Pseudotsuganol, a Biphenyl-linked Pinoresinol–Dihydroquercetin from Douglas-fir Bark: Isolation of the First True Flavonolignan

Lai Yeap Foo[a] and Joseph Karchesy[b]

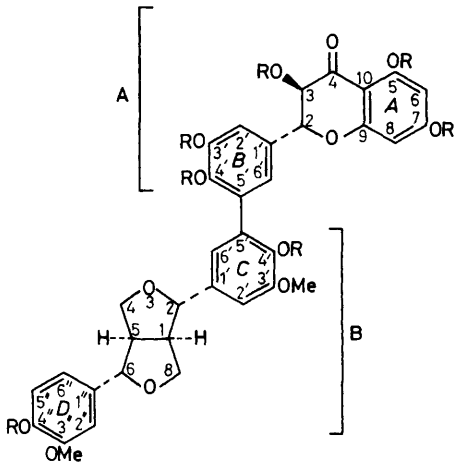
[a] *Chemistry Division, D.S.I.R., Private Bag, Petone, New Zealand*
[b] *Department of Forest Products, Oregon State University, Corvallis, Oregon 97331, U.S.A.*

Isolation of the biphenyl-linked pinoresinol–dihydroquercetin (**1**) from Douglas-fir bark represents the first example of a new class of compounds that are true flavonolignans.

Flavonolignans or flavolignans, an important class of phenolic natural products,[1—7] have aroused considerable interest in recent years because of their significant pharmacological properties,[8—10] particularly as antihepatotoxic agents for the treatment of liver cirrhosis.[11—15] Contrary to their title, these compounds are not true lignans, but are condensation products of a flavonoid and a phenylpropane unit, the latter being primarily coniferyl alcohol. Compound (**1**), isolated from the outer bark of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], consists of a dihydroquercetin flavonoid unit linked to a pinoresinol lignan unit by a biphenyl bond. This compound, named pseudotsuganol, is the first example of a new class of natural products that are true flavonolignans.

Compound (**1**), R_F 0.92 (cellulose t.l.c., Bu^{t}OH–HOAc–H_2O, 3 : 1 : 1 v/v), 0.28 (silica gel, EtOAc), $[\alpha]_{589}$ +20.2° (*c* 0.25; MeOH), Fast Atom Bombardment mass spectrometry (FAB MS): *m/z* 659 ($[M - H]^-$), was isolated from the ethyl acetate extract of the outer bark. Initial separation from the crude extract was carried out by elution from Sephadex LH-20, with ethanol. Final purification was

achieved by elution from a reversed phase (MCI-gel CHP-20P) column with MeOH–H_2O (3 : 7 v/v). The presence of the dihydroquercetin unit in (**1**) was evident from both the ^1H and ^{13}C n.m.r. spectra; the chemical shift values of the *A* and pyran rings and the magnitude of the proton-coupling constants between H-2 (δ 4.94, d, *J* 11.5 Hz) and H-3 (δ 4.52, d, *J* 11.5 Hz) closely paralleled those of dihydroquercetin. The nature of the lignan unit in (**1**) was elucidated by 2-D n.m.r. spectroscopy. Proton resonances at δ 3.07—3.30 (2H, m, H-1, H-5), 3.80 [2H, m, H-4 (β), H-8 (β)], 4.16—4.20 [2H, m, H-4 (α), H-8 (α)], 4.64 (1H, d, *J* 5.2 Hz, H-2), and 4.70 (1H, d, *J* 5.2 Hz, H-6) were assigned to the tetrahydrofuran moiety using proton–proton cross-peak correlation by COSY; aromatic (δ 6.74—7.0, 5H) and methoxy (δ 3.80 and 3.87, 3H each) resonances identified the guaiacyl rings. These assignments were corroborated by correlation of the ^1H and ^{13}C n.m.r. chemical shifts (HETCOR, Figure 1); the values of both types of resonances were consistent with published data on the pinoresinol structure.[15,16]



(**1**) R = H
(**2**) R = Ac

Treatment of (**1**) with acetic anhydride–pyridine gave the hepta-acetate (**2**), readily evident from ^1H and ^{13}C n.m.r. spectra and consistent also with FAB MS and elemental analysis. A better spread of the aromatic proton resonances in the ^1H n.m.r. spectrum of (**2**) facilitated assignment of the guaiacyl and catechol ring protons and hence location of the biphenyl linkage. Thus, the signals at δ 7.01 (1H, d, *J* 8.2 Hz), 6.99 (1H, d, *J* 1.7 Hz), and 6.88 (1H, dd, *J* 1.7, 8.2 Hz) were characteristic of the *ortho* and *meta* couplings in an ABX system of the unsubstituted guaiacyl ring (D). The other guaiacyl ring (C) protons were assigned as follows. The signal at δ 7.03 (1H, d, *J* 1.5 Hz) was assigned to H-2′ because of cross-peak correlation between this resonance and the methoxy signal at δ 3.88 in the NOESY (2-D nuclear Overhauser enhancement) map of (**2**). The resonance at δ 7.03 was also *meta*-coupled to the signal at δ 6.83 (1H, d, *J* 1.5 Hz), which therefore was assigned to H-6′. The remaining resonances in the low field region, at δ 7.31 and 7.32, were readily analysed as two sets of mutually *meta*-coupled doublets (*J* 1.9 Hz), which could only arise from the H-2′ and H-6′ of the dihydroquercetin ring (B). Thus the

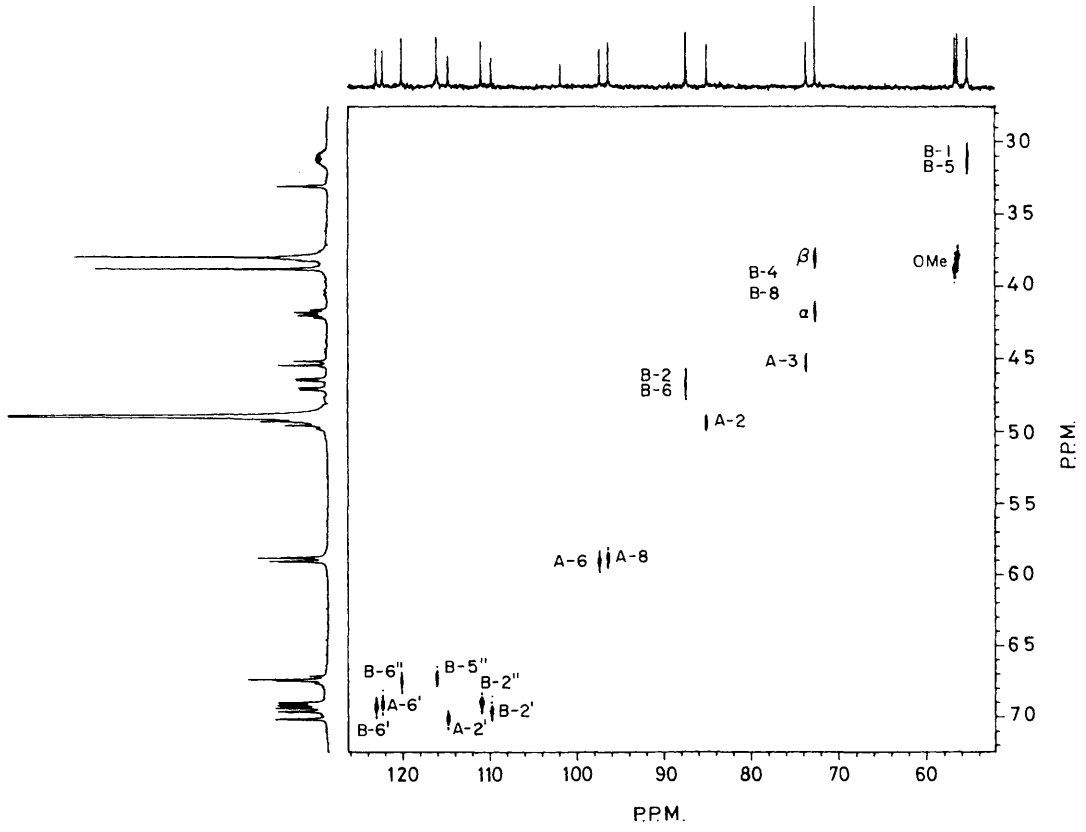


Figure 1. ^1H–^{13}C heteronuclear correlation (HETCOR) spectrum of pseudotsuganol (**1**) in CD_3OD.

biphenyl linkage between the dihydroquercetin and pinoresinol units was located at C-5′ of the former and C-5′ of the latter. The NOESY spectrum of the acetate (**2**) showed no cross-peak correlation between the protons of the two moieties, indicating that the acetate probably had the preferred 'unfolded' conformation.

Pseudotsuganol (**1**) is the first true flavonolignan and thus represents a new class of naturally occurring compounds.

We thank the USDA Competitive Research Grants Program (85-FSTY-9-0144) and the Chemistry Division, DSIR for support while L. Y. Foo was a Visiting Scientist at Oregon State University.

Received, 22nd July 1988; Com. 8/02985G

References

1 V. A. Kurkin and G. G. Zapesochnaya, *Chem. Nat. Compd.*, 1987, **23**, 7.
2 R. Cooper, H. E. Gottlieb, and D. Larie, *Isr. J. Chem.*, 1977, **16**, 12.
3 H. Wagner, O. Seligmann, L. Horhammer, and M. Seitz, *Tetrahedron Lett.*, 1971, 1895.
4 M. Fiebig and H. Wagner, *Planta Med.*, 1984, **50**, 310.
5 H. Wagner, O. Seligmann, M. Seitz, D. Abraham, and J. Sonnenbichler, *Z. Naturforsch.*, 1975, **331**, 876.
6 A. Pelter and R. Hänsel, *Chem. Ber.*, 1975, **108**, 790.
7 R. Hänsel, J. Schulz, and A. Pelter, *Chem. Ber.*, 1975, **108**, 1482.
8 G. K. Gupta, S. Raj, and P. R. Rao, *Res. Ind.*, 1982, 37.
9 H. Hikino, Y. Kiso, H. Wagner, and M. Fiebig, *Planta Med.*, 1984, **50**, 248.
10 H. Hikino, Y. Kiso, H. Taguchi, and Y. Ikeya, *Planta Med.*, 1984, **50**, 213.
11 G. Vogel in 'New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity,' eds. H. Wagner and P. Wolff, Springer-Verlag, New York, 1977, p. 249.
12 T. J. Mabry and A. Ulubelin, *J. Agric. Food Chem.*, 1980, **28**, 188.
13 J. Holzl, *Z. Naturforsch. C.*, 1974, **29**, 82.
14 V. R. Schrall and H. Becker, *Planta Med.*, 1977, **32**, 27.
15 T. Deyama, *Chem. Pharm. Bull.*, 1983, **31**, 2993.
16 F. Abe and T. Yamauchi, *Phytochemistry*, 1988, **27**, 575.