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SESQUITERPENE ALCOHOLS FROM *CHAMAECYPARIS OBTUSA* LEAF OIL

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Key Word Index—*Chamaecyparis obtusa*; Cupressaceae; sesquiterpene alcohol; 10-epi-cubebol; α-hinokienol; β-hinokienol.

Abstract—Three new sesquiterpene alcohols were found in the hexane extract of *Chamaecyparis obtusa* leaf oil and the structures were elucidated as 10-epi-cubebol, β-hinokienol and α-hinokienol. The absolute configuration of the last two alcohols was established by synthesis from (−)menthone.

INTRODUCTION

Chamaecyparis obtusa (Sieb. et Zucc.) Endl. (Japanese name Hinoki) is one of the most important timber trees in Japan, where it is planted widely in western parts. The leaf oil of hinoki has long been the target of investigation [1] and elemol, cedrol, α-, β- and γ-eudesmol, 10(15)-cadinen-4-ol, longicyclenyl alcohol and caryophylla-3,8(13)-dien-5α-ol have been identified among the sesquiterpene alcohols [1].

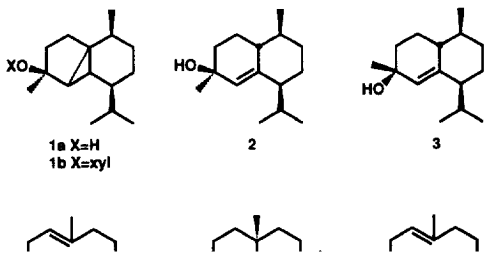
We have examined the hinoki leaf oil obtained by hexane extraction and found three new sesquiterpene alcohols (**1**, **2** and **3**) along with 4β-hydroxygermacra-1(10)-5-diene (**4**), thujopsan-2α-ol (**5**), hedycaryol (**6**), elemol, cedrol and eudesmols.

The new compounds were identified as 10-epi-cubebol (**1**), β-hinokienol (**2**) and α-hinokienol (**3**), respectively. The absolute configuration of **2** and **3** was established by synthesis.

RESULTS AND DISCUSSION

The ^{13}C NMR spectrum of compound **1** (Table 1), mp 100°, $[\alpha]_D$ −72.3° (C = 3.3), showed that it was a saturated tricyclic alcohol with four methyl, four methylene, five methine and two quaternary carbons. It was not cubebol [2] nor epi-cubebol [3] but the δ_H values of C-4 and C-15 (δ 80.1 and 28.2) were nearer those of cubebol (δ 80.3 and 28.0) than epi-cubebol (δ 81.0 and δ 25.0), thus the configuration of the hydroxy OH group was the same as in cubebol. The δ_H values of the C-7 and C-10 methines) (δ 42.4 and δ 29.3) were almost identical to those of 10-epi-cubebol xyloside (1b) (δ 42.1 and δ 29.4), but differed from those of cubebol (δ 44.2 and δ 30.9) and epi-cubebol (δ 44.6 and δ 30.2). Thus, **1** was 10-epi-cubebol.

Compound **2**, mp 78°, $[\alpha]_D$ +41.0° and **3**, oil, $[\alpha]_D$ +80.0°, appeared to be epimeric, as judged by their spectra. The ^{13}C NMR spectra (Table 1) showed that they were tert-alcohols of the $C(CH_3)$–OH type, having one double bond of the C=CH type and four methyl and four methylene carbons. The olefinic proton (sharp singlet) showed CH-LCOSY with $C(CH_3)OH$, three CH, and one CH_2; thus, it was an allylic alcohol. This was supported by the fact that the alcohol group was very labile and some methylation of the hydroxyl group took place during HPLC separation with methanol. Thus, the structures of **2** and **3** were deduced to be those of the C-4 epimeric forms of 7-isopropyl-4,10-dimethyl-bicyclo[4,4,0]dec-5-en-4-ol (**7**). As the amounts of the samples were insufficient for further work, we decided to synthesize the C-4 stereoisomers of **7**. The key intermediate was the α,β-unsaturated ketone **8**. One crystalline isomer was isolated by Gill and Lions [5] in the synthesis of cadalene from (−)-menthone via the Mannich base, the stereochemistry of which was established as **8a** by Wilson and Misra [6], who synthesized both isomers via intramolecular Diels–Alder reaction. We followed the procedure of Gill and Lions (Route 1, Scheme 1) and found that the product was a 87:13 mixture of **8a**:**8b**. When (−)menthone enamine was alkylated with methyl vinyl ketone [7]



1a X=H
1b X=xyl

2

3

Table 1. ^{13}C NMR data of Hinoki sesquiterpene alcohols

	Natural products			Synthetic products			
C	**1**	**2**	**3**	**10a** (=ent-**2**)	**10b** (=ent-**3**)	(**9a**)	**9b**
1	32.8	39.6	40.4	39.6	40.2	40.1	39.3
2	30.8	29.2	30.3	29.3	29.2	36.5	36.3
3	36.5	25.1	24.9	25.1	24.9	24.9	24.7
4	80.1	68.7	67.5	68.7	67.6	67.7	68.7
5	41.2	129.9	129.4	129.9	129.4	125.6	125.9
6	20.8	143.1	144.3	143.1	144.2	144.1	143.1
7	42.4	51.8	51.6	51.9	51.7	48.9	49.5
8	19.7	37.0	37.1	37.0	37.0	35.8	40.0
9	29.3	29.2	28.7	29.2	28.7	27.5	28.8
10	29.3	40.1	40.5	40.1	40.6	45.0	45.2
11	33.8	26.5	26.8	26.5	26.8	27.2	27.3
12	19.9	20.1	20.4	20.2	20.4	18.2	18.5
13	19.2	21.9	21.7	21.9	21.7	22.4	22.5
14	17.4	21.0	21.0	21.0	21.0	20.5	20.3
15	28.2	30.7	30.7	30.7	30.7	30.5	29.4

1: 125 MHz $CDCl_3$, **2**, **3**: 125 MHz C_6D_6 **9a,b**, **10a,b**: 22.4 MHz C_6D_6.

(Route 2), the product was a mixture of four diketone isomers instead of **8**. However, alkaline treatment gave a 19:81 isomeric mixture of **8a** and **8b** (equilibrium mixture). Crystalline **8a** was obtained via route 1 by recrystallization and the oily **8b** was obtained via route 2 by HPLC separation. Both **8a** and **8b** gave the alcohol mixtures **9** and **10**, respectively, on Grignard methylation. These were separated by alumina chromatography. The crystalline ketone **8a** gave an oily alcohol **9a**, $[\alpha]_D$ −97° and a crystalline alcohol **9b**, mp 45–48°, $[\alpha]_D$ −34.5°, and the oily ketone **8b** gave a crystalline **10a**, mp 76–78°, $[\alpha]_D$ −43.1° and an oily **10b**, $[\alpha]_D$ −78°. Thus, **2** was the enantiomer of **10a**, and **3** was that of **10b**. The configuration of these alcohols was determined by the lanthanoid-induced shift technique. Based on the X-ray analysis of **8a** [6], the ring containing the double bond was in the quasi chair form (C_1-H, $C_{2\alpha}$-H, and $C_{3\beta}$-H axial). Assuming the same conformation was retained in **9** and **10**, $C_{4\alpha}$ becomes the quasi *a* bond and $C_{4\beta}$ the quasi *e* bond. In this case, the β-alcohol group may induce a greater LIS shift to the C-5-H than the α-alcohol. The observed LIS shifts are shown in Table 2 and demonstrate that in both **9b** and **10a** (both crystalline) the alcohol group is β. Thus, **2** was the (1*R*,4*S*,7*S*,10*S*) form of 7-isopropy-4,10-

Table 2. Lanthanoid-induced shift (LIS) of ent-hinokienols

	9a	**9b**	**10a**	**10b**
C_5-H/C_{15}-H	0.964	1.080	1.002	0.928

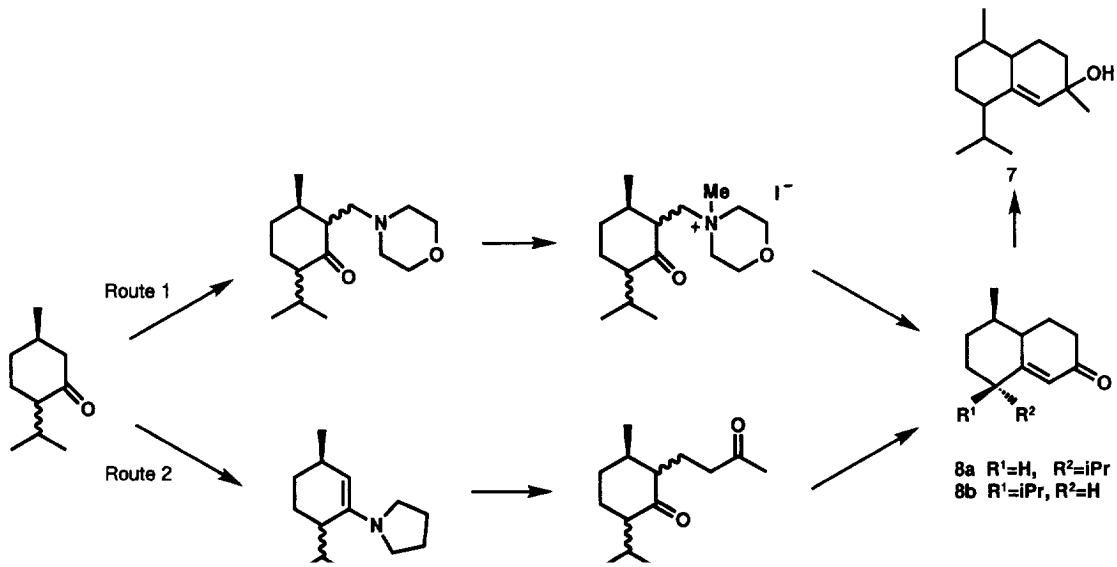


Table 3. Hinoki sesquiterpene alcohols from clones obtained from different habitats

	Kyushu Island			Shikoku Island		Fukushima p.
	Yamada 2		Aso 1†	Hata 12	Hata 13	Souma 1
	H	S	H	H	H	H
10-Epi-cubebol (**1**)	3	1	4	3	2	3
β-Hinokienol (**2**)	6	1	3	3	1	7
α-Hinokienol (**3**)	3	1	2	2	1	2
4-β-Hydroxygermacra 1(10)-5-diene (**4**)	6	1	–	2	–	5
Elemol	6	10	10	6	10	5
Thujopsan-2α-ol (**5**)	2	–	–	4	6	5
Cedrol	2	2	–	3	4	4
Eudesmols	1	7	5	5	7	3
Hedycaryol (**6**)	15	5	23	15	7	10
α-Cadinol	–	5	–	–	–	–

*H: hexane extraction, S: stream distillation.
†*Chamaecyparis obtusa* var. *Nangoa*.

dimethyl-bicyclo[4,4,0]dec-5-en-4-ol and **3** was the (1*R*,4*R*,7*S*,10*S*)-form. The names of the cadinane group depend on the relative stereochemistries at carbons 1,6, and 7. As the new alcohols have no chiral centre at C-6 and a new chiral centre at C-10 we abandoned the cadinane-muurolane nomenclature and proposed the new name hinokienol for **2** and **3**.

These new components were labile to steam distillation, as shown in Table 3. Hinoki leaves of six elite clones from Fukushima prefecture (northern limit), 73 from Shikoku Island and 44 from Kyushu Island include Kagoshima prefecture (southern limit) were analysed. They all contained **1**, **2**, **3**, **6** and eudesmols but some clones, in particular *C. obtusa* var. *Nangoa* [8], lack **4**, **5** and cedrol (Table 3).

EXPERIMENTAL

$[\alpha]_D$: 1% $CHCl_3$ soln unless stated otherwise; NMR: $CDCl_3$ or C_6D_6; FID-GC: OV-17, 1% on Gaschrom Q (1.5 m × 2.6 mm dia. packed column) and PEG 20M (25 m, fused silica capillary column).

Extraction. *Ca* 30 g of fresh hinoki leaves were chopped up in a kitchen mixer and extracted with hexane for 1 day or longer. The hexane extract was concd under reduced pressure and the residue dissolved in Et_2O was extracted with 5% NaOH soln. Work-up as usual gave *ca* 0.8 g of neutral and 0.1 g of acidic extract. Yields of extracts varied from 1.8 to 7.8% by season and individual.

Steam distillation. Fresh hinoki leaves (clone Yamada-2, 20 g) were placed in a 1-l three-necked flask with 800 ml H_2O and heated with an intermittent supply of water. After about 1 l of water had been distilled over, the distillate was extracted with hexane and the extract washed with 5% NaOH soln. Work-up as usual gave 302 mg (1.5%) neutral oil and 7.4 mg crystalline acid (Hinokiic acid).

Isolation of **1**, **2** *and* **3**. Leaves of *C. obtusa* var. fraction (3.3 g) was sepd by HPLC on an ODS-Silica column with 90% MeOH to give **1** 62.6 mg (83% pure), **2** 50.0 mg (94% pure), and **3** 26.4 mg (87% pure).

Compound **1**. 1 H NMR (500 MHz, $CDCl_3$): δ = 0.77 (2H, *m*), 0.92 (3H, *d*, *J* = 6.9 Hz), 0.96 (3H, *d*, *J* = 6.8 Hz), 0.98 (3H, *d*, *J* = 6.9 Hz), 1.27 (3H, *s*); IR ν_{max}^{KBr} cm^{-1}: 3200, 1315, 1300, 1190, 1148, 1125, 980, 928.

Compound **2**. 1H NMR (500 MHz, $CDCl_3$): δ 0.81 (3H, *d*, *J* = 6.35 Hz), 0.82 (3H, *d*, *J* = 5.62 Hz), 0.85 (3H, *d*, *J* = 6.47 Hz), 0.98–1.06 (2H, *m*), 1.10–1.22 (3H, *m*), 1.26 (3H, *s*), 1.27–1.40 (3H, *m*), 1.41–1.50 (2H, *m*), 1.54 (1H, *dd*, *J* = 3.1, 10.5 Hz), 1.60–1.69 (2H, *m*), 1.75–1.83 (2H, *m*), 5.32 (1H, *s*); IR ν_{max}^{KBr} cm^{-1}: 3286, 1663, 1458, 1371, 1002; MS *m/z* (rel. int.): 204 $[M - H_2O]^+$ (63), 189 (35), 162 (31), 161 (100), 159 (65), 133 (24), 190 (70), 105 (99), 93 (35), 91 (56), 81 (99), 79 (28), 77 (31), 69 (23), 67 (20), 55 (48), 53 (20), 43 (33), 41 (97).

Compound **3**. 1H NMR (500 MHz, C_6D_6): δ 0.72 (3H, *d*, *J* = 6.35 Hz), 0.85 (3H, *d*, *J* = 6.0 Hz), 0.87 (3H, *d*, *J* = 6.3 Hz), 1.04–1.26 (4H, *m*), 1.27 (3H, *s*), 1.3–1.45 (4H, *m*), 1.52–1.56 (1H, *m*), 1.59 (1H, *m*), 1.71–1.77 (2H, *m*), 1.82 (1H, *ddd*, *J* = 3.2, 5.3, 10.9 Hz), and 5.32 (1H, *s*); IR ν_{max}^{KBr} cm^{-1}: 3372, 1657, 1371, 909.

7-Isopropyl-10-methyl-bicyclo [4,4,0] *dec-5-en-4-one* (**8a** *and* **8b**). *Route* 1 *via* 2-*morpholinomethyl menthone methiodide*. The methiodide (73.4 g) was synthesized from (−)-menthone (68.7 g, $[\alpha]_D$ −6.8°, containing 15% isomenthone), morpholine hydrochloride (62.1 g), paraformaldehyde (total 16.0 g) and MeI (35.0 g) according to the procedure of ref. [5]. The methiodide (21.6 g) was reacted with Na (1.3 g) and EtOAc (8.1 g) to give 11.3 g of mixture 8, from which 6.2 g of crystalline **8a**, mp 71–72° (lit. 70°), was obtained by recrystallization from hexane.

Route 2 *via enamine.* (−)-*Menthone* (1.9 *g*) *was refluxed with excess pyrollidine* (8.5 *g*) *in the presence*

enamine mixture (1.5 g) was refluxed with methyl vinyl ketone (1.0 g) in dry benzene (30 ml) for 24 hr. After cooling to room temp., acetate buffer was added and the mixture refluxed for 4 hr. The reaction mixture was distilled after washing with 10% HCl, aq. $NaHCO_3$ and H_2O. The product, 845 mg (yield 59%), was a mixture of four isomers of 2-(3-oxobutyl)menthone **12** (1:4:3:1). The GC-MS of each component were identical: *m/z* (rel. int.) 224 $[M]^+$ (20), 209 (16), 166 (14), 153 (14), 139 (16), 124 (16), 111 (20), 95 (23), 83 (14), 69 (32), 55 (38) and 43 (100). The mixture (826 mg) was refluxed with aq. KOH (11%, 4 ml) for 4 hr. The product was a 19:81 mixture of **8a** and **8b**. They were separated by HPLC and 92% pure **8b** was obtained.

Compound **8a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, *d*, J = 6.6 Hz), 0.97 (3H, *d*, J = 6.6 Hz), 1.04 (3H, *d*, J = 6.6 Hz), 1.20–2.43 (12H, *m*), and 5.85 (1H, *s*); IR ν_{max}^{KBr} cm^{-1}: 2952 (m), 2926, 2868, 1673, and 1265.

Compound **8b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (3H, *d*, J = 6.2 Hz), 0.97 (3H, *d*, J = 6.2 Hz), 1.05 (3H, *d*, J = 5.9 Hz), 1.36–1.46 (2H, *m*), 1.50–1.67 (2H, *m*), 1.70 (1H, *m*), 1.82–1.93 (2H, *m*), 1.96–2.02 (2H, *m*), 2.17–2.28 (2H, *m*), 2.39 (1H, *m*), and 5.81 (1H, *d*, J = 2 Hz).

ent-Hinokienols. Oily enone **8b** (92% pure, 232 mg) was treated with methyl magnesium iodide in dry THF for 1 hr. The product was treated with satd aq. NH_4Cl and ice, then extracted with Et_2O. A yellow oil (245 mg) was obtained, yield 98%. The product was an 11:9 mixt. (by GC) and was sepd by alumina chromatography to give **10a**, mp 76–78°, $[\alpha]_D$ +41.0° and **10b**, oil, $[\alpha]_D$ +80°.

Compound **10a**. ^{1}H NMR (89.5 MHz, $CDCl_3$): δ 0.77 (3H, *d*, J = 6.2 Hz), 0.84 (3H, *d*, J = 5.3 Hz), 0.90 (3H, *d*, J = 5.9 Hz), 1.26 (3H, *s*), 3.49 (1H, OH), and 5.32 (1H, *s*).

Compound **10b**. ^{1}H NMR (89.5 MHz, $CDCl_3$): δ 0.71 (3H, *d*, J = 5.9 Hz), 0.90 (3H, *d*, J = 5.7 Hz), 0.95 (3H, *d*, J = 5.7 Hz), 1.28 (3H, *s*), and 5.34 (1H, *s*).

ent-7-epi-Hinokienols. Crystalline enone **8a** was treated with methylmagnesium iodide as above and a 1:1 mixture of alcohols was obtained in 95% yield. They were separated by alumina chromatography to give **9a**, oil, $[\alpha]_D$ −97.0° and **9b**, mp 45–48°, $[\alpha]_D$ −34.5°.

Compound **9a**. ^{1}H NMR (89.5 MHz, $CDCl_3$): δ 0.86 (3H, *d*, J = 6.6 Hz), 0.95 (6H, *d*, J = 6.8 Hz), 1.29 (3H, *s*), 5.29 (1H, *s*), 5.34 (1H, *bs*, OH).

Compound **9b**. ^{1}H NMR 89.5 MHz, $CDCl_3$): δ 0.86 (3H, *d*, J = 6.8 Hz), 0.95 (6H, *d*, J = 6.8 Hz), 1.28 (3H, *s*), 5.29 (1H, *s*).

Lanthanoid-induced shift (LIS) of **9a,b** *and* **10a,b**. ^{1}H NMR spectra were measured for *ca* 20 mg of each alcohol in 0.35 μl $CDCl_3$ with stepwise addition of $Eu(fod)_3$. The relative value of the LISs of the vinyl proton (1.00 for C-15-Me protons) was determined.

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