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TERPENOIDS FROM THE SEED OF *THUJOPSIS DOLABRATA* VAR. *DOLABRATA*

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Key Word Index—*Thujopsis dolabrata* var. *dolabrata*; Cupressaceae; seed; mono-, sesqui- and diterpenes; desoxypodophyllotoxin; abieta-8,11,13-trien-12,16-oxide; 16-hydroxyferruginol; chemotaxonomy.

Abstract—Fourteen mono-, 13 sesqui-, 16 diterpenes, dodecanal, sitosterol and desoxypodophyllotoxin were identified in the seed of *T. dolabrata* var. *dolabrata*. Sabinene and α-pinene were found to be the main components of the volatile oil. From the diterpenoid fraction, two new abietane-type compounds (ar-abietatrien-12,16-oxide and 16-hydroxyferruginol) were isolated and their structures were elucidated. Significant differences were observed in the seed diterpenoids when these results were compared with those obtained earlier with *T. dolabrata* var. *hondae*.

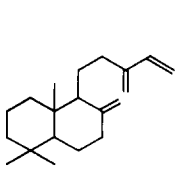
INTRODUCTION

In continuation of our previous work [1] on the chemical constituents of the seed of *Thujopsis dolabrata* Sieb. et Zucc. var. *hondae* Makino (Japanese name: Hiba), we investigated those of *Thujopsis dolabrata* Sieb. et Zucc. var. *dolabrata* (Japanese name: Asunaro) in order to compare the chemical constituents of the two from a viewpoint of chemotaxonomy. Most of the mono- and sesquiterpenes found were similar, while there were some differences in the diterpene fraction between the two. Two of the diterpenes isolated were new. This paper describes a comparison of the terpenoids of the two varieties as well as the elucidation of the structures of the new diterpenes.

RESULTS AND DISCUSSION

The distilled neutral oil of the *n*-hexane extract from the seed of Asunaro was analysed by GC. Table 1 shows the components identified and also the identification methods used. Sabinene and α-pinene were the main components in the volatile oil, which was the same as in that of the seed of Hiba. The differences in composition of the volatile oil between Asunaro and Hiba were slight. The unsaponifiable fraction of the distillation residue upon Si gel column chromatography gave 19 substances as listed in Table 2. All these compounds except **10** and **19** were identified by direct comparison of their IR and NMR spectra with those of authentic samples. Among them, **10** and **19** were new compounds and **3**, **5**, **6**, **7**, **8**, **9**, **14**, **15** and **16** were found only in Asunaro, not in Hiba. It was of interest that Asunaro contained some oxygenated diterpenoids (**10**, **15**, **16** and **19**) and methyl ethers of diterpene phenols (**5**, **6**, **7**, **8** and **14**) but not *trans*-communic acid and isocupressic acid, in comparison with Hiba.

Compound **19**, $C_{20}H_{30}O_2$, mp 141–142°, $[\alpha]_D$ +36.6°, showing in the UV (282.5 nm) and IR (3300,



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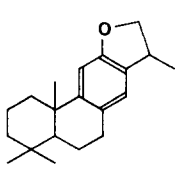

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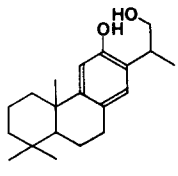

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Table 1. Constituents of the second distillate of the essential oil from the seed of *T. dolabrata* var. *dolabrata*

Peak No.	Compound*	Peak No.	Compound
1	α-Pinene	16	γ-Elemene
2	Sabinene	17	Sabinyl acetate
3	α-Terpinene	18	β-Acoradiene
4	Limonene	19	α-Terpinyl acetate
5	β-Phellandrene	20	Dodecanal
6	*p*-Cymene	21	β-Bisabolene
7	Terpinolene	22	γ-Cadinene
8	Unknown	23	α-Curcumene
9	Thujone	24	Cuparene
10	*trans*-Sabinene hydrate	25	$C_{15}H_{26}O$
	α-Cubebene		
11	α-Copaene	26	$C_{15}H_{26}O$
12	*cis*-Sabinene hydrate	27	Elemol
		28	$C_{15}H_{26}O$
13	Bornyl acetate	29	α-, β-Eudesmol
14	Terpinen-4-ol	30	Hibaene
	β-Elemene		
15	Thujopsene		

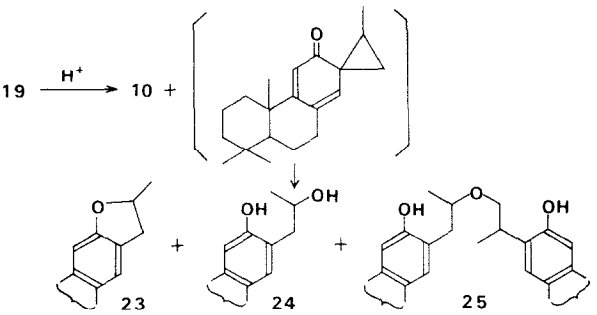
*All compounds initially identified by GC/MS. Compounds in peaks 10–12, 14, 16–24, 27 and 30 were further characterized by IR and NMR spectroscopy.

Table 2. Constituents of the unsaponifiable distillation residue from the seed of *T. dolabrata* var. *dolabrata*

Compound No.	Compound	Rel. %	Compound No.	Compound	Rel. %
1	*n*-Paraffin mixtures	0.42	**11**	Totarol	23.2
2	Hibaene	0.36	**12**	Ferruginol	6.80
3	Sclarene	1.05	**13**	Sempervirol	2.09
4	ar-Abietatriene	1.01	**14**	7-Hydroxytotaryl methyl ether	0.61
5	Totaryl methyl ether	0.39	**15**	7-Oxototarol	0.24
6	Ferruginyl methyl ether	0.15	**16**	7-Hydroxytotarol	19.9
7	6-Dehydroferruginyl methyl ether	0.10	**17**	Elemol	14.2
8	Semperviryl methyl ether	0.22	**18**	Sitosterol	2.34
9	Podototarin	0.17	**19**	16-Hydroxyferruginol	8.09
10	Ar-abietatrien-12,16-oxide	0.39			

1020 cm^{-1}) spectra the presence of a phenolic and primary alcoholic hydroxyl groups, gave an oily diacetate, which had a phenolic acetoxyl group [$\nu_{C=O}$ 1760 cm^{-1}, δ 2.3 (3H, *s*)] and a primary acetoxyl group [$\nu_{C=O}$ 1735 cm^{-1}, δ 2.0 (3H, *s*), 4.0 (2H, *d*, $J = 7.0$ Hz)]. ^{1}H NMR spectrum of **19** showed the presence of three tertiary methyl groups [δ 0.91, 0.93, 1.13 (each 3H, *s*)], one secondary methyl group [δ 1.25 (3H, *d*, $J = 7.5$ Hz)] and two aromatic protons [δ 6.70 (2H, *s*)] (see Table 3). A spin decoupling experiment indicated the presence of a 2-hydroxyisopropyl group, attached to an aromatic ring, since, by irradiation of the signal at 3.13 (Me–C<u>H</u>ϕ–CH_2OH), the doublet at 1.25 attributed to the secondary methyl collapsed to a singlet and two doublet of doublets at 3.82 and 3.59 became an AB quartet, centred at 3.72. The monomethyl ether of **19** was subjected to tosylation, followed by reduction with $LiAlH_4$ to give a desoxy compound, which was identical with (+)-ferruginyl methyl ether in all respects (IR, NMR, UV and $[\alpha]_D$). Thus, the structure of **19** was established as 16-hydroxyferruginol.

Compound **10**, $C_{20}H_{28}O$, mp 49–52°, $[\alpha]_D + 18.9°$, showing the presence of an aryl ether in the UV (289 nm) and IR (1245 and 1000 cm^{-1}) spectra, revealed ^{13}C NMR signals (Table 4) very similar to those of **19** except for signals for C-11, 12, 14, 16 and 20 in **19**. It was, therefore, presumed that the compound was a dehydrated derivative of **19** represented by formula **10**. Spin decoupling experiments also indicated that **10** had a similar proton system (Me–CHϕ–CH_2–O–) to **19**. Acid catalysed cyclization of **19** afforded the expected **10** together with **23**, **24** and **25**. As mentioned by Rüedi and Eugster[2], nucleophilic attack at C-15 on the spirocyclopropyl–cyclohexadienone system as an intermediate accounted well for the formation of **23**, **24** and **25**.



From the ether extract of the residue after extraction with *n*-hexane, desoxypodophyllotoxin was isolated, which was the same as in Hiba.

A comparison of the diterpenoids found in the two varieties of *Thujopsis dolabrata* seed is shown in

Table 3. ^{1}H NMR spectral data of compounds **10** and **19**

Compound	H-5	H-7	H-11	H-14	H-15	H-16	H-17	H-18	H-19	H-20	OH
10	2.20 *br d* ($J = 12.0$)	2.82 *m*	6.66 *s*	6.76 *d* ($J = 1.0$)	3.45 *dddq* ($J = 7.0$, 8.0, 1.0 and 7.5)	4.60 *t* ($J = 8.0$) 3.98 *dd* ($J = 7.0$ and 8.0)	1.28 *d* ($J = 7.5$)	0.91 *s*	0.93 *s*	1.16 *s*	—
19	2.14 *br d* ($J = 12.0$)	2.76 *m*	6.70*s*		3.13 *ddq* ($J = 4.0$, 7.0 and 7.5)	3.82 *dd* ($J = 9.0$ and 4.0) 3.59 *dd* ($J = 9.0$) and 7.0)	1.25 *d* ($J = 7.5$)	0.91 *s*	0.93 *s*	1.13 *s*	7.56 *br s* 2.92 *br s*

Coupling constants in Hz.

Table 4. ^{13}C NMR spectral data of compounds **10** and **19**

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
10	39.0 *t*	19.3 *t**	41.7 *t*	33.5 *s*	50.4 *d*	19.3 *t**	30.2 *t*	129.5 *s*†	150.1 *s*	38.1 *s*
19	38.7 *t*	19.2 *t*	41.8 *t*	33.4 *s*	50.4 *d*	19.2 *t*	29.7 *t*	127.7 *s**	149.7 *s*	37.6 *s*
	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
10	104.9 *d*	158.1 *s*	127.0 *s*†	123.9 *d*	36.4 *d*	78.6 *t*	24.8 *q*	33.3 *q*	21.6 *q*	19.2 *q*
19	112.5 *d*	152.3 *s*	127.1 *s**	128.0 *d*	36.5 *d*	69.2 *t*	24.7 *q*	33.3 *q*	21.6 *q*	15.8 *q*

*,†May be interchanged.

Table 5. Diterpenoids identified in *Thujopsis dolabrata* var. *dolbrata* and var. *hondae*

Compound	Var. *dolabrata* (Asunaro)	Var. *hondae* (Hiba)
Hibaene	+	+
Sclarene	+	—
ar-Abietatriene	+	+
Ferruginol	++	++
Totarol	+++	+++
Sempervirol	+	+
Ferruginyl methyl ether	+	—
Totaryl methyl ether	+	—
Semperviryl methyl ether	+	—
6-Dehydroferruginyl methyl ether	+	—
7-Hydroxytotarol	+++	—
7-Hydroxytotaryl methyl ether	+	—
7-Oxototarol	+	—
16-Hydroxyferruginol	++	—
ar-Abietatriene-12,16-oxide	+	—
Isoagatholal	—	+
trans-Communic acid	—	+++
Isocupressic acid	—	+
Isoagatholal-15-*O*-β-D-xylopyranoside	—	+

+, ++ and +++ denotes that the yield is below 5%, between 5 and 10% and above 10%, respectively, based on the unsaponifiables of the distillation residue, except *trans*-communic and isocupressic acids, which were isolated from the acidic fraction.

Table 5. It can be seen from this that there are marked differences between the two.

EXPERIMENTAL

Mps are uncorr. ^1H NMR (60 and 100 MHz) and ^{13}C NMR (25.1 MHz) spectra were recorded with TMS as an internal standard in $CDCl_3$. GC/MS was carried out with a 2 m × 3 mm stainless steel column with 10% PEG 20M; temp. programmed 50–250° at 5°/min; He at 60 ml/min; the mass spectrometer was operated at 15 eV.

Extraction. Seed (300 g), collected in Nagano Prefecture, Japan, in 1978, was homogenized in *n*-hexane and extracted with the same solvent (4.2 l.) to yield a *n*-hexane extract (116.7 g). A portion of the extract (30 g) was fractionated into a strongly acidic (35 mg), a less strongly acidic (237 mg), and a neutral oil (29.4 g). After extraction with *n*-hexane, the residue gave an Et_2O extract (16.5 g).

Fractionation of the volatile oil. The neutral oil was distilled *in vacuo* to give the following fractions; (a) the first fraction boiling below 145°/30 mm (8.7 g) and (b) the second fraction boiling between 145°/30 mm and 175°/6 mm (1.15 g). The first fraction consisted predominantly of a *ca* 1:5 mixture of α-pinene and sabinene. In addition, α-terpinene, limonene, β-phellandrene, *p*-cymene and terpinolene were identified by means of GC/MS as minor constituents. The second fraction was chromatographed on Si gel, eluting successively with *n*-hexane, C_6H_6, and Et_2O. The *n*-hexane eluate (202 mg) was further chromatographed on 5% $AgNO_3$–Si gel to give cuparene, α-cubebene, α-curcumene, β-acoradiene, γ-cadinene, β-bisabolene, β-elemene, γ-elemene and some diterpene hydrocarbons. The C_6H_6 eluate (320 mg) was subjected to prep. TLC (0.7 mm) on Si gel plates to give dodecanal (69 mg), mp 43–44°, sabinyl acetate (47 mg) and α-terpinyl acetate (145 mg). The Et_2O eluate (628 mg) offered elemol (356 mg) as well as *cis*-sabinene hydrate (50 mg) and terpinen-4-ol (91 mg) by means of prep. TLC on Si gel. All the compounds isolated were identified by direct comparison of their IR, NMR and mass spectra with those of authentic samples, respectively.

Fractionation of the distillation residue. The distillation residue (19.5 g) was refluxed for 2 hr with 2 N ethanolic KOH. The unsaponifiable matter (8.65 g) was chromatographed on Si gel (180 g), eluting successively with *n*-hexane (300 ml), C_6H_6 (600 ml), Et_2O–*n*-hexane (1:3; 400 ml, 1:2; 600 ml, 1:1; 400 ml) and EtOAc (400 ml). The *n*-hexane eluate (330 mg) was subjected to prep. TLC on Si gel impregnated with 5% $AgNO_3$ to give *n*-paraffin mixtures **1** (36 mg), hibaene **2** (31 mg) $[\alpha]_D^{25} - 46.0°$ ($CHCl_3$; *c* 1.2), sclarene **3** (53 mg), colourless oil, $[\alpha]_D^{29.5} + 31.6°$ ($CHCl_3$; *c* 1.26); IR ν_{max}^{neat} cm^{-1}: 3090, 2920, 1635, 1590, 1445, 1375, 990, 890; ^1H NMR (60 MHz): δ 6.35 (1H, *dd*, J = 17 and 11 Hz), 5.17 (1H, *d*, J = 17 Hz), 5.0 (1H, *d*, J = 11 Hz), 4.95 (2H, *s*), 4.82, 4.53 (each 1H, *s*), 0.87, 0.80, 0.67 (each 3H, *s*); UV λ_{max}^{EtOH} nm (log ϵ): 204 sh (4.16), 224.5 (4.23); MS (70 eV, direct inlet) *m/z* (rel. int.): 272 (M^+, 5.2), 257 (23), 204 (7), 189 (6), 175 (6), 161 (22), 137 (22), 119 (26), 105 (29), 93 (56), 81 (58), 69 (54), 55 (54), 41 (100), 29 (21), and ar-abietatriene (**4**) (87 mg). The C_6H_6 eluate (3.1 g) solidified was recrystallized from *n*-hexane to give totarol (**11**) (1.8 g), mp 126–127°. Column chromatography of the mother liquor on Si gel offered a mixture of totaryl methyl ether (**5**), ferruginyl methyl ether (**6**) and 6-dehydroferruginyl methyl ether (**7**) (55 mg), podototarin (**9**) (15 mg), mp 226–227°, totarol (**11**), ferruginol (**12**) (40 mg), sempervirol (**13**) (181 mg) and compound **10** (34 mg) as a colourless semisolid, mp 49–52°, $[\alpha]_D^{27} + 18.9°$ ($CHCl_3$; *c* 1.06); (Found: C, 83.71; H, 10.06. $C_{20}H_{28}O$ requires: C, 84.4; H, 9.9%); UV λ_{max}^{EtOH} nm (log ϵ): 289 (3.58); IR ν_{max}^{KBr} cm^{-1}: 3020, 1625, 1490, 1245, 1000; ^1H and ^{13}C NMR: see Tables 3 and 4; MS (70 eV, direct inlet) *m/z* (rel. int.): 284 (M^+, 100), 269 (84) 241 (7), 227 (10), 213 (14), 199 (44), 187 (59), 173 (66), 147 (18), 131 (12), 115 (6) 69 (34), 55 (12), 41 (19), 28 (10). The Et_2O–*n*-hexane (1:3) eluate (190 mg) was subjected to prep. TLC on Si gel to give 13-*O*-methyl-7-hydroxytotarol (**14**) (53 mg), mp 144–146° (*n*-hexane), and 7-oxototarol (**15**) (45 mg), mp 240–241°. The first fraction of the Et_2O–*n*-hexane (1:2) eluate (1.93 g) solidified was recrystallized from C_6H_6 to give 7-hydroxytotarol (**16**), mp 150–173°, which decomposed slowly to 6-dehydrototarol on standing at room temp. It seemed to exist as epimeric mixtures at the C-7 hydroxyl group because repeated recrystallization showed no sharp mp and Jones oxidation gave pure 7-oxototarol, mp 240–241°. The second fraction of the Et_2O–*n*-hexane (1:2) eluate (1.2 g) yielded elemol (950 mg) and sitosterol (**18**) (202 mg), mp 137.5–138.5°. The Et_2O–*n*-hexane (1:1) eluate (694 mg) was recrystallized from Et_2O–pentane to give compound **19** as a colourless prism, mp 141–142°, $[\alpha]_D^{29} + 36.6°$ ($CHCl_3$; *c* 0.87); (Found: C, 79.54; H, 10.01. $C_{20}H_{30}O_2$ requires: C, 79.4; H, 10.0); UV λ_{max}^{EtOH} nm (log ϵ): 282.5 (3.54); IR ν_{max}^{KBr} cm^{-1}: 3300, 1615, 1565, 1500, 1020, 890, 735; MS (70 eV, direct inlet) *m/z* (rel. int.): 302 (M^+, 50), 287 (M–Me, 15), 271 (M–CH_2OH, 100), 205 (9), 187 (11), 173 (9), 149 (77), 86 (14), 84 (22), 69 (17), 55 (11), 41 (17), 31 (10), 18 (6). ^1H and ^{13}C NMR: see Tables 3 and 4. The diacetate, colourless oil, $[\alpha]_D^{23} + 50°$ ($CHCl_3$; *c* 1.0); UV λ_{max}^{EtOH} nm (log ϵ): 268 (3.25) 276 sh; IR ν_{max}^{neat} cm^{-1}: 2935, 1760, 1735, 1500, 1220, 1038, 910; ^1H NMR (60 MHz): δ 6.79, 6.74 (each 1H, *s*), 4.0 (2H, *d*, J = 7 Hz), 3.13 (1H, *qt*, J = 7 and 6 Hz), 2.83 (2H, *m*), 2.27, 1.98, 1.17, 0.92 (each 3H, *s*), 1.22 (3H, *d*, J = 6 Hz).

Conversion of **19** *to ferruginyl methyl ether* (**6**). To a stirred suspension of dry K_2CO_3 (100 mg) in dry Me_2CO (5 ml) was added Me_2SO_4 (0.1 ml) and **19** (108 mg). The mixture was refluxed for 4 hr. After usual work-up, the crude product was chromatographed on Si gel (4 g) eluting with C_6H_6 and Et_2O successively. The Et_2O eluate gave 101 mg of pure 16-hydroxyferruginyl methyl ether (**21**) as a colourless oil; IR ν_{max}^{neat} cm^{-1}: 3300, 1035, 1250; ^1H NMR (60 MHz): δ 6.73, 6.66 (each 1H, *s*), 3.63 (2H, *d*, J = 7 Hz), 3.27 (1H, *qt*, J = 6 and 7 Hz), 2.80 (2H, *m*), 1.23 (3H, *d*, J = 6 Hz), 3.73, 1.18, 0.93 × 2 (each 3H, *s*). A mixture of **21** (60 mg) and dry pyridine (2 ml) containing *p*-toluenesulfonyl chloride (100 mg) was stirred for 21 hr at room temp. The reaction mixture was poured into aq. 5% HCl cooled to 0° and the products were isolated by extraction with Et_2O. Prep. TLC of the crude product (68 mg) on Si gel, and developing with Et_2O–*n*-hexane (1:2) gave 20 mg of 16-tosyloxyferruginyl methyl ether (**22**) as a colourless oil. To a stirred soln of **22** in dry Et_2O (2 ml) was added $LiAlH_4$ (17 mg) in limited amounts and the mixture was stirred for 5 hr at room temp. Chromatography of the product on Si gel eluting with C_6H_6–*n*-hexane (1:6) gave 12 mg of pure ferruginyl methyl ether (**6**) as a colourless, viscous oil, $[\alpha]_D^{23} +$ 48.4° (EtOH; *c* 0.516).

Dehydration of **19**. A mixture of **19** (200 mg) and dry *p*-toluenesulfonic acid (50 mg) in absolute C_6H_6 (10 ml) was refluxed for 4 hr. The reaction mixture was washed with aq. 5% $NaHCO_3$, brine, dried and removal of the solvent gave an oily residue. The crude products were chromatographed on Si gel (9 g). Elution with Et_2O–*n*-hexane (2:9) gave in order of elution a *ca* 1:3 mixture (69 mg) of **10** and **23**; ^1H NMR (100 MHz) of **23**: δ 2.18 (1H, *br d*, J = 12 Hz), 2.79 (2H, *m*), 6.63 (1H, *s*), 6.76 (1H, *s*), 2.72 (1H, *dd*, J = 15 and 8 Hz), 3.19 (1H, *dd*, J = 15 and 8.5 Hz), 4.80 (1H, *ddt*, J = 8, 7, and 8.5 Hz), 1.44 (3H, *d*, J = 7 Hz), 0.91, 0.93, 1.17 (each 3H, *s*); ^{13}C NMR ppm (multiplicity): 39.0 (*t*), 19.3 (*t*), 41.7 (*t*), 33.4 (*s*), 50.5 (*d*), 19.2 (*t*), 30.1 (*t*), 126.6 (*s*), 150.0 (*s*), 38.0 (*s*), 104.7 (*d*), 157.8 (*s*), 124.3 (*s*), 125.0 (*d*), 37.0 (*t*), 79.3 (*d*), 24.7 (*q*), 33.3 (*q*), 21.6 (*q*), **24** (49 mg); MS 70 eV) *m/z*: 302 (M^+, base peak); IR ν_{max}^{neat} cm^{-1}: 3150, 1500; ^1H NMR (100 MHz): δ 6.76 (1H, *s*), 6.64 (1H, *s*), 4.16 (1H, *m*), 2.84–2.67 (4H, *m*), 2.2 (1H, *br d*, J = 12 Hz), 1.25 (3H, *d*, J = 6 Hz) 0.92, 0.93, 1.17 (each 3H, *s*), **25** (35 mg); MS (70 eV) *m/z*: 586 (M^+); IR ν_{max}^{neat} cm^{-1}: 3150, 1500; ^1H NMR (100 MHz): δ 6.68 (2H, *s*), 6.64 (1H, *s*), 6.00 (1H, *s*), 3.77 (1H, *m*), 3.54 (2H, *m*), 3.17 (1H, *m*), 2.72 (6H, *m*), 2.17 (4H, *br d*, J = 12 Hz), 1.15, 0.93, 0.91 (each 6H, *s*) and unchanged **19**.

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