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STRUCTURAL STUDIES ON SANTALIN PERMETHYL ETHER*

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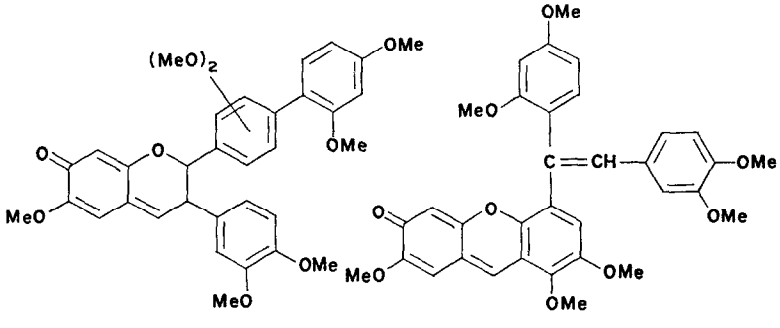
Key Word Index—*Pterocarpus santalinus*; Leguminosae; red sandalwood; santalin pigments; santalin-*A* and santalin-*B*; santalin permethyl ether; fluorone; naphthaldehyde derivative; 1,2-naphthaquinone derivative; biogenesis; biflavonoid nature; santarubin.

Abstract—The chloroform extract of the heartwood of *Pterocarpus santalinus* yielded a mixture of red pigments which could be separated by polyamide column chromatography into two major compounds, santalin-*A* and santalin-*B*. Both gave the same permethyl ether, $C_{38}H_{36}O_{10}$ which had 8 methoxyls and formed a number of derivatives typical of anhydrobenzopyranols. IR and UV spectra confirmed the same. NMR and MS suggested the presence of homoveratrayl group supported by the formation of veratraldehyde in alkali degradation. Permanganate oxidation gave 2,4-dimethoxy benzoic acid, veratric acid and 3,4,6-trimethoxy phthalic acid. On a basic fluorone skeleton, the substituents in the *A* ring are indicated by 2,4-dihydroxy-5-methoxy benzaldehyde, an alkali fission product and, further, 2,4-dimethoxy phenyl and homoveratryl units are located in ring *C* based on NMR, MS and biogenetic considerations. The residues constitute another benzene ring fused to ring *C* leading to the complete structures of the permethyl ether as (VII) which explains all its degradations and which constitutes a highly condensed biflavonoid of a new type.

INTRODUCTION

SANTALIN, the colouring matter of the heartwood of *Pterocarpus santalinus* (red sandalwood) was first isolated in impure form in 1832 by Pelletier.[1] Subsequently, a number of investigators have examined the pigment and over 30 papers have been published. A significant contribution to the structural problem, however, came only as late as 1954 from Robertson and Whalley.[2] On the basis of a series of degradative reactions, they proposed the tentative structure (I) for what they called 'santalin tetra-*O*-methyl ether'. In 1963, Dean[3] observed that the structure was unsatisfactory in view of some special reactions of the pigment and suggested the structure (II) for the compound. This was based mainly on biogenetic grounds and had no experimental support.



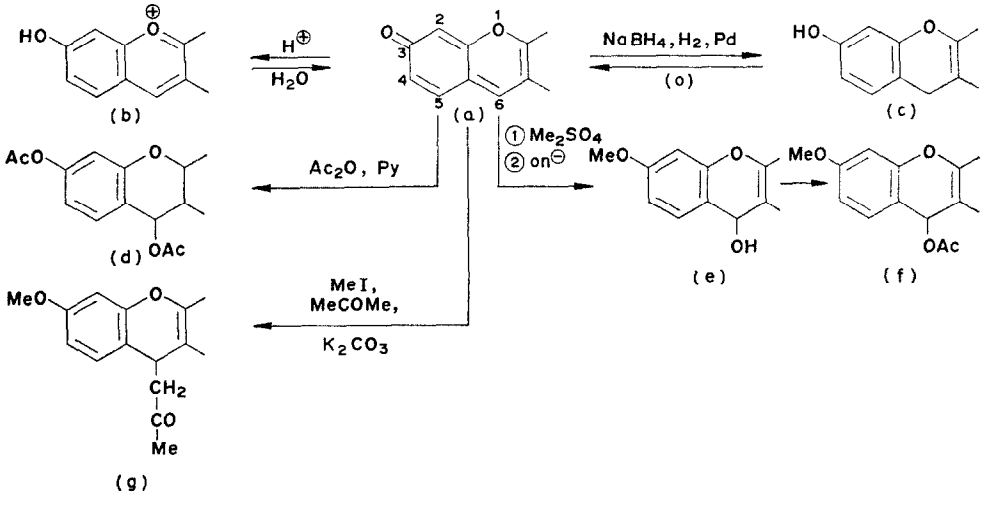
(I) (II)

* Part II in the series "Chemistry of Santalin Pigments". For Part I see Ref. 4.

[1] PELLETIER, J. (1832) *Ann. Chim. Phys.* **51**, 193.
[2] ROBERTSON, A. and WHALLEY, W. B. (1954) *J. Chem. Soc.* 2794.
[3] DEAN, F. M. (1963) *Naturally Occurring Oxygen Ring Compounds*, Butterworths, Oxford.

We have now examined the heartwood in detail and found that it contains a number of red pigments. Two of these, santalin-*A*, $C_{30}H_{17}O_7(OMe)_3$, and santalin-*B*, $C_{30}H_{16}O_6(OMe)_4$, are major components. Since both the compounds give the same complete (per) methyl ether on methylation, they are partial methyl ethers of the same polyphenol for which the name santalin may now be reserved. We have already proposed a new structure for santalin permethyl ether.[4] A detailed account of our structural studies is given here. Meanwhile, Merlini *et al.*[5] and Whalley *et al.*[6] have made reports of their work which confirms our findings.

Santalin permethyl ether, for which we now propose a new molecular formula, $C_{38}H_{36}O_{10}$ (M^+ 652), forms orange-yellow needles, melts first at 155–156°, resolidifies and melts again at 229–230°. Its anhydrobenzopyranol character (Scheme 1(a)) has now been firmly established by the preparation of a number of typical derivatives; the reactions are summarized in Scheme 1.

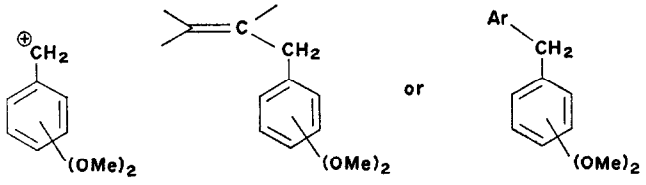


SCHEME 1. ANHYDROBENZOPYRANOL REACTIONS.

The compound corresponding to (g) (Scheme 1) was formed by an interesting participation of the solvent acetone during methylation; it was obtained as a colourless solid indicating aromatization of the quinonoid system. Its formulation as in (g) was based on its IR spectrum showing the aliphatic carbonyl band at 1710 cm^{-1} and its MS showing peaks at *m/e* 724 (M^+), 709 (M-Me), 681 (M-COMe) and 667 (M-CH_2COMe), the last mentioned peak being the base peak. The structure was supported by the observation that the synthetic model anhydrobase, anhydro-5,8-dimethoxy-7-hydroxybenzopyranol (M^+ 282) formed 5,7,8-trimethoxy-4-acetonylbenzopyranol which showed peaks at *m/e* 354(M^+), 339, 311 and 297 (base peak). The molecular formula of the compound corresponding to (g) (Scheme 1) was established as $C_{42}H_{44}O_{11}$ by high resolution MS (M^+ 724·2888), thus confirming the molecular formula now proposed for santalin permethyl ether.

[4] RAVINDRANATH, B. and SESHADRI, T. R. (1972) *Tetrahedron Letters* 1201.
[5] ARNONE, A., MERLINI, L. and NASINI, G. (1972) *Tetrahedron Letters* 3503.
[6] MATHIESON, D. W., MILLARD, B. J, POWELL, J. W. and WHALLEY, W. B. (1973) *J. Chem. Soc Perkin I* 184.

The UV and IR spectral data of the permethyl ether are given in the Experimental. Its NMR spectrum ($CDCl_3$) has been more useful; it showed eight separate signals in the region δ 3·80–4·20 indicating the presence of eight methoxyls in the molecule, instead of the seven methoxyls recorded by Robertson and Whalley.[2] In addition, the spectrum showed a multiplet for eight aromatic protons at δ 6·60–6·80, a one-proton doublet at δ 7·20 (*J* 9 Hz) and a one-proton singlet at δ 9·55 (C–6H); these signals account for the presence of ten aromatic protons including those on the anhydrobase unit. Thus 34 of the 36 protons of santalin permethyl ether have been accounted for. The nature of the remaining two protons was revealed by a careful examination of the MS of santalin permethyl ether and its derivatives. All the spectra invariably showed an intense peak at *m/e* 151. Since the NMR spectrum had already indicated that the compound is essentially aromatic with a number of methoxyls, this peak should be due to a dimethoxy benzyl (III) ion. The signals due to the methylene protons in the NMR seemed to have merged with one of the methoxyl signals. They could, however, be located at δ 4·05–4·10 in the spectra of santalin-*A* and *B* recorded in trifluoro-acetic acid. The chemical shift of the benzylic methylene indicated that it is attached either to a double bond or to an aryl moiety (IV).



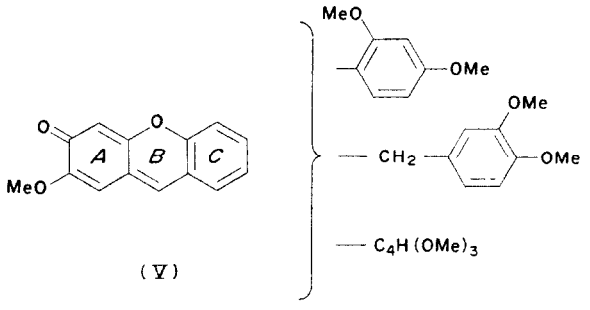
(III) (IV)

Alkali Degradation of Santalin Permethyl Ether

Alkali degradation of santalin permethyl ether gave a number of products, of which compounds-*A*, -*B*, -*C* and -*D* have been isolated in pure form and characterized. Compounds-*A* and -*B* have been identified as 2,4-dihydroxy-5-methoxy-benzaldehyde and 4-methoxy resorcinol respectively. Compounds-*C* and -*D* will be referred to later; but it should be mentioned here that compound-*C*, $C_{31}H_{32}O_9$ (M^+ 548), has been found to be an *O*-hydroxy aromatic aldehyde based on its sparing solubility in aqueous alkali, positive ferric chloride colour and NMR spectrum. Such formation of two *o*-hydroxy aromatic aldehydes by treatment with alkali requires the presence of a fluorone unit (see V) in the molecule. This has been unequivocally established when santalin permethyl ether was obtained by the condensation of compound-*C* with 4-methoxyresorcinol, using acetic acid and HCl.

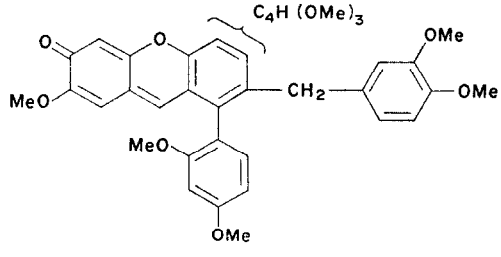
Permanganate Oxidation

Oxidation of the permethyl ether with $KMnO_4$ under neutral conditions yielded three acids *E*, *F* and *G*. The first two have been identified as 2,4-dimethoxybenzoic acid and veratric acid respectively. Acid *G* will be discussed later. The structure for the permethyl ether can now be written as (V), bearing in mind that veratraldehyde was isolated as one of the products of oxidation by Robertson and Whalley.[2]



(V)

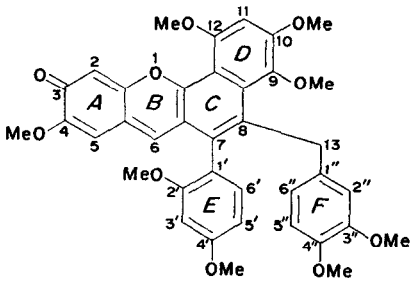
Substitution in rings *A* and *B* are defined by the isolation of 2,4-dihydroxy-5-methoxybenzaldehyde. Therefore, 2,4-dimethoxyphenyl, homoveratryl and C_4-$(OMe)_3$ residues have to be attached to ring *C*. Of the various ways in which this can be done, structure (VI) is favoured, in view of the observation that the NMR spectrum of the octa-*O*-methyl santalanol diacetate (compound corresponding to (d), Scheme 1) showed the alcoholic (C-6) acetoxyl signal at an unusually high field (δ1·2). This can only be explained as a result of the powerful shielding effect of an adjacent 2,4-dimethoxyphenyl ring. The homoveratryl unit is placed in the *ortho* position to the 2,4-dimethoxyphenyl unit on the basis of MS fragmentation and biogenetic considerations discussed below.



(VI)

Structure of Santalin Permethyl Ether

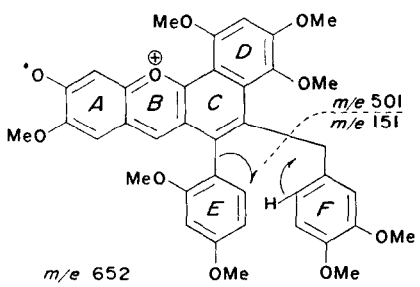
The molecular formula of santalin permethyl ether requires the presence of a total number of 21 double bond equivalents of which 18 are satisfied by the part structure (VI). The remaining three double bond equivalents, four carbon atoms and three methoxyls require the presence of another aromatic ring fused to ring *C* as in (VII). Permanganate oxidation of the permethyl ether should, therefore, give a trimethoxyphthalic acid derivable from ring *D*. The acid *G* mentioned earlier was identified with this on the basis of its chromatographic mobilities and colour reactions. Of the two possible trimethoxy phthalic acids, viz. 3,4,5- and 3,4,6-trimethoxyphthalic acids, the possibility of acid G being the former was ruled out by direct comparison with a synthetic sample. The choice between 9, 10, 12 and 9, 11, 12 substitution could be made on biogenetic grounds (see below). Accordingly, the methoxyls were located as in (VII).



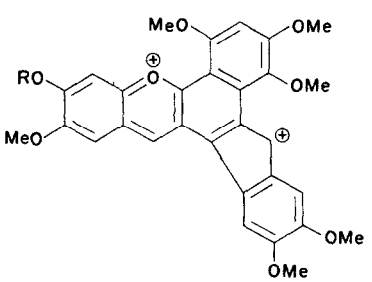
(VII)

MS of the Permethyl Ether

All the chemical and spectral data on santalin permethyl ether are in complete agreement with structure (VII). Thus, the MS showed four intense peaks at *m/e* 652 (M^+ 100%), 501, 257 and 151. The peaks at *m/e* 501 and 151 obviously arise from a cleavage at the benzylic methylene leading to complementary units. The peak at *m/e* 257 requires explanation. It would appear that ring *E* undergoes elimination along with an H atom from ring *F* and the resulting dibenzocyclopentadiene acquires resonance stability with rearrangement involving the migration of a proton to the C=O group leading to the doubly charged aromatic ring structure (IX) which has a mass of 514 and gives a peak at 257. This is confirmed by the isotope mass peak at 257·5 which represents a stable doubly charged ion with the charges localized in the pyrylium and cyclopentadienyl systems. The above explanation is also supported by the fact that the mass spectra of compounds corresponding to (e) and (g) (Scheme 1) which bear a methyl on the C_3–O show the corresponding peak at *m/e* 264 (a difference of only 14 m.u.) (X). Further, the observation of peaks due to doubly charged ions (IX and X) would indicate that 2,4-dimethoxy phenyl and homoveratryl units are in *ortho* positions with respect to ring *C* of (VIII).



(VIII)

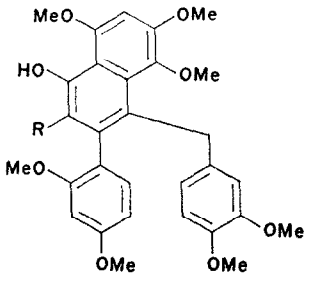


(IX) R = H
(X) R = Me

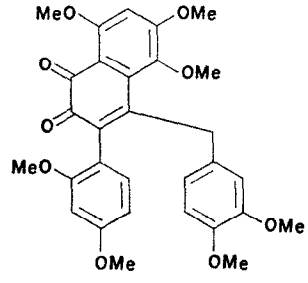
Structure of C

Structure (VII) for santalin permethyl ether indicates that the structure of compound-*C*, obtained by alkali degradation is (XI). Its spectral data are in complete agreement with this structure. Thus, the UV spectrum (see Experimental) indicated that it is a naphthalene derivative while the MS (M^+ 548) confirmed its formula as $C_{31}H_{32}O_9$. The NMR spectrum showed the presence of seven methoxyls (δ 3·50–4·00), a diarylmethane group (separated

from the methoxyls when the spectrum was recorded in C_6H_6), seven aromatic protons δ 6·50, 6H, broad; δ 6·90 1H, *d*, *J* 9 Hz), an aldehyde proton (δ 11·17, 1H, *s*) and a strongly chelated phenolic hydroxyl proton (δ 14·47, 1H, *s*).



(XI) R = CHO
(XIII) R = H
(XIV) R = OH



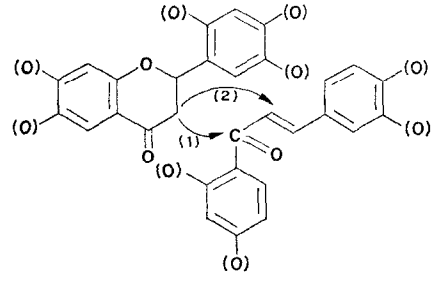
(XII)

Structure of D

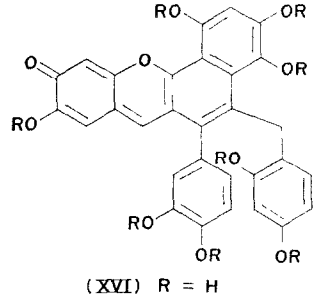
Compound-*D*, the fourth product of alkali degradation of santalin permethyl ether, was obtained as a red semi-solid, $C_{30}H_{30}O_9$ (M^+ 534, accompanied by a fairly intense M + 2 peak). Its red colour, absorption spectrum (λ_{max} 245 and 382 nm) and fragmentation pattern, particularly the appearance of peaks at *m/e* 506, 491 and 463 indicated that it is quinonoid in nature. Its NMR spectrum showed the presence of the diarylmethane group (δ 3·42, 2H, broad), seven methoxyls (δ 3·60–4·00, 21H), and seven aromatic protons (δ 6·00–7·00 6H, *m*; 7·50, 1H, *s*). These data agree with the *o*-quinone structure (XII), which is obviously formed by oxidation of (XI) during the alkali degradation via the intermediate (XIV). The structure of compound-*D* has been confirmed by its preparation from compound-*C* by Dakin's reaction.

Biogenesis

The proposed structure (VII) for santalin permethyl ether represents a new skeleton with many unique features. Thus, it is on one side a quinone methide and on the other a naphthalene, fused together to form a benzofluorone; the molecule also has an extra phenyl and benzyl groups and eight methoxyls located in the various rings. Accordingly, it would be of interest to discuss the probable biogenetic origin of the compound. It appears to be derivable from two C_6–C_3–C_6 units (XV) and can thus be a biflavonoid. The occurrence of chalcone, isoliquiritigenin, and flavanone, liquiritigenin, in the heartwood of *P. santalinus* may be significant in this connection.[7]



(XV)



(XVI) R = H
(XVII) R = Me

[7] SAWHNEY, P. L. and SESHADRI, T. R. (1956) *J. Sci. & Ind. Res.* **15C**, 154.

The reactive methylene of the flavanone part may be attached to the chalcone in two ways, i.e., (1) and (2) in structure XV. Path (1), followed by a series of steps which can easily be envisaged, would lead to santalin, while path (2) would give rise to a structure of the type (XVI). It may be mentioned here that Robertson and Whalley[2] found that the heartwood of *Baphia nitida* contained santalin, and another pigment which they called santarubin. The latter formed a methyl ether which was isomeric with that of santalin and showed very similar properties. However, santarubin methyl ether on oxidation gave 2,4-dimethoxy benzaldehyde while santalin methyl ether gave veratraldehyde. It is possible that structure (XVII) might represent santarubin methyl ether.

EXPERIMENTAL

Isolation of the pigments. The heartwood shavings (2 kg) were extracted with boiling C_6H_6 (which removed most of the less polar compounds) followed by boiling $CHCl_3$ containing 1–2% MeOH (4 × 5 l.). The $CHCl_3$ extract was concentrated and the residue crystallized from EtOAc yielding a mixture of pigments (10 g). Polyamide TLC (solvent system: $CHCl_3$–MeOH–MeCOEt–$MeCOCH_2COMe$, 100:10:5:1) revealed several red-coloured spots of which santalin-*A* and santalin-*B* are major. They were separated by polyamide column chromatography ($CHCl_3$:MeOH); santalin-*B* (being less polar) was first eluted with 3% MeOH in $CHCl_3$ while santalin-*A* was eluted with 4% MeOH in $CHCl_3$. Santalin-*A* formed red needles from aq. MeOH, m.p. 302–303°. λ_{max}^{MeOH} 241, 280, 319, 471 and 504 nm; ν_{max}^{KBr} 3636, 1639, 1613, 1538, 846 and 784 cm^{-1}. (Found: C, 68·1; H, 4·3; OMe 15·3. $C_{30}H_{17}O_7(OMe)_3$ requires: C, 68·2; H, 4·5; OMe, 16·0%.) Santalin-*B* was obtained as red needles from aq. MeOH, m.p. 292–294°. λ_{max}^{MeOH} 238, 280, 320, 472 and 504 nm; ν_{max}^{KBr} 3636, 1639, 1613, 1540, 846 and 784 cm^{-1}. (Found: C, 68·3; H, 4·8; OMe, 19·6. $C_{30}H_{16}O_6(OMe)_4$ requires: C, 68·5; H, 4·7; OMe, 20·8%)

Methylation of santalin-A. Santalin-*A* (100 mg) dissolved in dry acetone (10 ml) was refluxed for 6 hr with MeI (1 ml) over anhyd. K_2CO_3 (1 g). The acetone solution was filtered, evaporated and the residue crystallized from aq. MeOH, yielding the methyl ether as orange–yellow needles, m.p. 155–156°, resolidified and remelted at 229–230°.

Methylation of santalin-B Santalin-*B* (100 mg) was methylated as above and the methyl ether crystallized from aq. MeOH yielding orange–yellow needles, m.p. 155–156°, resolidified and melted again at 229–230°. M.m.p. with the methyl ether of santalin-*A* was undepressed. Their UV and IR spectra were superimposable. λ_{max}^{EtOH} 235, 269, 278, 320, 447, 475 and 508 nm. ν_{max}^{KBr} 1639, 1613, 1567, 847 and 826 cm^{-1}. (Found: C, 69·6; H, 5·7; OMe, 37·4. $C_{30}H_{12}O_2(OMe)_8$ requires: C, 69·9; H, 5·5; OMe, 38·0%). NMR($CDCl_3$, 60 MHz): δ 3·80 to 4·20 (26H, 8 signals), 6·60–6·80 (8H, *m*), 7·20 (1H, *d*, *J* 9 Hz) and 9·55 (1H, *s*).

Octa-O-*methyl santalylium hydrobromide.* The permethyl ether (50 mg) was dissolved in EtOH (2 ml), and 2 ml of 48% aq. HBr added. The clear deep-red solution slowly deposited dark-red prisms, m.p. 182–183° (decomp).

Octa-O-*methyl santalylium perchlorate.* It separated from a solution of permethyl ether (50 mg) in aq. HOAc–$HClO_4$ (5:1, 3 ml) in ruby-red plates, m.p. 213–215° (decomp).

6-*Hydroxy-dihydro-octa*-O-*methyl santalylium diacetate.* Santalin permethyl ether (100 mg) in dry pyridine (0·5 ml) was treated with Ac_2O (2 ml) and kept at 45–50° for 24 hr. The mixture was then poured onto ice and stirred, the solid product filtered and purified by precipitation from MeOH with H_2O. The diacetate was obtained as a white amorphous powder, m.p. 108–109°. (Found: C, 66·6; H, 5·7. $C_{42}H_{42}O_{13}$ requires: C, 66·8; H, 5·6%.)

Nona-O-*methyl*-6-*acetonyl dihydrosantalin.* Santalin-*A* or santalin-*B* (100 mg) was refluxed in dry acetone (10 ml) with CH_3I (1 ml) over anhyd. K_2CO_3 till the reaction mixture became colourless (36 hr). The acetone solution was filtered, evaporated and the residue crystallized from aq. MeOH, yielding colourless, prism shaped crystals of nona-*O*-methyl-6-acetonyl dihydrosantalin (50 mg), m.p. 98–99°; ν_{max}^{KBr} 1710 cm^{-1} (C=O) (Found: M^+ 724·2888. $C_{42}H_{44}O_{11}$ requires: MW = 724·2880.)

Octa-O-*methyl dihydrosantalin.* Santalin permethyl ether (20 mg) in EtOAc (10 ml) was hydrogenated over Pd–C (10 mg; 10%), yielding a colourless dihydroderivative which was soon oxidized to santalin permethyl ether; for this reason the compound could not be completely characterized. Reduction of santalin permethyl ether (10 ml) in MeOH using $NaBH_4$ gave the same product (TLC).

Degradation of santalin permethyl ether with alkali. Santalin permethyl ether (200 mg) in MeOH (5 ml) was refluxed with 3 g KOH in 2 ml H_2O for 3 hr. The mixture was diluted with H_2O and extracted with ether (neutral fraction). The aq. soln was then acidified and extracted with Et_2O (acidic fraction). This fraction on column chromatography ($CHCl_3$–MeOH) yielded two components, viz. *A* and *B*. Compound-*A* formed pale yellow plates from MeOH, m.p. 150–151°; it was identical with an authentic sample of 2,4-dihydroxy-5-methoxybenzaldehyde (TLC, m.m.p., and IR). Compound *B*, colourless needles from EtOH,

m.p. 72°, was identified as 4-methoxy resorcinol by comparison with an authentic sample. The neutral fraction was chromatographed on silica gel (C_6H_6:$CHCl_3$) yielding two compounds, C and D Compound C (XI) was obtained as pale yellow needles from hexane, m p. 69–70°, (M^+ 548); 2,4-dinitrophenyl hydrazone derivative formed orange-red plates, m.p. 232–233°. λ_{max}^{MeOH} 228 and 344 nm; ν_{max}^{Nujol} 1724, 1603, 1579, 840, 755 and 678 cm^{-1}. NMR (CCl_4, 60 MHz) δ 3·50–4 00 (23H, 7 signals), 6·30–6·70 (6H, envelope), 6·86 (1H, *d*, *J* 9 Hz), 11·17 (1H, *s*) and 14·47 (1H, *s*). Compound-*D* (XII) was a red-coloured semisolid, which could not be crystallized (M^+ 534) λ_{max}^{EtOH} 245 and 382 nm; ν_{max}^{Nujol} 1680, 1595, 1570, 840, 755 and 678 cm^{-1}. NMR (CCl_4, 60 MHz) δ 3·42 (2H, *b s*), 3·60–4·00 (21H, 5 signals); 6·10–6·70 (6H, *m*), and 7·50 (1H, *s*)

*Condensation of compound-*C *with 4-methoxy resorcinol* (Santalin permethyl ether). Compound-*C* (20 mg) and 4-methoxy resorcinol (10 mg) were dissolved in glacial AcOH (5 ml) and conc. HCl (1 ml) and heated at 100° for 1 hr. After keeping for 24 hr at room temp. it was diluted with satd aq. NaOAc. The product in ether was purified by column chromatography. It crystallized from MeOH as orange yellow needles, melted first at 155–156°, lost solvent, re-solidified and remelted at 229–230°. M. m p with santalin permethyl ether was undepressed and their IR spectra were superimposable.

*Conversion of compound-*C *into compound-*D. Compound-*C* (10 mg) in MeOH (2 ml) was treated with a drop of 10% aq. NaOH, followed by a few drops of 30% H_2O_2. The soln which immediately turned red, was extracted with Et_2O. The ether soln was washed with H_2O, dried and evaporated. The product was obtained as a red semi-solid. It had identical chromatographic and spectral properties as compound-*D*.

Permanganate oxidation of santalin permethyl ether. Santalin permethyl ether (200 mg) in acetone (10 ml) was treated with aq. $KMnO_4$ (1 g in 8 ml H_2O) and allowed to stand for 24 hr at room temp It was then decolourized with $NaHSO_3$ and HCl and extracted with Et_2O. The ether soln was extracted with satd aq. $NaHCO_3$ (3 × 10 ml). The bicarbonate soln was acidified, re-extracted with ether and the ether solution chromatographed on silica gel Elution with 2% MeOH in $CHCl_3$ yielded colourless needles of compound-*E* (10 mg), m.p 110–111°, identical with an authentic sample of 2,4-dimethoxybenzoic acid (TLC, m m p and IR) Further elution with the same solvent furnished compound-*F*, m p. 181–182°, identical with an authentic sample of veratric acid (TLC, m m p. and IR). Elution of the column with 5% MeOH in $CHCl_3$ afforded compound-*G* as a pale-brown solid m.p. 180–185°, and giving red colour on warming with conc. H_2SO_4 It had the same R_f (TLC) as 3,4,5-trimethoxyphthalic acid which, however, had m p 150–152°, and failed to give any colour with H_2SO_4.

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