HEXACOSYLFERULATE, A PHENOLIC CONSTITUENT OF PINUS ROXBURGHII

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Abstract—The occurrence of hexacosylferulate in *Pinus roxburghii* is reported. Its structure has been derived from spectral measurements, chemical reactions and finally from an unambiguous synthesis.

Earlier investigations on the genus *Pinus* revealed the presence of phenolic compounds and various terpenoids [1]. Extensive studies on the C_6H_6 extract of *Pinus* roxburghii Sargent (syn: *P. longifolia* Roxb.) which is used as a liniment in rheumatic pains, has resulted in the isolation of another phenolic constituent, hexacosylferulate (1).

Compound (1), $C_{36}H_{62}O_4$ could not be isolated in the pure state by any chromatographic procedure but could be purified by acetylation and subsequent chromatography of the crude acetate. It yielded a monoacetate (2) which exhibited a max at 273 and 326 nm, and IR absorptions at 1760 cm⁻¹ (acetoxy carbonyl) and at 1710 cm^{-1} (α,β -unsaturated ester carbonyl). The 270 MHz PMR spectrum of (2) showed signals for three aromatic protons at 6.90 (1H, d, J 8.0 Hz, H_a) and ca 7.00 (2H, m, H_b and H_c) and shows a pair of one-proton doublets appearing at 6.50 and 7.30 (J 18.0 Hz, He and Hd) typical of a trans olefine. The aromatic OMe group present resonated at 3.90 (3H, s) whereas the chemical shift for the acetoxy function appeared at 2.30 (3H, s). The monoacetate (2) on alkaline hydrolysis at room temp. [2] yielded ferulic acid and hexacosanol which were identified by comparison with authentic samples.

Structure (1) was further confirmed by its unambiguous synthesis from ferulic acid and hexacosanol in presence of dicyclohexylcarbodriimide (DCC) (yield: 75%) [3]. GC-MS analysis of the crude acetate also revealed the presence of *O*-acetyl-tetracosyl ferulate (M⁺ 572).

After completion of our work, the literature survey revealed that Soviet workers [4] isolated a mixture of ferulic acid esters from the family, Pinaceae. After hydrolysis and GLC analysis of the neutral fraction, they reported the presence of hexacosanol in trace amounts.



EXPERIMENTAL

Mp's were determined on a Kofler Block and are uncorrected. UV spectra were recorded in 95 % aldehyde-free EtOH and IR spectra in KBr. PMR spectra were studied with a 270 MHz instrument with TMS as internal standard. All chromatographic separations were carried out with Si gel.

Isolation of hexacosylferulate (1). Finely powdered stem-bark of P. roxburghii (5 kg) was percolated with C_6H_6 for 3 weeks at room temp. The C_6H_6 concentrate was chromatographed over Si gel and the column eluted with solvents of increasing polarity using petrol (bp 60-80°), petrol- C_6H_6 mixtures of various proportions, C_6H_6 , C_6H_6 -CHCl₃ mixtures of various proportions and CHCl₃. The C_6H_6 eluate furnished (1), $C_{36}H_{62}O_4$ (M⁺ 558.4681) (yield: 0.01 %), $R_f = 0.27$ (C_6H_6): v_{max}^{KBr} . 3310, 1710, 1630 and 980 (trans olefinic bond), 1590 cm⁻¹.

Acetylation. Hexacosylferulate (1) (0.2 g) dissolved in C_5H_5N (5 m]) was warmed with Ac_2O (15 m]) for 6 hr. The acetylated reaction mixture showed two M⁺ peaks at m/e 600 and 572 (GC-MS, 20 eV) corresponding with (2) and acetyltetracosylferulate. The monoacetate (2), the major component, was isolated by chromatography on Si gel using petrol- C_6H_6 (1:1) elution. It crystallised from a mixture of Et₂O-petrol (1:1) elution it crystallised from a mixture of Et₂O-petrol (1:1) as white flakes, $C_{38}H_{64}O_5$ (M⁺ 600), mp 93°, $R_f = 0.30$ (C_6H_6) (Found: C, 77.15; H, 10.75; O, 12.10% C_{38}H_{64}O_5 requires C, 77.12; H, 10.66; O, 12.12%); λ_{max}^{BBT} 1760, 1710, 1630, 985 cm⁻¹; PMR δ (CDCl₃) 0.90 (3H, t, J 7.0 Hz, terminal CH₃), 1.30 (48 H, br s, -(CH₂)₂₄--), 2.30 (3H, s, -CO₂-CH₃), 3.90 (3H, s, aromatic --OMe), 4.19 (2H, t, J 7.0 Hz, trans-olefinic protons, H_e and H_d respectively), 6.90 (1H, d J 8.0 Hz, aromatic H_b and H_d); m/e (%), 600 (M⁺, 1%), 558 (92%), 194 (98%), 177 (100%).

Hydrolysis of (2) [2]. (2) (0.1 g) dissolved in MeOH (50 ml) was warmed with 5% methanolic KOH (5 ml) and the reaction mixture was allowed to stand at room temp. for 2 hr. The neutral portion was taken up in Et₂O (3 × 50 ml), washed with H₂O and dried. Removal of the solvent afforded hexacosanol which crystallized from petrol as colourless flakes, mp 80° (yield: 0.03 g) (Found: C, 81.60; H, 14.20; O, 4.20, $C_{26}H_{34}O$ requires C, 81.67; H, 14.14; O, 4.19%). The aq portion obtained after removal of the neutral fraction was acidified with dil. H₂SO₄, coned *in vacuo* and extracted with CHCl₃ (3 × 50 ml). CHCl₃ extract was washed and dried, and on removal of solvent ferulic acid was obtained as colourless needles (aq. EtOH), mp 170° (yield: 0.02 g) (Found: C, 61.90; H, 5.20; O, 32.90; C₁₀H₁₀O₄ requires C, 61.85; H, 5.15; O, 33.00%).

Synthesis of (1) [3]. To a well-stirred soln of ferulic acid

(0.2 g) synthesized by Beyer's method [5] and hexacosanol (0.25 g) in dry CH_2Cl_2 (30 ml), a soln of dicyclohexylcarbodiimide (0.2 g) in dry CH_2Cl_2 (30 ml) was added dropwise for a period of 30 min and the reaction mixture was allowed to stand 18 hr. The solvent was removed and EtOAc (25 ml) was added to separate dicyclohexyl urea. The mother liquor, obtained after the separation of the urea derivative, on concentration yielded hexacosylferulate (1) which crystallized from cold petrol in colourless flakes, mp 70°, $R_f 0.27$ (C_6H_6) (yield: 75%) (Found: C, 77.50; H, 11.00; O, 11.50: $C_{36}H_{62}O_4$ requires C, 77.42; H, 11.11; O, 11.47%).

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REFERENCES

- 1. Norin, T. (1972) Phytochemistry 11, 1231.
- Shanbhag, S. N., Mesta, C. K., Maheshwari, M. L., Paknikar, S. K. and Bhattacharyya, S. C. (1964) Tetrahedron 20, 2605.
- 3. Fieser, L. F. and Fieser, M. (1967) Reagents for Organic Synthesis, p. 231. Wiley, New York.
- Leonteva, V. G., Gromova, A. S., Lutskii, V. I., Modonova, L. D. and Tyukavkina, N. A. (1974) Khim. Prir. Soedin. 10, 240.
- 5. Pearl, I. A. and Beyer, D. L. (1951) J. Org. Chem. 16, 219.

Phytochemistry, 1977, Vol. 16, pp 398-399, Pergamon Press, Printed in England

5,7-DIHYDROXY-6,2',4',5'-TETRAMETHOXYFLAVONE FROM THE LEAVES OF CHUKRASIA TABULARIS

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From the leaves of *Chukrasia tabularis* we have isolated a new flavone which is assigned the structure 5,7dihydroxy-6,2',4',5'-tetramethoxyflavone on the following evidence.

The flavone $C_{19}H_{18}O_8$, mp 213-214° [v_{max} 3400, 1640 cm⁻¹] readily formed a dimethyl ether with K_2CO_3 Me_2SO_4 and a diacetate both of which lacked hydroxyl absorption. The UV spectrum [λ_{max}^{EiOH} 257, 272, 360 (ε 16200, 14650, 19630); with NaOAc 240, 273, 364 (e 17600, 17140, 17760), with AlCl₃ 266, 278, 393 (£ 15270, 14490, 21190); with NaOEt 275, 383 (£ 20500, 18000) nm] indicated [1] the presence of phenolic hydroxyl groups at positions 5 and 7. The trisubstituted nature of both rings A and B and the lack of a substituent at position 3 was deduced from the mass spectrum of the dimethyl ether which had prominent peaks at 402, 387 (base peak), 357, 195, 192, 177 and 167 (cf. gardenin [2] and ref. [1]). These conclusions were reinforced by the NMR spectrum of the dimethyl ether which showed H-3 at the characteristic value [1] of 6.5 δ and three aromatic proton singlets (δ 6.72, 6.86 and 7.3). The 2',4',5'-substitution pattern in ring B followed from the singlet nature of the aromatic protons. The remaining problem of the position of attachment of the third aromatic proton (C-6 or C-8) was readily solved by the use of benzeneinduced shifts of the methoxyl groups [1]. In the dimethyl ether four methoxyl groups showed large upfield shifts (0.73, 0.67, 0.59, 0.32 ppm) in benzene while the remaining two moved by 0.13 and -0.11 ppm. In the diacetate the corresponding shifts were 0.71, 0.6, 0.34 and 0.13 ppm. These data confirmed the presence of a proton at position 8 and hence the structure of the flavone as 5,7-dihydroxy-6,2',4',5'-tetramethoxyflavone. Substantial benzene-induced shifts (>0.3 ppm) of five methoxyl groups would have been expected for 5,7,8,2',4',5'-hexamethoxyflavone.

EXPERIMENTAL

All mp's are uncorrected. NMR spectra were recorded on a Varian XL-100 instrument in $CDCl_3$ with TMS as internal standard. Shade dried leaves of *Chukrasia tabularis* A. Juss (2 kg) were exhaustively extracted with hexane in the cold. Concentration of the hexane afforded a solid which was crystd from EtOAc to give the flavone mp 213–214°. The mother liquors were chromatographed over Si gel in hexane. Elution with CHCl₃–EtOAc (19:1) yielded more flavone (0.5 g). MS:

(100 %), 359 (64), 356 (44), 331 (32), 301, 192, 178, 177, 167, 149, 144, 139 and 69 (52%). (Found: C, 60.8; H, 5.15. $C_{19}H_{18}O_8$ requires: C, 60.95; H, 4.8%). NMR δ 3.78, 3.82, 3.9, 3.96 (3H, s, each, 4 × OMe), δ 6.64 (1H, s, H-3), δ 6.89 (2H, s, 2 × Ar-H), δ 7.48 (1H, s, Ar-H). Acetylation with Ac₂O-C₃H₃N gave the diacetate ex ether, mp 182°. (Found: C, 60.05; H, 5.0. $C_{23}H_{22}O_{10}$ requires: C, 60.25; H, 4.8%). NMR δ 2.41, 2.52 (3H, s, each, 2 × OAc), δ 3.87, 3.89, 3.91, 3.95 (3H, s, each, 4 × OMe), δ 6.59 (1H, s, H-3), δ 6.99 (1H, s, Ar-H), δ 7.24 (1H, s, Ar-H), δ 7.35 (1H, s, Ar-H). Methylation of the flavone with K₂CO₃-Me₂SO₄ followed by crystn from CHCl₃-Et₂O afforded the dimethyl ether mp 184° (Found: C, 63.0; H, 5.7. $C_{21}H_{22}O_8$ requires: C, 62.7; H, 5.45%). NMR δ 3.99, 3.98, 3.97, 3.93 (3H, s, each), 3.92 (6H, s), (6 × OMe).