Diterpenoids from the Leaves of *Juniperus chinensis* var. *kaizuka*

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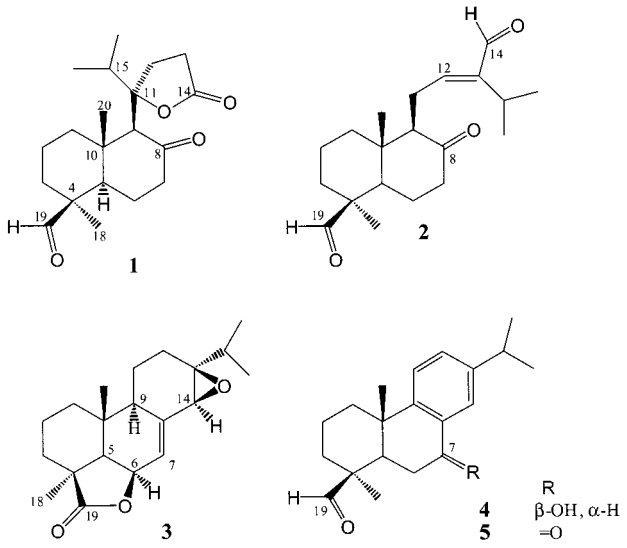
Two diterpenoids based on novel carbon skeletons, namely, 8,19-dioxo-8,14-*seco*-chinan-14,11-olide (**1**) and 8-oxo-8,14-*seco*-abiet-12-en-14,19-dial (**2**), have been isolated, together with two new abietane diterpenoids, 13*β*,14*β*-epoxyabiet-7-en-19,6*β*-olide (**3**) and 7*β*-hydroxyabieta-8,11,13-trien-19-al (**4**), from the leaves of *Juniperus chinensis*. Their structures were determined by spectroscopic methods, including 2D NMR, and, in the case of **1**, X-ray crystallographic analysis.

Juniperus chinensis var. *kaizuka* (Cupressaceae), called locally "long-bo", is a perennial shrub.[1] It has been used in traditional folk medicine for the treatment of various diseases, including urocystitis and uterine bleeding.[2] We become interested in the constituents of this plant due to the biological activity of related species in the same genus. In the present investigation on the constituents of the leaves of this species, two new diterpenoids based on new carbon skeletons have been isolated, 8,19-dioxo-8,14-*seco*-chinan-14,11-olide (**1**) and 8-oxo-8,14-*seco*-abiet-12-en-14,-19-dial (**2**), along with two new abietane diterpenoids, 13*β*,14*β*-epoxyabiet-7-en-19,6*β*-olide (**3**) and 7*β*-hydroxyabieta-8,11,13-trien-19-al (**4**). This paper deals with the isolation and structural elucidation of compounds **1**−**4**.



The acetone-soluble portion of the leaves of *J. chinensis* var. *kaizuka* was extracted with ethyl acetate. This extract was subjected to repeated gravity flow column chromatography and HPLC to give the four new diterpenoids, **1**−**4**.

Compound **1** was obtained as a colorless solid with the molecular formula $C_{20}H_{30}O_4$, as established by HREIMS. The IR absorption bands at 1706 and 1761 cm^{-1} confirmed the presence of a ketone (or aldehyde) and a *γ*-lactone carbonyl group, respectively. The ^1H NMR spectrum exhibited two singlet methyls (*δ* 0.91 and 1.09), an isopropyl group

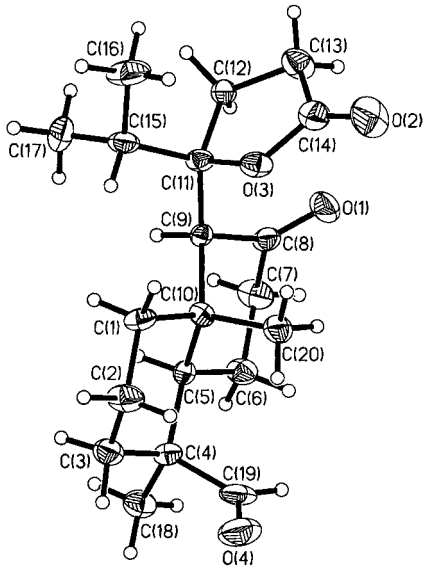


Figure 1. ORTEP diagram of the crystal structure of **1**.

[*δ* 0.83 (d), 0.83 (d), and 2.14 (sept.)], a singlet proton at *δ* 3.10 (s, H-9), and an aldehyde proton at *δ* 9.75. The ^{13}C NMR and DEPT data revealed the presence of four methyls, seven methylenes, three methines, and three quaternary carbons, in addition to three carbonyl carbons (*δ* 210.4, 204.4, and 177.2). 2D NMR experiments (COSY, HMQC, and HMBC) enabled the full assignments of all proton and carbon atoms (Tables 1 and 2). Of particular significance were the long-range correlations between C-15 (δ_C 37.4) and H-9 (*δ* 3.10) and H-12 (*δ* 2.49), as well as the correlations between C-11 (δ_C 91.7), H-9 (*δ* 3.10), H-12 (*δ* 2.49), H-16 (*δ* 0.83), and H-17 (*δ* 0.83). These data confirmed the location of the isopropyl group at C-11. In a NOE experiment on **1**, irradiation of H-18 (*δ* 1.09) caused a 7% enhancement of H-5 (*δ* 1.86). The structure and relative stereochemistry of **1** were confirmed by X-ray analysis and determined to be 8,19-dioxo-8,14-*seco*-chinan-14,11-olide. The occurrence of an isopropyl group at the C-11 position and bond cleavage between C-8 and C-14 is previously unknown among the structural class of diterpenoids represented by **1**. The most plausible biosynthesis pathway for the formation of compound **1** from chinanoxal (**6**)[3] is the oxidation at C-8 and C-14 to the diketone and then esterification to the novel secoditerpenal **1** (Scheme 1).

Analysis of the DEPT, ^{13}C NMR, and HREIMS spectra of **2** gave the molecular formula of $C_{20}H_{30}O_3$, indicating six degrees of unsaturation. The IR absorption bands at 1706

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**4** ($CDCl_3$, δ values in ppm)[a]

position	1	2	3	4
H-1	1.55 ddd (14,3,3)	1.03 n.r.[b]	1.18 m	1.34 ddd (13,13,4)
	1.72 br dd (14,2)	1.98 br dd (14,1)	1.50 n.r.[b]	2.23 n.r.[b]
H-2	1.46 qt (14,3)	1.55 m	1.49 n.r.[b]	1.63 m
	1.60 ddd (14,14,3)		1.68 n.r.[b]	1.74 m
H-3	1.10 n.r.[b]	1.11 ddd (14,14,2)	1.45 n.r.[b]	2.23 n.r.
	2.10 ddd (14,3,3)	2.09 br dd (14,2)	2.09 br dd (14,2)	
H-5	1.86 dd (14,3)	1.50 dd (11,9)	1.71 d (5)	2.00 n.r.[b]
H-6	2.02 ddd (14,14,4)	1.88 m	4.87 dd (5,2)	2.01 n.r.[b]
	2.36 n.r.[b]	2.04 m		2.58 dd (13,7)
H-7	2.45 n.r.[b]	2.40 ddd (17,11,6)	6.14 dd (4,2)	4.81 dd (10,7)
		3.01 ddd (17,8,5)		
H-9	3.10 s	2.01 n.r.[b]	1.68 n.r.[b]	
H-11		2.59 dd (11,7)	1.35 m	7.15 d (8)
H-12	2.49 n.r.[b]	6.78ddd (7,7,3)	1.59 n.r.[b]	7.08 dd (8,2)
			2.01 dt (12,3)	
H-13	2.25 n.r.[b]			
	2.45 n.r.[b]			
H-14		9.36 s	3.20 s	7.41 d (2)
H-15	2.14 sept. (7)	2.59 sept. (7)	1.62 sept. (7)	2.87 sept. (7)
H-16	0.83 d (7)	1.07 d (7)	0.97 d (7)	1.22 d (7)
H-17	0.83 d (7)	1.07 d (7)	0.93 d (7)	1.22 d (7)
H-18	1.09 s	1.04 s	1.27 s	1.09 s
H-19	9.75 s	9.87 s		9.79 s
H-20	0.91 s	0.67 s	0.79 s	1.09 s

[a] Coupling constants (Hz) are shown in parentheses. [b] Overlapping signal (n.r. = not resolved).

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**4** ($CDCl_3$, δ values in ppm)

carbon	1	2	3	4
C-1	40.6 t	38.2 t	32.6 t	38.3 t
C-2	19.2 t	18.4 t	17.9 t	19.1 t
C-3	34.0 t	35.2 t	27.8 t	33.7 t
C-4	48.4 s	47.3 s	42.5 s	48.1 s
C-5	56.7 d	50.7 d	51.9 d	49.8 d
C-6	23.9 t	21.2 t	72.8 d	29.8 t
C-7	43.6 t	42.0 t	125.1 d	71.3 d
C-8	210.4 s	215.1 s	141.2 s	137.5 s
C-9	65.1 d	48.0 d	47.3 d	144.8 s
C-10	46.5 s	36.9 s	32.8 s	38.8 s
C-11	91.7 s	24.2 t	15.9 t	124.9 d
C-12	29.0 t	151.2 d	23.5 t	124.8 d
C-13	27.1 t	144.0 s	64.1 s	146.8 s
C-14	177.2 s	194.6 d	59.6 d	126.0 d
C-15	37.4 d	40.7 d	33.7 d	33.7 d
C-16	16.0 q[a]	18.2 q[a]	17.7 q[a]	24.0 q[a]
C-17	17.3 q[a]	18.3 q[a]	18.1 q[a]	24.1 q[a]
C-18	24.9 q	24.2 q	24.0 q	24.2 q
C-19	204.4 d	205.5 d	182.2 s	205.1 d
C-20	17.4 q	14.3 q	17.9 q	23.8 q

[a] The assignments can be interchanged.

and 1689 cm^{-1} showed a ketone (or aldehyde) and a conjugated carbonyl group, respectively. The ^{1}H NMR spectrum (Table 1) revealed partial structural characteristics such as two aldehydes [δ 9.36 (s) and 9.87 (s)], a trisubstituted double bond [δ 6.78 (ddd)], an isopropyl group [δ 1.07 (d), 1.07 (d), 2.59 (sept.)], and two methyl groups [δ 1.04 (s) and 0.67 (s)]. Detailed analysis of HMBC data revealed correlations between C-12 (δ_C 151.2) and H-14 (δ 9.36) and H-15 (δ 2.59), as well as correlations of C-8 (δ 215.1) with H-11 (δ 2.59) and H-9 (δ 2.01). Irradiation of H-20 (δ 0.67) caused a 14% NOE at H-19 (δ 9.87), demonstrating that H-19 and H-20 have the same orientation. Irradiation of H-14 (δ 9.36) also caused a 23% enhancement of H-12 (δ 6.78), which established an *E* orientation of $\Delta^{12,13}$. The structure of **2** was thus assigned as 8-oxo-8,14-*seco*-abiet-12-en-14,19-dial. Compound **2** is the first example of a 8,14-*seco*-abietane type diterpenoid.

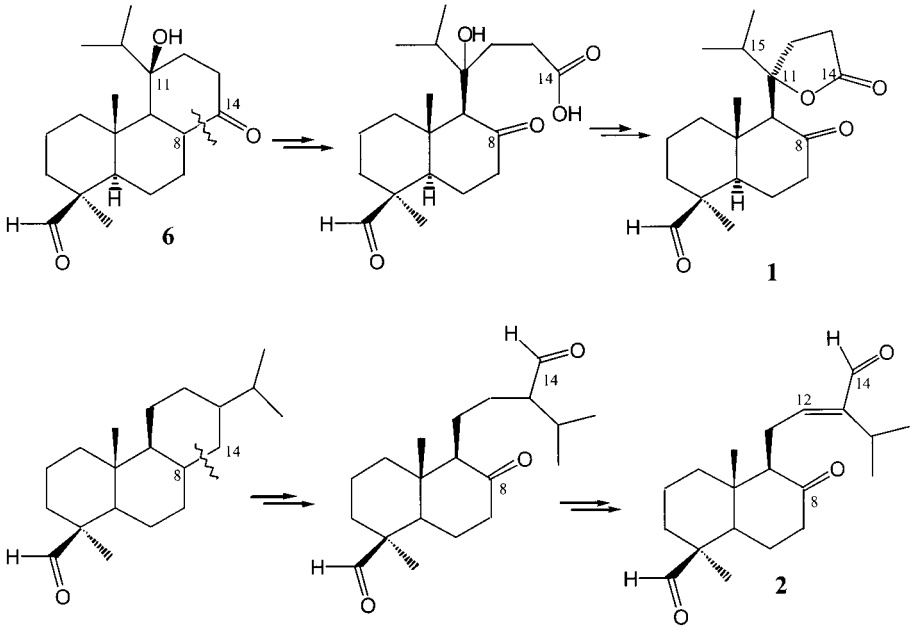
Compound **3** showed a molecular ion at *m*/*z* 316, and HREIMS indicated a molecular formula of $C_{20}H_{28}O_3$. The IR absorption bond at 1745 cm^{-1} indicated a γ-butyrolactone group. The ^{1}H NMR spectrum showed two methyl groups at δ 0.79 (s) and 1.27 (s), an isopropyl group at δ 0.93 (d), 0.97 (d), and 1.62 (sept.), and a proton on a trisubstitued double bond at δ 6.14 (dd). Comparison of the ^{1}H and ^{13}C NMR data of **3** with those of 13β-hydroxy-7-oxoabiet-8(14)-en-19,6β-olide[3] suggested that **3** is an abietane derivative. The ^{13}C NMR signals at δ 182.2 and 72.8 showed the characteristic resonances of a lactone moiety, and the signals at δ 64.1 and 59.6 were indicative of the presence of an epoxy group. The HMBC experiment revealed correlations of C-19 (δ_C 182.2) with H-18 (δ 1.27) and H-5 (δ 1.71) and C-7 (δ_C 125.1) with H-5 (δ 1.71) and H-14 (δ 3.20). The stereochemistry of **3** was determined by a NOE experiment as well as comparison with reported data.[4] In this genus, among naturally occurring abietane diterpenes whose configuration at C-13 is known, the isopropyl group is α-orientated.[4,6] Irradiation of H-7 (δ 6.14) caused a 5% enhancement of H-14 (δ 3.20), while irradiation of H-18 (δ 1.27) caused 8% and 9% enhancements of H-5 (δ 1.71) and H-6 (δ 4.87), respectively. Thus, the structure of **3** was determined as 13β,14β-epoxyabiet-7-en-19,6β-olide.

Compound **4**, $[M]^+$ *m*/*z* 300.2081, was assigned the molecular formula $C_{20}H_{28}O_2$. The proton resonance at δ 9.79 (s) was diagnostic of an aldehyde group. The compound was assigned as an abietane-type diterpene from the presence of three aromatic protons [δ 7.08 (dd, J = 8, 2 Hz), 7.15 (d, J = 8 Hz), and 7.41 (d, J = 2 Hz)] and an isopropyl group [δ 1.22 (d),1.22 (d), and 2.87 (sept.)]. The methine proton (H-7) geminal to the hydroxyl group as a double of doublets (J = 10, 7 Hz) indicated its axial orientation.[5] The structure of **4** was assigned, accordingly, as 7β-hydroxyabieta-8,11,13-trien-19-al. Compound **4** was unstable as it underwent autoxidation to give 7-oxoabieta-8,11,13-trien-19-al (**5**).[6]

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto (MP-500D) micro-melting point apparatus. Optical rotation measurements were conducted on a JASCO DIP-1000 instrument; a quartz cuvette (length 10 cm) was used. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. UV spectra were recorded on a

Scheme 1. Postulated Biosynthesis Pathways of **1** and **2**



Hitachi U-3210 spectrophotometer. ^{1}H and ^{13}C NMR spectra were run on a Bruker AM-300 spectrometer, with 2D NMR spectra run on a Bruker DMX-300 spectrometer, using $CDCl_3$ as solvent. The resonances of residual $CDCl_3$ at δ_H 7.24 and of $CDCl_3$ at δ_C 77.0 were used as internal references for the ^{1}H NMR and ^{13}C NMR spectra, respectively. Mass spectra were recorded (Finnigan TSQ-46C spectrometer) at an ionizing voltage of 70 eV. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-HX 110 spectrometer. The X-ray crystallographic data were collected on a Siemens Smart CCD diffractometer using graphic-monochromated Mo Kα radiation, and Waters M501 HPLC with Lichrosorb Si-60 (7 μm) columns (Waters R401 RI detector) were used. Merck 9385 silica gel (70−230 mesh, 230−400 mesh, ASTM) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis.

Plant Material. The leaves of *J. chinensis* var. *kaizuka* were collected on the campus of the National Taiwan University, Taipei, in April 1995. The plant was identified by Mr. Muh-Tsuen Gun, formerly of the Department of Botany, National Taiwan University. A voucher specimen (no. LB.001) is deposited in the Herbarium of the Department of Food Nutrition, Chung-Hwai College of Medical Technology, Tainan, Taiwan.

Extraction and Isolation. The fresh leaves of *J. chinensis* var. *kaizuka* (18 kg) were extracted with Me_2CO (70 L) at room temperature (7 days × 3). The Me_2CO extract was evaporated in vacuo to give 990 g of a residue, which was filtered through a column of activated charcoal, concentrated, and partitioned between EtOAc−H_2O (1:1 × 3). The combined EtOAc extracts were concentrated to give an oil (150 g), which was absorbed on 315 g of Si gel and then chromatographed on a column packed with 2.5 kg of Si gel by elution with gradients of hexane and EtOAc. Fractions of 500 mL were collected. Separation and purification of the components was carried out with HPLC: (i) hexanes−EtOAc (97:3), affording compounds **2** (12 mg) and **3** (3 mg); (ii) hexanes−EtOAc (96:4), affording compound **4** (8 mg); (iii) hexanes−EtOAc (50:50), affording compound **1** (21 mg).

8,19-Dioxo-8,14-*seco*-chinan-14,11-olide (1): colorless solid; mp 163−164 °C; $[\alpha]^{27}_D$ −101.5° (*c* 0.47, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 215 (1864), 284 (14) nm; IR (KBr) ν_{max} 2960, 1761, 1706, 1462, 1372, 1224, 1108, 984, 921, 768, 733 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 334.2160 (calcd for $C_{20}H_{30}O_4$, 334.2145); EIMS *m/z* 335 [M + 1]$^+$ (11), 307 (6), 291 (100), 273 (9), 245 (7), 193 (19).

8-Oxo-8,14-*seco*-abiet-12-en-14,19-dial (2): oil; $[\alpha]^{29}_D$ +7.1° (*c* 0.42, MeOH); UV (MeOH) λ_{max} (ϵ) 234.8 (3470) nm; IR (neat) ν_{max} 2926, 1706, 1689, 1629, 1458, 1380, 1185, 979, 898 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 318.2202 (calcd for $C_{20}H_{30}O_3$, 318.2196); EIMS *m/z* 318 [M]$^+$ (42), 275 (47), 229 (41), 201 (43), 109 (49), 44 (100).

13*β*,14*β*-Epoxyabiet-7-en-19,6*β*-olide (3): oil; $[\alpha]^{25}_D$ −8.8° (*c* 0.12, $CHCl_3$); IR (neat) ν_{max} 2933, 1745, 1455, 1379, 1192, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 316.2048 (calcd for $C_{20}H_{28}O_3$, 316.2039); EIMS *m/z* 316 [M]$^+$ (15), 273 (2), 253 (5), 229 (9), 187 (9), 159 (18), 147 (19), 109 (24), 43 (100).

7*β*-Hydroxyabieta-8,11,13-trien-19-al (4): oil; IR (neat) ν_{max} 3422, 2955, 1710, 1492, 1458, 1376, 1245, 1073, 1023, 911, 825 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 300.2081 (calcd for $C_{20}H_{28}O_2$, 300.2090); EIMS *m/z* 300 [M]$^+$ (41), 285 (5), 257 (7), 239 (16), 197 (18), 162 (33), 83 (90), 84 (100).

Autoxidation of 7*β*-Hydroxyabieta-8,11,13-trien-19-al (4) to 7-oxoabieta-8,11,13-trien-19-al (5). The compound **4** was unstable on standing in $CHCl_3$ solution at room temperature and underwent autoxidation to yield **5**. The spectral data ([α], NMR, MS) of **5** were in full agreement with those reported previously.[6]

X-ray Crystal Structure Analysis of 1. A colorless crystal of **1** with dimensions 0.45 × 0.40 × 0.30 mm was selected for X-ray analysis. The crystallographic data were collected on a Enraf-Nonius CAD4 diffractometer using graphite-monochromated Mo Kα radiation. Structure analysis was made using the SHELXTL program on a PC.[7] The compound crystallized in the space group $P2_12_12_1$, a = 6.569(2) Å, b = 11.267(2) Å, c = 24.625(9) Å, orthorhombic, V = 1822.4(9) Å^3, Z = 4, D_{calc} = 1.219 mg/m^3, λ = 0.71073 Å, μ(Mo Kα) = 0.083 mm^{-1}, F(000) = 728, and T = 295(2) K. A total of 1871 reflections were collected in the range 1.65° ≤ θ ≤ 24.97°, of which only 1871 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. Crystal decay was monitored every 60 min, and no decay was observed. An empirical absorption correction was applied. The structure was solved by the direct method and refined by full-matrix least-squares on F values. Non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0451, R_w = 0.0674 with goodness-of-fit = 0.958. Scattering factors were taken from the *International Tables for X-ray Crystallography*.[8]

Supporting Information Available: X-ray crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data center.

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