Plant Pinus strobus L (Eastern white pine). *Uses* Timber Pulp *Source* West Virginia and several Wisconsin locations. *Previous work* Wood resin [1,2] Cortex oleoresin [2,3]

Needles Needle samples were cut into small pieces (> 1 cm) and extracted with Et_2O. The extract was methylated (CH_2N_2) and analysed by GLC using DEGS [4] The peak eluting at $r_{pim} = 1\ 45$ was collected, this peak can be either anticopalate, isopimarate, or a mixture of the two, since they have the same retention times [6] After passing the collected eluant through alumina in pentane and evaporation of the solvent, an IR spectrum (CCl_4) was obtained The amount of anticopalate present in the mixture can be estimated from the ratio of absorbance at 1730 cm^{-1} (C=O) absorbance at 1650–1640 cm^{-1} (C=C stretching) by a calibration curve This procedure showed that anticopalic acid comprises 61–96 per cent of the total resin acids.[5]

Cortex oleoresin No anticopalic acid was found [5]

Wood Shavings of sapwood from mature trees were extracted with Et_2O and the resulting extract methylated Anticopalic acid represented 14–19 per cent of the resin acids as analysed by the above procedure

Plant Pinus monticola Dougl (Western white pine) *Uses* Timber Pulp *Source.* Lolo National Forest, Montana *Previous work* Bark [6] Wood [6,7]

Needles No anticopalic acid was found [5]

Cortex oleoresin No anticopalic acid was found [5]

Wood Anticopalic acid was previously reported[6] as 55 per cent of the resin acids

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[1] N M JOYE, JR and R V LAWRENCE, *J Chem & Eng Data* **12**, 279 (1967)
[2] F S SANTAMOUR, JR , *Morris Arboretum Bull* **18**, 82 (1967)
[3] D F ZINKEL and B P SPALDING, *Tetrahedron Letters* 2459 (1971)
[4] F H M NESTLER and D F ZINKEL, *Analyt Chem* **39**, 1118 (1967)
[5] Also found are several of the common resin acids (i e sandaracopimaric, levopimaric/palustric, isopimaric, abietic and neoabietic) The needles and cortex oleoresin of *Pinus strobus* also contain strobic acid [3]
[6] D F ZINKEL, J. K TODA and J W ROWE, *Phytochem* **10** 1161 (1971)
[7] A B ANDERSON, R RIFFER and A WONG, *Phytochem* **8**, 869 (1969)

Key Word Index—Pinus strobus, Pinus monticola, Pinaceae, anticopalic acid, resin acids

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NEW *C*-METHYLFLAVANONES FROM DOUGLAS-FIR*

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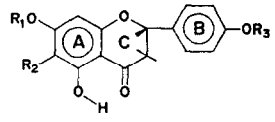
Abstract—Poria root rot of Douglas-fir [*Pseudotsuga menziesii* (Mirb) Franco], caused by *Poria weirii* Murr., infects most commercially important conifers in British Columbia and the northwestern United States, and is responsible for large annual losses in immature Douglas-fir In a search for naturally occurring anti-poria compounds in Douglas-fir roots, two new *C*-methylflavanones were discovered, namely, 4',5,7-trihydroxy-6-methylflavanone (poriol) and 7-*o*-β-D-glucosyl-3',4',5-trihydroxy-6-methylflavanone These structures were proved by colour reactions, UV, IR and NMR spectroscopy, mass spectrometry, chemical degradation, partial and unambiguous synthesis

INTRODUCTION

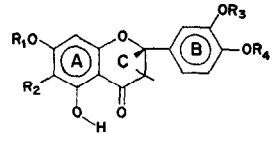
PORIA root rot of Douglas-fir trees [*Pseudotsuga menziesii* (Mirb.) Franco], caused by *Poria weirii* Murr., infects most commercially important conifers in British Columbia and the northwestern United States, and is responsible for large annual losses in immature Douglas-fir. In a search[1] for extractive differences between healthy and infected Douglas-fir roots, new *C*-methylflavanones were discovered.

RESULTS AND DISCUSSION

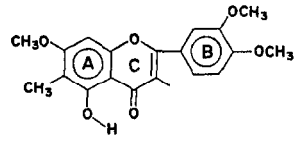
The first of these new *C*-methylflavanones called poriol (I)[2,3] (6-*C*-methylnaringenin) because of its association with diseased roots, was obtained in a yield of 0·2% from a methanolic extract of diseased root bark by preparative TLC Poriol gave a positive (violet) magnesium-hydrochloric acid[4] test for flavanones, but a negative zinc–hydrochloric acid test[5,6] for 3-hydroxyflavanones. It also gave a positive phenolic test with Barton's reagent (blue)[7] and Pauly's reagent (yellow) [8] IR and UV spectra indicated a substituted flavanone with a hindered carbonyl. NMR spectrum clearly showed the main features of structure I, namely, a phenolic hydroxyl group, which by virtue of its low field chemical shift is attached to C-5; a clearly defined ABX pattern, which could be assigned to protons of ring C; five aromatic protons which, on the basis of their splitting pattern, could be assigned to rings A and B; three protons of a methyl group and two protons corresponding to two phenolic hydroxyl groups The high value (12 5 c/s) for the coupling constant J_{AX} is indicative of an axial–axial coupling only. Therefore, the C-2 hydrogen is axial and ring B is equatorial. The mass spectrum showed a strong parent ion peak at *m/e* 286 and other fragments consistent with structure I.



Poriol (I) $R_1=R_3=H$, $R_2=CH_3$
Methylated poriol (Ia) $R_1=R_2=R_3=CH_3$
Poriolin (Ib) R_1 = glucose $R_2=CH_3$, $R_3=H$
Naringenin (II) $R_1=R_2=R_3=H$



(III) R_1 = glucose $R_2=CH_3$, $R_3=R_4=H$
(IIIa) $R_1=R_3=R_4=H$, $R_2=CH_3$
(IIIb) $R_1=H$ $R_2=R_3=R_4=CH_3$
(IIIc) $R_1=R_2=R_3=R_4=CH_3$
Hesperetin (V) $R_1=R_2=R_3=R_4=H$



6-Methylluteolin, 7', 3', 4' - trimethyl ether (IV)

[1] G M BARTON, *Can J Bot* **45**, 1545 (1967)
[2] G M BARTON, *Can J Chem* **45**, 1020 (1967)
[3] G M BARTON, Canada Department of Fisheries and Forestry, *Bi-Monthly Research Notes* **24**, No 5, 40 (1968)
[4] J SHINODA, *J Pharm Soc Japan* **48**, 214 (1928)
[5] J C PEW, *J Am Chem Soc* **70**, 3031 (1928)
[6] G M BARTON, *J Chromatog* **34**, 562 (1968)
[7] G M BARTON, R S EVANS and J A F GARDNER, *Nature, Lond* **170**, 249 (1952)
[8] IVOR SMITH, *Chromatographic Techniques, Clinical and Biochemical Applications*, p 145, Heinemann, London (1958)

Confirmation of structure I was obtained by preparing a known 4',7-dimethoxy derivative Ia by the nuclear methylation of naringenin (II) at carbon 6 with methyl iodide and sodium methoxide.[9] A comparison of chromatographic and spectral properties of this derivative with diazomethane-methylated poriol, showed that the two substances were identical Unambiguous proof of structure I was provided by Jain, Lal and Seshadri[10] who synthesized poriol starting with *C*-methylphloracetophenone

Shortly after the discovery of poriol, Hillis[11] discovered its 7-glucoside (poriolin) (Ib) in healthy Douglas-fir roots. It could be speculated, therefore, that enzymatic hydrolysis of poriolin due to poria infection accounted for the presence of the aglycone, poriol, in diseased roots

The second *C*-methylflavanone to be discovered in Douglas-fir roots was the new glucoside (III) (6 *C-methylhesperetin-7-o-β-D-glucoside*) In contrast to poriol, which was obtained in low yield (0·2%) from diseased root bark, III was obtained in high yield (2·6%) from healthy Douglas-fir root bark This glucoside gave identical color reactions to those already described for poriol. Its IR spectrum was similar to those obtained from flavone glucosides such as hesperitin rutinoside The NMR spectrum showed that it was a monoglucoside with 4 free aromatic protons The AB part of the ABX pattern of ring C protons was clearly discernible, centred at 7·2 τ

Mild hydrolysis of III gave two main fragments, aglycone (IIIa) and glucose which were resolved by TLC High-resolution NMR of the diazomethane–methylated aglycone (IIIc) enabled probable assignments to be made as follows a phenolic hydroxyl group, which by virtue of its low field chemical shift is attached to C-5, a clearly defined ABX pattern, which could be assigned to protons of ring C, three aromatic protons, which on the basis of their splitting pattern could be assigned to ring B, one aromatic proton assigned to ring A, C-8, nine protons which could be assigned to methoxyl groups on C-3', -4' and -7 and three protons of a methyl group assigned to C-6 As in the case of poriol, the high value (12·5 c/s) for the coupling constant J_{AX} is indicative of an axial–axial coupling only Therefore, the C-2 hydrogen is axial and ring B is equatorial

The mass spectrum of IIIb showed a strong parent ion peak at *m/e* 330 (100) and three main fragments at *m/e* 164 (99), 151 (86) and 133 (43) The abundance of the 164 fragment would be expected for the 3,4-dimethoxystyrene fragment ion arising from the cleavage between C-2 and the heterocylic oxygen and between C-3 and C-4 These fragments are consistent with structure IIIb

Since the NMR spectrum of IIIc had shown the presence of three methoxyl groups and color tests[12] indicated that parent glucoside (III) had vicinal phenolic hydroxyl groups, it must be concluded that the glucoside was attached at the non-vicinal hydroxyl group on C-7 The successful hydrolysis of compound III by emulsin showed that it was a β-glucoside

Final proof of structure III was obtained in two ways. It was shown that dehydrogenation of the methylated aglycone (IIIc) with iodine and glacial acetic acid, followed by purification on TLC, resulted in a spot with identical chromatographic and spectrometric properties to the known compound 6-methylluteolin 7,3',4'-trimethyl ether (IV) melting at 197·6° It was also shown that nuclear methylation of hesperetin (V) at C-6 with methyl iodide and sodium methoxide (5) resulted in the methylated aglycone (IIIc) This result was confirmed by quantitative IR comparison and mixture melting point 176 8°

[9] R N GOEL, A C. JAIN and T R SESHADRI, *Proc Indian Acad Sci* **48A**, 180 (1958)
[10] A C JAIN, P LAL and T R SESHADRI, *Tetrahedron* **25**, 283 (1969).
[11] W E HILLIS and N ISHIKURA, *Austral J Chem* **22**, 483 (1969)
[12] H A SCHROEDER, *J Chromatog* **30**, 537 (1967)

Both *C*-methylflavanones, poriol (I) and glucoside (III), have been submitted to extensive tests against *Poria weirii* without showing any anti-poria activity

EXPERIMENTAL

Poriol (I) obtained in a yield of 0 2% from a methanolic extract of diseased root bark by preparative TLC [R_f 0 88, silica gel, $CHCl_3$–MeOH (7 3)] It crystallized from $CHCl_3$ as light yellow platelets, with partial melting at 255–265° At 260° the melted material formed needle-like crystals which melted at 270° Color reactions violet with Mg–HCl test,[4] blue with Barton's reagent,[7] yellow with Pauly's reagent [8] The spectra gave λ_{max} in nm (log ϵ) 295 (4 22) ν_{max} 700, 730, 825, 900, 1110, 1162, 1170, 1240, 1300, 1330, 1450, 1500, 1600, 1625, 3000 cm^{-1} NMR (deuterioacetone) – 2 38 (1H, singlet), 2 72 (2H, doublet), 3 21 (2H, doublet), 4 04 (1H, singlet), 4 68 (1H, quartet, J_{BX} = 3 c/s), 6·74 (2H, singlet), AB spectrum 6 73–7 45 (2H, multiplet, J_{AB} = 17 5 c/s, J_{AX} = 12 5 c/s), 8 04 (3H, singlet) in τ units, mass spectrum, parent ion = 286 (80) ($C_{16}H_{14}O_5$), other main fragments at *m/e* 167 (100), 166 (65), 138 (42), 120 (38), 57 (40) and 55 (58)

Methylated poriol (Ia) Prepared with CH_2N_2 the spectra gave NMR (deuteriochloroform) – 1 99 (1H, singlet), 2 70 (2H, doublet), 3 14 (2H, doublet), 4 01 (1H, singlet), 6 28 (6H, singlet), 8 04 (3H, singlet) (ABX pattern similar to that of poriol)

Nuclear methylation of naringenin Naringenin (II) was methylated with MeI and NaOMe using the method of Goel and Seshadri [9] The resultant mixture was resolved by TLC (R_f 0 83, silica gel, Et_2O) to yield methylated poriol (Ia) A comparison of chromatographic and spectrometric properties showed the two substances were identical

Flavanone glucoside (III) Obtained from an EtOAc extract of healthy Douglas-fir root bark in a yield of 2 6% This bark had been previously Soxhlet extracted with $CHCl_3$ to remove interfering substances White crystals were obtained melting at 228 5° (Mettler FP1) After recrystallization from aq MeOH, the m p was 255 1° Color reactions violet with Mg-HCl,[4] blue with Barton's reagent,[7] yellow with Pauly's reagent [8] The spectra gave λ_{max} in nm (log ϵ) 288 (4 31) ν_{max} 530, 545, 565, 615, 625, 690, 720, 740, 760, 785, 815, 865, 880, 890, 915, 960, 1030, 1070, 1080, 1130, 1170, 1190, 1240, 1290, 1320–1350, 1435, 1450, 1490, 1540, 1580, 1605, 1635, 2920, 2940, 2960, 3000–3560 cm^{-1}

Hydrolysis of flavanone glucoside Mild hydrolysis of III (300 mg) with a mixture of conc HCl–HOAc (1 25) for 30 min at 100° gave glucose and aglycone (IIIa) This compound is also cleaved by treatment with emulsin

Hydrolysis of methylated glucoside A similar mild hydrolysis of CH_2N_2 methylated glucoside resulted in partially methylated aglycone (IIIb) Mass spectrum, parent ion = 330 (100) ($C_{18}O_6H_{18}$), other fragments at *m/e* 164 (99), 151 (86) and 133 (43)

Methylated aglycone (IIIc) Obtained by methylating IIIa with CH_2N_2 in MeOH The spectra gave NMR (deuteriochloroform) – 2 06 (1H, singlet), 3 10 (3H, multiplet), 3 95 (1H, singlet), 4 65 (1H, quartet, J_{BX} = 3 5 c/s), 6 10 (6H, singlet) 6 20 (3H, singlet) 7 07 (2H, multiplet, J_{AB} = 17 5 c/s, J_{AX} = 12 5 c/s) 8 01 (3H, singlet), in τ units

Dehydrogenation experiment Dehydrogenation of the methylated aglycone (IIIc) with I_2 and HOAc (reflux 2 hr) followed by purification on TLC (silica gel, CCl_4–MeOH (7 1), R_f 0 61) gave the known compound 6-methylluteolin-7,3′,4′-trimethylether (IV)

Nuclear methylation of hesperetin (V) Hesperetin (V) was methylated[9] in a similar manner to naringenin This resulted in the methylated aglycone (IIIc) confirmed by quantitative IR comparison and mixture melting point of 176 8°

Key Word Index—Pseudotsuga menziesii, Pinaceae, Douglas-fir, *C*-methyl flavones; 5,7,4′-trihydroxy-6-methylflavanone, 7-*O*-glucosyl-5,3′,4′-trihydroxy-6-methylflavanone