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SESQUITERPENE ALCOHOLS FROM *CRYPTOMERIA JAPONICA* AND *C. FORTUNEI* LEAF OIL

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Key Word Index—*Cryptomeria japonica*; *C. fortunei*; Taxodiaceae; sesquiterpene alcohols; 4β-hydroxygermacra-1(10),5-diene; thujopsan-2α-ol; hedycaryol.

Abstract—4β-Hydroxygermacra-1(10),5-diene, thujopsan-2α-ol and hedycaryol were found in the leaf oil of *Cryptomeria japonica* obtained by hexane extraction. Both the first and last were present in all races of *C. japonica* native in Kyushu main island, but thujopsan-2α-ol was found in about one-third and not found in *C. fortunei* so far examined. Moreover, some *C. fortunei* lacked 4β-hydroxygermacra-1(10),5-diene. On the other hand some contained only this as sesquiterpene alcohol. When the leaves were steam-distilled, 4β-hydroxygermacra-1(10),5-diene isomerized to α-cadinol and hedycaryol isomerized to elemol and eudesmols. Thujopsan-2α-ol was synthesized from mayurone.

INTRODUCTION

Two *Cryptomeria* species are known, one is *C. japonica* D. Don (Sugi) in Japan and the other is *C. fortunei* Hooibrenk ex Otto et Dietr. (Liŭ Shān) in China. It is not known whether they are independent [1]. Moreover, many cultivars or races of *C. japonica* are known and the classification of them was also uncertain.

Appleton *et al.* [2, 3] reported that *C. japonica* could be subdivided into four chemical varieties on the basis of the diterpene hydrocarbon content of leaf oil. Later, Yasue *et al.* [4] sub-divided Sugi into eight chemical varieties. However, the sesquiterpene alcohol fraction, another major component of Sugi leaf oil, was not examined from the standpoint of classification.

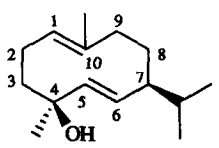
We have examined leaf oils of 42 cultivars and 27 elite clones of Sugi native in Kyushu Island and 34 individuals of Liŭ Shān originating in three different prefectures of China, both cultivated in Kyushu Forest Tree Breeding Institute, Kumamoto.

The neutral part of the hexane extract of Sugi leaf oil showed seven peaks by GC using an OV-17 column of which peaks 3 and/or 4 were missing in some races, in the sesquiterpene alcohol region. The compounds giving peaks 2, 3 and 7 were not reported as components of Sugi, and were identified as 4β-hydroxygermacra-1(10),5-diene (**1**) [5], thujopsan-2α-ol (**2**) [6], and hedycaryol (**3**) [7], respectively. Other components were elemol (peak 1), cedrol (peak 4) and eudesmols (γ-peak 5, α- and β-peak 6).

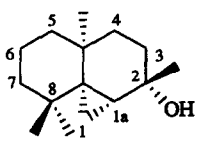
RESULTS AND DISCUSSION

The ^{13}C NMR spectrum of **1** showed the presence of two double bonds, while the ^{1}H NMR spectrum revealed the presence of an isopropyl, a methyl attached on a double bond, and a Me–C–OH group as well as a trans double bond. The alcohol was eventually shown to be 4β-hydroxygermacra-1(10),5-diene from the ^{1}H NMR spectrum [5]. The ^{13}C NMR spectrum of **1** was almost identical with that of the α-isomer [8], except for the value of C-15 (δ33.0 instead of 30.8).

The ^{1}H NMR spectrum of **2** revealed the presence of a three-membered ring, four *tert*-Me groups, one of which was Me–C–O–, thus suggesting the structure of thujopsanol. Thujopsan-2α- and 2β-ol were synthesized from mayurone (Scheme 1) and **2** was identified as the 2α-isomer. Grignard reaction of mayurone afforded α-alcohol as the main product [9], whereas dihydromayurone gave mainly the β-alcohol.



1



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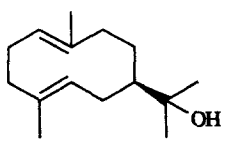

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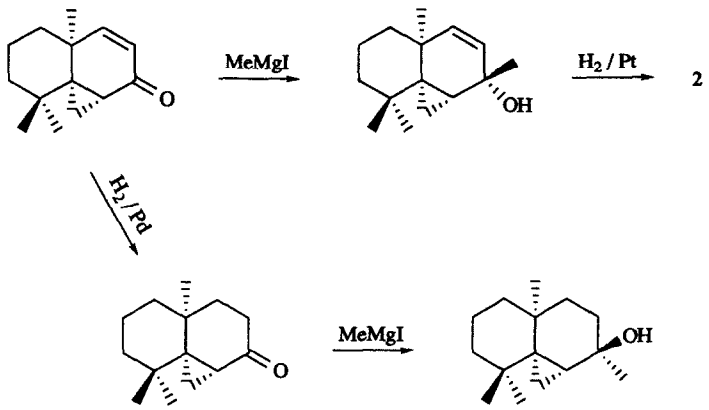

Scheme 1.

Compound **3** was converted to elemol by heating. The ^{1}H NMR spectrum showed the characteristic of hedycaryol reported by Wharton *et al.* [10], and was identified by direct comparison of the spectrum with that of an authentic sample [11]. Hedycaryol suffered acidic rearrangement to eudesmols [7]. Thus elemol, eudesmols and hedycaryol can be regarded as one group and referred to as group **3** hereafter.

Compounds of **1** and group **3** were found in all races of *C. japonica* and 28 individuals among 34 of *C. fortunei*. It is interesting that two individuals of *C. fortunei* contained only **1** as sesquiterpene alcohol, whereas the other two contained only group **3**. The former had phyllocladene as diterpene and the latter had kaurene. They could be two proto-types of *Cryptomeria* species. Compound **2** has not yet been found in *C. fortunei* and was found in about one-third of *C. japonica*. The distribution is distributed toward the southern part of Kyushu island; appearing mainly in the Obisugi group of Miyazaki prefecture. The presence of the third proto-type of *Cryptomeria* species having only **2** may be postulated, and if it exists, it may be found in Yakushima, a small island 60 km south of Kyushu main island, and may represent a primeval forest of Sugi.

Inability to detect components **1–3** by previous researchers may be attributed to the difference of sample preparation methods. Steam-distillation of the leaves was a conventional method for obtaining the leaf oil, but it may cause structural changes of some components as shown by comparative experiments of samples from the same tree by hexane extraction and steam-distillation. The results are summarized in Table 1. (i) The leaves of Liŭ Shān which contained only **1** as sesquiterpene alcohol component by hexane extraction were steam-distilled. Most **1** was isomerized or dehydrated. The main isomerization product was α-cadinol, the formation of which by acid catalysis was confirmed by isomerization of pure **1**. (ii) In four cultivars of different types, compounds **1–3** decreased markedly and elemol and eudesmols were increased by steam-distillation. The loss of **3** was prevented to some extent by distillation at pH 10 using buffer solution, but **1** was diminished markedly even under this condition. The presence of acid (tartarate buffer pH 3) caused severe loss of **1** and **3**, and γ-eudesmol became the main component. (iii) A neutral part of hexane extract was steam-distilled with or without addition of the acidic part of the same leaf extract. Considerable loss of **1** and **3** (*ca* 16% for **1** and 40% for **3**) was recognized even on steam-distillation of a neutral extract. Thus **1** and **3** were sensitive to heating. About 40% of **1** and 70% of **3** were lost in the presence of acidic extract. Therefore, steam distillation is unsuitable for chemical analysis of Sugi leaf oil, and this should be replaced by solvent extraction under mild conditions.

EXPERIMENTAL

The NMR spectra were measured in $CDCl_3$. GC was carried out using a 1.5 m glass column packed with OV-17 1% on Gas-Chrom Q or PEG-HT 5% on Uniport R, at 60–230°. A capillary column of PEG 20M 25 m was used for the analysis of α,β-eudesmol and α-cadinol.

Extraction. Ca 30 g of fresh Sugi leaves were chopped up by a kitchen mixer and extracted with hexane for 1 day or longer. Hexane extract was concd under red. pres. and the residue dissolved in hexane was extracted with 5% NaOH. Work-up as usual gave *ca* 0.5 g of neutral and 0.2 g of acidic extracts. Yields of extracts varied from 2.5 to 7.6% (dry base) by seasons and individuals.

Steam-distillation. Ca 20 g of fresh Sugi leaves were placed in a 1 l three-necked flask with 800 ml H_2O or buffer soln and heated with an intermittent supply of H_2O. *Ca* 1 l H_2O was distilled and the distillate extracted with hexane. The hexane extract was washed with 5% NaOH. Work-up as usual gave about 0.1 g of neutral distillate. Buffers were tartaric acid–Na_2HPO_4 for pH 3; Na_2HPO_4–KH_2PO_4 for pH 7; and $Na_2B_4O_7$–Na_2CO_3 for pH 10. Composition of distillates are shown in Table 1.

4β-Hydroxygermacra-1(10),5-diene (**1**). (i) A neutral extract (0.862 g) obtained from cultivar Yabukuguri was poured on an alumina column (30 g) deactivated by addition of 1% H_2O. Hexane eluted the hydrocarbon fraction (0.223 g, mainly phyllocladene), and

Table 1. Sesquiterpene alcohols of Sugi and Liŭ Shān

Cultivar	Method*	Buffer†	Sesquiterpene alcohol total %‡	Elemol 1	1 2	2 3	Cedrol 4	Eudesmol γ 5	Eudesmol α	Eudesmol β 6	3 7§	α-Cadinol	others %
(i) A Liŭ × Shān	H		25	—	25	—	—	—	—	—	—	—	—
	S	—	18	—	4	—	—	—	—	—	—	9	5
(ii) Yabukuguri	H		51	5	10	—	—	1	2	2	30	—	1
	S	—	40	10	1	—	—	8	5	3	8	3	2
Garin	H		27	3	4	9	3	2	3		3	—	—
	S	—	20	4	1	3	3	2	2	3	—	1	1
Tosaaka	H		60	4	11	—	6	—	1	1	33	—	4
	S	10	56	10	6	—	6	1	3	3	26	1	—
	S	—	52	8	2	—	6	5	5	5	18	3	—
Kagoshima-1	H		57	7	12	—	—	1	1	2	34	—	—
	S	10	54	13	6	—	—	1	1	2	28	1	2
	"	7	52	16	4	—	—	3	3	4	18	2	2
	"	—	51	7	2	—	—	11	3	3	22	3	—
	"	3	41	5	1	—	—	22	7	4	0	2	—
(iii) Kagoshima-1 Hexane neut.	S	—	53	11	10	—	—	3	3	4	20	2	—
"	"	7	51	10	9	—	—	2	3	3	23	1	—
" with acid	"	—	50	12	7	—	—	7	5	6	10	3	—

*H: Hexane extraction, S: steam distillation.
†—Without buffer solution.
‡—In neutral part.
§—Peak no. by OV-17 column.

hexane–CH_2Cl_2 (19:1) mixture eluted **1** (0.01 g). Hexane–CH_2Cl_2 (9:1–7:3) eluted the remaining alcoholic mixture (0.210 g). (ii) A neutral extract (0.587 g), obtained from a *C. fortunei* (dried residue 26.93 g, by origin of Línhāi pref., Zhéjiāng prov.) was chromatographed on 20 g of alumina. Hexane eluted 0.13 g of the phyllocladene fraction, and hexane–CH_2Cl_2 (4:1) eluted 0.070 g of **1**. Steam-distillation of 36.39 g (fresh) of leaves from the same tree gave 0.176 g of neutral oil.

^{13}C NMR (68 MHz) of **1**: δ140.1, 132.6, 128.9, 125.8, 73.1, 52.8, 41.3, 39.6, 33.0, 30.7, 26.0, 23.7, 20.6, 19.0 and 16.7. IR ν^{KBr} cm^{-1}: 3440, 1663, 1375, 1360, 1190, 978, 940, 902, 870, 835 and 733.

Isomerization of compound **1**. Compound **1** (50 mg) was treated with 3 ml of 85% HCO_2H for 1 hr at room temp. The hexane extract of the reaction mixture was evapd, and treated with excess $LiAlH_4$ in dry Et_2O to convert any formate esters to the corresponding alcohols. Evaporation of the ethereal soln after washing with H_2O afforded a product (47 mg) which was chromatographed on alumina (3 g). Hexane eluted a dehydrated product (5 mg), hexane–CH_2Cl_2 (2:1) eluted an alcohol (7 mg) and hexane–CH_2Cl_2 (1:1) eluted α-cadinol (27 mg, 54% yield), mp 73–74° (lit. 74.5° [12]); IR and ^{1}H NMR were identical to those reported in refs [12, 13].

Separation of other alcohols. An alcoholic mixture obtained by alumina chromatography was separated by HPLC on ODS-silica column with 90% MeOH. Compounds **2** and **3** were sepd, besides cedrol, elemol and eudesmols.

Thujopsan-2α-ol (**2**). Needles mp 58°; ^{13}C NMR (22 MHz): δ67.6 (C), 40.8 (CH_2), 36.3 (CH_2), 34.4 (C and CH_2), 33.7 (CH_3), 33.5 (C), 33.3 (CH_2), 31.9 (C), 31.5 (CH), 29.3 (CH_3), 29.2 (CH_3), 27.1 (CH_3), 18.8 (CH_2) and 8.6 (CH_2); ^{1}H NMR (90 MHz): δ0.04 (1H, *d*, J = 6.0 Hz), 0.32, 0.44 (each 1H, *d*, J = 5.4 Hz), 0.59, 0.98, 1.05, 1.23 (each 3H, *s*); IR ν^{KBr} cm^{-1}: 3400, 1170, 1090, 920; MS *m/z*: 123 (100), 222, 204, 189, 151, 135, 109, 95, 81, 69.

Synthesis of compound **2**. To a Grignard reagent from 101 mg (4.15 mmol) of Mg and 0.19 ml (3.0 mmol) of MeI in 15 ml Et_2O, 204 mg (1.0 mmol) of mayurone was added under cooling in a dry ice–MeOH bath. After 3 hr of reaction, excess reagent was decomposed by addition of 0.5 ml of MeOH, and 10% NH_4OAc soln was added under cooling. The reaction mixture was extracted with C_6H_6–Et_2O (1:1) and 223 mg of α-thujopsenol (91.8% pure) was obtained. Yield 93%. α-Thujopsenol (192 mg) was hydrogenated in MeOH (20 ml) with Pt black catalyst (20 mg). H_2 (20 ml) was absorbed. The product was purified through alumina chromatography. Yield 117 mg. NMR, IR, MS were identical with natural **2**.

Synthesis of thujopsan-2β-ol. Mayurone (408 mg) was hydrogenated in MeOH with Pd-C catalyst (41 mg). H_2 (45 ml) was taken up during 8 min. The catalyst was filtered off and H_2O added to the filtrate. The filtrate was extracted with Et_2O and the Et_2O evapd. The crude residue (631 mg from two runs) was added to a Grignard reagent prepared from 94 mg of Mg and 2.4 ml MeI under ice water. After stirring for one day, NH_4OAc soln was added and the reaction mixture was extracted with Et_2O. After removal of the Et_2O, the residue (570 mg) was chromatographed on alumina (10 g). Hexane eluted the dehydration product (429 mg, 72% thujopsene) and hexane–CH_2Cl_2 (1:1 to 1:3) eluted 82 mg of thujopsan-2β-ol (95% pure) mp 58–60°.

^{13}C NMR (22 MHz): δ69.7 (C), 40.1 (CH_2), 36.7 (CH_2), 35.6 (CH_2), 34.3 (CH_2), 33.4 (C), 32.9 (C), 31.9 (CH), 31.8 (C), 29.2 (CH_3), 29.2 (CH_3), 28.9 (CH_3), 27.3 (CH_3), 18.1 (CH_3) and 9.7 (CH_3); ^{1}H NMR (60 MHz) 0.1–0.6 (2H, *m*), 0.62, 1.00, 1.08 and 1.25 (each 3H, *s*); IR (oil) 3400, 1170, 1120, 940, 920 cm^{-1}; MS *m/z* 140 (100), 204, 189, 161, 151, 123, 109, 95, 81, 69.

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