Colouring Matters of the West African Red Woods *Pterocarpus osun* and *P. soyauxii*. Structures of Santarubins A and B

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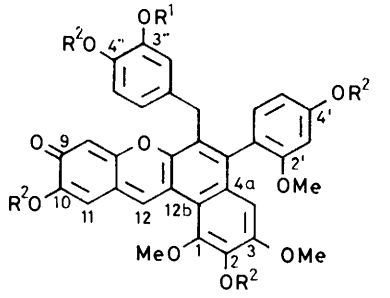
The red heartwood of the West African trees *Pterocarpus osun* and *P. soyauxii* contains the pigments santarubins A and B and the known santalin A. Santarubins A and B have been identified as 6-(2,4-dimethoxybenzyl)-2,10-dihydroxy-5-(4-hydroxy-3-methoxyphenyl)-1,3-dimethoxybenzo[*a*]xanthen-9-one (2a) and the corresponding 5-(3,4-dihydroxyphenyl) derivative (2b), respectively, on the basis of degradative and spectroscopic evidence, particularly the n.m.r. spectrum of the *O*-trideuteriomethyl derivatives in the presence of a lanthanoid shift reagent.

THE so-called ' insoluble red woods ' form a group of dyewoods consisting of some Asian [1] *Pterocarpus* species (padauk, sandalwood, and narrawood) and the West African red woods camwood and barwood. The origin of camwood and barwood has long been a source of confusion. One of us [2] has recently presented results which have helped to clarify the origin of the African timbers, and their description.

The colouring matters of these woods have been the subject of many investigations. A summary of the older results was given by Robertson and Whalley,[3] who also were the first to isolate in the pure state the main pigments, santalin and santarubin, from unspecified commercial red sandalwood and camwood, respectively. Reinvestigation of heartwood of the botanically identified Asian species *Pterocarpus santalinus* by Seshadri and his co-workers [4] and by ourselves [5] led to the isolation of santalins A, B, and C; no santarubin was found in this plant. As the only two West African *Pterocarpus* species which produce a red timber are *P. osun* and *P. soyauxii*,[2] we thought it interesting to investigate these species also. The present paper reports the isolation and identification of santarubins A and B, which occur together with santalin A in these woods. Both plants have the same content of pigments and other polyphenols.

Recent structural work [4,6,7] has led to different proposals for the structure of per-*O*-methylsantalin.§ Extensive spectroscopic and degradative work has however enabled us to establish unambiguously the structures (1a and b) for santalins A and B, respectively.[5]

The close similarity of santarubin and the santalins, especially in rings A and B, was established by Robertson and Whalley,[3] who prepared a per-*O*-methylsantarubin isomeric with the per-*O*-methylsantalins. Extraction of *P. osun* and *P. soyauxii* heartwoods afforded two main pigments, santarubin A (2a) and santarubin B (2b). Per-*O*-methylation of either gave the same product (2c), which also appeared identical with a sample of the per-*O*-methyl derivative prepared by Whalley. The two santarubins thus appeared to be different methyl ethers of the same parent phenol, differing only by the numbers of OMe and OH groups. Trideuteriomethylation afforded the two ethers (2d and e), and from their mass and n.m.r. spectra it could be inferred that santarubin A contains five OH and three OMe, and santarubin



(1)a; $R^1 = R^2 = H$
b; $R^1 = Me$, $R^2 = H$
c; $R^1 = R^2 = Me$

B four OH and four OMe groups. Whalley and his coworkers,[7] on the basis of previous degradative work and new spectroscopic data, recently proposed a structure for per-*O*-santarubin. The suggested arrangement of carbon atoms was consistent with a common biosynthetic origin of the two classes of natural pigment.[5,7] However, comparison of the ^1H n.m.r. spectrum of per-*O*-methylsantarubin in the presence of the shift reagent $Eu(dpm)_3$ with that of per-*O*-methylsantalin (1c) indicated that the structure should be revised with regard to the substitution of ring D, so that the definitive formulation for per-*O*-methylsantarubin is (2c).[5]

In order to confirm this structure and to establish that of santarubin A, the latter was ethylated with ethyl iodide to give the triethyl ether (2f). Degradation of this compound with permanganate gave a mixture of acids, from which 3-methoxy-4-ethoxybenzoic acid (3),[5] 2,4-dimethoxybenzoic acid (4), and the benzo-

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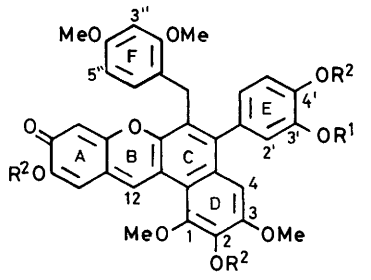
§ It is unfortunate that in his recently published review [4a] the late Professor T. R. Seshadri has not taken into account our and Whalley's structural proposals.

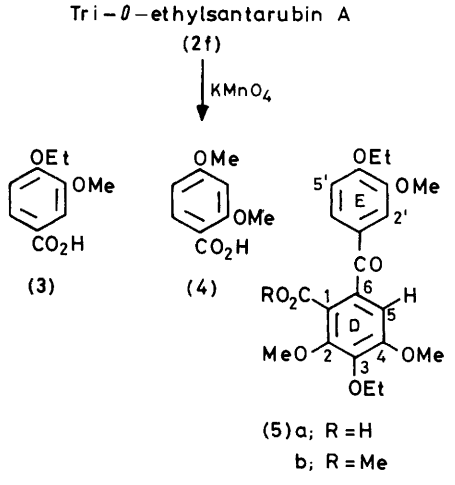
[1] T. R. Seshadri, *Phytochemistry*, 1972, **11**, 881.
[2] D. A. H. Taylor, *J. West. African Sci. Assoc.*, 1972, **15**, 21.
[3] A. Robertson and W. B. Whalley, *J. Chem. Soc.*, 1954, 2794.
[4] (*a*) T. R. Seshadri, in ' Chemistry of Naturally Occurring Carbon Compounds,' vol. 7, Akadémiai Kiadò, Budapest, 1976; (*b*) B. Ravindranath and T. R. Seshadri, *Tetrahedron Letters*, 1972, 1801; *Phytochemistry*, 1973, **12**, 2781; K. N. Gurudutt and T. R. Seshadri, *ibid.*, 1974, **13**, 2845.
[5] A. Arnone, L. Camarda, L. Merlini, and G. Nasini, *J.C.S. Perkin I*, 1975, 186.
[6] A. Arnone, L. Merlini, and G. Nasini, *Tetrahedron Letters*, 1972, 3503.
[7] D. W. Mathieson, B. J. Millard, J. W. Powell, and W. B. Whalley, *J.C.S. Perkin I*, 1973, 184.

phenone (5a) were obtained. The structures of (3) and (4) were established by direct comparison with authentic material. Compound (5a) was converted into the



(2) a; R^1 = Me, R^2 = H
b; R^1 = R^2 = H
c; R^1 = R^2 = Me
d; R^1 = Me, R^2 = CD_3
e; R^1 = R^2 = CD_3
f; R^1 = Me, R^2 = Et

methyl ester, which was identified from its mass spectrum, from comparison of the n.m.r. spectrum with that of the corresponding derivative of santalin B (where



the OMe group on ring E is in position 2'), and above all by decoupling of all the aromatic proton signals from those of the OMe and OEt groups. These results establish with certainty the connection between rings D and E of (2f). Together with previous data [7] concerning rings A and B, they confirm the structure (2f) for the triethyl ether and therefore the skeletons of (2a and b) and the structure of santarubin A (2a).

The previous ^{1}H n.m.r. study of the permethyl ether (2c) with addition of the shift reagent trisdipivaloylmethanatoeuropium(III) [Eu(dpm)$_3$] allowed us to assign the signals of the methoxy-groups of this compound.[5] Now comparison of the n.m.r. spectra of the trideuteriomethyl ethers (2d and e) of santarubins A and B under the same conditions with that of (2c) has established the substitution pattern and therefore the structure of (2b); this also represents an independent proof for the structure of (2a). The Eu(dpm)$_3$-induced shifts for the eight methoxy-groups of (2c) are given in the Table.

Eu(dpm)$_3$-induced shifts

OMe	Δδ [a] for (2c)	Δδ [a] for (2d)	Δδ [a] for (2e)
1-	3.3	3.4	3.5
2-	10.3		
3-	2.7	3.0	3.0
10-	10.4		
3'-	7.5 [b]	8.5	
4'-	6.2 [b]		
2''-	0.6	0.8	0.8
4''-	0.1	0.1	0.1

[a] Shifts induced by adding Eu(dpm)$_3$ (4 mol. equiv.) to (2c) (20 mg) in 1 : 1 CCl_4–$CDCl_3$ (0.5 ml) or in similar ratio to (2d and e). [b] Respectively assigned by comparison with (2d).

The signals had already been assigned on the basis of the chemical shifts of the compound itself, of the close agreement with the data of Wright and Tang Wei [8] for model methoxybenzenes, and (for H-2'' and -4'') of consideration of the effect of the lanthanoid on other protons. As there are significant differences amongst the induced shifts, inspection of the data for (2d and e) makes the assignment of the positions of the OMe and OCD_3 groups straightforward. The structure of (2b) is thus easily assigned. The distinction between the OMe in position 3' and 4' of (2c) was previously not unambiguous: [5] now the presence of the 3'-OMe group in (2d) has been established by the observed decoupling of H-2' (and not H-5') on irradiation of the OMe region, and confirmed by the chemical degradation.

The structures given here are also consistent with the results of a ^{13}C n.m.r. analysis.[9]

EXPERIMENTAL

U.v. spectra were measured for solutions in 95% ethanol with a Beckman DK-2 apparatus, and n.m.r. spectra with a Varian XL-100 instrument. Where not otherwise stated, column chromatography was performed with Merck silica gel (0.05—0.20 mm) and t.l.c. with Merck HF_{254} silica gel.

Pterocarpus osun heartwood was collected in Western Nigeria. Specimens are in the Forest Herbarium, Ibadan, with reference number F.H.I.45825. Samples of *P. soyauxii*, collected in North-East Zaire, were obtained from the Musée Royal de l'Afrique Centrale, Tervuren (Belgium).

Isolation of the Pigments.—The shavings of the heartwood of *P. osun* were extracted with hexane, then with chloroform, ethyl acetate, and finally methanol. The ethyl acetate and the chloroform extracts were concentrated and the combined residues were chromatographed on a Woelm polyamide column to give (i) pterocarpin, pterostilbene, and santal [10] (with hexane–chloroform as eluant), (ii) santarubin A (with chloroform), (iii) santarubin B (with chloroform–methanol, 100 : 1), and (iv) santalin A (with chloroform–methanol, 50 : 1). The methanol extract similarly afforded santalin A (eluted with chloroform–methanol).

Similar extraction of *P. soyauxii* samples gave extracts containing the same main components (t.l.c.).

Santarubin A (2a) crystallised from dichloromethane as

[7] G. E. Wright and T. Y. Tang Wei, *Tetrahedron*, 1973, **29**, 3775.

[8] A. Arnone, L. Camarda, L. Merlini, and G. Nasini, following paper.

[9] A. Akisanya, C. W. L. Bevan, and J. Hirst, *J. Chem. Soc.*, 1959, 2679.

orange needles, m.p. 283—285°, $\lambda_{max.}$ 242, 270, 279, 308, 320, 447sh, 475, and 508 nm (ε 35 700, 26 400, 26 100, 9 400, 10 400, 12 500, 20 800, and 20 000), $\lambda_{max.}$ (KBr) 6.1 μm (conj. CO), *m/e* 610 (Found: C, 68.9; H, 4.95. $C_{35}H_{30}O_{10}$ requires C, 68.85; H, 4.95%), δ ($CDCl_3$) 4.06, 3.78, 3.78, 3.74, and 3.66 (5 OMe), 4.01 (CH_2), 9.66 (H-12), and 6.1—7.1 (9 aromatic protons and 3 OH).

Santarubin B (2b) crystallised from acetone–ether as orange needles, m.p. >300° (decomp.), $\lambda_{max.}$ 270.5, 279, 308, 319, 445sh, 473, and 505 (ε 40 200, 41 000, 16 400, 16 000, 14 100, 22 700, and 22 300), $\lambda_{max.}$ (Nujol) 6.1 μm (conj. CO).

Methylation of Santarubins A (2a) *and B* (2b).—To santarubin A (2a) (80 mg) in dry acetone was added methyl iodide (1.5 ml), and the solution was refluxed for 6 h over anhydrous potassium carbonate (100 mg). Filtration, evaporation, and preparative t.l.c. of the residue (ethyl acetate) yielded santarubin permethyl ether (2c). Santarubin B gave the same permethyl ether (2c) δ ($CDCl_3$–CCl_4, 1 : 1) 3.64 (3′-OMe), 3.70 (4″-OMe), 3.70 (3-OMe), 3.75 (2″-OMe), 3.94 (10-OMe), 3.94 (4′-OMe), 4.02 (CH_2), 4.02 (2-OMe), 4.09 (1-OMe), 6.18 (H-5″, dd, *J* 8.5 and 2.0 Hz), 6.30 (H-8, d, $J_{8,12}$ 1.3 Hz), 6.38 (H-3″, d, *J* 2.0 Hz), 6.52 (H-6″, d, *J* 8.0 Hz), 6.62br (H-11, s, $J_{11,12}$ 0.5 Hz), 6.62 (H-2′, d, *J* 2.0 Hz), 6.76 (H-4, s), 6.80 (H-6′, dd, *J* 8.0 and 2.0 Hz), 6.96 (H-5′, d, *J* 8.0 Hz), and 9.46br (H-12, s).

Trideuteriomethylation of Santarubin A (2a).—Santarubin A was similarly methylated with trideuteriomethyl iodide to give 2,4′,10-tris-*O*-trideuteriomethylsantarubin A (2d) as orange needles (50%), m.p. 160°, *m/e* 661; the ^1H n.m.r. spectrum ($CDCl_3$–CCl_4, 1 : 1) shows the same pattern of aromatic proton signals as (2c) and δ 3.70 (4″-OMe), 3.70 (3-OMe), 3.75 (2″-OMe), and 4.09 (1-OMe).

Trideuteriomethylation of Santarubin B (2b).—Santarubin (2b) similarly gave 2,3′,4′,10-tetrakis-*O*-trideuteriomethylsantarubin B (2e) as orange needles, m.p. 160°, *m/e* 664; the ^1H n.m.r. spectrum ($CDCl_3$–CCl_4, 1 : 1) shows the same pattern of aromatic proton signals as (2c) and δ 3.64 (3′-OMe), 3.70 (4″-OMe), 3.70 (3-OMe), 3.75 (2″-OMe), and 4.09 (1-OMe).

Ethylation of Santarubin A (2a).—Santarubin (2a) (300 mg) was dissolved in dry acetone (25 ml) and refluxed for 6 h with ethyl iodide (3 ml) over anhydrous potassium carbonate (400 mg). Filtration, evaporation, preparative t.l.c. (ethyl acetate), and crystallisation of the residue from methanol yielded 2,4′,10-tri-*O*-ethylsantarubin A (2f) as orange needles (40%), m.p. 250—251°, $\lambda_{max.}$ 269, 278, 300, 307sh, 322, 418sh, 448, 475, and 508 nm (ε 52 000, 55 500, 14 600, 14 400, 16 800, 16 300, 24 500, 39 400, and 33 800), *m/e* 694.

Oxidation of 2,4′,10-Tri-O-ethylsantarubin A (2f).—2,4′,10-Tri-*O*-ethylsantarubin A (2f) (200 mg) in acetone (50 ml) was treated with saturated aqueous potassium permanganate and stirred for 3 days at room temperature and then for 1 h on a water-bath. Filtration, concentration, acidification, and extraction with ethyl acetate gave a mixture which was separated by preparative t.l.c. [benzene–ether–formic acid (50 : 50 : 1) then chloroform–methanol (9 : 1)] into 3-methoxy-4-ethoxybenzoic acid (3),[7] 2,4-dimethoxybenzoic acid (4) (both identified by direct comparison), and 3-ethoxy-6-(4-ethoxy-3-methoxybenzoyl)-2,4-dimethoxybenzoic acid (5a). The acid (5a) with diazomethane in ether gave the methyl ester (5b), m.p. 124°, *m/e* 418.158 ± 0.004 (Calc. for $C_{22}H_{26}O_8$: *M*, 418.1628), $\lambda_{max.}$ 283 and 314 nm (ε 12 300 and 12 100), $\lambda_{max.}$ (KBr) 5.74 and 6.12 μm, δ ($CDCl_3$) 1.22 and 1.30 (O·CH_2·CH_3), 3.57, 3.87, 3.94, and 3.99 (OMe), 4.18 (O·CH_2·CH_3), 6.85 (s, H-5 decoupled from OMe), 6.87 (d, H-5′, J_{ortho} 8.0 Hz; decoupled from O·CH_2·CH_3), 7.29 (dd, H-6′, J_{ortho} 8.0, J_{meta} 2.0 Hz), and 7.51 (d, H-2′, J_{meta} 2.0, decoupled from OMe).

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