The Constituents of the Bark of Armand Pine

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Twenty three compounds were isolated from the bark of *Pinus armandii*. These compounds include nine serratenes (1-9), a norserratenone 10, eight phenols 11-18, two flavonoids 19-20, one lignan 21, a diterpene 22 and a novel triterpentiol acetone 23. Their structures were determined from their physical and spectral properties.

INTRODUCTION

Pinus armandii Franchet var. *mastersiana* Hayata, the armand pine (華山松), is an economically important conifer indigenous to the southwest region of China.¹ The heartwood of the plant is frequently used in building and for paper making. It has been shown that the chemical constituents of the heartwood are rich in flavonoids and stilbenes.² As a continuing study, we report thirteen compounds in addition to the ten serratenes (1-10) isolated from the bark of *P. armandii*.

RESULTS AND DISCUSSION

The bark of *P. armandii*, collected in the Central Range of Taiwan, was exhaustively extracted with acetone. The concentrated extract was subjected to column chromatography to give twenty three constituents 1-23 after purification by HPLC. The structures of 1-23 were mainly determined from their spectral and physical properties (mp, $[\alpha]$, CD, IR, ^1H and ^{13}C NMR, MS, HRMS). Serratenes 1-10 are a group of pentacyclic triterpenes bearing central seven-membered C rings. Detailed data of these compounds will be published elsewhere.³ Concurrent isolation of the serratenone 6 ($\text{R}^5 = \text{CH}_3$), the alcohol 7 ($\text{R}^5 = \text{CH}_2\text{OH}$), the aldehyde 8 ($\text{R}^5 = \text{CHO}$) and the 30-norserratenone 10 ($\text{R}^5 = \text{H}$) from the same plant source suggested a possible biogenetic pathway from 6-7-8-10.

Compounds 11-13 were *para*-substituted phenols which can be characterized by the AB patterns for four aromatic protons in the NMR spectra. Compound 11, $[\text{M}]^+$ at m/z 122, is identified as 4-hydroxybenzaldehyde,⁴ which showed the aldehyde proton at δ 9.83. Compound 12, $[\text{M}]^+$ at m/z 136, is 4-hydroxyphenylethanone,⁵ whose acetyl group appears at δ 2.54. Compound 13, $[\text{M}]^+$ at m/z 166,

- 1 $\text{R}^1 = \text{R}^4 = \text{OH}$; $\text{R}^2 = \text{R}^3 = \text{H}$; $\text{R}^5 = \text{Me}$
- 2 $\text{R}^1 = \text{R}^3 = \text{OH}$; $\text{R}^2 = \text{R}^4 = \text{H}$; $\text{R}^5 = \text{Me}$
- 3 $\text{R}^1 = \text{R}^4 = \text{H}$; $\text{R}^2 = \text{R}^3 = \text{OH}$; $\text{R}^5 = \text{Me}$
- 4 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{R}^3 = \text{H}$; $\text{R}^4 = \text{OH}$; $\text{R}^5 = \text{Me}$
- 5 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{R}^3 = \text{H}$; $\text{R}^4 = \text{OAc}$; $\text{R}^5 = \text{Me}$
- 6 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{H}$; $\text{R}^3, \text{R}^4 = \text{O}$; $\text{R}^5 = \text{Me}$
- 7 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{H}$; $\text{R}^3, \text{R}^4 = \text{O}$; $\text{R}^5 = \text{CH}_2\text{OH}$
- 8 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{H}$; $\text{R}^3, \text{R}^4 = \text{O}$; $\text{R}^5 = \text{CHO}$
- 9 $\text{R}^1 = \text{OMe}$; $\text{R}^2, \text{R}^3 = \text{H}$; $\text{R}^4 = \text{OAc}$; $\text{R}^5 = \text{CHO}$
- 10 $\text{R}^1 = \text{OMe}$; $\text{R}^2 = \text{R}^5 = \text{H}$; $\text{R}^3, \text{R}^4 = \text{O}$

was identified as 3-hydroxy-1-(4-hydroxyphenyl)propan-1-one,⁶ which showed seven ^{13}C signals at δ 40.3, 57.3, 114.7, 129.1, 130.1, 161.6 and 196.9. Compound 14, $[\text{M}]^+$ at m/z 154, has three aromatic protons at δ 6.88 ($d, J = 8$ Hz), 7.46 ($dd, J = 8, 2$ Hz) and 7.51 ($d, J = 2$ Hz). The structure is assigned as 3,4-dihydroxybenzoic acid⁷ as its ^{13}C NMR spectrum exhibits seven signals at δ 115.2, 116.9, 122.6, 123.1, 145.1, 150.2 and 167.3. Compound 15 is an aldehyde as indicated by the base peak at m/z 151, $[\text{M}]^+ - 1$. From the ^1H NMR data of its aromatic protons at δ 7.02 (1 H, $d, J = 8$ Hz) and 7.38-7.42 (2 H, m), compound 15 was inferred to be isovanilline (3-hydroxy-4-methoxybenzaldehyde)⁸ rather than vanillin. This assignment was supported by the ^{13}C NMR spectrum which displayed resonances at δ 56.1,

108.8, 114.4, 127.5, 129.9, 147.1, 151.7 and 190.9 (CHO). Compound 16 showed the parent ion at m/z 166 and the base peak at m/z 151 ($M^+ - CH_3$), which was assigned as (4-hydroxy-3-methoxyphenyl)ethanone.⁹ Irradiation of the methoxy resonance yields a 15% enhancement of the H-2 signal at δ 7.52 ($d, J = 1.2$ Hz). A colorless crystalline compound 17, mp 167-167.5°C, showing $[M]^+$ at m/z 194, was identified as ferulic acid (4-hydroxy-3-methoxycinnamic acid). The trans vinylic protons appear at δ 6.36 ($d, J = 16$ Hz) and 7.59 ($d, J = 16$ Hz). The carbons of the methoxyl and carboxylic groups occurred at δ 56.3 and 168.9, respectively. The structure of compound 18, $[M]^+$ at m/z 196, was inferred as 3-hydroxy-(4-hydroxy-3-methoxyphenyl)propan-1-one¹¹ by comparison of the 1H and ^{13}C NMR spectra with those of compound 13. A nuclear Overhauser enhancement of the H-2 signal was observed on saturation of the methoxyl group.

- 11 X = OH, Y = H, R = CHO
 12 X = OH, Y = H, R = COMe
 13 X = OH, Y = H, R = COCH₂CH₂OH
 14 X = Y = OH, R = CO₂H
 15 X = OMe, Y = OH, R = CHO
 16 X = OH, Y = OMe, R = COMe
 17 X = OH, Y = OMe, R = CH=CHCO₂H
 18 X = OH, Y = OMe, R = COCH₂CH₂OH

Analysis of the 1H and ^{13}C NMR spectra revealed that compounds 19 and 20 were flavane ($[M]^+$ at m/z 290) and flavone ($[M]^+$ at m/z 304), respectively.^{12a} Besides five aromatic protons, the resonances of H-2, H-3 and two H-4 in compound 19 was found to occur at δ 4.55 ($d, J = 7.5$ Hz), 3.96 (m), 2.84 ($dd, J = 16, 5$ Hz) and 2.49 ($dd, J = 16, 8$ Hz). Thus, 19 was assigned as (+)-catechin, $[\alpha]_D^{25} + 10^\circ$ (MeOH, c 1.0).¹² Compound 20 has a carbonyl carbon at δ 198. The H-2 and H-3 resonances which appeared at δ 5.02 ($d, J = 12$ Hz) and 4.61 ($d, J = 12$ Hz) indicated a trans configuration. Thus, 20 was assigned as (+)-2,3-trans-dihydroquercetin, $[\alpha]_D^{25} + 19^\circ$ (MeOH, c 0.7).¹³ Compound 21 (mp 136-139°C) showed the parent ion at m/z 346. It was

identified as a known lignan, cedrusin,¹⁴ by comparison of the 1H and ^{13}C NMR data.^{14b} The optical rotation, $[\alpha]_D^{25} - 7.2^\circ$ (MeOH, c 0.8), was in agreement with the (2*S*, 3*S*)-configuration.^{14c}

- 19 X = H, H
 20 X = O

21

Compound 22, mp 130-132°C, $[\alpha]_D + 44.3^\circ$ (MeOH, c 0.75), was found to be a diterpenoidal acid, pinusolidic acid.¹⁵ The IR absorptions at 3000-2500, 1690 and 1743 cm^{-1} were attributable to the carboxylic group and the α, β -unsaturated γ -lactone moiety. Three olefinic protons appeared as broad singlets at δ 4.53 (H-17), 4.82 (H-17) and 7.08 (H-14). The C-10 methyl group at high field, δ 0.53, indicated that it was strongly shielded by the axial carboxylic group at C-4. The mass spectrum of 22 shows the parent ion at m/z 332 and the characteristic fragments at m/z 167 and 121 (base peak) for the labdane-type skeleton.

The 1H NMR spectrum of compound 23 showed the presence of ten methyl groups in the region between δ 0.6-1.4, which included nine singlets for the tertiary methyls and a doublet for the secondary methyl group. An olefinic proton was found at δ 5.20 ($d, J = 6$ Hz). An equatorial methoxyl group was indicated by the resonances at δ 3.35 (3H, s) and 2.63 (1 H, $dd, J = 11, 4$ Hz, CHOMe). The resonance at δ 3.59 ($dd, J = 7.5, 5$ Hz) was attributable to the other carbinol proton (R_2CHOR'). Interestingly, compound 23 has 34 carbon atoms, as revealed by the ^{13}C NMR spectrum, and gave an intense fragment at m/z 456 due to elimination of a molecule of acetone, $[M]^+ - Me_2CO$. Thus, compound 23 was considered to be the acetone of a triterpendiol. Hydrolysis of 23 in an 80% acetic acid solution (room temperature, 24 h) yielded a diol, which was

22

23

identified as 3β -methoxy-lanost-9(11)-en-24,25-diol by comparison of the ^1H NMR data with literature.¹⁶ An NOE experiment of **23** indicated that H-24 (δ 3.59) was in the proximity of H-26 (δ 1.23) but far apart from H-27 (δ 1.08). Thus, the new compound **23** (exact mass 514.4366) was unambiguously determined to be (24*S*)-24,25-isopropylidenedioxy- 3β -methoxy-5 α -lanost-9(11)-ene. However, it was unclear whether **23** is a natural product or an artifact.

EXPERIMENTAL SECTION

Plant material

The bark of *Pinus armandii* Fr. var. *masterisana* Hay. was collected in Tayulin mountain (大禹嶺, 2600 m altitude) in August 1985. A voucher specimen, identified by Dr. Ta-Wei Hu (胡大維), has been deposited in the herbarium of the Taiwan Forestry Institute. The bark was air-dried (760 g) and extracted (6 L x 3) with Me_2CO . The combined extracts (37 g) were subjected to column chromatography on 380 g of SiO_2 by elution with the gradients of hexane and EtOAc. The components of each fraction were combined for further purification by HPLC with either the normal phase (μ -Porasil or LiChrosorb Si 60) or the

reverse phase column (LiChrosorb RP-18) to give, according to the ascending order of polarity, compounds **23** (12 mg), **5** (13 mg), **6** (20 mg), **10** (28 mg), **9** (26 mg), **8** (29 mg), **4** (3 mg), **7** (13 mg), **1** (3 mg), **2** (800 mg), **3** (6 mg), **22** (23 mg), **11** (7 mg), **12** (13 mg), **15** (10 mg), **16** (5 mg), **17** (10 mg), **20** (12 mg), **14** (6 mg), **13** (14 mg), **18** (10 mg), **19** (23 mg) and **21** (5 mg).

Instrumentation

Yanagimoto (or MP-500D) micro melting point apparatus, Jasco Dip-180 digital polarimeter, Jasco J-20 circular dichroism spectropolarimeter, Finnigan TSQ-46c mass spectrometer, Perkin-Elmer 983G infrared spectrophotometer, Bruker AM-300 WB (or AC 200) nuclear magnetic resonance spectrometer, and Waters M-45 high pressure liquid chromatograph were used.

(24*S*)-24,25-Isopropylidenedioxy- 3β -methoxy-5 α -lanost-9(11)-ene (**23**)

Colorless solid, mp 183–184°C; $[\alpha]_D^{25} +85^\circ$ (CHCl_3 ; c 0.2); IR (KBr) 1688 (C=C), 1101 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.63 (3 H, *s*, H-18), 0.72 (3 H, *s*, H-28), 0.76 (3 H, *s*, H-30), 0.88 (3 H, *d*, $J = 6$ Hz, H-21), 0.94 (3 H, *s*, H-29), 1.02 (3 H, *s*, H-19), 1.08 (3 H, *s*, H-27), 1.23 (3 H, *s*, H-26), 1.30 (3 H, *s*, H-31), 1.39 (3 H, *s*, H-32), 2.63 (1 H, *dd*, $J = 11, 4$ Hz, H-3), 3.35 (3 H, *s*, OCH_3), 3.59 (1 H, *dd*, $J = 7.5, 5$ Hz, H-24), 5.20 (1 H, *d*, $J = 6$ Hz, H-11). ^{13}C NMR (CDCl_3) δ 14.4 (C-18), 16.4 (C-30), 18.3 (C-21), 18.5 (C-28), 21.2 (C-6), 22.2 (C-19), 22.5 (C-2), 22.8 (C-27), 26.3 (C-22, C-26), 26.9 (C-31), 28.0 (C-16), 28.1 (C-7), 28.3 (C-29), 28.6 (C-32), 33.2 (C-23), 33.9 (C-15), 36.0 (C-1), 36.3 (C-20), 37.2 (C-12), 39.0 (C-4), 39.4 (C-10), 41.8 (C-8), 44.3 (C-13), 47.0 (C-14), 50.9 (C-17), 53.0 (C-5), 57.5 (OCH_3), 80.2 (C-25), 84.1 (C-24), 88.6 (C-3), 106.3 ($\text{O-CMe}_2\text{-O}$), 114.7 (C-11), 148.7 (C-9); MS (70 eV) m/z (rel. int.) 514 (70, M^+), 499 (21, $\text{M}^+ - \text{CH}_3$), 467 (4, $\text{M}^+ - \text{CH}_2\text{OH}$), 456 (43, $\text{M}^+ - \text{Me}_2\text{CO}$), 441 (91), 423 (18), 409 (96), 391 (19), 327 (100), 295 (24); HRMS Exact mass $\text{C}_{34}\text{H}_{58}\text{O}_3$ requires 514.4371, found 514.4366.

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Key Words

Pinus armandii; Pinaceae; Bark; Serratenes;
Phenols; Flavonoids; Cedrusin; Pinusolidic acid;
Triterpendiol acetone.

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