DITERPENES FROM LEAVES OF *JUNIPERUS CHINENSIS*

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Key Word Index—*Juniperus chinensis*; Cupressaceae; leaves; diterpenes; norditerpene; secoditerpene; sempervirane-type; abietane-type; totarane-type; chinane-type.

Abstract—Sixteen diterpenes, a norditerpene and a secoditerpene were isolated from the leaves of *Juniperus chinensis*. The structures of new compounds abieta-8,11,13-trien-7 β -ol, 13 β -hydroxy-7-oxoabiet-8(14)-en-19,6 β -olide (juniperolide), 6,13 β -dihydroxy-7-oxoabiet-5,8(14)-dien-19-al (juniperal), 15-oxo-16-norabiet-7,13-dien-19,6 β -olide (norjuniperolide), 13 β ,14 β -epoxy-8-oxo-7,8-secoabietan-7,19-olide (secojuniperolide) and 11 β -hydroxy-14-oxochinan-19-al (chinanoxal) were determined by spectral methods and X-ray crystallography. Besides the sempervirane, abietane and totarane types having the isopropyl group, respectively, at the C-12, C-13 and C-14 positions, the remaining chinane-type diterpene having an isopropyl group at C-11 is first reported. Occurrence of all four types of diterpenes in this plant is significant.

INTRODUCTION

Juniperus chinensis is a common ornamental tree [1]. The components of hinokiflavone and kayaflavone have been previously reported [2, 3]. We recently isolated umbelliferone, 13 lignans and an unusual compound 2-(3,4-methylenedioxyphenyl)propane-1,3-diol from the leaves of this plant [4]. We report here a variety of diterpenoid components found in the leaves.

RESULTS AND DISCUSSION

The acetone-soluble part of the leaves of *J. chinensis* *kaizuka* was extracted with EtOAc and the extract subjected to chromatography to give diterpenoid compounds 1–18. Compound 1 was readily identified as phytol by comparison of the IR, ^1H , ^{13}C NMR and mass spectra with those of an authentic sample [5]. The structures of two known diterpenes, sandaracopimaric acid (2) [6, 7] and isocupressic acid (3) [8, 9] were also determined from their spectral and physical properties (NMR, MS, mp and $[\alpha]$). Semperviol (4) [10] and totarol (5) [11, 12] having the isopropyl group at C-12 and C-14, respectively, were isolated in addition to ferruginol (6) [13, 14] having the isopropyl group at C-13. Other known abietane-type diterpenes included hinokiol (7) [7], abieta-8,11,13-trien-7 α -ol (8) [15], abieta-8,11,13-trien-7-one (10) [15], abieta-8,11,13-trien-12,19-diol (11) [12, 16], 4-epiabietinal (12) [17] and 4-epiabietic acid (13) [17]. It is noted that the C-19 oxygenated compounds such as 11–13 (the epi-series) are generally found in plants of *Juniperus* species, whereas the C-18 oxygenated compounds are found in *Pinus* species.

By comparison of the spectral properties of 8 and 9, the new compound 9 was readily assigned as an epimer of 8. The axial 7-H of 9 exhibited a double doublet signal ($J = 10, 7$ Hz) while the equatorial 7-H of 8 showed a triplet ($J = 3$ Hz). Reduction of the ketone 10 with LiAlH_4 (THF, 0°, 2 hr) gave exclusively the alcohol 9 as a consequence of hydride attack from the less hindered α -

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face. The structure of **9** is thus confirmed as abieta-8,11,13-trien-7 β -ol.

Five novel polyoxygenated diterpenes **14–18**, namely juniperolide, juniperol, norjuniperolide, secojuniperolide and chinanoxal, were isolated. The exact mass $[M]^+$ of **14** at m/z 332.196 indicated a molecular formula $C_{20}H_{28}O_4$. The IR absorptions at 3527, 1754 and 1694 cm^{-1} were, respectively, attributed to the functionalities of an alcohol, a γ -lactone and a conjugated ketone. The 1H NMR spectrum (Table 1) showed some readily assignable signals such as two methyl singlets (δ 0.71 and 1.29), an isopropyl group [δ 0.83 (*d*), 0.94 (*d*) and 1.79 (*sept*)], a γ -H of lactone (δ 4.78) and a β -H of enone (δ 6.90). Detailed analysis of the ^{13}C NMR spectrum (Table 2) and the COLOC experiment revealed the correlation between C-13 (δ 72.3) and H-15 (δ 1.79) as well as the correlation of C-19 (δ 180.0), H-5 (δ 2.10) and H-18 (δ 1.29). The structure of **14** was thus assigned as 13 β -hydroxy-7-oxoabieta-8(14)-en-19,6 β -olide. Irradiation of H-18 caused nuclear Overhauser enhancements on H-5 and H-6; this experiment indicates that these protons are on the same face. The isopropyl group was considered to be on the α -face because the signal of H-12 α (δ 1.53) was enhanced when the methyl resonance (δ 0.94) was irradiated. The stereochemistry of **14** was finally proved by an X-ray diffraction analysis.

Compound **15** ($C_{20}H_{28}O_4$), $[M]^+$ at m/z 332.199, was readily recognized as a diterpene aldehyde from an NMR resonance at δ 9.42 (*s*) and an IR absorption at 1710 cm^{-1} . The moieties of alcohol and enone were also inferred from the IR absorptions at 3417 and 1658 cm^{-1} . The proton resonance at δ 6.46 (*s*) was attributable to an

Table 1. 1H NMR spectral data of new compounds **14–18** ($CDCl_3$ solution, δ values in ppm, *J* values in Hz)

H	14	15	16	17	18
1	1.23, 1.64	1.43, 1.82		1.22, 1.47	
2	1.59, 1.69	1.72		1.50	
3	1.47, 2.18	1.25 (<i>ddd</i> , <i>J</i> = 14, 14, 7), 2.02 (<i>ddd</i> , <i>J</i> = 14, 7, 7)		1.24, 1.75	
5	2.10 (<i>d</i> , <i>J</i> = 6)		1.80 (<i>d</i> , <i>J</i> = 5)	2.03 (<i>dd</i> , <i>J</i> = 8, 4)	
6	4.78 (<i>d</i> , <i>J</i> = 6)		4.98 (<i>dd</i> , <i>J</i> = 5, 4)	2.33 (<i>dd</i> , <i>J</i> = 18, 4) 2.53 (<i>dd</i> , <i>J</i> = 18, 8)	
7			6.15 (<i>d</i> , <i>J</i> = 4)		
8					2.84 (<i>ddd</i> , <i>J</i> = 12, 12, 3)
9	2.08 (<i>ddd</i> , <i>J</i> = 11, 4, 2)	2.41 (<i>ddd</i> , <i>J</i> = 11, 5, 3)	1.99	2.27 (<i>dd</i> , <i>J</i> = 12, 8)	
11	1.36, 1.72	1.74, 1.85	1.61, 1.22	1.41 (<i>dd</i> , <i>J</i> = 12, 4), 1.92 (<i>qd</i> , <i>J</i> = 8, 4)	
12	1.53 (<i>ddd</i> , <i>J</i> = 14, 3), 1.82 (<i>dddd</i> , <i>J</i> = 14, 7, 7, 2)	1.47, 1.76	2.01, 2.74	1.78, 2.10 (<i>dt</i> , <i>J</i> = 14, 3)	
14	6.90 (<i>dd</i> , <i>J</i> = 2, 2)	7.00 (<i>dd</i> , <i>J</i> = 3, 3)	7.00 (<i>d</i> , <i>J</i> = 2)	3.19 (<i>s</i>)	
15	1.79 (<i>sept</i> , <i>J</i> = 7)	1.85 (<i>sept</i> , <i>J</i> = 7)		1.73 (<i>sept</i> , <i>J</i> = 7)	1.52 (<i>sept</i> , <i>J</i> = 7)
16	0.83 (<i>d</i> , <i>J</i> = 7)	0.87 (<i>d</i> , <i>J</i> = 7)	2.34 (<i>s</i>)	1.00 (<i>d</i> , <i>J</i> = 7)	0.83 (<i>d</i> , <i>J</i> = 7)
17	0.94 (<i>d</i> , <i>J</i> = 7)	0.96 (<i>d</i> , <i>J</i> = 7)		0.93 (<i>d</i> , <i>J</i> = 7)	0.95 (<i>d</i> , <i>J</i> = 7)
18	1.29 (<i>s</i>)	1.45 (<i>s</i>)	1.31 (<i>s</i>)	1.14 (<i>s</i>)	1.02 (<i>s</i>)
19		9.42 (<i>s</i>)		3.90 (<i>d</i> , <i>J</i> = 12), 4.34 (<i>d</i> , <i>J</i> = 12)	9.77 (<i>s</i>)
20	0.71 (<i>s</i>)	1.04 (<i>s</i>)	0.84 (<i>s</i>)	1.07 (<i>s</i>)	0.98 (<i>s</i>)

Table 2. ^{13}C NMR spectral data of new compounds 14–17 (CDCl_3 solution, δ values in ppm)

C	14	15	16	17
1	32.5	32.0	33.0	32.8
2	17.7	15.5	17.8	17.6
3	28.3	27.9	28.1	31.0
4	41.8	50.0	42.4	40.3
5	52.6	138.7	51.7	41.3
6	76.0	144.6	73.3	28.2
7	193.1	182.6	137.9	171.1
8	136.8	135.4	141.3	210.6
9	49.5	47.5	47.1	53.0
10	33.1	37.6	32.8	31.7
11	18.9	18.6	21.4	19.1
12	29.8	28.6	23.2	22.9
13	72.3	71.3	141.9	69.3
14	143.0	141.5	125.4	63.5
15	37.6	37.8	195.5	33.3
16	17.3	17.3	24.4	17.9
17	16.1	16.3		17.6
18	25.3	20.8	181.7	29.0
19	180.0	201.0	25.4	76.7
20	16.6	23.1	17.5	19.9

Compound 18 showed the ^{13}C signals at δ 14.2, 16.7 (q), 16.9 (q), 19.0 (q), 20.8, 24.9 (q), 27.2, 33.8, 34.5, 35.6, 36.0, 39.2, 41.6, 47.3, 48.7, 56.7, 57.3, 77.4, 205.7, 215.1.

intramolecularly bonded OH. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 71 (s), two carbonyl carbons at δ 183 (s) and 210 (d) as well as four olefinic carbons at δ 135 (s), 139 (s), 141.5 (d) and 147 (s). From these spectral data and by comparison with those of 14, we assigned 15 as 6,13 β -dihydroxy-7-oxoabieta-5,8(14)-dien-19-al. The stereochemistry of 15 was shown as such by analogy to that of 14.

The ^{13}C NMR spectrum of 16 showed 19 signals. Its molecular formula $\text{C}_{19}\text{H}_{24}\text{O}_3$ was deduced from the exact mass $[\text{M}]^+$ at m/z 300.170. The IR absorptions at 1761 and 1657 cm^{-1} were attributable to the functionalities of a γ -lactone and an enone. This norditerpene displayed a resonance for an acetyl group at δ 2.34 (s) instead of signals for an isopropyl group. The ^1H NMR spectrum also showed a lactonic proton (H-6) at δ 4.98 and two olefinic protons at δ 6.16 and 7.00. Analysis of the ^{13}C NMR spectrum along with the UV absorption at 274 nm led to the assignment of 16 as 15-oxo-16-norabieta-7,13-dien-19,6 β -olide.

The ^1H and ^{13}C long-range correlations obtained from an HMBC experiment were useful in determining the uncommon structure of 17 ($\text{C}_{20}\text{H}_{30}\text{O}_4$) as 13 β ,14 β -epoxy-8-oxo-7,8-secoabietan-7,19-olide. The carboxy carbon (C-7) at δ 171 was found to correlate with the proton resonances at δ 3.90 (d, $J=12$ Hz) and 4.34 (d, $J=12$ Hz), indicating the moiety of a lactone ($\text{CH}_2\text{OC}=\text{O}$). Two oxy carbons occurring at δ 63.5 (s, R_3CO) and 69.3 (d, R_2CHO) were correlated with the isopropyl group. The proton resonance for R_2CHO appearing at δ 3.19 (s) was correlated with a carbonyl carbon (C-8, δ 211). Taking the supportive evidence of IR

absorptions at 1726 and 1682 cm^{-1} for a δ -lactone and the epoxycyclohexanone, we were able to connect a secoabietane skeleton for 17. The stereochemistry in 17 could be inferred by analogy to those of 14–16 and it was actually established by X-ray crystallography.

A small amount of 18 was isolated. It was readily recognized as a diterpene aldehyde from its $[\text{M}]^+$ at m/z 320 ($\text{C}_{20}\text{H}_{32}\text{O}_3$) and the proton resonance at δ 9.77 (s). The ^1H NMR spectrum also revealed the presence of an isopropyl group and two methyl singlets. The resonances at δ 77.4 (s) and 205.7 (s) were attributable to the carbons R_3COH and $\text{R}_2\text{C}=\text{O}$, respectively. The structure of 18 was unambiguously determined to be 11 β -hydroxy-14-oxochinan-19-al by X-ray analysis. The occurrence of an isopropyl group at the C-11 position was previously unknown. Thus, all sempervirane-, abietane-, totarane- and chinane-type diterpenes were found in the leaves of *J. chinensis*. It has been suggested that these four types may interconvert through acid-catalysed processes in biogenesis [18].

EXPERIMENTAL

Plant material. The leaves of *Juniperus chinensis* Linn. var. *kaizuka* Hort. were collected from plants grown in the surroundings of the Department of Chemistry of the National Taiwan University. A voucher specimen has been deposited in the Herbarium of our University. The leaves (1.83 kg) were soaked in Me_2CO (7 l) for a week. The Me_2CO extract was concd to give 90 g of a residue, which was diluted with H_2O and extracted $\times 3$ with EtOAc . The combined EtOAc extracts were concd to give an oil (25.5 g), which was absorbed by 31 g of silica gel and then chromatographed on a column packed with 250 g of silica gel. By elution with gradients of hexane, EtOAc and CHCl_3 , 12 (11 mg), 10 (23 mg), 8 (14 mg), 9 (10 mg), 6 (6 mg), 5 (54 mg), 4 (32 mg), 1 (32 mg), 13 (640 mg), 2 (4 mg), 3 (3 mg), 17 (5 mg), 18 (2 mg), 11 (8 mg), 15 (8 mg), 16 (6 mg), 7 (2 mg) and 14 (35 mg) were obtained in the ascending order of polarity.

Phytol (1). An oil. $[\text{M}]^+$ m/z 296. Sandaracopimaric acid (2). Mp 173.5–175°. $[\alpha]_{\text{D}}^{25} -14^\circ$ (EtOH ; c 1.1). Isocupressic acid (3). Mp 117–120°. $[\alpha]_{\text{D}}^{25} +44.3^\circ$ (CHCl_3 ; c 1.2). Semperviol (4). An oil. $[\text{M}]^+$ m/z 286. $[\alpha]_{\text{D}}^{25} +23.6^\circ$ (EtOH ; c 2.5). Totarol (5). Mp 131–132°. $[\alpha]_{\text{D}}^{25} +40.2^\circ$ (EtOH ; c 2.2). Ferruginol (6). Mp 52–53°. $[\alpha]_{\text{D}}^{25} +34.8^\circ$ (EtOH ; c 0.63). Hinokiol (7). Mp 232–233.5°. $[\alpha]_{\text{D}}^{25} +62.8^\circ$ (EtOH ; c 0.8). Abieta-8,11,13-trien-7 α -ol (8). An oil. $[\text{M}]^+$ m/z 286. $[\alpha]_{\text{D}}^{30} -16.2^\circ$ (CHCl_3 ; c 0.98). Abieta-8,11,13-trien-7-one (10). Mp 88–89°. $[\alpha]_{\text{D}}^{25} +17.2^\circ$ (CHCl_3 ; c 1.6). 12-Hydroxydehydroabietinol (11). Mp 186.5–188°. $[\alpha]_{\text{D}}^{25} +56.8^\circ$ (EtOH ; c 0.8). 4-Epiabietinal (12). Mp 182–183°. $[\alpha]_{\text{D}}^{25} -142^\circ$ (CHCl_3 ; c 1.2). 4-Epiabietic acid (13). Mp 156–157°. $[\alpha]_{\text{D}}^{25} -74^\circ$ (CHCl_3 ; c 1.5).

Abieta-8,11,13-trien-7 β -ol (9). An oil. $[\alpha]_{\text{D}}^{30} +33.5^\circ$ (CHCl_3 ; c 0.66). IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 3398 (OH). ^1H NMR (CDCl_3): δ 0.91 (s, H-19), 0.93 (s, H-18), 1.22 (d, $J=6.9$ Hz, H-16, H-17), 1.23 (s, H-20), 2.85 (sept, $J=6.9$ Hz, H-15), 4.79 (dd, $J=10.3, 7.2$ Hz, H-7), 7.07 (dd, $J=8.3, 1.7$ Hz, H-12), 7.15 (d, $J=8.3$ Hz, H-11), 7.36 (br s, H-14). ^{13}C NMR

(CDCl₃): δ 19.2 (t, C-2), 21.5 (q, C-19), 23.9 (q, C-16), 24.1 (q, C-17), 25.3 (q, C-20), 30.3 (t, C-6), 33.2 (s, C-4), 33.2 (q, C-18), 33.6 (d, C-15), 38.2 (s, C-10), 38.8 (t, C-1), 41.3 (t, C-3), 49.2 (d, C-5), 71.4 (d, C-7), 124.4 (d, C-12), 125.0 (d, C-11), 125.8 (d, C-14), 137.7 (s, C-8), 146.2 (s, C-13), 147.4 (s, C-9). EIMS (70 eV) m/z (rel. int.): 286 [M]⁺ (23), 271 (4), 253 (55), 243 (11), 211 (41), 197 (13), 183 (74), 162 (100). Exact mass [M]⁺ for C₂₀H₃₀O₄ requires 286.2298. Found: 286.2293.

13 β -Hydroxy-7-oxoabiet-8(14)-en-19,6 β -olide (14). Mp 191.5–193° (from hexane–EtOAc–PhH–Et₂O, 20:3:3:1). $[\alpha]_D^{25} + 8.5$ (MeOH; c 3.2). IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 3527 (OH), 1754, 1694. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 254 (875), 344 (65). CD (MeOH): $[\theta]_{343.5} - 2690$, $[\theta]_{289.5} 0$, $[\theta]_{278.5} + 534$, $[\theta]_{259} 0$. EIMS (70 eV) m/z (rel. int.): 333 [M + 1]⁺ (13), 315 (47), 289 (100), 261 (18), 123 (19), 109 (20). CIMS (CH₄) m/z 333 [M + 1]⁺. Exact mass [M]⁺ for C₂₀H₂₈O₄ requires 332.1988. Found: 332.1958.

6,13 β -Dihydroxy-7-oxoabieta-5,8(14)-dien-19-al (15). Yellow oil. $[\alpha]_D^{25} + 3.2^\circ$ (MeOH; c 1.6). IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 3417 (OH), 1710, 1658. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 266 (4060), 314 (4647). CD (MeOH): $[\theta]_{426} - 73$, $[\theta]_{364.5} - 3198$, $[\theta]_{326.5} - 101$. EIMS (70 eV) m/z (rel. int.): 333 (3) [M + 1]⁺, 315 (1), 304 (7), 303 (5), 289 (3), 273 (2), 262 (20), 261 (100), 84 (100). CIMS (CH₄) m/z 333 [M + 1]⁺. Exact mass [M]⁺ for C₂₀H₂₈O₄ requires 332.1988. Found: 332.1994.

15-Oxo-16-norabieta-7,13-dien-19,6 β -olide (16). Mp 176.5–178° (from MeOH). $[\alpha]_D^{25} - 114.7^\circ$ (MeOH; c 0.34). IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 1761, 1657. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 207 (2800), 274 (7800). CD (MeOH): $[\theta]_{395.5} - 51$, $[\theta]_{348.5} - 860$, $[\theta]_{318.5} - 208$, $[\theta]_{306} - 827$, $[\theta]_{284} + 558$, $[\theta]_{269.5} + 24$, $[\theta]_{264} - 63$, $[\theta]_{248.5} - 5040$. EIMS (70 eV) m/z (%): 300 [M]⁺ (26), 272 (4), 256 (5), 241 (4), 229 (5), 185 (9), 137 (100), 109 (27). Exact mass [M]⁺ for C₁₉H₂₄O₃ requires 300.1726. Found: 300.1699.

13 β ,14 β -Epoxy-8-secoabietan-7,19-olide (17). Mp 96–98°. $[\alpha]_D^{25} + 20.6^\circ$ (MeOH; c 0.16). IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 1726, 1682. CD (MeOH): $[\theta]_{370.5} + 163$, $[\theta]_{310.5} + 7443$, $[\theta]_{275} - 47$, $[\theta]_{252} - 1128$. EIMS (70 eV) m/z (rel. int.): 334 (5), 316 (4), 300 (22), 181 (100). CIMS (CH₄) m/z 335 [M + 1]⁺. Exact mass for C₂₀H₃₁O₄ [M + 1]⁺ requires 335.2222. Found: 335.2214.

11 β -Hydroxy-14-oxochinan-19-al (18). Solid. EIMS (70 eV) m/z (rel. int.): 320 [M]⁺ (42), 302 (5), 292 (8), 277 (16), 259 (20), 206 (20), 149 (23), 135 (29), 127 (49), 109 (42), 43 (100).

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REFERENCES

1. Ying, S. S. (1985) in *Coloured Illustrated Flora of Taiwan*, Vol. 1, p. 168. Nat. Taiwan Univ, Taipei.
2. Hsu, C. M. (1960) in *Chemical Constituents of the Medicinal Plants in Taiwan*, Vol. 1, p. 174. Nat. Res. Inst. Chin. Med., Taipei.
3. Sawada, T. (1958) *Yakugaku Zasshi* **78**, 1020.
4. Fang, J. M., Lee, C. K. and Cheng, Y. S. (1992) *Phytochemistry* **31**, 3659.
5. Fang, J. M., Hsu, K. C. and Cheng, Y. S. (1989) *Phytochemistry* **28**, 1173.
6. Edwards, O. E., Nicolson, A. and Rodger, M. N. (1960) *Can. J. Chem.* **38**, 663.
7. Fang, J. M., Sheu, C. M. and Cheng, Y. S. (1986) *J. Chin. Chem. Soc., Taipei* **33**, 245.
8. Cambie, R. C., Grigor, B. A. and Tjong, M. I. (1981) *Aust. J. Chem.* **34**, 1073.
9. Fang, J. M., Lang, C. I., Chen, W. L. and Cheng, Y. S. (1991) *Phytochemistry* **30**, 2793.
10. Mangoni, L. and Caputo, R. (1967) *Tetrahedron Lett.* 673.
11. Cambie, R. C. and Mander, L. N. (1962) *Tetrahedron* **18**, 465.
12. Nishida, T., Wahlberg, I. and Enzell, C. R. (1977) *Org. Mag. Res.* **9**, 203.
13. Brandt, C. W. and Neubauer, L. G. (1939) *J. Chem. Soc.* 1031.
14. Lin, Y. T., Kuo, Y. H. and Chang, B. H. (1975) *J. Chin. Chem. Soc., Taipei* **22**, 331.
15. Conner, A. H., Nagasampagi, B. A. and Rowe, J. W. (1980) *Phytochemistry* **19**, 1121.
16. Fukushima, I., Sayama, Y., Kyogoku, K. and Murayama, H. (1968) *Agric. Biol. Chem.* **32**, 1103.
17. Tabacik, C. and Poisson, C. (1971) *Phytochemistry* **10**, 1639.
18. Wenkert, E. and Jackson, B. G. (1958) *J. Am. Chem. Soc.* **80**, 211.