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SHORT COMMUNICATION

EXTRACTIVES OF JACK PINE BARK: OCCURRENCE OF (+)-13-EPIMANOYL OXIDE AND RELATED LABDANE DITERPENES[1]

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Abstract—A new natural product, (+)-13-epimanoyl oxide, has been isolated from the benzene extract of the bark of jack pine (*Pinus banksiana*), together with the diterpenes, manoyl oxide, torulosol, 13-epitorulosol, and agathadiol; four serratenediol-related triterpenes; esters of ferulic acid; pinosylvin dimethyl ether; a series of phytosterols; waxes; fatty acids; and resin acids.

In the course of our investigation on softwood bark extractives, we examined the benzene extract (4·2 per cent) of the bark of jack pine (*Pinus banksiana* Lamb.). Two-thirds of the extract consisted of acidic materials which were found to consist of homologous wax acids, the usual fatty and resin acids, and wax alcohol esters of ferulic acid. Saponification of the neutrals yielded homologous wax acids, polyphenols (phlobaphenes) insoluble in water and benzene, and a neutral fraction (19 per cent).[3] This neutral fraction, upon treatment with urea, digitonin, picric acid, and finally by crystallization, was divided into: *n*-aliphatics (4 per cent) consisting of homologous wax alcohols together with a trace of paraffins; sterols (2 per cent) consisting predominantly of β-sitosterol together with lesser amounts of campesterol and the corresponding dihydrosterols;[4] 3,5-dimethoxystilbene[5] (pinosylvin dimethyl ether) (1·5 per cent); the triterpenes (0·7 per cent), serratenediol, 21-episerratenediol, diepiserratenediol, and episerratenediol 21-methyl ether;[1, 6] and a terpene-rich residue (7 per cent). From this residue was isolated by chromatography Δ^4-stigmasten-3-one (0·04 per cent), Δ^4-campesten-3-one (0·002 per cent), and the labdane diterpenes agathadiol[7] (0·3 per cent), torulosol (0·01 per cent) (not previously reported in the Pinaceae), 13-epitorulosol[7] (0·01 per cent) and an oily nonpolar diterpene (0·2 per cent). Although this latter material appeared homogeneous, thin-layer chromatography on silver nitrate–alumina[8] showed it to be a mixture. Chromatography on a column of 40 per cent silver nitrate on alumina succeeded in sharply separating this material into two main compounds. The first compound (0·07 per cent) was shown to differ from the second compound (0·1 per cent) only in the orientation of a vinyl group; the more readily eluted compound possessed a hindered axial vinyl while the second compound with an equatorial vinyl was more tightly absorbed.

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[1] Previous paper in this series: *Triterpenes of Pine Barks: Naturally Occurring Derivatives of Serratenediol.* J. W. Rowe and C. L. Bower, *Tetrahedron Letters* No. 32, 2745 (1965).

[2] Maintained at Madison, Wis., in co-operation with the University of Wisconsin.

[3] This and subsequent percentages relate to the benzene extract.

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[5] No trace of pinosylvin phenols was found in a previous examination of jack pine bark. G. Lindstedt and A. Misiorney, *Acta Chem. Scand.* 5, 121 (1951).

[6] J. W. Rowe, *Tetrahedron Letters* No. 34, 2347 (1964).

[7] J. W. Rowe and G. W. Shaffer, *Tetrahedron Letters* No. 30, 2633 (1965).

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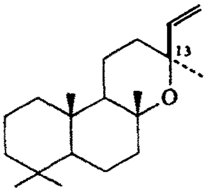
The second compound was readily shown to be (+)-manoyl oxide with physical properties and i.r. and NMR spectra identical to those of an authentic sample. This is the first reported occurrence of manoyl oxide in the genus *Pinus*. It has been previously reported in the trees, *Abies amabilis*,[9] *Xylia dolabriformis*,[10] and *Dacrydium* spp.[11-13]

The first compound was analogously shown to be (+)-13-epimanoyl oxide (I) (8α, 13α-epoxy-Δ^{14}-labdene with a 13S configuration), a new natural product. Although it has not been found previously in nature, it has been isolated as a minor product from the acetylation of sclareol.[14, 15] Moreover, its antipode (−)-13-epimanoyl oxide (olearyl oxide) has been reported to occur in the shrub *Olearia paniculata*,[16] the fungus *Gibberella fujikuroi*,[17] and the herb *Beyeria* sp.[18]



(I)

In analogy with our results on the closely related lodgepole pine bark,[19] it might be expected that a mixture of manool and 13-epimanool would also be present in jack pine bark. Further fractionation of the oily terpenoids from jack pine bark will be carried out in an attempt to confirm this.[20]

EXPERIMENTAL[21]

The fraction remaining, 43·9 g (7 per cent),[3] after treating the benzene extract of jack pine bark with base to remove free and combined acids, with urea to remove the bulk of the *n*-aliphatics, with digitonin to remove the sterols, with picric acid to remove the bulk of the 3,5-dimethoxystilbene, and with hexane at −20° to separate the insoluble polar terpenes, was

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[18] P. R. JEFFERIES and T. G. PAYNE, *Australian J. Chem.* **18**, 1441 (1965).

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[20] A further paper on the benzene extractives of jack pine bark and on the triterpenes of pine bark is being prepared.

[21] Melting points are determined in a copper block and are corrected. Nuclear magnetic resonance spectra are run on a Varian A60 in deuterochloroform with tetramethylsilane as an internal standard. Optical rotations are run in chloroform on a Rudloph Model 80 polarimeter. Infrared spectra are run on a Baird Atomic spectrophotometer. Gas chromatograms are run on an F & M Model 700 using 2·5 per cent SE-30 on 70/80 Anakrom ABS and 10 per cent DEGS on 1 per cent polyvinylpyrrolidinone pretreated 70/80 Anakrom ABS in ¼ in. O.D., 6 ft stainless-steel columns at about 203° and a flow rate of about 60 ml of helium per minute. Under these conditions, manool has an elution time of about 4 min on either column.

chromatographed on Woelm neutral alumina, activity II.[20] Elution with petroleum ether yielded a mixture of hydrocarbons, which were immediately followed by 2·7 g of a yellow oil that appeared homogeneous. Rechromatography of a purified center cut on alumina yielded 516 mg of white low-melting crystals which gave only a single peak on gas chromatography on both SE-30 ($r_{\mathrm{manool}}=0{\cdot}84$) and DEGS ($r_{\mathrm{manool}}=0{\cdot}42$). However, thin-layer chromatography on 40 per cent silver nitrate on alumina[8] developed with petroleum ether showed two major and one minor distinct spots. The product was therefore absorbed onto 82 g of activated 40 per cent silver nitrate on neutral alumina in a multibore column. Petroleum ether eluted first a small amount of a complex mixture and then 108 mg of *(+)-epimanoyl oxide.* Anhydrous ether later eluted 167 mg of (+)-manoyl oxide.

The (+)-epimanoyl oxide was recrystallized twice from methylene chloride–methanol for analysis, m.p. 97–99·5°, $[\alpha]_D^{22^\circ}+38^\circ$ (*c*, 0·9). Reported: 94–96°, $[\alpha]_D+37{\cdot}5^\circ$,[14, 15] and 100·5–101·5°, $[\alpha]_D+35{\cdot}3^\circ$.[25] Found: C, 82·80; H, 12·06. $C_{20}H_{34}O$ required: C, 82·69; H, 11·80%.

The i.r. spectrum (KBr) showed a manoyl oxide-type ring (1073 and 1121 cm^{-1})[22] and a vinyl group (3049, 1818, 1629, 1412, 987, and 923 cm^{-1}). The NMR spectrum showed a typical AB_2 pattern, τ 4·02 and 5·05 ($J=13{\cdot}2$ c/s) for three vinylic protons, five tertiary methyls τ 8·78, 8·88, 9·14, 9·22, and 9·28, and integrated for thirty-four protons in agreement with the data reported previously.[17, 23, 25] Gas chromatography showed one peak on both SE-30 ($r_{\mathrm{manool}}=0{\cdot}91$) and DEGS ($r_{\mathrm{manool}}=0{\cdot}47$). Comparison with a sample of (−)-13-epimanoyl oxide showed identical behavior on thin-layer chromatography (silver nitrate–alumina) and gas chromatography; and they had identical i.r. spectra.

The (+)-manoyl oxide was a clear, colorless oil which crystallized on cooling. It was distilled for analysis, b.p. 130–137° (bath) at 0·05 mm Hg, m.p. 22·5–26·5°, $[\alpha]_D^{22^\circ}+19^\circ$ (*c*, 1·2). Reported: b.p. 135–137° at 0·3 mm Hg, m.p. 25·5–29°, $[\alpha]_D+18^\circ$ to $+23^\circ$.[10, 11, 24] Found: C, 82·82; H, 11·97. Calc. for $C_{20}H_{34}O$: C, 82·69; H, 11·80%.

The i.r. spectrum (film) showed a manoyl oxide-type ring (1076 and 1116 cm^{-1})[22] and a vinyl group (3077, 1637, 1406, 996, and 917 cm^{-1}). The NMR spectrum[25] showed a typical AB_2 pattern, τ 4·16 and 4·87 ($J=14{\cdot}6$ c/s) for three vinylic protons, four sharp singlets τ 8·72, 8·74, 9·14, and 9·19 (double intensity) for five tertiary methyls, and integrated for thirty-four protons. Gas chromatography showed one peak on both SE-30 ($r_{\mathrm{manool}}=0{\cdot}74$) and DEGS ($r_{\mathrm{manool}}=0{\cdot}41$). The i.r. and NMR spectra were identical to those of an authentic sample, and they behaved identically on thin-layer chromatography (silver nitrate–alumina) and gas chromatography.

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