Three Labdane-Type Diterpenes from the Bark of *Juniperus formosana* HAY. var. *concolor* HAY.

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Three new labdane-type diterpenes, (13*S*)-15-hydroxylabd-8(17)-en-19-oic acid, (13*S*)-15-acetoxylabd-8(17)-en-19-oic acid, and (13*S*)-15-octadecanoyloxylabd-8(17)-en-19-oic acid, together with one known compound, *enantio*-oliveric acid, were found from the bark of *Juniperus formosana* HAY. var. *concolor* HAY. Their structures were elucidated on the basis of spectral data and chemical transformation.

Key words *Juniperus formosana* var. *concolor*; Cupressaceae; bark; (13*S*)-15-hydroxylabd-8(17)-en-19-oic acid; (13*S*)-15-acetoxylabd-8(17)-en-19-oic acid; (13*S*)-15-octadecanoyloxylabd-8(17)-en-19-oic acid

Ten species of *Juniperus* (*J*) are indigenous to Taiwan. From these species, we have studied the chemical components of the heartwood of *J. squamata* LAMB. var. *morrisonicola* (HAY.),[1] the heartwood of *J. formosana* HAY.,[2] the roots of *J. chinensis* LINN.,[3] and the bark of *J. chinense* LINN. var. *kaizuca* HORT. ex ENDL.[4] In a continuation of our investigation in this area, we have investigated the methanol extract of the bark of *J. formosana* HAY. var. *concolor* HAY. A new lignan (formosalactone)[5] was described in a previous report. We have now reinvestigated the same extract from the bark of *J. formosana* HAY. var. *concolor* HAY. and isolated three new diterpenes. In this paper we describe in detail the structural elucidation of three labdane-type diterpenes, (13*S*)-15-hydroxylabd-8(17)-en-19-oic acid (**1a**), (13*S*)-15-acetoxylabd-8(17)-en-19-oic acid (**1b**), and (13*S*)-15-octadecanoxylabd-8(17)-en-19-oic acid (**1c**), together with a known compound, *enantio*-oliveric acid (**1d**).

enantio-Oliveric acid (**1d**) was isolated as its dimethyl ester (**1e**) after treatment with diazomethane. The stereochemistry at C-13 and the absolute configuration of this dimethyl ester were established by comparison of the proton nuclear magnetic resonance (^{1}H-NMR) data of **1e** with those reported for *enantio*-dimethyl oliverate ($[\alpha]_D^{25}$ +49.5° in $CHCl_3$),[6] which were identical, and of the optical rotation, which showed the same sign. Hence **1e** must be dimethyl (13*S*)-labd-8(17)-en-15,19-dioate. The reduction of diester **1e** with lithium aluminum hydride yielded a diol, **1f**, which was identified as that reported in literature[6] by comparison of their ^{1}H-NMR and optical rotation data. Haeuser[7] has claimed that compound **1f** and imbricatadiol (**2**)[8] are distinguishable from their X-ray powder diffraction and IR spectra. Meanwhile, compound **1f** (mp 99—100 °C) also has a distinguishable melting point with imbricatadiol (mp 113—113.5 °C).[8]

(13*S*)-15-Hydroxylabd-8(17)-en-19-oic acid (**1a**) was obtained as an amorphous solid. It was deduced to have the molecular formula $C_{20}H_{34}O_3$ on the basis of its elemental analysis and mass spectrum (MS) [M^+ peak at 322]. It shows infrared (IR) absorption bands at 3370 (–OH), 3300—2500, 1689 (–COOH), and 3060, 1639, 888 cm^{-1} (terminal methylene). The ^{1}H-NMR spectrum (Table 1) of **1a** revealed that **1a** has two singlet methyl groups (δ 0.58 and 1.20), one doublet methyl group [δ 0.86 (d, J=6.2 Hz)], a primary alcohol [δ 3.64 (2H, m)], and a terminal methylene [δ 4.46 and 4.79 (each 1H, br s)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) data (Table 2) of **1a** indicated that it is a diterpene. The MS fragment ions at 304 ($M^+ - H_2O$), 276 ($M^+ - HCOOH$), 221 (structure **3**) and 121 (100%, structure **4**)[3b,4,9,10] and the presence of the C-10 methyl signal at a

Table 1. ^{1}H-NMR Data for **1a**—**c** and **1f** in $CDCl_3$

H	1a	1b	1c	1f
15	3.64 m	4.06 t (6.0)	4.06 t (6.0)	3.63 m
16	0.86 d (6.2)	0.89 d (6.0)	0.90 d (6.0)	0.87 d (6.4)
17	4.46 br s	4.47 br s	4.46 br s	4.45 br s
	4.79 br s	4.81 br s	4.81 br s	4.76 br s
18	1.20 s	1.22 s	1.23 s	0.94 s
19				3.34 d (10.9)
				3.72 d (10.9)
20	0.58 s	0.58 s	0.52 s	0.61 s
OAc		2.02 s		

Figures in parentheses are coupling constants.

Table 2. ^{13}C-NMR Data for **1a**, **1b** and **1c** in $CDCl_3$

C	1a	1b	1c
1	39.1	38.7	38.7
2	19.9	19.8	19.9
3	36.4	36.0	36.1
4	44.1	44.1	44.1
5	56.6	56.5	56.6
6	26.0	26.0	25.1
7	38.0	37.9	38.0
8	148.2	148.1	148.2
9	56.3	56.3	56.4
10	40.5	40.4	40.5
11	21.1	20.9	21.0
12	39.4	39.1	39.1
13	30.2	30.4	30.6
14	38.7	35.1	35.3
15	61.1	63.0	62.8
16	19.8	19.6	19.7
17	106.3	106.3	106.4
18	29.0	28.9	28.8
19	183.3	183.9	183.4
20	12.7	12.6	12.8
OC(O)R		171.2	174.0
OC(O)CH$_3$		20.9	

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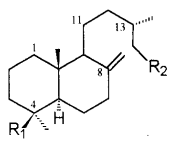

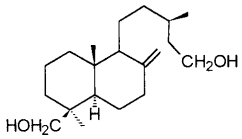

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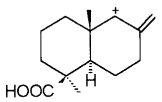
1a R_1=COOH, R_2=CH_2OH
1b R_1=COOH, R_2=CH_2OAc
1c R_1=COOH, R_2= $CH_2OC(O)(CH_2)_{16}CH_3$
1d R_1= R_2=COOH
1e R_1= R_2=$COOCH_3$
1f R_1=R_2=CH_2OH
1g R_1=COOMe, R_2=CH_2OH
1h R_1=COOMe, R_2=CH_2OAc



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higher field at δ 0.58 (it received the shield effect from C-4 carboxylic acid) showed that **1a** is a labdane-type diterpene with carboxylic acid attached at the C-4 axial orientation.[4,9] Comparison of the ^{1}H-NMR spectra between **1a** and (13*S*)-labd-8(17)-en-15,19-diol (**1f**) suggested that **1a** possessed of same carbon skeleton as **1f** with a carboxylic acid group instead of a hydroxymethyl group. In order to give the chemical correlation to compound **1f**, **1a** was methylated with diazomethane to yield an ester, **1g** [amorphous solid; 3376 (–OH), 1721 (–COOMe); δ 3.57 (3H, s)]. Reduction of **1g** with lithium aluminum hydride in dry tetrahydrofuran (THF) afforded **1f**. Therefore, compound **1a** can be assigned as (13*S*)-15-hydroxylabd-8(17)-en-19-oic acid but not imbricatalic acid.

The second labdane derivative is compound **1b**. It is an amorphous solid. MS *m/z* (%) 364 (M^+, 40) and elemental analysis gave the molecular formula $C_{22}H_{36}O_4$. The ^{1}H-NMR signals (Table 1) of **1b** showed it is a derivative of **1a** with an extra acetyl group. The ^{13}C-NMR data (Table 2) of **1b** also confirmed the assigned structure. The chemical correlation between **1a** and **1b** was described as follows. Methylation of **1b** with diazomethane yielded methyl ester **1h** [1737 cm^{-1}; δ 3.56 (3H, s, $-COOCH_3$)], which was subsequently reduced with lithium aluminum hydride in dry THF to afford **1f**. Compound **1b** was also obtained from **1a** by acetylation with acetic anhydride in pyridine. These results deduced the structure of **1b** as (13*S*)-15-acetoxylabd-8(17)-en-19-oic acid.

Compound **1c** is also a labdane-type diterpene. FAB-MS (positive) [*m/z*: 589 $(M+H)^+$, 100%] and elemental analysis gave the molecular formula $C_{38}H_{68}O_4$. It is an amorphous solid and contains a carboxyl group (3300—2500, 1697 cm^{-1}), ester group (1731, 1260, 1180 cm^{-1}), and terminal methylene (3050, 1640, 888 cm^{-1}) referring to its IR spectrum. The ^{1}H-NMR spectrum (Table 1) of **1c** revealed that **1c** is also a derivative of **1a** with an extra octadecanoyl group [δ 0.90 (3H, J=6.0 Hz), 1.23 (about 28H, br s), 1.50 (2H, quintet, J=6.0 Hz), 2.29 (t, 2H, J=6.0 Hz), and 4.06 (t, 2H, J=6.5 Hz)]. The ^{13}C-NMR data (Table 2) of **1c** also coincided with the assigned structure. When **1c** was heated in 1 N NaOH in MeOH and H_2O (1 : 1) solution at 70 °C, the products were **1a** and stearic acid after purification. Basic hydrolysis is sufficient to confirm the structure of **1c**. Therefore, the structure of **1c** can be assigned as (13*S*)-15-octadecanoyloxylabd-8(17)-en-19-oic acid.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. 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 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ-value, and coupling constants (J) are given in hertz (Hz). Electron impact-MS (EIMS) was taken on a JEOL-JMS-100 spectrometer.

Extraction and Isolation The bark of *J. formosana* HAY. var. *concolor* HAY. (980 g) was extracted with methanol (20 l) five times (for 4 d each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a residue (48 g), which was subsequently subjected to chromatography on silica gel using a gradient (hexane–ethyl acetate) system. Compounds **1c** (52 mg), **1b** (34 mg), **1a** (47 mg), and *enantio*-oliveric acid (**1d**) were eluted with 10% ethyl acetate in hexane, 20% ethyl acetate in hexane, 40% ethyl acetate in hexane, and 10% methanol in ethyl acetate, respectively. *enantio*-Oliveric acid (**1d**) was purified as its dimethyl ester (**1e**).

(13*S*)-15-Hydroxylabd-8(17)-en-19-oic Acid (**1a**): An amorphous solid, $[\alpha]_D^{25}$ +17.9° (c=1.0, $CHCl_3$). IR (KBr) cm^{-1}: 3370, 3300—2500, 3060, 1689, 1639, 1229, 1149, 1050, 888. MS *m/z* (rel. int. %): 322 (8), 304 (10), 276 (35), 221 (13), 129 (33), 121 (100). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. *Anal.* Calcd for $C_{20}H_{34}O_3$: C, 74.49; H, 10.63. Found C, 74.65; H, 10.69.

(13*S*)-15-Acetoxylabd-8(17)-en-19-oic Acid (**1b**): An amorphous solid, $[\alpha]_D^{25}$ +19.7° (c=0.8, $CHCl_3$). IR (KBr) cm^{-1}: 3300—2500, 3045, 1732, 1690, 1639, 1237, 1040, 889, 800, MS *m/z* (rel. int. %): 364 (40), 318 (100), 304 (43), 235 (15), 221 (17), 207 (30), 166 (50), 136 (100), 121 (78). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. *Anal.* Calcd for $C_{22}H_{36}O_4$: C 72.49; H, 9.96. Found C, 72.68; H, 9.88.

(13*S*)-15-Octadecanoyloxylabd-8(17)-en-19-oic acid (**1c**): An amorphous solid, $[\alpha]_D^{20}$ +23.4° (c=0.7, $CHCl_3$). IR (KBr) cm^{-1}: 3300—2500, 3050, 1731, 1697, 1640, 1420, 1260, 1180, 988, 800, 725. FAB-MS (positive) *m/z* (rel. int. %): 589 [$(M+1)^+$, 100)], 543 [$(M+1)^+$–HCOOH, 36], 365 (12), 285 (60), 319 (27), 270 (75). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. *Anal.* Calcd for $C_{38}H_{68}O_4$: C, 77.49; H, 11.64. Found C, 77.78; H 11.56.

enantio-Dimethyl Oliverate (**1e**): A colourless gum, $[\alpha]_D^{15}$ +47.5° (c=1.1, $CHCl_3$) (lit. +49.5°).[6] IR (KBr) cm^{-1}: 1720, 1640, 1175, 890. MS *m/z* (rel. int. %): 364 (M^+, 18) 304 (60), 235 (37), 121 (100). ^{1}H-NMR: ($CDCl_3$) δ: 0.48 (s, 3H), 1.15, 3.58, 3.63 (s, each 3H), 0.93 (d, 3H, J=6.2 Hz), 4.45 and 4.80 (br s, each 1H).

Reduction of 1e by Lithium Aluminum Hydride $LiAlH_4$ (55 mg, 1.4 mmol) was added to a solution of **1e** (20 mg, 0.04 mmol) in dry THF (10 ml) and the reaction mixture was left at room temperature for 1 h. The reaction mixture was quenched with 0.06 ml of water. 10% Aqueous NaOH (0.06 ml) was subsequently poured into the reaction mixture and stirred for 5 min. Then, 0.1 ml of water was added, and the reaction mixture was stirred until the white precipitation appeared. After filtration, the filtrate was purified on silica gel chromatography and yielded **1f** (15 mg) [mp 99—100 °C] (lit. 98—100 °C).[6] $[\alpha]_D^{25}$ +32.6° (c=0.9, $CHCl_3$) (lit. +35°).[6] IR (KBr) cm^{-1}: 3292, 1638, 1060, 1035, 896. ^{1}H-NMR ($CDCl_3$) δ: 0.61, 0.94 (s, each 3H), 0.87 (d, 3H, J=6.4 Hz), 3.34, 3.72 (d, 1H, J=10.9 Hz, each), 3.63 (m, 2H), 4.45, 4.76 (br s, each 1H).

Methylation of 1a and 1b with Diazomethane Excess diazomethane in ether was added dropwise to a solution of compound **1a** (10 mg) or **1b** (10 mg) in methanol (3 ml), and the reaction mixture was left to stand for 30 min. The yellow reaction solution was evaporated and gave the product **1g** (10 mg) [amorphous solid: IR (KBr) cm^{-1}: 3376, 3062, 1721, 1638, 1330, 1246, 1153, 988, 888. ^{1}H-NMR ($CDCl_3$) δ: 0.45, 1.14, 3.57, (s, each 3H), 0.85 (d, 3H, J=6.4 Hz), 3.62 (m, 2H, H-15), 4.45, 4.78 (br s, each 1H)] or **1h** (10 mg) [amorphous solid. IR (KBr) cm^{-1}: 3056, 1737, 1639, 1365, 1240, 1152, 1091, 986, 888, 820. ^{1}H-NMR ($CDCl_3$) δ: 0.45, 1.13, 1.98, 3.56 (s, each 3H), 0.85 (d, 3H, J=6.0 Hz), 4.03 (m, 2H, H-15), 4.43, 4.78 (br s, each 1H)].

Reduction of 1g or 1h by Lithium Aluminum Hydride $LiAlH_4$ (50 mg, 1.3 mmol) was added to a solution of **1d** (8 mg, 0.02 mmol) or **1e** (9 mg, 0.02 mmol) in dry THF (10 ml), and the reaction mixture was left at room temperature for **1h**. The reaction mixture was quenched with 0.05 ml of water. 10% Aqueous NaOH (0.05 ml) was subsequently poured into the reaction mixture and stirred for 5 min. Then, 0.1 ml of water was added and the reaction mixture was stirred until the white

precipitation appearance. After filtration, the filtrate was purified using silica gel chromatography. Both yielded the same product, **1f** (each 7 mg) (mp 99—100 °C).

Acetylation of 1a Compound **1a** (10 mg) was allowed to react with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight. The usual work-up gave **1b** (10 mg).

Hydrolysis of 1c with Aqueous Sodium Hydroxide Compound **1c** (40 mg) and powdered NaOH (20 mg) were added to a solution of methanol and H_2O (each 5 ml), and then the mixture was heated at 70 °C for 6 h. H_2O (50 ml) was poured into the reaction mixture and then the mixture was acidified with 1 N HCl to pH 2. The aqueous solution was extracted with ethyl acetate (30 ml) three times. The combined extracts were purified on silica gel chromatography to yield stearic acid (13 mg) and compound **1a** (16 mg).

Preparation of 1c from 1a Stearic acid (7.1 g, 0.025 mol) was dissolved in 50 ml of $CHCl_3$ at 30 °C, and then 2.3 ml of fresh $SOCl_2$ (0.03 ml) was added dropwise to the solution for 30 min. To a solution of 20 mg of compound **1a** in 1 ml of dry pyridine at 5 °C, the above prepared stearyl chloride (3 ml) was added dropwise under a nitrogen atmosphere. After 30 min, the reaction mixture was quenched with ice water, and then the product was worked up as usual to afford **1c** (23 mg).

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