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Two New Sesquiterpenes, (−)-15-Hydroxycalamenene and (−)-1-Hydroxy-1,3,5-bisabolatrien-10-one, from the Heartwood of *Juniperus formosana* HAY. var. *concolor* HAY.

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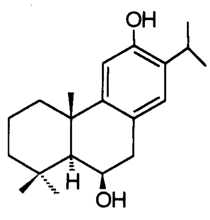
The following constituents were isolated from the heartwood of *Juniperus formosana* HAY. var. *concolor* HAY.: (−)-sesquichamaenol, (−)-7-hydroxycalamenene, (−)-15-hydroxycalamenene, and (−)-1-hydroxy-1,3,5-bisabolatrien-10-one. The latter two compounds are new cadinane- and bisabolane-type sesquiterpenes, and their structures were elucidated on the basis of spectral and chemical evidence.

Key words *Juniperus formosana* var. *concolor*; cadinane; bisabolane; sesquiterpene; (−)-15-hydroxycalamenene; (−)-1-hydroxy-1,3,5-bisabolatrien-10-one

Natural products with many kinds of skeletons are distributed in *Juniperus species.*[1] The chemical components 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. *concolor* HAY.[6] were studied in our laboratory. Recently, we have examined the chemical principles of the methanolic extract of the heartwood of the last species, and three new ferruginol derivatives, 6β-hydroxyferruginol (**1**), formosaninol (**2a**), and formosanin (**2b**), were isolated by Si gel chromatography with 10% to 20% ethyl acetate in hexane.[7]

From the same eluate fraction, we have isolated two new sesquiterpenes, (−)-15-hydroxycalamenene (**3a**) and (−)-1-hydroxy-1,3,5-bisabolatrien-10-one (**4a**), together with two known sesquiterpenes, (−)-3-hydroxycalamenene (**3b**)[8] and sesquichamaenol (**5**).[9] In this paper, we describe the structural elucidation of the two new sesquiterpenes.

15-Hydroxycalamenene (**3a**), an amorphous solid, was formulated as $C_{15}H_{22}O$ from high-resolution (HR)-EI-MS and elemental analysis. It showed infrared (IR) absorption bands at 3366 (–OH), 3035, 1605, 1485 (aromatic absorption), and 1385, 1370 cm^{-1} (geminal dimethyl absorption). The ^{1}H- and ^{13}C-NMR spectra (Table 1) revealed that **3a** has an isopropyl group, a secondary methyl group [δ 1.25 (d, 3H, J=6.9 Hz)], four methylene protons, three methine protons, a primary alcohol attached to a phenyl group, and a 1,2,4-trisubstituted benzene ring. From heteronuclear multiple quantum coherence (HMQC) experiments, proton and



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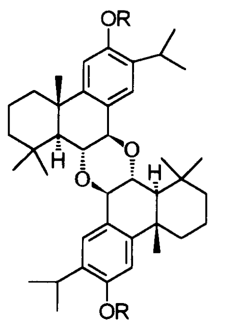
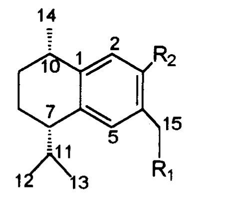
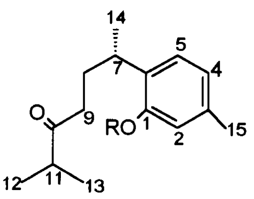


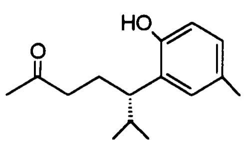
2a R=H
2b R=Me



3a R_1=OH, R_2=H
b R_1=H, R_2=OH
c R_1=OAc, R_2=H
d R_1=R_2=H



4a R=H
b R=Ac



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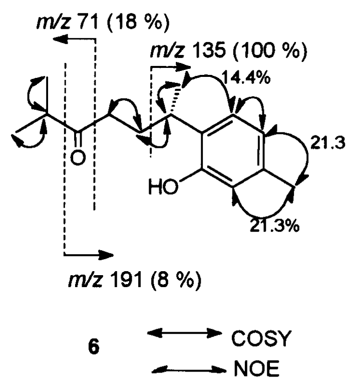

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Table 1. ^{1}H- and ^{13}C-NMR (δ-Values) Data for **3a** and **3b** and HMBC Correlations of **3a** (300 and 75 MHz in $CDCl_3$)

H	3a	3b	C	3a	Protons correlated
			1	142.7	H-14, H-2, H-3
2	7.21 d (7.3)	6.63 s	2	124.2	H-15, H-3
3	7.12 br d (7.3)		3	126.9	H-15, H-2
			4	137.7	H-15, H-3, H-5
5	7.19 br s	6.92 s	5	127.2	H-15
			6	140.4	H-5
7	2.71 m	2.60 m	7	31.9	H-12, H-13
8	1.39 m	1.31 m	8	21.3	H-7, H-11
	1.85 m	1.78 m			
9	1.32 m	1.42 m	9	30.6	H-14
	1.96 m	1.92 m			
10	2.77 m	2.70 m	10	32.7	H-14, H-2
11	2.20 m	2.18 m	11	43.9	H-12, H-13, H-7
12	0.69 d (6.7)	0.67 d (6.8)	12	21.4	H-11, H-7
13	0.98 d (6.7)	0.97 d (6.8)	13	17.4	H-11, H-12
14	1.25 d (6.9)	1.22 d (6.8)	14	22.3	H-9, H-10
15	4.62 br s	2.19 s	15	65.6	H-5

Figures in parentheses are coupling constants in Hz.

carbon correlations of **3a** were assigned as shown in Table 1. The comparison of ^{1}H- and ^{13}C-NMR data between **3a** and 3-hydroxycalamenene (**3b**) (Table 1)[8] suggested that **3a** possesses the same carbon skeleton as **3b** with a hydroxy group at C-15 instead of C-3. The acetylation of **3a** with Ac_2O in pyridine at room temperature yielded a monoacetate (**3c**) [1733 cm^{-1}, no hydroxy absorption band , δ 2.07 (s, 3H), 5.03 (s, 2H)]. The structure of **3a** was deduced to be as shown on the basis of the HMQC, heteronuclear multiple bond conectivity (HMBC) (Table 1) and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra. Compound **3a** was reduced on catalytic hydrogenation (10%Pd–C in MeOH) to yield a known compound, (−)-calamenene (**3d**) {$[\alpha]_D^{27}$ −32.1° (c=0.8, $CHCl_3$)}.[10] Thus, **3a** is (−)(7*S*,10*S*)-15-hydroxycalamenene.[10]

1-Hydroxy-1,3,5-bisabolatrien-10-one (**4a**), an oil, was formulated as $C_{15}H_{22}O_2$ from the HR-EI-MS and showed IR absorption bands at 3403 (–OH), 3035, 1616, 1586, 1518, 1506 (aromatic absorption), 1698 (C=O) and 1384, 1372 cm^{-1} (geminal dimethyl absorption). The ^{1}H- and ^{13}C-NMR spectra (Table 2) revealed that **4a** has an isopropyl group attached to a carbonyl group, an aromatic methyl group, a secondary methyl group, a 1,2,4-trisubstituted benzene ring, a phenolic proton [δ 6.80 (br s, disappeared on D_2O exchange)], a benzylic methine, four methylenes, and a carbonyl carbon. Acetylation of **4a** with Ac_2O in pyridine at room temperature yielded a monoacetate **4b** [1753 cm^{-1}; δ 2.28, 2.29 (s, each 3H)]. The structure of **4a** was deduced on the basis of HMQC, HMBC, ^{1}H–^{1}H COSY and nuclear Overhauser effect (NOE) (see structure **6**). The absolute configurations at C-7 and C-10 in (−)-15-hydroxycalamenene (**3a**) and (−)-3-hydroxycalamenene (**3b**) {$[\alpha]_D^{25}$ −29.6° (c=1.0, $CHCl_3$, lit.[8] −33°)} have been assigned as *S*. (−)-Sesquichamaenal (**5**) {$[\alpha]_D^{25}$ −4.3° (c=0.6, $CHCl_3$ lit.[9] 0°)} and (−)-1-hydroxy-1,3,5-bisabolatrien-10-one (**4a**) {$[\alpha]_D^{25}$ −1.2° (c=0.8, $CHCl_3$)} are considered to be derived from (−)-calamenene *via* biological oxidative cleavage; cleavage between C-1 and C-10 would give

Table 2. ^{1}H- and ^{13}C-NMR (δ-Values) Data for **4a** and HMBC Correlations (300 and 75 MHz in $CDCl_3$)

H		C		Protons correlated
		1	154.0	H-2, H-5, H-7
2	6.69 br s	2	116.9	H-4, H-15
		3	137.1	H-5, H-15
4	6.68 br d (8.3)	4	121.0	H-2, H-15
5	6.99 d (8.3)	5	125.8	H-7
		6	128.8	H-2, H-4, H-7, H-8, H-14
7	2.88 sex (6.7)	7	30.5	H-5, H-8, H-9
8	1.55 m	8	31.8	H-7, H-9, H-14
	1.84 m			
9	2.43 dt (18.1, 5.6)	9	37.5	H-8
	2.56 ddd			
	(18.1, 8.9, 5.1)			
		10	217.4	H-8, H-9, H-11, H-12, H-13
11	2.59 sep (6.8)	11	40.9	H-12, H-13
12	1.08 d (6.8)	12	18.3	H-11, H-13
13	1.09 d (6.8)	13	18.3	H-11, H-12
14	1.23 d (6.7)	14	19.1	H-7, H-8
15	2.26 s	15	20.9	H-2, H-4

Figures in parentheses are coupling constants in Hz.

(−)-sesquichamaenol (**5**), and between C-6 and C-7 would yield (−)-1-hydroxy-1,3,5-bisabolatrien-10-one (**4a**). Therefore the absolute configurations in **4a** and **5** may be *S*.

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. The eluate with 20% AcOEt in hexane gave a 6.5 g residue, part of which (3.2 g) was separated repeatedly by Si gel column chramatography. Four sesquiterpenes, sesquichamaenol (**5**) (15 mg), 1-hydroxy-1,3,5-bisabolatrien-10-one (**4a**) (15 mg), 3-hydroxycalamenene (**3b**) (9 mg), and 15-hydroxycalamenene (**3a**) (5 mg), were isolated in that order (eluted with 10% to 20% EtOAc in hexane).

(−)-15-Hydroxycalamenene (**3a**): An amorphous solid; $[\alpha]_D^{20}$ −46.5° (c=0.8, $CHCl_3$). IR (KBr) cm^{-1}: 3366, 3035, 1605, 1485, 1385, 1370, 1016, 822. EI-MS (70 eV) *m/z* (rel. int. %) 218 (M^+, 18), 175 (M^+ −isopropyl, 100%), 145 (70), 128 (22), 11 (18), 105 (15), 90 (20), 55 (15). HR-MS Calcd for $C_{15}H_{22}O$: 218.1671. Found: 218.1671 (M^+, 28%). ^{1}H- and ^{13}C-NMR: Table 1. *Anal.* Calcd for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found: C, 82.73; H, 10.21.

(−)-7-Hydroxycalamenene (**3b**): An amorphous solid. IR (KBr) cm^{-1}: 3393, 3035, 1600, 1588, 1480, 1378, 1365, 1261, 1181, 1026, 885. EI-MS (70 eV) *m/z* (rel. int. %) 218 (M^+, 22), 201 (13), 175(M^+ −isopropyl, 100%), 145 (77), 128 (21), 117 (19), 91 (20). ^{1}H-NMR: Table 1.

(−)-1-Hydroxy-1,3,5-bisabolatrien-10-one (**4a**): Liquid. IR (neat) ccm^{-1}: 3403, 3035, 1698, 1616, 1586, 1518, 1506, 1384, 1372, 1290, 1227, 1126, 1094, 947, 861, 810. HR-MS Calcd for $C_{15}H_{22}O_2$: 234.1620, Found: 234.1623 (M^+, 100%). ^{1}H- and ^{13}C-NMR: Table 2. *Anal.* Calcd for $C_{15}H_{22}O_2$: C, 76.87; H, 9.47. Found: C, 76.99 ; H, 9.43.

(−)-Sesquichamaenol (**5**): mp 109—111 °C. IR (KBr) cm^{-1}: 3376, 1693, 1604, 1502, 1480, 1380, 1361, 1254, 1202. EI-MS (70 eV) *m/z* (rel. int. %) 234 (M^+, 70), 191 (40), 176 (38), 163 (65), 147 (20), 133 (100), 121 (95), 105 (28), 91 (40), 77 (32), 55 (26). ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.99 (each 3H, d, J=6.6 Hz), 1.67—1.89 (3H, m), 2.02 (3H, s), 2.04—2.17 (2H, m), 2.23 (3H, s), 2.58 (1H, td, J=8.5, 5.2 Hz), 5.23 (1H, br s, –OH), 6.65 (1H, d, J=8.0 Hz), 6.81 (1H, br s), 6.85 (1H, br d, J=8.0 Hz).

Acetylation of 3a or 4a with Acetic Anhydride in Pyridine Compound **3a** (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 **3c** (11 mg) viscid liquid. IR (neat) cm^{-1}: 3035, 1733, 1605, 1490, 1225, 1022. ^{1}H-NMR ($CDCl_3$) δ: 0.69, 0.98 (each 3H, d, J=6.8 Hz), 1.25 (3H, d, J=6.9 Hz), 2.07 (3H, s), 2.19 (1H, m, J=6.8 Hz), 2.62—2.82 (2H, m, H-7, H-10), 5.03 (2H, s), 7.10 (1H, br d, J=7.8 Hz), 7.16 (1H, br s), 7.21 (1H, d, J=7.8 Hz). On similar treatment, compound **4a** (13 mg) afforded the monoacetate **4b** (13 mg) viscid liquid. IR (neat) cm^{-1}: 3035, 1753, 1701, 1605, 1501, 1204, 1087, 1016, 895, 817. ^{1}H-NMR ($CDCl_3$) δ: 1.01, 1.02, 1.21 (each 3H, d, J=6.8 Hz), 1.68—1.90 (2H, m, H-8), 2.12—2.30 (2H, m, H-9), 2.28, 2.29 (each 3H, s), 2.45 (1H, sept, J=6.8 Hz, H-11), 2.80 (1H, m, H-7), 6.77 (1H, br s, H-2), 7.00 (1H, br d, J=8.1 Hz, H-4), 7.11 (1H, d, J=8.1 Hz, H-5).

Catalytic Hydrogenolysis of 3a A solution of **3a** (10 mg) and TsOH (5 mg) in 15 ml of MeOH was hydrogenated in the presence of 10% Pd–C (10 mg). After 10 h, the catalyst was removed by filtration and washed several times with MeOH. The combined filtrate and washings gave a product (6 mg) which was identical with (−)-calamenene.[11]

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