

Fungus Pigments

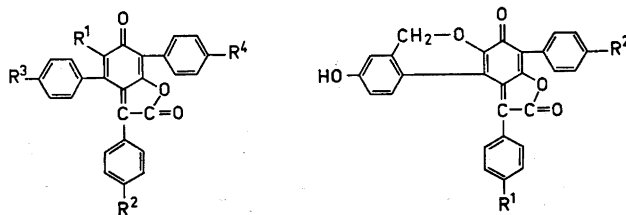
XXII.* Peniosanguin and Its Methyl Ether

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Peniosanguin, one of the pigments produced by the wood-attacking fungus *Peniophora sanguinea* Bres., has been found to be either 8,9-dihydroxy-1-(*p*-hydroxyphenyl)-4-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (7) or 8,9-dihydroxy-4-(*p*-hydroxyphenyl)-1-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (8). Another pigment from the same source is a monomethyl derivative of peniosanguin, either 8,9-dihydroxy-1-(*p*-methoxyphenyl)-4-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (9) or 8,9-dihydroxy-4-(*p*-methoxyphenyl)-1-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (10). The proposed structures are based on the spectral properties of the pigments and their derivatives, as well as on the products of oxidative degradation.

The isolation of four pigments, designated A, B, C, and D, from wood, attacked by the fungus *Peniophora sanguinea* Bres. has been reported in a preliminary communication.¹ Two more pigments, B₁ and B₂, were reported in a later paper.² The structures 1 and 2 have been definitely established for A (5-*O*-methylxylerythrin) and B (xylerythrin), respectively.³ Two structures, 3 and 4, are possible for pigment C (peniophorin),⁴ and there are likewise two possible structures, 5 and 6, for pigment B₂ (peniophorinin).²

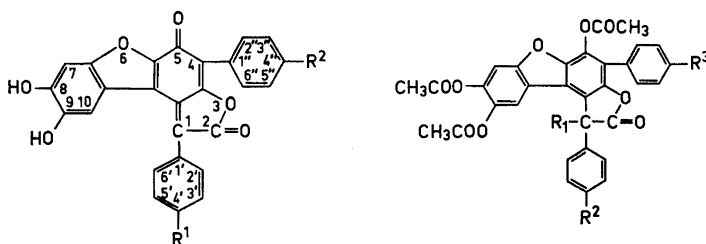


- 1 R¹ = OCH₃; R² = OH; R³ = R⁴ = H
 2 R¹ = R³ = OH; R² = R⁴ = H
 3 R¹ = R² = R³ = OH; R⁴ = H
 4 R¹ = R² = R⁴ = OH; R³ = H

- 5 R¹ = OH; R² = H
 6 R² = OH; R¹ = H

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The present paper deals with the two remaining pigments, D and B₁. Evidence will be presented which shows that pigment D, for which the trivial name peniosanguin is now proposed, is either 8,9-dihydroxy-1-(*p*-hydroxyphenyl)-4-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (7) or 8,9-dihydroxy-4-(*p*-hydroxyphenyl)-1-phenylbenzofuro[5,4-*b*]benzofuran-2,5-dione (8). Pigment B₁ is the corresponding *p*-methoxyphenyl derivative (9 or 10).



- 7 R¹ = OH; R² = H
 8 R² = OH; R¹ = H
 9 R¹ = OCH₃; R² = H
 10 R² = OCH₃; R¹ = H

- 11 R¹ = R² = H; R³ = OCOCH₃
 12 R¹ = R² = H; R³ = OCOCH₃
 13 R¹ = R² = OCOCH₃; R³ = H
 14 R¹ = R² = OCOCH₃; R³ = H

Peniosanguin has the composition C₂₆H₁₄O₇, and gives a trimethyl ether (C₂₉H₂₀O₇) and a triacetate (C₃₂H₂₀O₁₀). There are thus three hydroxyl groups in peniosanguin. The strong absorption at 1780 cm⁻¹ by peniosanguin, and that at 1770 cm⁻¹ by its trimethyl ether, indicate the presence of a lactone group. Prolonged treatment of peniosanguin with acetic anhydride and pyridine gives a colourless pentaacetate (C₃₆H₂₆O₁₃) (13 or 14), formed by addition of one molecule of acetic anhydride to the triacetate. In this respect, peniosanguin is similar to xylyerythrin,³ peniophorin,⁴ and peniophorinin.² Reductive acetylation of peniosanguin gives a dihydrotetraacetate (11 or 12), which has a one-proton singlet at τ 4.74 in its NMR spectrum. Also this reaction is analogous to the similar reactions of the other pigments mentioned.

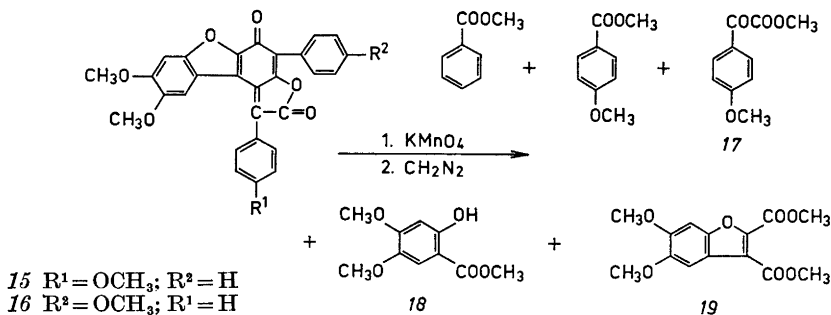
All these facts strongly indicate that peniosanguin has the same quinone methide system as the other pigments from the same source.¹⁻⁴

The composition of peniosanguin shows that it contains one oxygen atom more than are present in the three hydroxyls, the quinone methide moiety, and the lactone group. It also contains two hydrogens less than xylyerythrin and peniophorin. A cyclic ether explains these facts. The formation of such an additional ring can most easily be visualised as having taken place between a hydroxyl group, attached to the quinone methide system and the adjacent aromatic ring, whereupon the ring system of structures 7-10 results.

Support for this proposal is found in the UV spectra of the leucoacetate (11 or 12) and the colourless pentaacetate (13 or 14), which have absorption maxima at 302 nm (log ε 4.52) and 308 nm (log ε 4.47), respectively. These wavelengths agree well with the wavelength 303 nm (log ε 4.42), reported for 3-phenyldibenzofuran.⁵

Definite proof of the presence of a benzofuran ring system in peniosanguin and of the positions of the hydroxyl groups in the compound was obtained

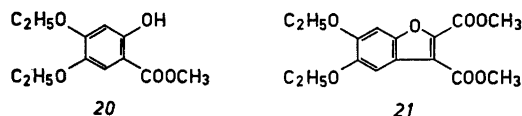
by oxidation of peniosanguin trimethyl ether (*15* or *16*) with potassium permanganate. The acids formed in the oxidation were separated by preparative TLC of their methyl esters. The following esters were identified by comparing their physical properties with those of the authentic compounds: methyl benzoate, methyl anisate, methyl *p*-methoxyphenylglyoxylate (*17*),⁶ methyl 2-hydroxy-4,5-dimethoxybenzoate (*18*),⁷ and dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate (*19*).⁸



Although the formation of *p*-methoxyphenylglyoxylic acid from *15* is easy to understand, the presence of this acid among the oxidation products cannot be taken as definite evidence in favour of *15* over *16*, because even the latter could conceivably give rise to the same acid, particularly in view of the observation of Kögl and Becker,⁶ that atromentin tetramethyl ether (2,5-dimethoxy-3,6-di(*p*-methoxyphenyl)-*p*-benzoquinone) upon oxidation gives *17*. The question of which of the two phenyl groups is the *p*-hydroxy-substituted one must therefore be left open.

The locations of the hydroxyl groups revealed by the oxidation experiment are also supported by the NMR spectra of the derivatives of peniosanguin. Most clearly, this is demonstrated by the spectrum of the trimethyl ether (*15* or *16*), which shows a five-proton singlet at τ 2.94, two two-proton doublets at τ 2.70 and τ 3.19 ($J = 9$ Hz), and two one-proton singlets at τ 3.33 and τ 4.10. The last-mentioned signal is at a very high field for an aromatic proton, and is assigned to the proton in position 10, which in addition to being *ortho* to a methoxyl group is in the shielding zone of the benzene ring attached to carbon atom 1. A similar signal was encountered in the NMR spectrum of peniophorin acetate.² In the spectrum of peniosanguin triacetate, this signal has shifted downfield to τ 3.40.

Pigment B₁ has the composition C₂₇H₁₆O₇ and contains one methoxyl group. Methylation of the pigment with dimethyl sulphate gave a dimethyl ether, which was identical with peniosanguin trimethyl ether. Pigment B₁ is thus a monomethyl ether of peniosanguin. The position of the methoxyl group was determined by oxidation of the diethyl ether of the pigment. After methylation of the acidic oxidation products, methyl anisate was isolated. Two other oxidation products are considered to be *20* and *21*, although they



could not be fully characterised because of the small amounts available. Their UV spectra are almost identical with those of 18 and 19, respectively, and their mass spectra show that they contain two ethoxyl groups. The formation of these oxidation products defines the position of the methylated hydroxyl group as the one in the *para* position. Pigment B₁ is thus either 4'-*O*-methyl- or 4''-*O*-methylpeniosanguin (9 or 10), depending on the structure of peniosanguin.

This conclusion is also supported by a comparison of the NMR spectra of the leucoacetates of peniosanguin and *O*-methylpeniosanguin. In the spectrum of the latter, there is a two-proton doublet at τ 3.14, assignable to the protons *ortho* to the methoxyl. In the spectrum of the former, this signal is at τ 2.91, the protons now being *ortho* to an acetoxy group.

EXPERIMENTAL

UV and visible spectra were recorded 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 purities of all compounds were checked by TLC. Silica gel, impregnated with monopotassium phosphate, was used for the compounds with free hydroxyl groups. Benzene, chloroform, or chloroform-methanol (9:1) was used as solvent, depending on the polarity of the compound in question. The elementary analyses were done by Alfred Bernhardt, Mikroanalytisches Laboratorium Elbach, West Germany, or by Ilse Beetz, Mikroanalytisches Laboratorium, Kronach, West Germany.

Isolation of peniosanguin (8,9-dihydroxy-1(or 4)-(p-hydroxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2,5-dione) (7 or 8). The extraction of the wood and the treatment of the extract has been described before.³ Peniosanguin was isolated from the part of the extract soluble in chloroform by further elution with ethyl acetate of the column after successive elution of xylyerythrin and peniophorin. More of the peniosanguin was recovered from the part of the original extract, soluble in ethyl acetate³ by chromatography on silica gel using ethyl acetate as eluent. The crude peniosanguin was purified by dissolving it in pyridine and carefully adding water to the solution. It crystallized as almost black needles, which decompose at 310° without melting. (Found: C 71.34; H 3.48. C₂₆H₁₄O₇ requires C 71.23; H 3.22.) Mass spectrum: *m/e* 438 (M⁺). IR maxima: 3390, 1780, 1640, 1600, 1290, 1215, 1175, 1125, 1025, 920, 830, 745, 695 cm⁻¹. UV spectrum (dioxan): λ_{\max} 261(4.37), 299(4.15), 420(4.12); λ_{\min} 237(4.15), 283(4.04), 350(3.55) nm (log ϵ).

Peniosanguin triacetate (8,9-diacetoxy-1(or 4)-(p-acetoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2,5-dione) was prepared by acetylation of peniosanguin with acetic anhydride, containing a drop of pyridine at room temperature. Addition of water to the reaction mixture, after it had stood overnight, precipitated the acetate as yellow crystals, which were recrystallized from ethanol. M.p. 270–272°. (Found: C 68.48; H 3.69. C₃₂H₃₀O₁₀ requires C 68.08; H 3.57.) Mass spectrum: *m/e* 564 (M⁺), 522, 480, 438, 409, 392. IR maxima: 1785, 1645, 1200, 1022, 930, 825, 745, 693 cm⁻¹. UV spectrum (dioxan): λ_{\max} 259(4.46), 278 inf. (4.41), 385(4.33); λ_{\min} 237(4.27), 317(3.83) nm (log ϵ).

1,5,8,9-Tetraacetoxy-1(or 4)-(p-acetoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2(IH)-one (13 or 14). When an acetylation mixture, prepared as described in the preceding paragraph, was allowed to stand for one month, the original orange colour faded to a faint yellow. Addition of water caused the precipitation of the colourless

pentaacetate. M.p. 262–263°. (Found: C 64.74; H 4.27. $C_{36}H_{26}O_{13}$ requires C 64.82; H 3.94.) Mass spectrum: m/e 564 ($M^+ - (CH_3CO)_2O$), 522, 480, 438, 409, 309. IR maxima: 1815, 1775, 1620, 1500, 1455, 1395, 1365, 1300, 1200, 1115, 1050, 1005, 965, 742, 730, 700 cm^{-1} . UV spectrum (dioxan): λ_{max} 247(4.56), 308(4.47), 317 inf. (4.43); λ_{min} 238(4.52) 277(3.73) nm (log ϵ).

Peniosanguin leucoacetate (5,8,9-triacetoxy-1(or 4)-(p-acetoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2(IH)-one). (11 or 12). Zinc powder was added to a mixture of peniosanguin, acetic anhydride, and pyridine. The dark colour of the mixture disappeared almost at once. The remaining zinc powder was removed by filtration, and water was added to the filtrate until crystallization began. The leucoacetate was recrystallized from chloroform-ethanol. M.p. 227–229°. (Found: C 67.74; H 3.80. $C_{34}H_{24}O_{11}$ requires C 67.10; H 3.98.) Mass spectrum: m/e 608(M^+), 566, 524, 482, 440, 411, 395. IR maxima: 1810, 1775, 1460, 1370, 1200, 1160, 1050, 1010, 900, 755, 700 cm^{-1} . UV spectrum (dioxan): λ_{max} 240 inf. (4.60), 265 inf. (4.22), 302 (4.52), 315 inf. (4.41); λ_{min} 276(3.89) nm (log ϵ). NMR spectrum ($CDCl_3$): τ 2.54 (5 H, br s), 2.58 (1 H, s), 2.64 (2 H, d; $J = 8.5$ Hz), 2.91 (2 H, d; $J = 8.5$ Hz), 3.27 (1 H, s), 4.74 (1 H, s), 7.72 (6 H, s), 7.76 (6 H, s).

Peniosanguin trimethyl ether (8,9-dimethoxy-1(or 4)-(p-methoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2,5-dione (15 or 16). Potassium carbonate was added in small portions to a stirred solution of peniosanguin (100 mg) in acetone containing dimethyl sulphate (2 g). The dark red solution first became green, but the colour slowly changed to dark brown and a brown-violet precipitate formed. When further addition of potassium carbonate did not effect any change in the colour, the precipitate was filtered off and washed with water to dissolve the potassium salts. Peniosanguin trimethyl ether, which is sparingly soluble in acetone, was thus obtained in a pure state, m.p. 289–293°. A further amount of less pure product was obtained by dilution of the acetone solution with water. (Found: C 72.13; H 4.33; OCH_3 18.77. $C_{29}H_{20}O_7$ requires: C 72.49; H 4.20; 3 OCH_3 19.38.) Mass spectrum: m/e 480(M^+). IR maxima: 1770, 1640, 1605, 1477, 1300, 1280, 1020, 820, 770, 750, 695 cm^{-1} . UV spectrum (dioxan): λ_{max} 263(4.43) 295(4.38), 413(4.28); λ_{min} 242(4.29), 278(4.26), 330(3.71) nm (log ϵ). NMR spectrum (CF_3COOH): τ 2.70(2H, d; $J = 9$ Hz), 2.94(5 H, s), 3.19(2 H, d; $J = 9$ Hz), 3.32(1 H, s), 4.10(1 H, s), 6.42(3 H, s), 6.48(3 H, s), 6.83(3 H, s).

Oxidation of peniosanguin trimethyl ether. Peniosanguin trimethyl ether (110 mg) was dissolved in warm acetone (100 ml). Portions of a solution of potassium permanganate in acetone were added, until the permanganate colour persisted for some time. The mixture was diluted with water and the manganese dioxide removed by filtration. The acetone was evaporated from the filtrate and the aqueous solution extracted with ether. Only traces of ether soluble material were found. The aqueous solution was then acidified and again extracted with ether. The solutes in the ether solution were methylated with diazomethane. Evaporation of the ether left a semisolid mass, which according to TLC was a mixture of several compounds. It was first fractionated by preparative TLC on Kieselgel HF₂₅₄ from Merck, using chloroform as eluent. Inspection of the plate in ultra-violet light revealed the presence of three zones, two dark ones and one with a strong white fluorescence, in order of increasing polarity. The zones were separated and eluted with acetone. The least polar zone gave a liquid, the IR spectrum of which was identical with that of *methyl benzoate*. The second zone was a mixture, according to TLC using benzene as eluent, as it was separated by this solvent into three zones. The least polar zone contained *methyl anisate*, m.p. 47–48°, identified by mixture m.p. and IR and NMR spectra. The second zone contained *methyl p-methoxyphenylglyoxylate (17)*, m.p. 50° (lit.⁶, m.p. 54°). No depression of the melting point was observed when it was mixed with a sample prepared by methylation of authentic *p-methoxyphenylglyoxylic acid* with diazomethane.⁹ The IR spectra with maxima at 1730, 1670, 1600, 1270, 1226, 1170, 1025, 1010, and 845 cm^{-1} , and the NMR spectra (CCl_4) (τ 2.10 (2 H, d; $J = 9$ Hz), 3.12(2 H, d; $J = 9$ Hz), 6.10(3 H, s), 6.13(3 H, s)) were identical for both methyl ester preparations. The third zone contained *methyl 2-hydroxy-4,5-dimethoxybenzoate (18)*, m.p. 94° (lit.⁷ 95°). Mass spectrum: m/e 212(M^+), 180, 165, 152, 137, 124, 109. IR maxima: 1655, 1615, 1510, 1435, 1355, 1260, 1235, 1210, 1165, 1005, 955, 860, 835, 785, 780 cm^{-1} . UV spectrum (ethanol): λ_{max} 225(4.46), 260(4.29), 318(4.02); λ_{min} 215(4.31), 240(3.76), 278(3.17) nm (log ϵ). NMR spectrum (CCl_4): τ -0.40(1 H, s, exch. D_2O), 2.95 (1 H, s), 3.70(1 H, s), 6.12(3 H, s), 6.16(3 H, s), 6.26(3 H, s). The zone with a white fluorescence in the first preparative TLC gave *dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate (19)*, m.p. 156°.

No depression of the melting point was observed when it was mixed with the authentic compound. Its mass, IR, UV, and NMR spectra were also identical with those of the authentic compound. The synthesis of authentic dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate described below was carried out essentially according to Holton *et al.*⁸

Dimethyl chlorooxaloacetate was prepared by condensation of dimethyl oxalate and methyl chloroacetate with sodium.⁸ Holton *et al.*⁸ report a boiling point of 113–115°/0.3 mm for this compound. This value is very high in comparison with the value reported for the corresponding diethyl ester, namely 139–142°/12 mm.⁸ In fact, a boiling point of 95–96°/5 mm was recorded in this work, and it must be assumed that there is a typographical error in the value reported by Holton *et al.*⁸

Methyl 3-chloro-6,7-dimethoxycoumarin-4-carboxylate was prepared by saturating a solution of methyl chlorooxaloacetate (3 g) and 3,4-dimethoxyphenol¹⁰ (2.3 g) in methanol (15 ml) with hydrogen chloride at 0°. After the mixture had stood for 24 h in a refrigerator and for 12 h at room temperature, yellow crystals of methyl 3-chloro-6,7-dimethoxycoumarin-4-carboxylate (2.15 g) were collected and recrystallized from methanol-chloroform; m.p. 198°. (Found: C 52.18; H 3.67. C₁₃H₁₁ClO₆ requires: C 52.26; H 3.72.) IR maxima: 1730, 1610, 1510, 1275, 1220, 1200, 1155, 1040, 975, 842 cm⁻¹. UV spectrum (dioxan): λ_{\max} 235(4.50), 261(4.13), 304(4.00), 358(4.40); λ_{\min} 224(4.35), 253(4.08), 281(3.74), 314(3.98) nm (log ϵ).

5,6-Dimethoxybenzofuran-2,3-dicarboxylic acid. Methyl 3-chloro-6,7-dimethoxycoumarin-4-carboxylate (1 g) was boiled with 10% potassium hydroxide solution (10 ml) for 1 h. Acidification precipitated 5,6-dimethoxybenzofuran-2,3-dicarboxylic acid as pale yellow crystals (0.85 g). After recrystallization from acetic acid, the compound melted at 274–275° (lit.,¹¹ m.p. 264°). IR maxima: 2500(broad), 1700, 1590, 1575, 1450, 1275, 1230, 1140, 1000 cm⁻¹. UV spectrum (dioxan): λ_{\max} 269(3.93), 347(4.39); λ_{\min} 250(3.83), 278(3.88) nm (log ϵ).

Dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate was prepared by treating the acid with diazomethane and recrystallized from dilute methanol. M.p. 156° (lit.,^{9,11} m.p. 156°). Mass spectrum: m/e 294(M⁺), 279, 263, 219. IR maxima: 1735, 1705, 1430, 1335, 1260, 1225, 1205, 1130, 1055, 1005, 905, 855, 815 cm⁻¹. UV spectrum (dioxan): λ_{\max} 270 inf.(4.02), 295 inf.(4.22), 329(4.51); λ_{\min} 245(3.74) nm (log ϵ). NMR spectrum (CDCl₃): τ 2.68(1 H, s), 3.02(1 H, s), 6.04(6 H, s), 6.07(6 H, s).

Isolation of 4' (or 4'')-O-methylpeniosanguin (8,9-dihydroxy-1(or 4)-(p-methoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2,5-dione) (9 or 10). The chromatographic separation of *O*-methylpeniosanguin (pigment B₁) from peniophorin (pigment B₂) has already been described.³ The material recovered from the fractions, which according to TLC contained *O*-methylpeniosanguin, was still contaminated by a colourless impurity, from which it could be freed by recrystallization from pyridine-water. *O*-Methylpeniosanguin melts at 290–295° with decomposition. (Found: C 71.56; H 3.48; OCH₃ 6.70. C₂₇H₁₈O₇ requires C 71.68; H 3.56, 1 OCH₃ 6.86.) Mass spectrum: m/e 452(M⁺), 423. IR maxima: 3500, 3350, 1785, 1635, 1600, 1300, 1260, 1180, 1020, 915, 830, 740 cm⁻¹. UV spectrum (dioxan): λ_{\max} 262(4.47), 297(4.36), 420(4.33); λ_{\min} 241(4.29), 281(4.23), 334(3.72) nm (log ϵ).

4' (or 4'')-O-Methylpeniosanguin diacetate (8,9-diacetoxy-1(or 4)-(p-methoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]benzofuran-2,5-dione). *O*-Methylpeniosanguin was allowed to react with acetic anhydride containing a drop of pyridine overnight. Addition of water precipitated the orange-yellow diacetate, which was recrystallized from ethanol; m.p. 261–263°. (Found: C 69.20; H 3.66. C₃₁H₂₀O₉ requires C 69.40; H 3.76.) Mass spectrum: m/e 536(M⁺), 494, 452, 423. IR maxima: 1790, 1620, 1600, 1215, 1020, 925, 825, 770, 690 cm⁻¹. UV spectrum (dioxan): λ_{\max} 259(4.50), 275 inf.(4.43), 3.62(4.05), 438 (4.22); λ_{\min} 237(4.31), 318(3.88), 3.87(4.00) nm (log ϵ). NMR spectrum (CDCl₃): τ 2.3–3.1(10 H, m), 3.49(1 H, s), 6.09 (3 H, s), 7.70(3 H, s), 7.79(3 H, s).

4' (or 4'')-O-Methylpeniosanguin leucoacetate (5,8,9-triacetoxy-1-(or 4)-(p-methoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]-benzofuran-2(1H)-one). *O*-Methylpeniosanguin was dissolved in acetic anhydride to which a drop of pyridine had been added. Addition of zinc powder in small portions gave a colourless solution, which was then filtered. The leucoacetate was precipitated by adding water to the filtrate. When recrystallized from ethanol, it melted at 236–238°. (Found: C 68.42; H 4.39. C₃₃H₂₄O₁₀ requires C 68.27, H 4.17.) Mass spectrum: m/e 580(M⁺), 538, 496, 454, 425, 411, 395. IR maxima: 1805, 1775, 1605, 1510, 1455, 1385, 1365, 1205, 1190, 1160, 1100, 1045, 985 cm⁻¹. UV spectrum

(dioxan): λ_{\max} 230(4.66), 262 infl.(4.25), 303(4.52); λ_{\min} 276(3.97) nm (log ϵ). NMR spectrum (CDCl₃): τ 2.55(5 H, br s), 2.60(1 H, s), 2.75 (2 H, d; $J=9$ Hz), 3.14(2 H, d; $J=9$ Hz), 3.23(1 H, s), 4.82 (1 H, s), 6.21(3 H, s), 7.72(3 H, s), 7.76(6 H, s).

8,9-Di-O-ethyl-4' (or 4'')-O-methylpeniosanguin (8,9-diethoxy-1(or 4)-(p-methoxyphenyl)-4(or 1)-phenylbenzofuro[5,4-b]-benzofuran-2,5-dione. This compound was prepared from *O*-methylpeniosanguin by the method described above for the methylation of peniosanguin, but using diethyl sulphate instead of dimethyl sulphate. M.p. 242–244°. (Found: C 73.69; H 5.26. C₃₁H₂₄O₇ requires C 73.22; H 4.76.) Mass spectrum: m/e 508(M⁺), 479, 451, 423. IR maxima: 1775, 1640, 1595, 1460, 1280, 1255, 1200, 1170, 1130, 1030, 925, 835, 825, 765, 740, 690 cm⁻¹. UV spectrum (dioxan): λ_{\max} 263(4.46), 295(4.40), 414(4.26); λ_{\min} 242(4.31), 279(4.28), 332(3.73) nm (log ϵ).

Oxidation of 8,9-di-O-ethyl-4' (or 4'')-O-methylpeniosanguin. Diethylmethylpeniosanguin (100 mg) was oxidized in the same way as peniosanguin trimethyl ether. Preparative TLC of the methyl esters using chloroform as eluent gave five main zones. The first (least polar) had the odour of methyl benzoate, but was not investigated further. When the material from the second zone was rechromatographed using benzene as eluent, *methyl anisate*, identified by mixture m.p. determination, and IR and NMR spectra, was isolated. Recrystallization of the material in the third zone from dilute ethanol gave *methyl 4,5-diethoxy-2-hydroxybenzoate (20)*, m.p. 93–94°. Mass spectrum: m/e 240(M⁺), 208, 183, 180, 152, 151, 69. IR maxima: 1665, 1620, 1510, 1445, 1380, 1260, 1235, 1210, 1190, 1035, 810, 775 cm⁻¹. UV spectrum (ethanol): λ_{\max} 226(4.26), 262(4.08), 316(3.86); λ_{\min} 217(4.13), 241(3.64), 279(3.08) nm (log ϵ). The fourth zone contained a small amount of non-crystalline coloured material, which was not studied further. The fifth and hence most polar zone showed a strong white fluorescence in UV light, similarly as dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate. Treatment of the material from this zone with a little methanol led to the formation of crystals of *dimethyl 5,6-diethoxybenzofuran-2,3-dicarboxylate (21)*, m.p. 107–108°. Mass spectrum: m/e 322(M⁺), 294, 291, 266, 265. IR maxima: 1725, 1715, 1570, 1460, 1310, 1270, 1200, 1155, 1145, 1035 cm⁻¹. UV spectrum (dioxan): λ_{\max} 270 infl.(4.03), 295 infl.(4.22), 329(4.50); λ_{\min} 246(3.76) nm (log ϵ).

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