

Fungus Pigments

XIX.* Xylerythrin and its 5-O-Methyl Derivative

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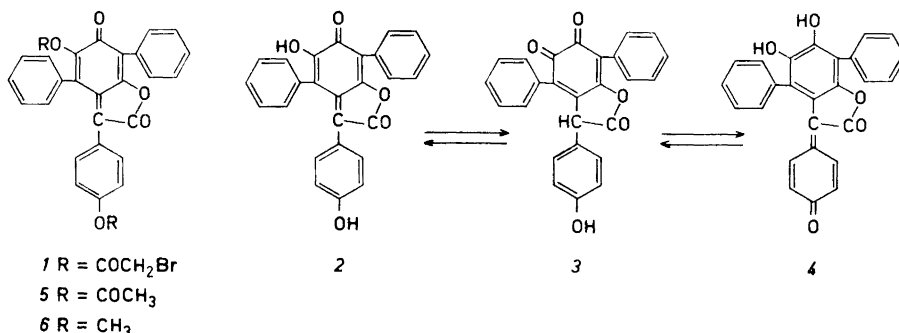
Xylerythrin, one of the pigments isolated from decaying wood, attacked by the fungus *Peniophora sanguinea* Bres., has been shown to be 5-hydroxy-3-*p*-hydroxyphenyl-4,7-diphenyl-benzofuran-2,6-dione (2). It is accompanied by 5-*O*-methylxylerythrin. The compounds have been synthesised by condensing *p*-hydroxyphenylacetic acid with polyporic acid and its monomethyl ether, respectively.

In a preliminary communication¹ the isolation of four pigments, provisionally termed A, B, C, and D, from wood attacked by the fungus *Peniophora sanguinea* Bres. (*Corticium sanguineum* Fr.) was described. The present paper gives a full account of the isolation of pigments A and B (xylerythrin), the determination of their structures and their syntheses.

Xylerythrin has the composition $C_{26}H_{16}O_5$ and gives a dimethyl ether ($C_{28}H_{20}O_5$) and a diacetate ($C_{30}H_{20}O_7$). In the acetylation of xylerythrin the formation of the orange coloured diacetate is always accompanied by the formation of a colourless product. Upon extension of the reaction time the colourless product becomes the main product. The composition of this, $C_{34}H_{26}O_{10}$, indicates addition of one molecule of acetic anhydride to the diacetate. Acid hydrolysis converts the substance back into xylerythrin. Reductive acetylation of xylerythrin gives a dihydro triacetate.

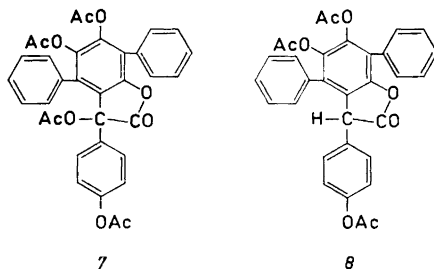
An X-ray analysis of xylerythrin bisbromoacetate carried out by Abrahamsen and Innes^{2,3} led to the structure 1 for this derivative. The structure 2 for xylerythrin is thus indicated, but the two tautomeric structures 3 and 4 are also possible. These can, however, be dismissed by a comparison of the spectral properties of xylerythrin and its derivatives. The electronic spectrum of xylerythrin dimethyl ether (λ_{\max} 245(4.38), 358(4.01), 443(4.21); λ_{\min} 317(3.80), 387(3.84) nm (log ϵ)) is very similar to that of xylerythrin (λ_{\max} 255(4.37), 360(3.94), 450(4.18); λ_{\min} 318(3.71), 388(3.76) nm (log ϵ)), showing that they have the same chromophore. This fact excludes 3 which can only

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give a monomethyl ether. In the IR-spectrum of xylyerythrin there is a band at 1625 cm⁻¹, which is attributed to the carbonyl group of the quinone methide system. In the methyl ether this is shifted to 1635 cm⁻¹ and in the acetate to 1645 cm⁻¹, indicating chelation of the carbonyl group in xylyerythrin. Clearly, in 4 there is no possibility for chelation, and 2 is thus the correct structure for xylyerythrin, namely 5-hydroxy-3-*p*-hydroxyphenyl-4,7-diphenyl-benzofuran-2,6-dione. The diacetate and the dimethyl ether are accordingly formulated as 5 and 6, respectively.

For the colourless acetate and the leucoacetate the structures 7 and 8 can be derived. 7 is thus formed by addition of acetic anhydride to the quinone



methide group, which is the result of the well known tendency of this system to undergo 1,6-addition.⁴ The structure 8 for the leucoacetate is supported, both by analysis and the similarity of its IR- and UV-spectra to those of 7, and by the appearance in the NMR-spectrum of an one proton singlet at τ 5.12. Wanzlick and Jahnke⁵ report τ 5.20 for a proton in an almost identical environment

The only reaction of xylyerythrin, which is not readily explained on the basis of structure 2 is its behaviour in dilute alkaline solution. Xylyerythrin dissolves very readily in 2 N sodium hydroxide with a red colour. The colour, however, soon turns into a dull brown. When the solution is acidified, benzoic acid and a yellow acidic compound C₁₉H₁₄O₆ are obtained. This readily loses one molecule of water giving a red compound C₁₉H₁₂O₅. The formation of

benzoic acid and the compound $C_{19}H_{14}O_6$ in a single reaction requires in addition to hydrolysis an oxidation. Whether this is caused by the air, or is the result of a disproportionation is not known at present. Neither are we prepared to suggest any structural formulae for the compounds $C_{19}H_{14}O_6$ and $C_{19}H_{12}O_5$. Experiments to settle these points are in progress.

It has already been pointed out¹ that xylerythrin can formally be derived from a condensation of polyporic acid (2,5-dihydroxy-3,6-diphenyl-1,4-benzoquinone) and *p*-hydroxyphenylacetic acid.

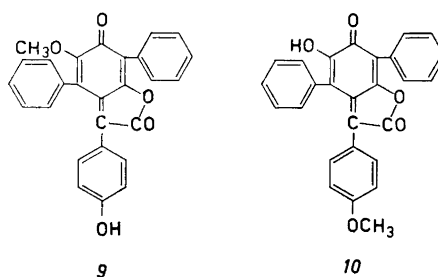
In this connection it should be mentioned that polyporic acid has been isolated from *Peniophora filamentosa* (B. & C.) Burt.⁶

Taking into account that it has recently been established in our laboratory^{7,8} that quinones can be condensed with acetic anhydride in a Perkin-type reaction, a synthesis of xylerythrin from polyporic acid and *p*-hydroxyphenylacetic acid seemed feasible.

A number of solvents and catalysts for this condensation were tried, but only in acetic anhydride with sodium acetate as catalyst could any reaction be achieved. For the successful use of this system it had to be assumed that the methylene group of *p*-hydroxyphenylacetic acid will react in preference to the methyl group of acetic acid. Chromatographic separation of the reaction mixture gave a small amount of xylerythrin diacetate (5). As it was to be expected that xylerythrin under the reaction conditions would be converted mainly into the colourless acetate (7), the crude reaction mixture was submitted to acid hydrolysis, which gave xylerythrin in a fair yield.

Recently a synthesis of xylerythrin by a different route has been reported.⁵

Pigment A has the composition $C_{27}H_{18}O_5$ with one methoxyl group. It gives a monomethyl ether and a monoacetate and the spectral properties of the pigment itself and of its derivatives are very close to those of xylerythrin and its derivatives. It was therefore not at all surprising to find that the methyl ether was identical with xylerythrin dimethyl ether. Pigment A is thus either 5-*O*-methylxylerythrin (9) or 4'-*O*-methylxylerythrin (10).



A decision between these two alternatives was sought by a synthesis. First polyporic acid was condensed with *p*-methoxyphenylacetic acid, which reaction should have given a derivative of 10. When an analysis by TLC of the crude reaction product failed to show any sign of the presence of the acetate of pigment A this approach was not pursued any further. Instead

polyporic acid monomethyl ether was condensed with *p*-hydroxyphenylacetic acid. Chromatography of the reaction product gave the acetate of pigment A, identified by TLC and IR-spectra. Pigment A is thus 5-*O*-methylxylerythrin (9).

EXPERIMENTAL

The UV-spectra were recorded on a Beckman DK-2 instrument for dioxan solutions, unless stated otherwise, the IR-spectra for KBr-discs on Perkin Elmer 125 and Beckman IR-5 instruments and the NMR-spectra on a Varian A 60 instrument. The analyses have been performed partly by Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach, Germany, partly by Miss R. Puurula of this Department on an F & M Scientific 185 CHN-analysator.

Isolation of xylerythrin and its methyl ether. Wood, attacked by the fungus *Peniophora sanguinea* Fr., is easily detected by the, sometimes, very strong red colour which the wood has attained. Such wood was collected in different parts of Southern Finland and extracted with cold acetone. Most of the acetone was distilled off and ether was added to the residue. A dark brown amorphous precipitate that was formed was discarded. The ether solution was washed several times with water, whereby more of the brown precipitate was formed. The ether was then removed from the remaining dark red solution and the residue treated with several portions of light petroleum.

Chloroform was added to the resinous mass, insoluble in light petroleum, giving a dark red solution and a chloroform insoluble residue, which was dissolved in ethyl acetate. The chloroform solution was then poured on a column of silica gel (Merck Kieselgel 0.05–0.20 mm) and the elution carried out by more chloroform. The first dirty brown fraction which was discarded was followed by a yellow fraction, which has not yet been investigated. This was again followed by a large dark red eluate, which according to TLC-analysis contained mainly two pigments. Pigment A was enriched in the first fractions and appeared in TLC-analysis as an orange red spot. Pigment B which appeared in TLC-analysis as a dark green spot of lower R_F -value than A was the major pigment of the last fractions of the chloroform eluate.

Xylerythrin (Pigment B) (2). The fractions which according to TLC contained mainly pigment B were pooled, the solvent removed and the residue recrystallised from chloroform/benzene giving almost black crystals with a green lustre, m.p. 265–268°. (Found: C 75.74; H 4.03. $C_{26}H_{16}O_5$ requires C 76.46; H 3.95). IR-maxima: 3500, 3330, 1750, 1625, 1600, 835, 751, 688 cm^{-1} .

Xylerythrin dimethyl ether (6). Xylerythrin (50 mg) was dissolved in acetone containing dimethyl sulphate (3 ml). Potassium carbonate was then added in small portions and the mixture stirred at room temperature. The solution first turned dark brown-green and then slowly red. When addition of more potassium carbonate did not change the colour, the salt was filtered off and water was carefully added to the acetone solution until crystallisation set in. The xylerythrin dimethyl ether (40 mg) was further purified by recrystallisation from acetone-water. M.p. 213–215°. (Found: C 76.37; H 4.56; OCH_3 13.90. $C_{26}H_{14}O_3(OCH_3)_2$ requires C 77.05; H 4.62; OCH_3 14.22). IR-maxima: 1775, 1635, 1600, 835, 745, 695 cm^{-1} . NMR-spectrum ($CDCl_3$): τ 2.45 (5H,m), 2.85 (5H,s), 3.03 (2H,d; $J = 9$ Hz), 3.47 (2H,d; $J = 9$ Hz) 6.15 (3H,s), 6.23 (3H,s).

Xylerythrin diacetate (5). Xylerythrin in acetic anhydride with a drop of pyridine was refluxed for 2 h. The reaction mixture was treated with water and the product recrystallised from chloroform/light petroleum. M.p. 228–230° (Found: C 72.73; H 3.94. $C_{30}H_{20}O_7$ requires C 73.16; H 4.09). UV-spectrum (EtOH): λ_{max} 242 (4.31), 366 (4.14), 400 inf. (4.09); λ_{min} 221(4.24), 312(3.84) nm (log ϵ). IR-maxima: 1785, 1775, 1645, 1595, 1195, 697 cm^{-1} . When the mother liquor from the crystallisation was allowed to evaporate, colourless crystals of (7), m.p. 192–193°, were obtained. This substance was the main product when the acetylation mixture was refluxed for 24 h. (Found: C 68.82; H 4.38. $C_{34}H_{28}O_{10}$ requires C 68.68; H 4.41). UV-spectrum (EtOH): λ_{max} 225(4.54), 292(3.64); λ_{min} 283(3.60) nm (log ϵ). IR-maxima: 1820, 1780, 1205, 753, 698 cm^{-1} .

Xylerythrin leucoacetate (8). Xylerythrin was treated with zinc-powder and acetic anhydride in the presence of a drop of pyridine. The colour disappeared in a few minutes. The solution was filtered from the zinc and then treated with water and the precipitate

crystallised from ethanol. M.p. 200–202°. (Found: C 70.94; H 4.72; CH₃CO 25.05. C₂₆H₁₅O₂(OCOCH₃)₃ requires C 71.63; H 4.51; CH₃CO 22.95). UV-spectrum (EtOH): λ_{\max} 220 inf. (4.64), 250 inf. (4.30), 290 inf. (3.75) nm (log ϵ). IR-maxima: 1810, 1780, 1200, 755, 698 cm⁻¹. NMR-spectrum (CDCl₃): τ 2.50 (5H,s, (broad)), 2.65–3.10 (4H,m), 3.18 (5H,s) 5.12 (1H,s) 7.74 (3H,s) 7.93 (3H,s) 8.06 (3H,s).

Xylerythrin bisbromoacetate. Xylerythrin (50 mg) was dissolved in acetone to which bromoacetyl bromide (0.5 ml) and potassium carbonate were added and the mixture stirred at room temperature until the solution had acquired a bright red colour. To the filtered solution water was added until the solution became slightly clouded. Upon standing red crystals were obtained which after recrystallisation from ethanol had m.p. 183–185° and were used for X-ray analysis. (Found: C 55.84; H 2.93; Br 24.31. C₃₀H₁₈O₇Br₂ requires C 56.71; H 2.79; Br 24.58). When this procedure for the preparation of the bisbromoacetate was tried on a later occasion no reaction could be achieved. The only difference was that the potassium carbonate was from a new batch. Evidently the originally used potassium carbonate, whose origin is not known, contained some impurity, probably alkali, which caused the reaction.

Treatment of xylerythrin with alkali. Xylerythrin (50 mg) was dissolved in 2 N sodium hydroxide. The originally wine-red solution slowly turned brown-yellow. After standing at room temperature for 5 days the solution was acidified and extracted with ether. The ether extract was then extracted with sodium hydrogen carbonate and again acidified. The precipitate was treated with chloroform leaving yellow crystals (22 mg) undissolved. These have no m.p. but decompose between 240 and 260°. (Found: C 67.50; H 4.49. C₁₉H₁₄O₆ requires C 67.45; H 4.17). UV-spectrum: λ_{\max} 249(4.09), 265 inf. (4.03) 346(4.06); λ_{\min} 226(4.04), 298(3.83) nm (log ϵ). IR-maxima: 3450, 2500, 1650, 740, 700 cm⁻¹. When a sample of this compound was sublimed at 220°/0.1 mm it was transformed into a dark red compound which was recrystallised from acetone/benzene. It decomposes at 260–280° without melting. (Found: C 70.97; H 3.75. C₁₉H₁₂O₅ requires C 71.25; H 3.78). UV-spectrum (EtOH): λ_{\max} 408(4.33); λ_{\min} 302(3.67) nm (log ϵ). IR-maxima: 3300, 1790, 1720, 1610 cm⁻¹. Evaporation of the chloroform solution gave benzoic acid, identified by mixed m.p. and IR-spectrum.

5-O-Methylxylerythrin (Pigment A) (9). The fractions from the chloroform eluate obtained in the chromatographic separation as described above, which according to TLC contained mainly pigment A, were pooled, evaporated and recrystallised from a small amount of chloroform giving almost black crystals, m.p. 250–256°. (Found: C 76.67; H 4.52; OCH₃ 6.79. C₂₆H₁₅O₄OCH₃ requires C 76.77; H 4.30; OCH₃ 7.31). UV-spectrum: λ_{\max} 246(4.37), 357(4.03), 448(4.24); λ_{\min} 319(3.83), 388(3.83) nm (log ϵ). IR-maxima: 1780, 1640, 1590, 835, 748, 695 cm⁻¹.

Methylation of 5-O-methylxylerythrin. 5-O-Methylxylerythrin was methylated by the procedure described above for the methylation of xylerythrin. The product had m.p. 213–215°, undepressed on admixture of xylerythrin dimethyl ether. Its IR-spectrum was superimposable upon that of xylerythrin dimethyl ether.

5-O-Methylxylerythrin acetate. The acetylation was carried out as described above for the acetylation of xylerythrin. M.p. 208–210°. (Found: C 73.77; H 4.04. C₂₈H₂₀O₅ requires C 74.99; H 4.34). UV-spectrum (EtOH): λ_{\max} 246(4.33), 384(4.18); λ_{\min} 316(3.71) nm (log ϵ). IR-maxima: 1780, 1640, 1215, 840, 750, 693 cm⁻¹.

Synthesis of xylerythrin. 2,5-Dihydroxy-3,6-diphenyl-1,4-benzoquinone (290 mg) and *p*-hydroxyphenylacetic acid (150 mg) were refluxed for 3 h in acetic anhydride containing a little sodium acetate. The acetic anhydride was decomposed by water and a mixture of hydrogen bromide (48 %) and acetic acid (1:1) added. After refluxing for half an hour the precipitate (180 mg) was filtered. It was identified as xylerythrin by TLC, UV- and IR-spectra.

Synthesis of 5-O-methylxylerythrin. 2-Hydroxy-5-methoxy-3,6-diphenyl-1,4-benzoquinone⁹ and *p*-hydroxyphenylacetic acid were refluxed for 2 h in acetic anhydride containing a little sodium acetate. Water was then added to decompose the acetic anhydride and the precipitate was purified by preparative TLC. Elution of the orange-yellow band gave the acetate of 5-O-methylxylerythrin, identified by its IR-spectrum which was superimposable upon that of authentic 5-O-methylxylerythrin acetate.

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