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TERPENOIDS FROM THE SEED OF *CHAMAECYPARIS OBTUSA*

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Key Word Index—*Chamaecyparis obtusa*; Cupressaceae; mono-, sesqui- and diterpenes; 1,2-dehydrohinokione, 1,2-epoxy-hinokione.

Abstract—Investigation of the extract from the seed of *Chamaecyparis obtusa* revealed the presence of 14 monoterpenoids, four sesquiterpenoids and 10 diterpenoids including one novel compound and a lignan. The structure of the novel diterpenoid was elucidated. Sabinene, α-terpinyl acetate, terpinen-4-ol, *trans*-sabinene hydrate, longifolene and thujopsene were main components of mono- and sesquiterpenoids. Ferruginol and hinokione were major diterpenoid constituents.

INTRODUCTION

The terpenoid constituents of wood, leaves and bark of *Chamaecyparis obtusa* (Cupressaceae) have been investigated by many workers, while those of the seed have not been studied. This paper deals with the identification of the terpenoids from the seed of this species and describes the structural elucidation of a new diterpenoid.

RESULTS AND DISCUSSION

The distilled neutral oil of the ethyl acetate extract from the seed of *C. obtusa* was analysed by GC, and then fractionated by means of CC and prep. TLC on Si gel. All the isolated compounds were identified by direct comparison of their IR, ^{1}H NMR and mass spectra with those of authentic samples. The minor components which were not isolated were identified by GC/MS. Table 1 shows the compounds identified.

The distillation residue was saponified with 2 N ethanolic potassium hydroxide. The unsaponifiable matter obtained was chromatographed on Si gel to give ferruginol (**1**), hinokiol (**2**), hinokione (**3**), 12-methoxy-6,8,11,13-abieatatetraen-11-ol (**4**), 12-methoxy-8,11,13-abietatriene-7β,11-diol (**5**), 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**6**), 1,2-dehydrohinokione (**7**), 1,2-epoxyhinokione (**8**), 12-methoxy-8,11,13-abietatriene-7β,11-diol-3-one (**9**) and savinin (**10**). Compounds **1–3** and **10** were identified by direct comparison of their IR and ^{1}H NMR spectra with those of authentic samples. Compounds **4–6**, all of which had previously been isolated from the heartwood of *Juniperus rigida* [1], were also identified by direct comparison of their IR and ^{1}H NMR spectra.

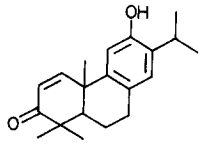
Compound **7**, $C_{20}H_{26}O_2$, mp 167–170°, $[\alpha]_D^{25}$ + 165°, was identified as 1,2-dehydrohinokione by its mass spectrum [2], IR, and ^{1}H NMR spectra and by conversion of it to hinokione on catalytic hydrogenation. This compound was isolated for the first time from natural resources, although it has been synthesized by Chow and Erdtman [3].

Compound **8**, a new compound, $C_{20}H_{26}O_3$, mp 157–159°, $[\alpha]_D^{25}$ + 224°, MS *m/z* 314 $[M]^+$, indicated the presence of a phenolic hydroxyl, a carbonyl and an

Table 1. Constituents of the distilled neutral oil of *C. obtusa*

No.	Compound	No.	Compound
1	α-Pinene	14	*Bornyl acetate
2	Sabinene	15	$C_{15}H_{24}$
3	β-Myrcene	16	*Terpinen-4-ol
4	α-Terpinene	17	*Thujopsene
5	Limonene	18	*α-Terpinyl acetate
6	β-Phellandrene	19	*p*-Cymene-8-ol
7	*p*-Cymene	20	$C_{20}H_{26}O$
8	Terpinolene	21	Elemol
9	**trans*-Sabinene hydrate	22	Cedrol
10	$C_{15}H_{24}$	23	$C_{15}H_{26}O$
11	$C_{10}H_{16}O$	24	$C_{15}H_{24}O$
12	**cis*-Sabinene hydrate	25	*ar*-Abietatriene
13	*Longifolene		

*Isolated compound.



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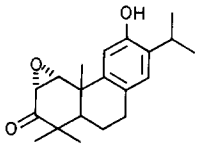

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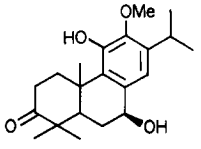

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epoxide ring in the UV (284 nm, log ε 3.52), IR (3350, 1680, 1220, 1030 cm^{-1}) and ^1H NMR (δ 4.81, exchangable 1H, *s*; δ 4.00, 3.52 1H, each *d*, J = 4.2 Hz, AB quartet) spectra. The ^1H NMR spectrum offered the signals at δ 1.07 (3H, *s*), 1.18 (6H, *s*), 1.22 (6H, *d*, J = 7.0 Hz), 2.8 (2H, *t*, J = 5.0 Hz), 3.1 (1H, *m*), 6.85, 6.74 (1H, each *s*) showing the presence of three tertiary methyls, one isopropyl, one benzylic methylene and a 1,2,4,5-tetrasubstituted benzene ring. These spectral data suggested that **8** was a phenolic diterpenoid of the abietane type possessing a carbonyl group at C-3 and an 1,2-epoxide at C-1 and C-2, since (a) the signal assigned to the geminal dimethyl group at C-4 appeared at δ 1.18, which was close to that in hinokione, and (b) the signals of two protons on an epoxide ring showed as an AB quartet in the ^1H NMR spectrum. To establish this structure, 1,2-dehydrohinokione was reacted with hydrogen peroxide in a basic medium to give 1,2-epoxy-hinokione, the IR and ^1H NMR spectra of which agreed with those of **8**. Therefore, **8** was identified as 1,2-epoxyhinokione.

Compound **9**, $C_{21}H_{30}O_4$ (M^+ at *m/z* 346), mp 224–225°, $[\alpha]_D^{25}$ + 225°, showed the following spectral data. UV: 284 nm (log ε 3.24) (phenolic OH), IR: 3350, 1690, 1060 cm^{-1} (alcoholic OH and carbonyl), ^1H NMR: δ 1.18 (6H, *s*, geminal dimethyl), 1.26 (6H, *d*, J = 7.2 Hz, isopropyl), 1.35 (3H, *s*, angular methyl), 1.60 (1H, *s*, alcoholic OH), 2.4–2.8 (2H, *m*, α-methylene to a carbonyl group), 3.2 (1H, *m*, methine of isopropyl), 3.77 (3H, *s*, –OMe), 4.7 (1H, *t*, J = 5.0 Hz, benzylic methine attached to OH), 6.00 (1H, *s*, phenolic OH), 7.05 (1H, *s*, aromatic proton). The ^1H NMR spectrum was very similar to that of the known 12-methoxy-8,11,13-abietatriene-7β,11-diol, except for the signals at δ 1.18 (6H, *s*) and 2.4–2.8 (2H, *m*) due to the geminal dimethyl at C-4 and α-methylene to a carbonyl group, respectively. The chemical shift of the geminal dimethyl and the multiplicity of the methylene α to a carbonyl group in the ^1H NMR spectrum suggested that the structure of **9** was 12-methoxy-8,11,13-abietetriene-7β,11-diol-3-one, which, to the best of our knowledge, is a new compound.

EXPERIMENTAL

^1H 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 packed with 10% PEG 20M; temp. programmed 60° to 250° at 5°/min; He 60 ml/min; the mass spectrometer was operated at 15 eV.

Extraction. Seeds (450 g), collected in Chiba Prefecture, Japan, in 1979, were ground and extracted with EtOAc (3 l. × 2, 1 l. × 1). The extract (75 g) was separated into a basic (138 mg), a strongly acidic (442 mg), a less strongly acidic (3.3 g), a weakly acidic (1.2 g) and a neutral oil (69.5 g) in the usual manner.

Fractionation of the distilled neutral oil. The neutral oil was distilled *in vacuo* to give the following fractions: Fraction 1 bp 120°/30 mmHg (2.4 g), fraction 2 bp 150°/10 mmHg (4.0 g), fraction 3 bp 180°/5 mmHg (1.9 g) and residue (54.0 g). Fraction 2 was found to include the components of fractions 1 and 3 by means of GC (stainless steel column 2 × 3 m packed with 5% OV-17 and 10% PEG 20M; temp. programmed 80° to 250° at 5°/min; N_2 60 ml/min).

Fraction 2 was chromatographed on Si gel (40 g), eluting successively with *n*-hexane, C_6H_6, Et_2O and MeOH (each 100 ml). The *n*-hexane eluate (1.4 g) was subjected to prep. TLC (0.7 mm) on Si gel to give longifolene (372 mg) and thujopsene (400 mg). The C_6H_6 eluate (1.1 g) gave bornyl acetate (112 mg), α-terpinyl acetate (534 mg) and terpinen-4-ol (106 mg) by means of prep. TLC on Si gel and 10% $AgNO_3$–Si gel. The Et_2O eluate (1.3 g) yielded terpinen-4-ol (357 mg), *trans*-sabinene hydrate (316 mg) and *cis*-sabinene hydrate (226 mg) after prep. TLC on Si gel.

Fractionation of the distillation residue. The distillation residue (54.0 g) was refluxed with 2 N ethanolic KOH for 4.5 hr, to give an unsaponifiable matter (44.9 g).

A portion of the unsaponifiable matter (10.6 g) was chromatographed on Si gel (Si gel 50.0 g), eluting successively with *n*-hexane, C_6H_6, Et_2O–*n*-hexane (1:1) and MeOH (each 300 ml). The *n*-hexane eluate (2.4 g) was further chromatographed on Si gel (86 g) to give 12-methoxy-6,8,11,13-abietatetraen-11-ol (**4**, 120 mg), oil, UV λ_{max}^{EtOH} nm (log ε): 270 (3.86), 220 (4.27); IR ν_{max} cm^{-1}: 3500, 1610, 1560, 1240, 1050 and ferruginol (**1**, 378 mg), oil. Rechromatography of the C_6H_6 eluate (2.8 g) on Si gel gave ferruginol (**1**, 38 mg); 12-methoxy-8,11,13-abietatriene-7β,11-diol (**5**, 111 mg), oil, UV λ_{max}^{EtOH} nm (log ε): 282 (3.25); IR ν_{max} cm^{-1}: 3350, 1240, 1050, 1020; 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**6**, 32 mg), oil, UV λ_{max}^{EtOH} nm (log ε): 288 (3.90), 234 (4.12), IR ν_{max} cm^{-1}: 3300, 2750, 1720, 1680, 1280, 1180. The Et_2O–*n*-hexane eluate (3.5 g) was rechromatographed on Si gel (100 g), eluting with Et_2O–*n*-hexane (1:2) to yield hinokione (**3**, 460 mg), prisms, mp 188–189° (lit. 191–192° [3]), 1,2-epoxy-hinokione (**8**, 90 mg), colourless needles, mp 157–159° (Et_2O–*n*-hexane), $[\alpha]_D^{25}$ + 224° ($CHCl_3$; *c* 0.66); UV λ_{max}^{EtOH} nm (log ε): 220 (shoulder), 284 (3.52); IR ν_{max}^{KBr} cm^{-1}: 3350, 1680, 1220, 1030; EIMS 70 eV *m/z* (rel. int.): 314 $[M]^+$ (100), 299 $[M-Me]^+$ (55), 285 (6), 271 (6), 243 (8), 229 (11), 187 (9), 175 (10), 149 (23), 115 (7), 43 (13); 1,2-dehydrohinokione (**7**, 73 mg), colourless needles, mp 167–170° (Et_2O–*n*-hexane), $[\alpha]_D^{25}$ + 165° ($CHCl_3$; *c* 0.52); UV λ_{max}^{EtOH} nm (log ε): 223 (shoulder), 284 (3.52); IR ν_{max}^{KBr} cm^{-1}: 3250, 1630, 1510, 1240; EIMS 70 eV *m/z* (rel. int.): 298 $[M]^+$ (86), 283 $[M-Me]^+$ (100), 255 (15), 241 (31), 229 (20), 216 (12), 213 (12), 199 (12), 187 (18), 185 (14), 159 (19), 141 (14), 128 (11), 115 (11), 69 (15), 43 (17); ^1H NMR: δ1.18 (6H, *s*), 1.25, 1.23 (3H, each *d*, J = 6.6 Hz), 1.38 (3H, *s*), 3.2 (1H, *m*), 4.83 (exchangable 1H, *s*), 6.00, 7.49 (1H, each *d*, J = 12 Hz, AB quartet), 6.79, 6.90 (1H, each *s*), and savinin (**10**, 11 mg).

The MeOH eluate (900 mg) was rechromatographed on Si gel (45 g), eluting with Et_2O–*n*-hexane (1:1), to give hinokiol (**2**, 171 mg), needles, mp 232–233° (lit. 234–235° [4]), and 12-methoxy-8,11,13-abietatriene-7β,11-diol-3-one (**9**, 162 mg), colourless needles, mp 224–225° (Me_2CO–*n*-hexane), $[\alpha]_D^{25}$ + 225° ($CHCl_3$; *c* 0.127); UV λ_{max}^{EtOH} nm (log ε): 225 (shoulder), 284 (3.24); IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1060; EIMS 70 eV *m/z* (rel. int.): 346

$[M]^+$ (100), 303 (12), 289 (9), 271 (26), 259 (13), 239 (10), 229 (32), 215 (8), 208 (26), 197 (8), 193 (8), 187 (9), 149 (7), 55 (8), 43 (13).

Epoxidation of 1,2-*dehydrohinokione.* To a soln of 1,2-dehydrohinokione (8.3 mg) in dioxane (0.35 ml) was added 30% H_2O_2 (0.35 ml), and 1 N NaOH (0.08 ml) added dropwise at 0°. After stirring for 45 min, additional 30% H_2O_2 (0.35 ml) was added and stirring continued for 55 min. The reaction mixture was extracted with Et_2O (5 ml × 2), and the Et_2O extract was washed with $NaHSO_3$ (1 ml), brine and dried to give 1,2-epoxy-hinokione (8.55 mg, yield 98%).

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