerythrin had been eluted by chloroform, elution was continued with chloroform-ethyl acetate (9:1). The dark red effluent contained mainly peniophorin according to TLC. On concentration of the solution, peniophorin crystallised in an almost pure state. It was purified further by recrystallisation from pyridine-water or acetone-water. M.p. 300–305° (dec.). (Found: C 72.91; H 4.03. $C_{26}H_{16}O_6$ (424.4) requires C 73.58; H 3.80.) *m/e* 424. IR maxima: 3400, 1775, 1630, 1600, 1510, 1410, 1240, 1150, 1005, 925, 827, 687 cm^{-1}. UV spectrum (in dioxan): λ_{max} 265 (4.42), 395 infl. (4.07), 453 (4.18); λ_{min} 228 (4.29), 319 (3.79) nm (log ε).

Peniophorin trimethyl ether. The methylation of peniophorin was carried out in the same way as the methylation of xylerythrin.[3] Peniophorin trimethyl ether was obtained as orange-red plates, m.p. 215–217°. (Found: C 74.75; H 4.64; OCH_3 19.41. $C_{29}H_{22}O_6$ (466.5) requires C 74.67; H 4.75; 3 OCH_3 19.95.) *m/e* 466, 438. Ir maxima: 1775, 1635, 1605, 1510, 1305, 1255, 1180, 1150, 1130, 1030, 1020, 922, 828, 795, 745, 690 cm^{-1}. UV spectrum: λ_{max} 260 (4.36), 395 (4.09), 447 (4.12); λ_{min} 226 (4.31), 330 (3.82), 414 (4.08) nm (log ε). NMR spectrum (in $CDCl_3$): τ 2.3–3.5 (13H,m), 6.17 (3H,s), 6.23 (6H,s).

Acetylation of peniophorin. Peniophorin was allowed to react with acetic anhydride containing a small amount of pyridine overnight. Peniophorin triacetate was recovered from this mixture after careful decomposition of the acetic anhydride with water until crystallisation began. When purified by recrystallisation from ethanol, the acetate melted at 217–218°. (Found: C 69.53; H 4.26. $C_{32}H_{22}O_9$ (550.5) requires C 69.81; H 4.03.) *m/e* 550, 508, 466, 424, 395, 379. IR maxima: 1785, 1645, 1605, 1505, 1375, 1200, 1170, 1125, 1020, 930, 910, 847, 693 cm^{-1}. UV spectrum: see Table 1.

Addition of more water to the mother liquor from which the first crop of crystals had separated, yielded a mixture of red and colourless crystals. A higher yield of the colourless acetate was obtained when the acetylation mixture was allowed to stand for a longer time or when it was warmed on a steam bath. The solution was then almost colourless. When the acetic anhydride was decomposed with water, the colourless acetate (*8*) separated. After recrystallisation from ethanol it melted at 218–230°. Although the sample was homogeneous according to TLC, it had a broad melting interval. This may have been a case of dimorphism with incomplete conversion of the low-melting form into the high-melting form. On one occasion, a sample melting at 242–244° was obtained. (Found: C 65.95; H 4.21. $C_{36}H_{28}O_{12}$ (652.6) requires C 66.25; H 4.32.) *m/e* 652, 610, 568, 526, 510, 498, 466, 456, 439, 424, 395, 379, 320. IR maxima: 1825, 1775, 1370, 1200, 1165, 1105, 1080, 1015, 970, 910, 895, 850, 695 cm^{-1}. UV spectrum: see Table 1. NMR spectrum (in $CDCl_3$): τ 2.51 (5H, br s), 2.90 (4H, A_2B_2-quartet; $\Delta\nu \sim 5$ Hz; $J \sim 9$ Hz), 3.14 (4H, A_2B_2-quartet; $\Delta\nu = 9.5$ Hz; $J = 8.5$ Hz) 7.75 (6H,s), 7.97 (6H,s), 8.11 (3H,s).

Peniophorin leucoacetate (*7*). Zinc powder was added to a mixture of peniophorin, acetic anhydride, and pyridine, whereupon the initially red solution became colourless. After filtration and decomposition of the acetic anhydride with water, the leucoacetate separated as colourless crystals melting at 224–226°. (Found: C 68.61; H 4.44. $C_{34}H_{26}O_{10}$ (594.6) requires C 68.68; H 4.41.) *m/e* 594, 552, 510, 468, 439, 426, 423, 397, 381. IR maxima: 1810, 1780, 1500, 1370, 1200, 1060, 1010, 910, 860, 750, 700 cm^{-1}. UV spectrum: see Table 1. NMR spectrum: ($CDCl_3$): τ 2.53 (5H,br s), 3.08 (4H,br s), 3.16 (4H,br s), 5.17 (1H,s), 7.73 (3H,s), 7.77 (3H,s), 7.95 (3H,s), 8.07 (3H,s).

*3,5,6-Triacetoxy-4,7-di(*p*-acetoxyphenyl)-3-phenylbenzofuran-2(3*H*)-one* (*6*). A solution of atromentin tetra-acetate [5] (500 mg) and phenylacetic acid (140 mg) in acetic anhydride (10 ml) containing a little sodium acetate was refluxed for 30 h. The acetic anhydride was decomposed by adding water, and the solution was extracted with chloroform. The extract was chromatographed on silica gel plates, impregnated with potassium dihydrogen phosphate (a 0.5 M solution being used in the preparation of the plates), using chloroform as solvent. Most of the material migrated in two slightly overlapping zones, one red and one colourless. There were also light to dark brown zones of lower mobility, but these were not investigated. Upon treatment with methanol, the material eluted from the red zone, separated as orange plates, melting at 247–248°. It proved to be identical with the triacetate of 5-hydroxy-4,7-di(*p*-hydroxyphenyl)benzofuran-2,6-dione (*5*) described below. The colourless zone contained two compounds, crystals of which could easily be separated mechanically. One crystallised as compact crystals, melting at 237–240°, and was identical with atromentin leucoacetate (Lit.[5] m.p. 235–240°). The other (*6*) crystallised as colourless feather-like crystals, melting at 226–228°.

(Found: C 65.83; H 4.29. $C_{36}H_{28}O_{12}$ requires C 66.25; H 4.32.) IR maxima: 1815, 1760, 1735, 1365, 1185, 1010, 965, 905, 885, 835, 755, 690 cm^{-1}. UV spectrum (in dioxan): λ_{max} 230 infl. (4.59), 260 infl. (4.32), 290 infl. (3.85) nm (log ε). NMR spectrum (in $CDCl_3$): τ 2.61 (4H, A_2B_2-quartet (partly hidden); $\Delta\nu = 20.0$ Hz; $J = 9.0$ Hz), 2.72 (5H,br s), 3.13 (4H, A_2B_2-quartet; $\Delta\nu = 8.5$ Hz; $J = 8.5$ Hz), 7.68 (3H,s), 7.72 (3H,s), 7.92 (3H,s), 7.96 (3H,s), 8.10 (3H,s).

*5-Hydroxy-4,7-di(*p*-hydroxyphenyl)-3-phenylbenzofuran-2,6-dione* (*5*). 3,5,6-Triacetoxy-4,7-di(*p*-acetoxyphenyl)-3-phenylbenzofuran-2(3*H*)-one (*6*), 40 mg, were dissolved in 2 ml of acetic acid. A few drops of hydrobromic acid (48 %) were added, and the mixture was heated on a steam bath for half an hour. The dark brown solution was poured into water. When the brown precipitate was recrystallised from dilute methanol, brown crystals of *5* separated which decomposed above 300° without a sharp melting point. IR-maxima: 3340, 1770, 1625, 1600, 1508, 1435, 1400, 1310, 1270, 1240, 1178, 1137, 1000, 990, 928, 835, 787, 688, 660 cm^{-1}. UV spectrum (in dioxan): λ_{max} 263 (4.48), 412 (4.28), 525 infl. (2.96); λ_{min} 234 (4.19), 346 (3.75) nm (log ε). Treatment of the brown crystals with acetic anhydride containing pyridine gave the triacetate, m.p. 247—248°, which was identical with the acetate obtained directly from the condensation of atromentin tetraacetate with phenylacetic acid as described above. IR maxima: 1775, 1760, 1633, 1596, 1500, 1462, 1185, 1158, 1120, 1025, 1008, 925, 908, 852, 831, 685 cm^{-1}. UV spectrum (in dioxan): λ_{max} 250 (4.38), 374 (4.21), 460 infl. (3.39); λ_{min} 226 (4.23), 309 (3.83) nm (log ε).

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REFERENCES

1. Gripenberg, J. *Acta Chem. Scand.* **19** (1965) 2242.
2. Abrahamsson, S. and Innes, M. *Acta Cryst.* **21** (1966) 948.
3. Gripenberg, J. and Martikkala, J. *Acta Chem. Scand.* **23** (1969) 2583.
4. Gripenberg, J. *Acta Chem. Scand.* **24** (1970) 3449.
5. Gripenberg, J. *Acta Chem. Scand.* **10** (1956) 1111.

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