

FLAVONOIDS FROM *PINUS MORRISONICOLA*

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Key Word Index—*Pinus morrisonicola*; Pinaceae; heartwood; flavonoids; (2*R*,3*R*)-3-acetoxy-5,7-dihydroxy-6-methylflavanone; (2*R*,3*R*)-3,5,7-trihydroxy-6-methylflavanone.

Abstract—Two dihydroflavonols, (2*R*,3*R*)-3-acetoxy-5,7-dihydroxy-6-methylflavanone and (2*R*,3*R*)-3,5,7-trihydroxy-6-methylflavanone, accompanied by 12 known flavonoids were isolated from the acetone extract of the heartwood of *Pinus morrisonicola*. The major flavonoids include chrysin, pinobanksin and tectochrysin.

INTRODUCTION

Pinus morrisonicola Hay., also known as *P. formosana* and *P. parviflora* Matsum. et Hay., is a native conifer in Taiwan [1]. The wood is frequently used in building and for paper making. The resin and essential oil are used medicinally. *P. morrisonicola* is morphologically considered a variety of the Japanese white pine, *Pinus parviflora* Sieb. et Zucc. The flavonoids of the heartwood of *P. parviflora* Sieb. et Zucc. are chrysin, cryptostrobin, pinobanksin, pinocembrin and strobopinin, but pinostrobin and tectochrysin are absent [2]. On the other hand, a preliminary study has shown that *P. morrisonicola* Hay. contains pinostrobin and tectochrysin [3]. We here describe a detailed investigation of the flavonoids of the heartwood of *P. morrisonicola*.

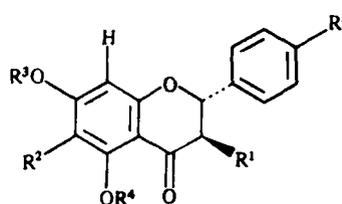
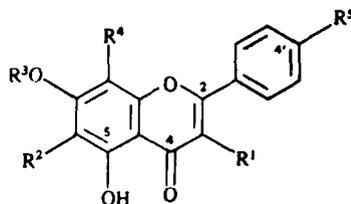
RESULTS AND DISCUSSION

By means of repetitive chromatography and crystallization, 14 flavonoids (Table 1) were isolated from the acetone extract of the heartwood of *P. morrisonicola*. Besides two dihydroflavonols 13 and 14, other com-

Table 1. Relative percentage contents and R_f values* of 1–14

Compound	Percentage	R_f
1	50.10	0.29
2	3.75	0.55
3	0.13	0.48
4	0.03	0.49
5	0.10	0.08
6	0.13	0.15
7	0.12	0.57
8	0.18	0.34
9	0.15	0.43
10	0.26	0.65
11	0.08	0.12
12	3.13	0.27
13	0.32	0.38
14	0.46	0.35

*The samples were developed on a SiO₂ plate (Merck Co. Art. 5554) by elution with EtOAc-hexane (3:7).



- | | | | |
|---|---|-----|--|
| 1 | $R^1 = R^2 = R^3 = R^4 = R^5 = H$ | 9 | $R^1 = R^2 = R^3 = R^4 = R^5 = H$ |
| 2 | $R^1 = R^2 = R^4 = R^5 = H, R^3 = Me$ | 10 | $R^1 = R^2 = R^4 = R^5 = H, R^3 = Me$ |
| 3 | $R^1 = R^2 = R^4 = R^5 = H, R^3 = Me$ | 11 | $R^1 = R^3 = R^4 = H, R^2 = Me, R^5 = OH$ |
| 4 | $R^1 = R^2 = R^3 = R^5 = H, R^4 = Me$ | 12 | $R^2 = R^3 = R^4 = R^5 = H, R^1 = OH$ |
| 5 | $R^1 = R^2 = R^3 = R^4 = H, R^5 = OH$ | 13 | $R^3 = R^4 = R^5 = H, R^1 = OAc, R^2 = Me$ |
| 6 | $R^1 = R^2 = R^4 = H, R^3 = Me, R^5 = OH$ | 13a | $R^1 = OAc, R^2 = Me, R^3 = R^4 = Ac, R^5 = H$ |
| 7 | $R^2 = R^3 = R^4 = R^5 = H, R^1 = OH$ | 13b | $R^1 = OAc, R^2 = R^3 = Me, R^4 = R^5 = H$ |
| 8 | $R^2 = R^4 = R^5 = H, R^1 = OH, R^3 = Me$ | 14 | $R^3 = R^4 = R^5 = H, R^1 = OH, R^2 = Me$ |

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pounds (1–12) were identified as chrysin (1) [4], tectochrysin (2) [4], strobachrysin (3) [5], cryptochrysin (4) [5, 6], apigenin (5) [7, 8], genkwanin (6) [9, 10], galangin (7) [5, 11], izalpinin (8) [12], pinocembrin (9) [11, 13], pinostrobin (10) [11, 14], poriol (11) [15], and pinobanksin (12) [16] from their physical and spectroscopic properties (mp, $[\alpha]$, IR, MS, UV and $^1\text{H NMR}$). Chrysin, pinobanksin and tectocrysin are the major components. Since *P. morrisonicola* contains both flavones and flavanones, it belongs to the subgenus *Haploxylon* [17].

Compound 13 was isolated as colourless crystals, mp 196–197°. The mass spectrum showed the parent peak at m/z 328 and the base peak at m/z 268 ($\text{M} - \text{CH}_3\text{CO}_2\text{H}$)⁺. The IR absorptions at 3400, 1723 and 1640 cm^{-1} were attributable to the hydroxyl, ester and conjugated carbonyl groups. The UV spectrum exhibited characteristic absorptions of flavonols at 340 nm (sh, Band I) and 296 nm (Band II). Addition of AlCl_3 or AlCl_3/HCl resulted in bathochromic shifts, ~20 nm for Band I and ~8 nm for Band II, indicating the presence of hydroxyl groups at C-5 and C-7 [18]. The $^1\text{H NMR}$ spectrum revealed one aromatic proton on the A ring (δ 6.00, s) and five aromatic protons on the B ring. The resonances of two phenolic protons at C-5 and C-7 appeared at δ 11.67 and 6.14, respectively. The resonances at δ 2.02 (3H, s) and 2.04 (3H, s) corresponded to an acetyl group and an aromatic methyl group. The *trans* configuration of H-2 and H-3 was inferred by a large coupling constant of 12 Hz. Acetylation of 13 (Ac_2O , pyridine, 25°, 48 hr) afforded a crystalline triacetate 13a, mp 84–86°. The presence of an acetyl and two hydroxyl groups in 13 was thus confirmed. Compound 13 formed a monomethyl ether 13b, MW 342, on treatment with CH_3N_2 . Irradiation of the aromatic methyl resonance of 13b (at δ 1.98) caused a nuclear Overhauser effect on the signal of the phenolic proton at C-5, indicating that the methyl group orients at C-6 rather than at C-8. Therefore, 13 is 3-acetoxy-5,7-dihydroxy-6-methylflavanone. (This assignment has been confirmed by an X-ray diffraction of 13. Crystallographic data will be published elsewhere.) The CD measurement of 13 and comparison of the curve with that of reported dihydroflavanols [19] established the absolute configuration to be (2R,3R).

Compound 14, mp 110–112°, exhibited the parent peak at m/z 286 in the mass spectrum. Interpretation of the IR, UV and $^1\text{H NMR}$ spectra revealed the structure of 14 as 3,5,7-trihydroxy-6-methylflavanone. (While the structure of compound 14 was rigorously determined, relevant references to strobobanksin (mp 177–178°) have not clearly indicated the position of the methyl group at C-6 or C-8 [20].) The *trans* configuration at C-2 and C-3 was inferred by the large coupling constant $J_{2,3}$ of 12 Hz. The CD measurement also demonstrated that 14 has the same configuration (2R,3R) as that of 13. Acetylation of 14 gave a triacetate 13a. This chemical correlation confirms the structural relationship between 13 and 14.

EXPERIMENTAL

Plant material. The heartwood (1.1 kg) of *Pinus morrisonicola* Hay. was collected in Ho-Ping County, Taichung, in September 1984. A voucher specimen, identified by Dr Ta-Wei Hu, has been deposited in the herbarium of the Taiwan Forestry Institute. The heartwood was sliced, air-dried and extracted $\times 4$ with Me_2CO . The extracts (14.5 g) were combined and subjected to CC on

200 g of SiO_2 by elution with the gradients of hexane, EtOAc and MeOH. The components of each fraction were analysed by TLC, and the appropriate fractions were combined for further purification, by recrystallization or by the flash chromatography, to give compounds 1–14 in a total yield of 9.4 g.

3-Acetoxy-5,7-dihydroxy-6-methylflavanone (13). Colourless crystals from EtOAc, mp 196–197°, $[\alpha]_{\text{D}}^{25} + 58.6^\circ$ (Me_2CO ; c 0.34). CD (MeOH): $[\theta]_{317} + 6150$, $[\theta]_{284} - 22550$. MS m/z (rel. int.): 328 [M]⁺ (64), 286 (36), 268 (100), 222 (21), 180 (49), 167 (50), 166 (33). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 340 (2530, sh) 296 (44 200), in the presence of AlCl_3 , 360 (4 310, sh), 302 (14 460); in the presence of AlCl_3/HCl , 360 (3 300, sh), 304 (12 680). $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 2.02 (3H, s), 2.04 (3H, s), 5.31 (1H, d, $J = 12$ Hz), 5.81 (1H, d, $J = 12$ Hz), 6.00 (1H, s, H-8), 6.14 (1H, br s), 6.40–7.48 (5H, m), 11.67 (1H, s).

Triacetate (13a). Colourless crystals from Et_2O -hexane, mp 84–86°. MS m/z (rel. int.): 412 [M]⁺ (0.7), 370 (28), 328 (9), 310 (11), 268 (27), 222 (12), 166 (24), 43 (100). $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 1.96 (3H, s), 2.02 (3H, s), 2.31 (3H, s), 2.40 (3H, s), 5.39 (1H, d, $J = 12$ Hz), 5.71 (1H, d, $J = 12$ Hz), 6.74 (1H, s), 7.38–7.44 (5H, m).

Methyl ether (13b). Colourless crystals, mp 126–128°. MS m/z (rel. int.): 342 [M]⁺ (16), 300 (4), 282 (33), 194 (39), 181 (42), 180 (49), 43 (100). $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 1.98 (3H, s), 2.00 (3H, s), 3.83 (3H, s), 5.30 (1H, d, $J = 11.7$ Hz), 5.77 (1H, d, $J = 11.7$ Hz), 6.04 (1H, s), 7.37–7.45 (5H, m), 11.45 (1H, OH, $W_{1/2} = 2$ Hz).

3,5,7-Trihydroxy-6-methylflavanone (14). Colourless crystals from EtOAc, mp 110–112°, $[\alpha]_{\text{D}}^{25} + 20.6$ (Me_2CO ; c 1.94). CD (MeOH): $[\theta]_{325} + 5580$, $[\theta]_{286} - 20590$. MS m/z (rel. int.): 286 [M]⁺ (100), 257 (44), 167 (57), 152 (48). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 336 (1 340), 292 (7 250); in the presence of AlCl_3 , 390 (1 140), 312 (7 060); in the presence of AlCl_3/HCl , 380 (1 140), 308 (6 100). $^1\text{H NMR}$ (CDCl_3 , 90 MHz): δ 2.00 (3H, s), 3.50 (1H, br s, OH), 4.60 (1H, d, $J = 12$ Hz), 4.98 (1H, d, $J = 12$ Hz), 5.90 (1H, s, H-6), 6.15 (1H, br s, OH), 7.30–7.62 (5H, m), 11.30 (1H, s, OH).

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REFERENCES

1. *Flora of Taiwan* (1975) Vol. 1, p. 523. Epoch, Taiwan.
2. Lindstedt, G. and Misiorny, A. (1951) *Acta Chem. Scand.* **5**, 121.
3. Wang, K. T., Das, K. C., Lin, Y. Y. and Weinstein, B. (1970) *Experientia* **26**, 930.
4. Erdtman, H., Kimland, B. and Norin, T. (1966) *Phytochemistry* **5**, 927.
5. Mukerjee, S. K. and Seshadri, T. R. (1953) *Proc. Indian Acad. Sci.* **38A**, 207. *Chem. Abstr.* (1955) **49**, 1025h.
6. Venturella, P. (1961) *Ann. Chim.* **51**, 759. *Chem. Abstr.* (1962) **57**, 9793d.
7. Baker, W., Hemming, R. and Ollis, W. D. (1951) *J. Chem. Soc.* 691.
8. Reed, R. I. and Wilson, J. M. (1963) *J. Chem. Soc.* 5949.
9. Devon, T. K. and Scott, A. I. (1975) *Handbook of Naturally Occurring Compounds* Vol. 1, p. 122. Academic Press, New York.
10. Brieskorn, C. H. and Michel, H. (1968) *Tetrahedron Letters* 3447.
11. Suga, T., Iwata, N. and Asakawa, Y. (1972) *Bull. Chem. Soc. Jpn* **45**, 2058.
12. Hottori, S. (1955) *J. Pharm. Soc. Jpn* **55**, 1330.

13. Bick, I. R. C., Brown, R. B. and Hills, W. E. (1972) *Aust. J. Chem.* **25**, 449.
14. Mongkolsuk, S. and Dean, F. M. (1964) *J. Chem. Soc.* 4654.
15. Barton, G. M. (1972) *Phytochemistry* **11**, 426.
16. Jefferis, P. R., Knox, J. R. and Middleton, E. J. (1962) *Aust. J. Chem.* **15**, 532.
17. Lindstedt, G. (1951) *Acta Chem. Scand.* **5**, 129.
18. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
19. Herz, W., Gibaja, S., Bhat, S. V. and Srinivasan, A. (1972) *Phytochemistry* **11**, 2859.
20. Lindstedt, G. and Misiorny, A. (1951) *Acta Chem. Scand.* **5**, 1.