the MS and the retention data (Table 1). The percentage content of polyacetylenic compounds **1** and **2** in the essential oil of *E. naudini* was very high (35–40%), as in all *Erigeron* species. Therefore both esters **1** and **2** may testify to the accidental or fraudulent presence of the essential oil of *E. naudini* in geranium essential oil. Indeed, the IR spectrum of the latter, when pure, presents no absorption band at 2200–2220 cm^{-1}, the area where the very intense sharp absorption band of the acetylenic bond is usually found.

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TERPENOIDS FROM THE SEED OF *THUJOPSIS DOLABRATA*

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Key Word Index — *Thujopsis dolabrata* var. *hondae*; Cupressaceae; seed; mono-, sesqui-, and diterpenes; sabinene; hedycaryol.

Abstract—Sabinene and hedycaryol were found to be the main components of the seed oil of *Thujopsis dolabrata*. Nine mono- and three sesquiterpenes were also isolated from the distilled, neutral oil. Hedycaryol rearranged to elemol during GLC at higher temperatures. The distillation residue contained *n*-paraffins, eight diterpenes, sitosterol, and *trans*-communic acid and isocupressic acid.

INTRODUCTION

Although terpenoid constituents of woods, barks, and leaves of conifer species have been investigated by many workers, very few studies appear to have been reported on those of seeds. It is well known that considerable differences in chemical constituents between woods, barks, and leaves exist. Therefore, it seemed to be of interest from a biogenetic viewpoint to examine components of seeds.

Thujopsis dolabrata Sieb. et Zucc. var. *hondae* Makino (Cupressaceae) is one of the most valuable trees endemic to Japan. The wood contains predominantly sesquiterpenes, such as thujopsene, cedrol [1], γ-cuparenol, α-, β-costal, β-elemenal, mayurone, widdrol, elemol, sesquibenihiol, sesquibenihidiol and selinadiol [2] together with various tropolones [3, 4] and phenols [5], while the leaf oil is composed of monoterpenes, such as *d*-sabinene, borneol, sabinol, and dipentene [6], together with a small amount of the diterpene hydrocarbons, dolabradiene, hibaene, and ar-abietatriene [7].

In a previous paper [8], we described the isolation of a new diterpene glycoside and two lignans from the ether extract of the seed. This paper deals with terpenoid components from the *n*-hexane extract.

RESULTS AND DISCUSSION

α-Pinene, sabinene, β-myrcene, sabinyl acetate, terpinen-4-ol, α-terpinyl acetate, β-eudesmol and elemol were isolated and identified by their IR and NMR spectra. Small amounts of limonene, β-phellandrene, *p*-cymene, terpinolene, and cuparene were identified by GC–MS (Table 1).

GLC analyses of the undistilled neutral oil under various conditions indicated variations of the relative concentrations between elemol and another compound (compound X) whose retention time was longer than that of elemol. The relative concentration of elemol decreased, while that of compound X increased, when the injection and column temperature was lower. Hence it was suspected that compound X was transformed to elemol during GLC. Compound X was subsequently isolated by column chromatography and found to be hedycaryol, which is known to rearrange to elemol [9, 10].

Quantitative GLC analysis of the undistilled neutral oil at 110° on 5% SE 30 showed that hedycaryol was the main component of the terpene fraction (21.7% calculated from the peak area), while elemol was present in a small amount. From this result a more widespread co-occurrence of hedycaryol with elemol in nature is to be anticipated.

The unsaponifiable fraction of the distillation residue on silica gel column chromatography gave *n*-paraffins, hibaene, ar-abietatriene, nonacosan-10-ol, totarol, ferruginol, sempervirol, isoagatholal, sitosterol, and cryptomeridiol, all of which were identified by physical and chemical properties.

From the NaOH-soluble portion of the *n*-hexane extract, *trans*-communic acid and isocupressic acid were isolated and identified by comparison of their IR spectra and physical data with those of authentic samples after conversion to their methyl esters [11].

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded with TMS as an int. standard in $CDCl_3$. GC–MS was carried out with a 2 m × 3 mm stainless steel column with 10% PEG-20M; temp. programmed 100–200° at 5°/min; He at 30 ml/min; the mass spectrometer was operated at 70 eV with the ion source at 200°. CC, unless otherwise stated, was carried out on Si gel Wakogel C-200. Kieselgel HF_{254} (Merk) was used for analytical and prep. TLC.

Extraction. Seed (380 g), collected in Aomori prefecture, in autumn 1977, was homogenized in *n*-hexane and extracted with *n*-hexane. The *n*-hexane extract (160 g), thus obtained, was fractionated into a strongly acidic (32 mg), a less strongly acidic (19 g) and a neutral oil (130 g).

Fractionation of the volatile oil. A portion of the neutral oil (36 g) was distilled *in vacuo* to give the following fractions; (1) the first fraction, boiling below 120°/30 mm (5.3 g), (2) the second fraction, boiling between 120°/30 mm and 200°/10 mm (3.9 g). The first fraction was subjected to prep. GLC, 10% DEGS 1.5 m × 6 mm, isothermal 40°, N_2 at 60 ml/min, and α-pinene, sabinene, and β-myrcene were isolated and identified by direct comparisons of their IR spectra with those of authentic samples. In addition, limonene, β-phellandrene, *p*-cymene, and terpinolene were identified by means of GC–MS. The second fraction was chromatographed on 5% $AgNO_3$–Si gel, eluting with *n*-hexane–Et_2O mixtures. The *n*-hexane–Et_2O (5:1) eluate gave a mixture of hydrocarbons (298 mg), containing cuparene (identified by GC–MS), α-terpinyl acetate (958 mg), $[\alpha]_D^{24}$ + 57° (EtOH, *c* 3.0), and sabinyl acetate (192 mg), $[\alpha]_D^{29}$ + 73° ($CHCl_3$, *c* 0.75). The *n*-hexane–Et_2O (3:1) eluate gave terpinen-4-ol (156 mg), $[\alpha]_D^{19}$ + 20° (EtOH, *c* 1.0) and a mixture of α- and γ-eudesmols. The *n*-hexane–Et_2O (2:1) eluate gave β-eudesmol (39 mg), mp 80–81°. The *n*-hexane–Et_2O (1:1) eluate gave elemol (1.87 g), mp 52.5–53°, $[\alpha]_D^{28}$ − 15° ($CHCl_3$, *c* 5.0). The IR and NMR spectra were identical with those of authentic materials.

Isolation of hedycaryol. The undistilled neutral oil (5 g) was chromatographed on neutral alumina (activity I, 50 g), eluting successively with *n*-hexane, C_6H_6, $CHCl_3$, and Et_2O. The last of the three eluates (1.0 g) was rechromatographed on 5% $AgNO_3$–Si gel (30 g), eluting with Et_2O and then Me_2CO. The Me_2CO eluate was washed with satd NaCl soln and gave pure hedycaryol (80 mg), $[\alpha]_D^{28}$ + 30° ($CHCl_3$, *c* 1.2) which was converted to the *p*-nitrobenzoate, mp 112–113.5°. IR and NMR spectra were in good agreement with those of an authentic sample, kindly provided by Professor Itō.

Fractionation of the unsaponifiable matter. The distillation residue (27.3 g) was refluxed for 2 hr with 2 N methanolic KOH. The unsaponifiable matter (5.9 g) was chromatographed on Si

Table 1. Composition of the *n*-hexane soluble neutral terpenic fraction from the seed of *Thujopsis dolabrata* Sieb. et Zucc. var. *hondae* Makino

Compound	Relative percentage*	Identification method
α-Pinene	8.7	IR, GC–MS
Sabinene	38.9	IR, NMR, GC–MS
β-Myrcene	4.2	NMR, GC–MS
Limonene	1.5	GC–MS
β-Phellandrene	0.3	GC–MS
p-Cymene	0.1	GC–MS
Terpinolene	0.4	GC–MS
$C_{10}H_{18}O$	0.6	GC–MS
$C_{15}H_{24}$		GC–MS
$C_{12}H_{20}O_2$	1.0	GC–MS
$C_{15}H_{24}$	0.1	GC–MS
Terpinen-4-ol	1.1	IR, NMR, MS
$C_{15}H_{24}$		GC–MS
Sabinyl acetate	1.2	IR, NMR, MS
$C_{15}H_{24}$		GC–MS
α-Terpinyl acetate	5.9	IR, NMR, MS
Unidentified	0.6	
Cuparene	0.2	GC–MS
$C_{15}H_{26}O$	0.3	GC–MS
$C_{15}H_{26}O$		GC–MS
Elemol	0.04	IR, NMR, MS
γ-Eudesmol	0.6	IR
Unidentified		
α,β-Eudesmol	0.7	IR
Hibaene		IR, NMR, MS
Hedycaryol	21.7	IR, NMR, MS
Ar-abietatriene	0.2	IR, NMR, MS
Cryptomeridiol	0.1	IR, NMR, MS
Unidentified	0.3	
Sempervirol	0.4	IR, NMR, MS
Totarol	7.8	IR, NMR, MS
Ferruginol	2.3	IR, NMR, MS
Unidentified	0.5	
Nonacosan-10-ol	0.1	IR, NMR, MS

* Based on relative peak areas of the GLC analysis of *n*-hexane soluble neutral oil before distillation. GLC on PEG 20M, 50° to 250° at 5°/min. The amounts of elemol and hedycaryol were recalculated from the result of GLC on SE 30, isothermal at 100°.

gel, eluting successively with *n*-hexane, C_6H_6, Et_2O and EtOAc. The *n*-hexane eluate (312 mg) was subjected to PLC (Si gel, 0.75 mm, *n*-hexane) to give *n*-paraffin mixtures (33 mg), hibaene (20 mg), $[\alpha]_D^{24}$ − 45° ($CHCl_3$, *c* 1.0) and ar-abietatriene (39 mg), $[\alpha]_D^{24}$ + 0.38° ($CHCl_3$, *c* 1.0); UV λ_{max}^{EtOH} nm: 275 (log ε 2.97).

The C_6H_6 eluate (2.3 g) was rechromatographed on Si gel (*n*-hexane–Et_2O, 50:4) to give totarol (1.1 g), mp 127–8°; $[\alpha]_D^{24}$ + 45° ($CHCl_3$, *c* 2.0); UV λ_{max}^{EtOH} nm: 279–285 (log ε 3.29), totarol benzoate, mp 145–146°, nonacosan-10-ol (182 mg), mp 82.5–83°; ferruginol (399 mg), ferruginol acetate, $[\alpha]_D^{27}$ + 65° (EtOH, *c* 1.0), UV λ_{max}^{EtOH} nm: 276 (log ε 3.03), ferruginol benzoate, mp 156–157°, and sempervirol (79 mg), sempervirol acetate, mp 90–91°; $[\alpha]_D^{27}$ + 50° ($CHCl_3$, *c* 1.3); UV λ_{max}^{EtOH} nm: 276 (log ε 3.01).

The Et_2O eluate (2.8 g) was rechromatographed on Si gel, eluting with *n*-hexane–EtOAc mixtures. The *n*-hexane–EtOAc (12:1) eluate contained elemol (2.1 g), mp 52.5–53°, $[\alpha]_D^{28}$ − 15° ($CHCl_3$, *c* 5.0) and a small amount of eudesmol isomers, identified by GLC. The *n*-hexane–EtOAc (10:1) eluate gave sitosterol (80 mg), mp 138–139°. The *n*-hexane–EtOAc (8:1) gave isoagatholal, $[\alpha]_D^{27}$ + 22.5° ($CHCl_3$, *c* 2.0), MS *m/e* 304.2404 (calc. for $C_{20}H_{32}O_2$, 304.2404); IR ν_{max} cm^{-1}: 3300, 3090, 2725, 1715, 1640, 1000, 895; ^1H NMR: δ 9.52 (*s*, 1H), 5.27 (*t*, 1H, *J* = 6.8 Hz), 4.78, 4.47 (each *s*, 1H), 4.05 (*d*, 2H, *J* = 6.8 Hz), 1.65, 1.00, 0.56 (each *s*, 3H); the semicarbazone, mp 172–173° (recrystallized from EtOAc), $[\alpha]_D^{26}$ + 15° ($CHCl_3$, *c* 1.0) (lit [12] mp 157–159°, $[\alpha]_D^{21}$ + 22°). Reduction of this compound with $LiAlH_4$ afforded agathadiol, mp 109–110°, identical with the product prepared from methyl isocupressate (see below) with $LiAlH_4$. The EtOAc eluate (258 mg) gave cryptomeridiol, mp 137–138°, $[\alpha]_D^{27}$ − 35° (EtOH, *c* 1.0).

Fractionation of the n*-hexane soluble acidic portion.* The *n*-hexane soluble acidic portion (2.7 g) was chromatographed on Si gel, eluting with *n*-hexane–Et_2O mixtures. The *n*-hexane–Et_2O (7:2) eluate gave *trans*-communic acid (1.54 g), which was converted to the methyl ester, mp 107–108°, $[\alpha]_D^{25}$ + 56.7° ($CHCl_3$, *c* 1.5); UV λ_{max}^{EtOH} nm: 231 (log ε 4.4) (lit. [11] mp 107–108°, $[\alpha]_D$ 47.2°, UV λ_{max} nm: 232 [log ε 4.4]); MS *m/e* 316 (M^+); IR ν_{max} cm^{-1}: 3100, 1715, 1640, 1610, 995, 895, 880; ^1H NMR: δ 6.28 (*dd*, 1H, *J* = 10 and 17 Hz), 5.34 (*t*, 1H, *J* = 6.0 Hz), 5.00 (*d*, 1H, *J* = 17 Hz), 4.84 (*d*, 1H, *J* = 10 Hz), 4.79 and 4.40 (each *s*, 1H), 3.57, 1.73, 1.18, 0.55 (each *s*, 3H). The *n*-hexane–Et_2O (7:3) eluate gave a fatty acid mixture (204 mg) which was not further investigated. The *n*-hexane–Et_2O (3:5) eluate gave isocupressic acid (460 mg), $[\alpha]_D^{25}$ + 50° ($CHCl_3$, *c* 2.0). The methyl ester, $[\alpha]_D^{25}$ + 42.8° ($CHCl_3$, *c* 2.0); IR ν_{max} cm^{-1}: 3350, 3090, 1720, 1640, 888; ^1H NMR: δ 5.27 (*t*, 1H, *J* = 6.4 Hz), 4.67 and 4.40 (each *s*, 1H), 4.02 (*d*, 2H, *J* = 6.4 Hz), 3.85, 1.63, 1.16, 0.50 (each *s*, 3H); MS *m/e* 334 (M^+), was reduced with $LiAlH_4$ to afford agathadiol, mp 109–110°.

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