

## FLAVONOIDS AND STILBENES FROM ARMAND PINE

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**Key Word Index**—*Pinus armandii*, Pinaceae, heartwood, flavonoids, stilbenes, chemotaxonomy.

**Abstract**—Seven flavonoids and seven stilbenes were isolated from the heartwood of *Pinus armandii* Fr. var. *mastersiana* Hay. Among them, *trans*-3,5-dimethoxystilbene oxide is a new compound and 3-acetyloxy-5,7-dihydroxyflavanone is the first report of its occurrence in nature. Comparison of the flavonoid and stilbene components in *P. armandii*, *P. morrisonicola* Hay. and *P. parviflora* Sieb. et Zucc. supports the chemotaxonomy of three species.

### INTRODUCTION

*Pinus armandii* Franchet var. *mastersiana* Hayata. The armand pine, is an economically important conifer indigenous to the southwest region of China. *Pinus armandii* is morphologically as the Taiwan endemic *P. morrisonicola* Hay. and Japanese white pine, *Pinus parviflora* Sieb. et Zucc. [1]. We have recently shown that the flavonoid constitution of the heartwood of *P. morrisonicola* is different from that of *P. parviflora* [2]. A preliminary paper chromatographic study of chemical constituents of *P. armandii* has been reported [3]. We here describe a detailed investigation of the flavonoids and stilbenes of the heartwood of *P. armandii*.

### RESULTS AND DISCUSSION

The heartwood of *P. armandii*, collected in the Central Range of Taiwan [3], was exhaustively extracted with acetone. The concentrated extract was separated by chromatography to give seven flavonoids (1–7) and seven stilbenes (8–14). Compounds 1–7 were identified as chrysin (1) [4], tectocrysin (2) [4], apigenin (3) [5, 6], pinocembrin (4) [7, 8], pinostrobin (5) [7, 9], pinobanksin (6) [10] and pinobanksin 3-acetate (7) [11] from their physical and spectroscopic properties (mp,  $[\alpha]$ , IR, MS, UV, and  $^1\text{H NMR}$ ). Compound (7),  $[\alpha]_{\text{D}}^{25} + 42^\circ$ , is the first report of its occurrence in nature while the synthetic sample (the stereochemistry not defined) has been used as an anti-inflammatory agent [11]. Compound 7 should have the *trans* configuration as that of pinobanksin by evidence of a large coupling constant  $J_{2,3}$  of 12 Hz. Since *P. armandii* contains both flavones and flavanones, it belongs to the subgenus *Haploxylon* [12]. The flavonoids of *P. armandii*, *P. morrisonicola* [2] and *P. parviflora* [13] are listed in Table 1. This comparison supports the chemotaxonomic basis of three species.

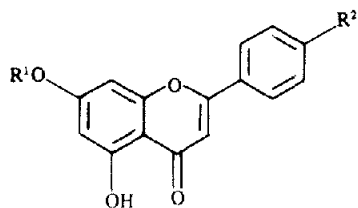
Two major stilbene compounds (8), mp 122–123°, and (9), mp 55–56°, were recognised as mono- and dimethyl ethers of *trans*-pinosylvin [14, 15] as supported by their compatible spectral data (IR, MS, UV,  $^1\text{H}$  and  $^{13}\text{C NMR}$ ). A *cis* isomer (10), three dihydro derivatives (11–13) and an oxide (14) were also isolated. The struc-

tures (10–14) were determined by spectroscopic analysis and chemical correlation as follows. The *cis* configuration of compound (10) was inferred by a relatively small coupling constant of 12 Hz between two vinyl protons [15]. Hydrogenation (Pd/C, EtOAc, 25°, 4 hr) of stilbene (8) afforded a dibenzyl (11) [13]. Similarly, either stilbene (9) or the *cis* isomer (10) absorbed one equivalent of  $\text{H}_2$  to give compound (13). Treatment of (9) with *m*-chloroperbenzoic acid ( $\text{CH}_2\text{Cl}_2$ , 25°, 2 hr) produced the oxide (14) and *trans*-2-hydroxy-3,5-dimethoxystilbene. The new compound of oxide (14) was also found in the acetone extract of *P. morrisonicola* [16]. However, whether the oxide (14) is a natural product or artifact remains uncertain. Comparison of the stilbene constitution in *P. armandii*, *P. morrisonicola* and *P. parviflora* also indicates the species difference (Table 2). While flavonoids (1, 2 and 6) are major components in the acetone extract of heartwood of *P. morrisonicola*, the stilbenes (8, 9 and 12) are richly present in *P. armandii*.

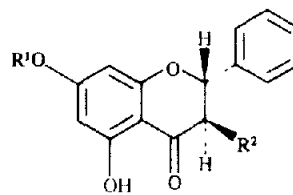
### EXPERIMENTAL

**Plant material** The heartwood of *Pinus armandii* Fr. var. *mastersiana* Hay. was collected in Gu-Kuang County, Taichung, in August 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 (1.2 kg) and extracted  $\times 4$  with  $\text{Me}_2\text{CO}$ . The combined extracts (39.0 g) were subjected to CC on 350 g of  $\text{SiO}_2$  by elution with the gradients of hexane, EtOAc and  $\text{Me}_2\text{CO}$ . The components of each fraction were analysed by TLC, and the appropriate fractions were combined for further purification, by HPLC or by recrystallisation, to give flavonoids 1–7 (4.1 g) and stilbenes 8–14 (16.2 g).

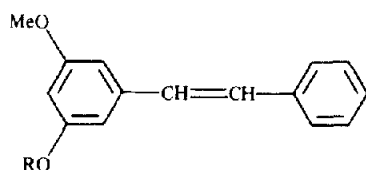
**3-Acetyloxy-5,7-dihydroxyflavanone (7)** Colourless crystals, mp 173–175°.  $[\alpha]_{\text{D}}^{25} + 42^\circ$  (MeOH,  $c$  1.7). MS  $m/z$  (rel. int.) 314 [ $\text{M}^+$ ] (28), 272 (11), 255 (13), 254 (62), 243 (12), 208 (7), 195 (6), 166 (21), 153 (100), 152 (23), 124 (9), 120 (60), 118 (27), 103 (4), 91 (32). IR  $\nu_{\text{KBr}}$   $\text{cm}^{-1}$  3420, 1750, 1630.  $\lambda_{\text{max}}^{\text{MeOH}}$  nm ( $\epsilon$ ): 328 (4300), 290 (17000), 223 (16000), 209 (25500), in the presence of  $\text{AlCl}_3$ , 378 (2860), 314 (21000), 215 (24000), 205 (26000), in the presence of  $\text{AlCl}_3\text{-HCl}$ , 380 (3570), 314 (23000), 215 (23600), 205 (26000).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  2.00 (3H, s), 5.30 (1H, d, J



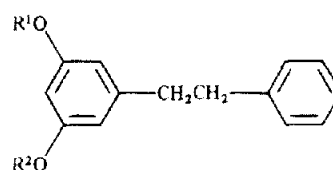
- 1** R<sup>1</sup> = R<sup>2</sup> = H  
**2** R<sup>1</sup> = Me, R<sup>2</sup> = H  
**3** R<sup>1</sup> = H, R<sup>2</sup> = OH



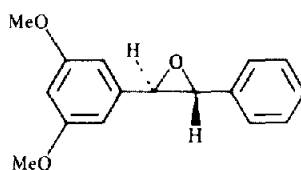
- 4** R<sup>1</sup> = R<sup>2</sup> = H  
**5** R<sup>1</sup> = Me, R<sup>2</sup> = H  
**6** R<sup>1</sup> = H, R<sup>2</sup> = OH  
**7** R<sup>1</sup> = H, R<sup>2</sup> = OAc



- 8** R = H (*trans*)  
**9** R = Me (*trans*)  
**10** R = Me (*cis*)



- 11** R<sup>1</sup> = R<sup>2</sup> = H  
**12** R<sup>1</sup> = Me, R<sup>2</sup> = H  
**13** R<sup>1</sup> = R<sup>2</sup> = Me



**14**

Table 1 Flavonoids in the heartwood of *P. armandu* Fr var *mastersiana*, *P. morrisonicola* Hay and *P. parviflora* Sieb et Zucc

Compound	<i>P. armandu</i>	<i>P. morrisonicola</i>	<i>P. parviflora</i>
Chrysin ( <b>1</b> )	*(9.51)	*(50.10)	*
Tectochrysin ( <b>2</b> )	*(0.59)	*(3.75)	*
Strobochrysin		*(0.13)	
Cryptochrysin		*(0.03)	
Apigenin ( <b>3</b> )	*(0.30)	*(0.10)	-
Genkwanin		*(0.13)	
Gelangin		*(0.12)	
Izalpinin		*(0.18)	
Pinocembrin ( <b>4</b> )	*(2.61)	*(0.15)	*
Pinostrobin ( <b>5</b> )	*(0.18)	*(0.26)	
Poriol		*(0.08)	
Pinobanksin ( <b>6</b> )	*(2.85)	*(3.13)	*
Pinobanksin 3-acetate ( <b>7</b> )	*(3.56)		
6-Methyl-pinobanksin		*(0.46)	
6-Methyl-pinobanksin 3-acetate		*(0.32)	
Strobopinin			*
Cryptostrobin			*

\* Indicates presence and — indicates absence of the specified flavonoid. The number in parenthesis indicates relative percentage content.

Table 2. Stilbenes in the heartwood of *P. armandu*, *P. morrisonicola*, and *P. parviflora*

Compound	<i>P. armandu</i>	<i>P. morrisonicola</i>	<i>P. parviflora</i>
<i>trans</i> -Pinosylvin	—	*(0.09)	*
<i>trans</i> -Pinosylvin monomethyl ether (8)	*(42.80)	*(38.87)	*
<i>trans</i> -Pinosylvin dimethyl ether (9)	*(14.20)	*(0.22)	—
<i>cis</i> -Pinosylvin	*(0.12)	*(0.01)	—
<i>cis</i> -Pinosylvin dimethyl ether (10)	*(0.12)	*(0.01)	—
Dihydropinosylvin (11)	*(0.15)	—	*
Dihydropinosylvin monomethyl ether (12)	*(19.02)	—	*
Dihydropinosylvin dimethyl ether (13)	*(0.35)	*(0.08)	—
<i>trans</i> -Pinosylvin oxide dimethyl ether (14)	*(0.12)	*(0.07)	—

\* Indicates presence and — indicates absence of the specified stilbene. The number in parenthesis indicates relative percentage content.

=12 Hz, H-2), 5.77 (1H, *d*, *J*=12 Hz, H-3), 5.97 (1H, *d*, *J*=2 Hz), 6.00 (1H, *d*, *J*=2 Hz, H-8), 7.42 (5H, *br s*)

*trans* 3,5-Dimethoxystilbene oxide (14) Oily sample, *R<sub>f</sub>* 0.53 (4% EtOAc-hexane),  $[\alpha]_D^{25}$  0° (CHCl<sub>3</sub>, *c* 1.0) MS *m/z* (rel. int.) 256 [M<sup>+</sup>] (100), 255 (27), 241 (11), 239 (25), 238 (27), 227 (14), 225 (10), 208 (3), 165 (11), 91 (17) IR (neat) 1602, 1470, 1205, 1160, 1085 cm<sup>-1</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 3.78 (6H, *s*), 3.81 (2H, *s*), 6.41 (1H, *t*, *J*=2 Hz), 6.49 (1H, *d*, *J*=2 Hz), 7.34 (5H, *m*)

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