Two New Sesquiterpenes, 12-Hydroxy-α-longipinene and 15-Hydroxyacora-4(14),8-diene, from the Heartwood of *Juniperus chinensis* LINN. var. *tsukusiensis* MASAM.

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The following constituents were isolated from the heartwood of *Juniperus chinensis* LINN. var. *tsukusiensis* MASAM.: cedrol, thujopsenal, widdrol, sandaracopimaric acid, hinokiic acid, 12-hydroxy-α-longipinene and 15-hydroxyacora-4(14),8-diene. The latter two compounds are new tricyclic and spirobicyclic sesquiterpenes, and their structures were elucidated on the basis of spectral and chemical evidence.

Key words *Juniperus chinensis* var. *tsukusiensis*; tricyclic sesquiterpene; spirobicyclic sesquiterpene; 12-hydroxy-α-longipinene; 15-hydroxyacora-4(14),8-diene

The components of *Juniperus* species contain many natural products with various skeletons.[1] We have examined the chemical principles of the heartwood of *J. squamata* LAMB. var. *morrisonicola* HAY.,[2] heartwood of *J. formosana* HAY.,[3] roots of *J. chinensis* LINN.,[4] bark of *J. chinense* LINN. var. *kaizuca* HORT. ex ENDL.,[5] and bark of *J. formosana* HAY. var. *concolar* HAY.,[6] which are indigenous in Taiwan. We have now investigated the other indigenous species of *Juniperus*, *Juniperus chinensis* LINN. var. *tsukusiensis* MASAM., and found five known components, cedrol (**1**),[2d] thujopsenol (**2**),[7] widdrol (**3**),[1d] sandaracopimaric acid (**4**),[3b] and hinokiic acid (**5**),[1d] together with a new tricyclic sesquiterpene, 12-hydroxy-α-longipinene (**6a**) and a new spirobicyclic sesquiterpene, 15-hydroxyacora-4(14),8-diene (**7a**) in the heartwood. The known compounds were identified by comparison with authentic samples, and in this paper we describe the structural elucidation of the two new sesquiterpenes (Chart 1).

12-Hydroxy-α-longipinene (**6a**), a viscid liquid, has the molecular formula $C_{15}H_{24}O$ on the basis of exact mass of *m/z* 220.1829 and elemental analysis. It shows infrared (IR) absorption bands at 3330 (–OH), 1641 and 810 cm^{-1} (–C=C–H). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table 1) revealed that **6a** has three singlet methyl groups [δ 0.81, 0.81, 0.89 (s, each 3H)], a trisubstituted olefinic proton [δ 5.43 (br s, 1H)], and a primary alcohol attached to olefin [δ 3.96 (br s, 2H)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) data (Table 1) of **6a** show that it contains three methyl groups

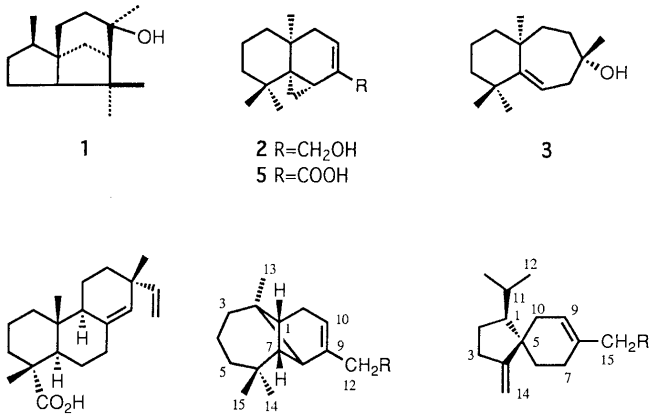

Chart 1

Table 1. ^{1}H- and ^{13}C-NMR Data (δ-Value) for **6b**[8] and **6a**, and HMBC Correlation for **6a**

H	**6b**[a]	**6a**[b]	C	**6b**[a]	**6a**[b]	Protons correlated
1	2.09	2.10 m	1	40.0	40.1 d	H-8, H-11
3	1.36	1.34 m	2	39.9	39.8 s	H-1, H-3, H-7, H-8, H-11, H-13
	1.60	1.59 m	3	41.3	40.9 t	H-4, H-13
4	1.32	1.33 m	4	21.8	21.7 t	H-3, H-5
	1.56	1.57 m	5	39.3	39.0 t	H-3, H-4, H-14, H-15
5	1.36	1.31 m	6	32.7	32.8 s	H-5, H-7, H-8, H-14, H-15
	1.56	1.55 m	7	58.9	59.1 d	H-5
7	1.46	1.45 m	8	46.5	42.6 d	H-7, H-12
8	2.00	2.21 d (6.0)	9	147.6	151.0 s	H-8, H-10, H-11, H-12
10	5.17	5.43 br s	10	117.2	118.9 d	H-8, H-11, H-12
11	2.22	2.28 br s	11	34.3	34.1 t	H-7, H-10
12	1.66	3.96 br s	12	22.8	65.9 t	H-8, H-10
13	0.83	0.81 s	13	23.6	22.9 q	H-3
14	0.90	0.89 s	14	28.1	28.0 q	H-5, H-7, H-15
15	0.83	0.81 s	15	27.8	27.8 q	H-5, H-7, H-14

Figure in parentheses is coupling constant. *a*) 200, 50 MHz in $CDCl_3$. *b*) 300, 75 MHz in $CDCl_3$.

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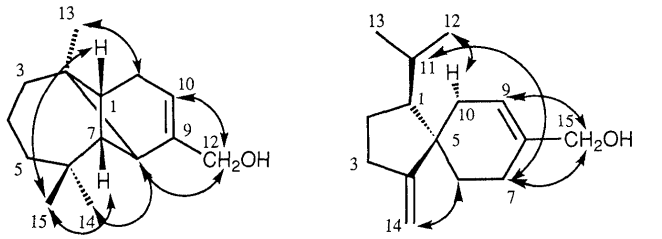

Fig. 1. NOE's Observed in the NOESY Spectra of **6a** and **7a** (in $CDCl_3$, 400 MHz)

(δ 22.9, 27.8, 28.0), a trisubstituted olefin (δ 118.9, 151.0), a hydroxymethyl group (δ 65.9), four secondary carbons, three tertiary carbons, and two quaternary carbons, based on a distortionless enhancement by polarization transfer (DEPT) experiment. From heteronuclear multiple quantum coherence (HMQC) experiments, proton and carbon correlation of **6a** can be assigned as in Table 1. From the above evidence, **6a** is a tricyclic sesquiterpene containing an endocyclic double bond. A comparison of ^{1}H- and ^{13}C-NMR data between **6a** and α-longipinene (**6b**)[8] suggested that **6a** possesses the same carbon skeleton as **6b** with a hydroxymethyl group instead of a methyl group. The acetylation of **6a** with Ac_2O in pyridine at room temperature yielded a monoacetate **6c** [1735 cm^{-1}, no hydroxyl absorption band; δ 1.99 (3H, s), 4.38, 4.47 (d, J=12.4 Hz, each 1H)]. The structure of **6a** was deduced to be as shown (12-hydroxy-α-longipinene) on the basis of HMQC, heteronuclear multiple-bond correlation spectroscopy (HMBC) (Table 1) and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra (^{1}H–^{1}H COSY correlation between H-1 and H-11, H-8 and H-10,-12, H-10 and H-11, -12). The nuclear Overhauser effect (NOE) correlations (Fig. 1) of **6a** confirmed the stereochemistry.

15-Hydroxyacora-4(14),8-diene (**7a**), a viscid liquid, has the molecular formula $C_{15}H_{24}O$ based on its elemental analysis and exact mass of m/z 220.1827 . The absorption bands at 3394, 1636, 890, and 840 cm^{-1} show that it contains a hydroxyl group, and trisubstituted and 1,1-disubstituted double bonds. Compound **7a** has an isopropyl group [δ 0.84, 0.93 (d, J=6.6 Hz, each 3H), 1.72 (m, 1H)], a trisubstituted olefinic proton [δ 5.66 (br s, 1H)], two terminal olefinic protons [δ 4.71 (br s, 2H)], and a hydroxymethyl group attached to a double bond [δ 3.98 (br s, 2H)] as deduced from the ^{1}H-NMR data (Table 2). The ^{13}C-NMR data (Table 2) of **7a** show that it contains two methyl groups (δ 21.0, 23.8), a trisubstituted olefin (δ 122.5, 137.3), a cyclopentane methylidene (δ 160.2, 103.8), a hydroxymethyl group (δ 67.3), five secondary carbons, two tertiary carbons, and one quaternary carbon, based on a DEPT experiment. The assignments of protons, carbons, proton–carbon correlations and long-range correlations were made by comparison with those of **7b**, with the aid of HMQC and HMBC experiments (Table 2). Comparison of the ^{1}H-NMR data of **7a** and acora-4(14),8-diene(**7b**)[9] suggested that compound **7a** is a derivative of acora-4(14),8-diene with a hydroxyl group located at C-15. The monoacetate **7c** [1735 cm^{-1}, no hydroxyl absorption band; δ 2.04 (s, 3H), 4.42 (br s, 1H) and 4.70 (m, 1H)] was obtained by acetylation of compound **7a**. Based on the HMQC, HMBC and ^{1}H–^{1}H COSY correlations (H-2 and H-3, H-6 and H-7, H-9 and H-10, H-11 and H-12, H-13), the structure of **7a** can be assigned as 15-hydroxyacora-4(14),8 or 7-diene. The NOE correlations (Fig. 1) of **7a** suggested that the diene was located at the 4 (14) and 8 positions.

Table 2. ^{1}H- and ^{13}C-NMR Data (δ-Value) for **7b**[9] and **7a**, and HMBC Correlation for **7a**

H	**7b**[a]	**7a**[b]	C	**7a**[b]	Protons correlated
1	1.45	1.46 m	1	54.0 d	H-2, H-3, H-11, H-12, H-13
2	1.45	1.43 m	2	24.4 t	H-1, H-3
	1.75	1.76 m	3	30.1 t	H-2, H-14
3	2.29	2.30 m	4	160.2 s	H-2, H-3, H-14
	2.46	2.37 m	5	46.6 s	H-3, H-6, H-7, H-9, H-11, H-14
6	1.42	1.43 m	6	24.1 t	H-7, H-10
	1.98	1.90 m	7	22.9 t	H-6
7	1.88	1.90 m	8	137.3 s	H-6, H-7, H-10, H-15
	1.98	2.02 m	9	122.5 d	H-7, H-10, H-15
9	5.35	5.66 br s	10	36.0 t	
10	1.73	1.78 br d (12.9)	11	27.9 d	H-12, H-13
	2.31	2.38 br d (12.9)	12	23.8 q	H-13
11	1.75	1.72 m	13	21.0 q	H-12
12	0.94	0.93 d (6.6)	14	103.8 t	H-3
13	0.84	0.84 d (6.6)	15	67.3 t	H-9
14	4.71	4.71 br s			
	4.76				
15		3.98 br s			

Figures in parentheses are coupling constant. *a*) 400 MHz. *b*) 300, 75 MHz in $CDCl_3$.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus without correction. IR spectra were recorded on a Perkin-Elmer 781 spectrometer. ^{1}H- and ^{13}C-NMR spectra were run on a Bruker AM 300 at 300 and 75 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ-values and coupling constants (J) in hertz (Hz). Electron impact (EI)-MS was taken on a JEOL JMS-100 spectrometer.

Extraction and Isolation The heartwood of *Juniperus chinensis* LINN. var. *tsukusiensis* MASAM. (2.5 kg) was extracted with methanol (30 l) four times (4 d for every time) at room temperature. The combined extracts were concentrated *in vacuo* to give a residue (98 g), which was subjected to chromatography on silica gel with a gradient solvent system (hexane–ethyl acetate) to give the following products: **1** (1.2 g) (with 10% ethyl acetate in hexane), **6a** (20 mg), **2** (35 mg), **7a** (12 mg), **3** (45 mg), **4** (75 mg), and **5** (450 mg) (with 20% ethyl acetate in hexane) in that order. The physical characteristics of the new compounds are as follows.

12-Hydroxy-α-longipinene (**6a**): Viscid liquid. $[\alpha]_D^{25}$ +30.0° (c=0.6, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3330, 2917, 2833, 1641, 1446, 1427, 1355, 1273, 1137, 1115, 1044, 993, 902, 850, 810. EI-MS (70 eV) m/z (rel. int. %): 220 (M^+, 15), 205 (5), 202 (10), 189 (23), 159 (20), 135 (100), 121 (26), 119 (48), 105 (72). ^{1}H- and ^{13}C-NMR: Table 1. *Anal.* Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found. C, 81.83; H, 10.91. HR-MS m/z: 220.1829. $C_{15}H_{24}O$ requires 220.1827.

15-Hydroxyacora-4(14),8-diene (**7a**): Viscid liquid. $[\alpha]_D^{25}$ −34.4° (c=0.6, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3394, 2950, 1636, 1458, 1379, 1043, 890, 840. EI-MS (70 eV) m/z (rel. int. %): 220 (M^+, 15), 189 (16), 177 (81), 159 (100), 131 (28), 117 (60), 94 (74). ^{1}H- and ^{13}C-NMR: Table 2. *Anal.* Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.69; H, 11.04. HR-MS m/z: 220.1827. $C_{15}H_{24}O$ requires 220.1827.

Acetylation of 6a with Acetic Anhydride in Pyridine Compound **6a** (10 mg) was allowed to react with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature overnight. Usual work-up gave **6c** (10 mg) [viscid liquid. IR ν_{max}^{neat} cm^{-1}: 3050, 1735, 1647, 1369, 1229, 1019, 956, 796. ^{1}H-NMR ($CDCl_3$) δ: 0.81, 0.83. 0.87, 1.99 (s, each 3H), 4.38, 4.47 (d, J=12.4 Hz, each 1H), 5.53 (br s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 20.9, 21.6,

21.6, 23.7, 27.6, 27.8, 32.7, 34.2, 39.0, 39.8, 40.8, 42.9, 59.0, 67.0, 122.7, 146.2, 171.0.

Acetylation of 7a with Acetic Anhydride in Pyridine Compound **7a** (10 mg) was acetylated under the same conditions as mentioned above. Usual work-up gave **7c** (10 mg) [viscid liquid. IR ν_{max}^{neat} cm^{-1}: 3045, 1735, 1677, 1364, 1225, 1022, 960, 886. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.92 (d, each 3H, J=6.6 Hz), 2.04 (s, 3H), 4.42 (br s, 1H), 4.70 (m, 1H), 5.71 (br s, 1H).

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