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Four New Sesquiterpenes from the Heartwood of *Juniperus formosana* var. *concolor*

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Four new sesquiterpenes, *cis*-4,5-dihydroxycorocalane (1), *trans*-4,5-dihydroxycorocalane (2), calamenen-10α-ol (3), and 15-hydroxycadalene (4) together with two know compounds, calamenen-10β-ol (5) and cadalene (6) were isolated from the heartwood of *Juniperus formosana* HAY. var. *concolor* HAY.

Key words *Juniperus formosana* var. *concolor*; Cupressaceae; *cis*-4,5-dihydroxycorocalane; *trans*-4,5-dihydroxycorocalane; calamenen-10α-ol; 15-hydroxycadalene

Only ten species of *Juniperus* (Cupressaceae) are indigenous to Taiwan. The chemical components of the heartwood of *J. squamata* LAMB. var. *morrisonicola* HAY.,[1)] *J. formosana* HAY.,[2)] *J. chinensis* LINN.,[3)] *J. chinense* LINN. var. *kaizuca* HORT. *ex* ENDL.,[4)] *J. chinensis* var. *tsukusiensis*,[5)] and the bark of *J. formosana* HAY. var. *concolor* HAY.[6)] have been studied in our laboratory. Recently, we reported the isolation of new diterpenes and sesquiterpenes from the heartwood of the latter species.[7)] Using the same extract, we have now purified in detail and have isolated four new sesquiterpenes, *cis*-4,5-dihydroxycorocalane (**1**), *trans*-4,5-dihydroxycorocalane (**2**), calamenen-10α-ol (**3**), and 15-hydroxycadalene (**4**) together with two known compounds, calamenen-10β-ol (**5**)[8,9)] and cadalene (**6**).[8)] This paper deals with the structural elucidation of these compounds.

Compound **1** was isolated as an amorphous solid with the molecular formula $C_{15}H_{22}O_2$, on the basis of the exact mass peak at *m/z* 234.1617. It showed infrared (IR) absorption bands at 3388 (–OH), 3035, 1609, 1504 (aromatic), 1385 and 1372 cm^{-1} (geminal dimethyl). The ^1H-NMR spectrum (Table 1) revealed one methyl (δ 2.17, s) and one isopropyl group [δ 1.19 and 1.28 (d, each 3H, J=6.8 Hz), 3.43 (sep, 1H, J=6.8 Hz)] attached to a phenyl group, a singlet methyl group (δ 1.14) geminal to a hydroxyl group, a methine proton (δ 4.55, s) bearing a hydroxyl group, and two 1,2,3,4-tetrasubstituted phenyl protons [δ 7.13 and 7.10 (d, each 1H, J=8.0 Hz)]. The remaining ^1H-NMR signals were present at δ 2.56 (ddd, 1H, J=18.0, 12.4, 6.8 Hz, H_α-2), 2.82 (dd, 1H, J=18.0, 6.8 Hz, H_β-2), 1.76 (dd, 1H, J=12.4, 6.8 Hz, H_α-3) and 2.07 (td, 1H, J=12.4, 6.8 Hz, H_β-3). From the heteronuclear multiple quantum coherence (HMQC) experiments, proton and carbon correlation of **1** was assigned as shown in Table 1. The signals at δ 2.56 and 2.82 were assigned as benzylic protons due to the chemical shift and larger geminal coupling constant (J=18.0 Hz). Nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlations were apparent between δ 2.17 (H_3-14) and 2.82 (H_β-2), 2.82 and 1.76 (H_α-3) and 1.76 and 1.14 (H_3-15). From these results the structure of **1** can be assigned as 4,5-dihydroxycorocalane. The heteronuclear multiple bond connective (HMBC) spectrum of **1** also confirmed the assigned structure. Relative stereochemistry was determined by the nuclear Overhauser effect (NOE) technique (see structure **7**). H-5 was determined to be in an equatorial orientation, due to 18.3% NOE with H-11. H_3-15 exhibited 12.7% NOE with H_α-5 and 17.5% NOE with H_α-2, which suggested that H_3-15 was located in an axial orientation. Therefore, compound **1** is a *cis*-4,5-dihydroxycorocalane.

Compound **2** was shown to be an isomer of **1** (the molecular formula $C_{15}H_{22}O_2$ was derived from HR-MS), and had hydroxyl, aromatic, and geminal dimethyl absorption bands in the IR spectrum. The ^1H- and ^{13}C-NMR data (Table 1) of **2** were closely related to those of **1**, the only slight difference being the signal for H_3-15, which resonated at lower field (δ 1.47, s) than the corresponding proton in **1**. The evidence indicated that **2** was a stereoisomer of **1**, a conclusion that was also proven by HMQC and HMBC techniques. The H-5 signal of **2** was present at δ 4.53 (H-5) and had 28.2% NOE with H-11. This indicated that H-5 was in an equatorial orientation. H_3-15 was assigned as equatorial, due to 9.6% NOE with H-5 and no NOE with H_α-2 (see structure **8**). Hence, compound **2** was identified as *trans*-4,5-dihydroxycorocalane.

Compounds **3** and **5** were isomers with the same formula $C_{15}H_{22}O$, as deduced from their electron impact-mass spectra (EI-MS) and ^{13}C-NMR spectral data (Table 1). Hydroxyl, aromatic, and geminal absorption bands were present in their IR spectra. In their ^1H-NMR spectra, the C-10 methyl was a singlet at δ 1.48 in **3**, and at δ 1.53 in **5**. Both compounds had the same chemical shift signal at δ 2.29 (3H, s), which was assigned to H_3-15. Two geminal dimethyl signals were present at δ 0.69 and 1.01 (d, each 3H, J=6.8 Hz) in **3** and at δ 0.75 and 1.05 (d, each 3H, J=7.4 Hz) in **5**. The aromatic region showed a typical 1,2,4-substitution pattern in the both compounds. Compound **3** spontaneously dehydrated in $CDCl_3$ solution over one month to give the known compound α-calacorene but **5** afforded α-calacorene on the same solution in only three days. This evidence supported the conclusion that both compounds are calamenen-10-ol. It was assumed that **3** and **5** have the 7*S*-configuration, since all calamenene derivatives isolated from the same source show 7*S*-configuration.[7b,d)] The NOE correlation between H-5 and H-7 in both **3** and **5** is about 14.5%. The major difference between **3** and **5** was the NOE between H-2 and H_3-14; 10.6% for **3** and 24.3% for **5**. A product named calamenen-10-ol has been isolated from *Siparuna macrotepala* by El-Seedi.[8)] Daniewski[9)] assigned the C-10 hydroxyl group of calamenen-10-ol as β-configuration, based on the biosynthetic assumption that the product is an aromatized derivative of T-cadinol (**9**) (both compounds were isolated from the same plant, *En-*

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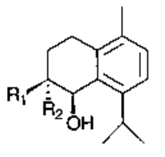
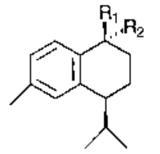
Table 1. ^{1}H- and ^{13}C-NMR Data for **1**—**5** (300 MHz and 75 MHz in $CDCl_3$)

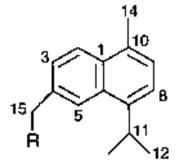
No.	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	133.9		134.0		141.5		132.0		140.2	
2	26.2	2.56 ddd (18.0, 12.4, 6.8) 2.82 dd (18.0, 6.8)	23.5	2.72 dd (9.3, 4.3)	128.2	7.48 d (8.5)	124.4	8.01 d (8.7)	128.4	7.46 d (7.9)
3	30.0	1.76 dd (12.4, 6.8) 2.07 td (12.4, 6.8)	28.5	1.82 dt (13.6, 4.3) 2.05 dt (13.6, 9.6)	125.7	7. 01 br d (8.5)	125.5	7.50 br d (8.7)	126.0	7.00 br d (7.9)
4	71.1		71.1		136.3		137.6		136.5	
5	71.1	4.55 s	71.0	4.53 s	126.9	7.00 s	121.6	8.10 br s	126.9	7.04 br s
6	147.3		147.4		138.8		132.5		139.9	
7	133.4		133.8		43.3	2.67 m	142.9		43.3	2.60 m
8	123.5	7.10 d (8.0)	123.7	7.10 d (8.1)	20.3		121.8	7.31 d (7.4)	19.5	
9	130.1	7.13 d (8.0)	130.0	7.14 d (8.1)	38.2		126.6	7.24 d (7.4)	37.6	
10	133.1		132.6		70.8		131.4		70.1	
11	27.8	3.43 sep (6.8)	26.5	3.42 sep (6.8)	21.1	2.03 m	28.3	3.73 sep (6.6)	21.2	1.98 m
12	23.7	1.19 d (6.8)	24.0	1.21 d (6.8)	17.1	0.69 d (6.8)	23.6	1.37 d (6.6)	17.5	0.75 d (7.4)
13	25.4	1.28 d (6.8)	25.3	1.28 d (6.8)	21.2	1.01 d (6.8)	23.7	1.37 d (6.6)	21.3	1.05 d (7.4)
14	19.6	2.17 s	19.6	2.20 s	30.5	1.48 s	19.4	2.65 s	30.8	1.53 s
15	22.4	1.14 s	27.7	1.47 s	30.8	2.29 s	66.0	4.87 s	31.2	2.29 s



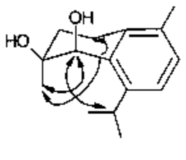
1 R_1=OH, R_2=CH_3
2 R_1=CH_3, R_2=OH



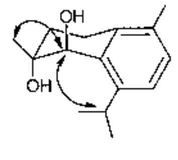
3 R_1=CH_3, R_2=OH
5 R_1=OH, R_2=CH_3



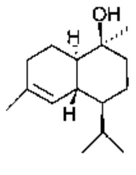
4 R=OH
6 R=H



7 NOE ←→



8 NOE ←→



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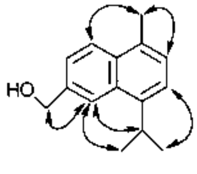

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tandrophragma cyclindricum). The physical data of calamenen-10β-ol[8] were identical with those of compound **5**. Therefore, the structure of compound **3** can be assigned as calamenen-10α-ol.

The fourth new compound **4** was also a sesquiterpene with the formula $C_{15}H_{18}O$ based on its high resolution (HR)-MS. Hydroxyl and aromatic absorption bonds were present in its IR spectrum. Signals for methyl (δ 2.65, s), isopropyl [δ 1.37 (d, 6H, J=6.6 Hz), 3.73 (sep, 1H, J=6.6 Hz)], and hydroxymethyl groups (δ 4.87, 2H, s) attached to a naphthalene nucleus were apparent in the ^{1}H-NMR. The appearance of low field signals due to [δ 7.24 and 7.31 (d, each 1H, J=7.4 Hz)] and [δ 7.05 (br d, 1H, J=8.7 Hz), 8.01 (d, 1H, J=8.7 Hz), 8.10 (br s, 1H)] suggested naphthalenic protons. Comparison of ^{13}C-NMR data of **4** with cadalene (**6**)[8] showed that **4** is a derivative of cadalene (**6**) except for an additional hydroxyl located at C-14 or C-15. Catalytic hydrogenation of **4** with Pd–C in acidic methanol solution yielded **6**. The hydroxyl group was located on C-15 by the NOESY technique (see structure **10**).

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AM 300 spectrometer. EI-MS and specific rotations were taken on a JEOL-JMS-HX300 spectrometer and a JASCO DIP-180 polarimeter, respectively.

Extraction and Isolation The heartwood of *J. formosana* Hay. var. *concolor* Hay. (2 kg) was extracted with MeOH (20 l) at room temperature 3 times. The MeOH extract was evaporated *in vacuo* to leave a black residue (189 g), which was chromatographed on silica gel (2 kg) with hexane/EtOAc, EtOAc, and EtOAc/MeOH gradient solvent systems. Six sesquiterpenes, cadalene (**6**) (5 mg) (2% EtOAc in hexane), calamenen-10β-ol (**5**)[8,9] (5 mg) and calamenen-10α-OH (**3**) (7 mg) (10% EtOAc in hexane), *trans*-4,5-dihydroxycorocalane (**2**) (6 mg) (10% EtOAc in hexane), *cis*-4,5-dihydroxycorocalane (**1**) (16 mg) (10% EtOAc in hexane), and 15-hydroxycadalene (**4**) (8 mg) (30% EtOAc in hexane) were obtained.

cis-4,5-Dihydroxycorocalane (**1**): Amorphous solid, $[\alpha]_D^{20}$ $-3.7°$ (c=0.3, $CHCl_3$). IR (KBr) cm^{-1}: 3388, 3035, 1609, 1504, 1385, 1372, 1039. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) m/z (rel. int. %) 234 (M^+, 20), 216 (40), 198 (18), 176 (52), 163 (85), 145 (100), 131 (46), 91 (50). HR-MS Calcd for $C_{15}H_{22}O_2$: 234.1620. Found 234.1618.

trans-4,5-Dihydroxycorocalane (**2**): Amorphous solid, $[\alpha]_D^{20}$ $+1.5°$ (c=0.3, $CHCl_3$). IR (KBr) cm^{-1}: 3432, 3041, 1605, 1510, 1384, 1370, 1245,

1111. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m*/*z* (rel. int. %) 234 (M^+, 30), 216 (65), 176 (50), 163 (90), 145 (100), 131 (45), 91 (40). HR-MS Calcd for $C_{15}H_{22}O_2$: 234.1620. Found 234.1617.

Calamenen-10α-ol (**3**): Amorphous solid, $[\alpha]_D^{20}$ $-95°$ (c=0.2, $CHCl_3$). IR (KBr) cm^{-1}: 3400, 3035, 1615, 1505, 1375, 1360. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m*/*z* (rel. int. %) 218 (M^+, 18), 201 (100), 175(20), 157 (70), 145 (8), 121 (10).

15-Hydroxycadalene (**4**): Oil. IR (neat) cm^{-1}: 3404, 3045, 1599, 1500, 1385, 1369, 1265, 1161, 888. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m*/*z* (rel. int. %) 214 (M^+, 52), 199 (100), 169 (30), 154 (30), 73 (52). HR-MS Calcd for $C_{15}H_{18}O$: 214.1358. Found: 214.1360.

Catalytic Hydrogenolysis of **4**: A solution of **4** (4 mg) and TsOH (3 mg) in 5 ml of MeOH was hydrogenated in the presence of 10% Pd–C (5 mg). After 8 h, the catalyst was removed by filtration and washed several times with MeOH. The combined filtrate and washings gave a product (3 mg) which was identical with cadalene.[8)]

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