[Chem. Pharm. Bull. 20(9)1993—1999(1972)]

UDC 547.597.02 : 581.192

Triterpenoids of *Echinodontium tsugicola*

Akiko Kanematsu and Shinsaku Natori

National Institute of Hygienic Sciences[1]

(Received March 16, 1972)

Seven triterpenoids were isolated from fruit bodies of *Echinodontium tsugicola* Imaz. and the structures (Ib, Ie, Ig, Ih, IIa, IIc, IIe) were elucidated.

Triterpene-carboxylic acids of lanostane series are characteristic constituents of Basidiomycetes.[2] In our course of studies on the distribution of triterpenoids and related compounds among higher fungi, especially in Polyporaceae and related families,[3] the fruit bodies of *Echinodontium tsugicola* (P. Henn. et Shirai) Imaz. (Japanese name, man-nenharitake) (Echinodontiaceae) were proved to contain several triterpenoids devoid of the carboxyl group. This paper concerns the structures of the seven compounds.

The fungus is indigenous to Japan and saprophytic to *Tsuga diversifolia* Mast (Pinaceae). The dried fruit bodies of the fungus were extracted with ether and the benzene soluble part of the extract showed the presence of several kinds of triterpenes or related compounds by thin-layer chromatography (TLC) and gas chromatography (GLC). After repeated chromatographic separation seven triterpenoids (Ib, Ie, Ig, Ih, IIa, IIc, IIe) and one diterpene ester (IVa) were isolated.

The compound (Ib), mp 225—227°, $C_{32}H_{48}O_4$, contains an acetoxyl group (ν_{max} 1748, 1225 cm^{-1}, δ 1.98 (3H, s)) and a carbonyl group (λ_{max} 278 mμ (log ε 1.8), ν_{max} 1705 cm^{-1}). Reduction of Ib with lithium aluminum hydride afforded diol (Id), mp 226—228°, while that with sodium borohydride monoacetate of the diol (Ia), mp 230—232°.

The compound (Ie), mp 186—187°, $C_{30}H_{46}O_3$, is a monohydroxy-monoketone (λ_{max} 278 mμ (log ε 1.8), ν_{max} 3520, 1710 cm^{-1}). Acetylation of Ie gave Ib and hydrolysis of Ib gave Ie. Thus Ib is the acetate of Ie.

The third compound (Ig), mp 230—231°, $C_{32}H_{50}O_4$, contains a hydroxyl group (ν_{max} 3480 cm^{-1}) and an acetoxyl group (ν_{max} 1750, 1228 cm^{-1}, δ 1.98 (3H, s)). Oxidation of Ig with chromium trioxide-pyridine afforded the ketone (Ib).

The fourth compound (Ih), mp 232—233°, $C_{30}H_{48}O_3$, is a diol (ν_{max} 3480 cm^{-1}) and forms diacetate (Ii) (ν_{max} 1748, 1730, 1240 cm^{-1}, δ 1.98, 2.05 (each 3H, s)), which was also obtained by the acetylation of Ig. Ih itself was obtained from Ig by hydrolysis. Oxidation of Ih with chromium trioxide-pyridine under a controled condition resulted in the formation of a monohydroxyketone, which was proved to be identical with Ie. On the other hand oxidation of Ih with Jones' reagent gave a diketone (If) (ν_{max} 1710 cm^{-1}) which was also obtained from Ie by the same treatment.

Thus the four compounds were correlated with each other (Chart 1) and were proved to contain a common ring system. The nuclear magnetic resonance (NMR) spectra[4] (Table I) of all these compounds showed the presence of five angular methyl groups, one secondary methyl, two vinyl methyls (δ 1.7) and one vinyl proton (δ 5.1). In the NMR spectrum of dihydro derivative (Ic) prepared from Ib the two vinyl methyls now appear as two secondary

1) Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo.*
2) G. Ourisson, P. Crabbé, and O.R. Rodig, "Tetracyclic Triterpenes," Hermann, Paris, 1964.
3) A. Kanematsu, S. Natori, and K. Aoshima, the paper presented at the Annual Meeting of Japanese Society of Pharmacognosy, Mukogawa, October 1970, Abstracts of Papers, p. 18.
4) A.I. Cohen, D. Rosenthal, G.W. Krakower, and J. Fried, *Tetrahedron*, **21**, 3171 (1965).

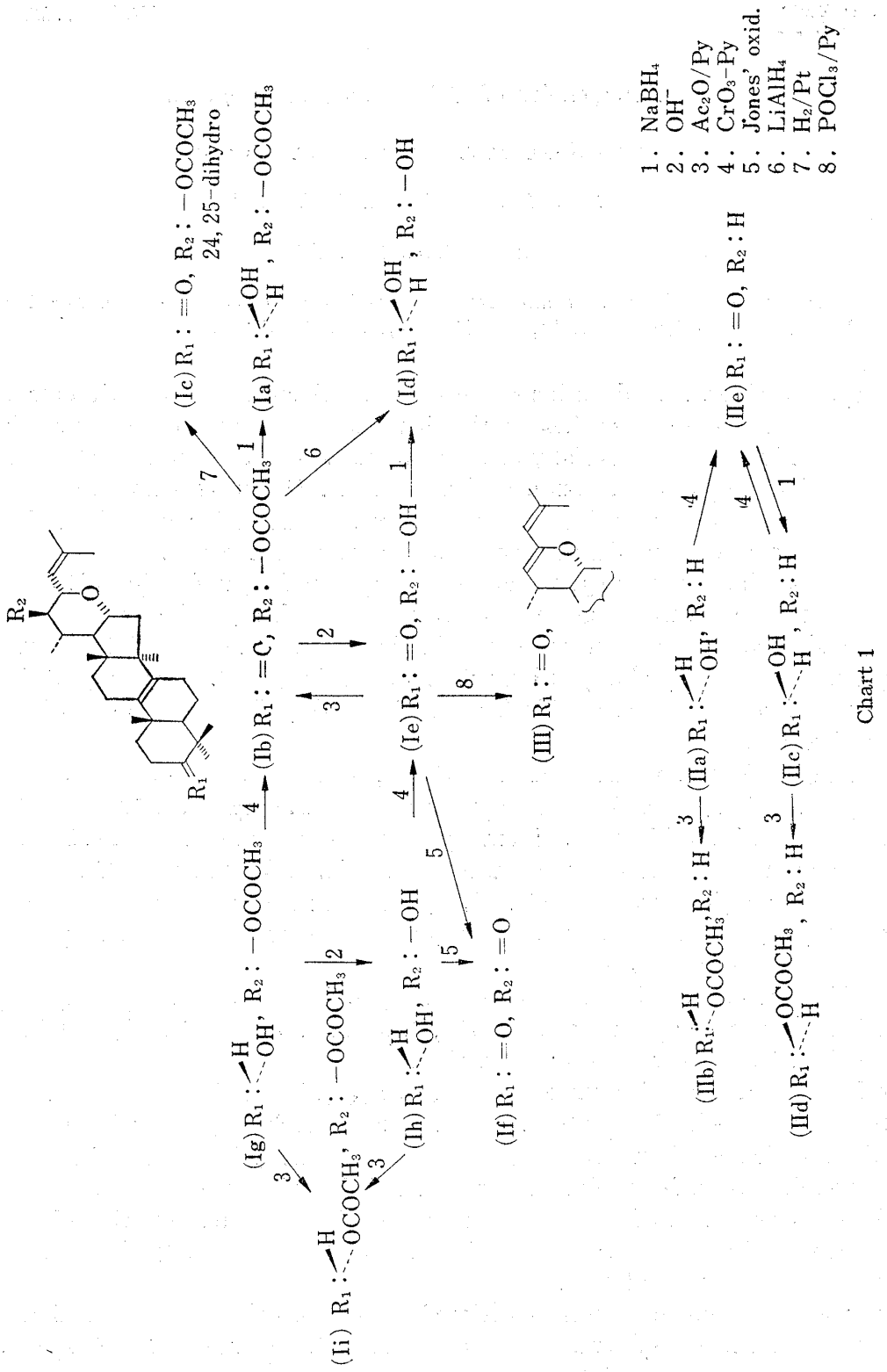

Chart 1

methyls and the vinylic proton disappears, the fact indicating the presence of an isopropylidene group. The NMR spectra also indicated that the remaining oxygen function exists in the form of ether bridge flanked by two methin groups (in Ib, δ 3.72 (1H, m), 4.03 (1H, t)).

These results showed that the four triterpenoids are tetracyclic triterpenes with two double bonds, one probably at the 8-position as in most of the lanostane group and the other at the terminal isopropylidene group, an ether bridge, and two oxygen functions (carbonyl, secondary hydroxyl, and/or secondary acetoxyl groups).

Table I. NMR Spectra of the Triterpenoids (δ in ppm in $CDCl_3$ Solution)

	H					CH_3						-OCOCH$_3$	
	C-3	C-16	C-22	C-23	C-24	C-18	C-19	C-21	C-26,27	C-30,31	C-32	C-3	C-22
Ia	3.20 (m)	3.72 (m)	4.52 (t)[a]	4.03 (t)[a]	5.12 (bd)[a]	0.68	0.98	0.90 (d)[b]	1.69 (bs)	0.80, 0.98	1.03		1.98
Ib		3.72 (m)	4.50 (t)[a]	4.03 (t)[a]	5.10 (bd)[c]	0.71	1.10	0.90 (d)[b]	1.69 (bs)	1.05	1.07		1.98
Ic		3.45 (m)	4.33 (t)[a]	3.82 (bt)[a]		0.68	1.12	0.86 (d)[d]	0.82 (d)[b]	1.06	1.07		2.06
Id	3.20 (m)	3.75 (m)	2.90 (t)[a]	3.88 (t)[a]	5.12 (bd)[a]	0.68	0.98	*e*)	1.77 (bs)	0.80, 0.98	1.02		
Ie		3.75 (m)	2.96 (t)[a]	3.95 (t)[a]	5.18 (bd)[a]	0.72	1.12	*e*)	1.77 (bs)	1.05	1.07		
If		4.23 (m)		4.42 (d)[c]	5.22 (bd)[c]	0.76	1.07	*e*)	1.65 (bs)	1.07	1.09		
Ig	3.42 (m)[f]	3.79 (m)	4.52 (t)[a]	4.03 (t)[a]	5.12 (bd)[a]	0.70	0.98	0.91 (d)[b]	1.70 (bs)	0.98, 0.87	1.05		1.98
Ih	3.42 (m)[f]	3.75 (m)	2.92 (t)[a]	3.90 (t)[a]	5.14 (bd)[c]	0.69	0.97	*e*)	1.75 (bs)	0.97, 0.88	1.03		
Ii	4.65 (m)[f]	3.79 (m)	4.52 (t)[a]	4.03 (t)[a]	5.12 (bd)[a]	0.70	0.98	0.91 (d)[b]	1.70 (bs)	0.92, 0.88	1.10	2.05	1.98
IIa	3.39 (m)[f]	3.63 (m)		4.12 (bt)[a]	5.12 (bd)[a]	0.68	1.00	0.95 (d)[b]	1.73 (bs)	1.02, 0.91	1.07		
IIb	4.62 (m)[f]	3.62 (m)		4.13 (bt)[a]	5.15 (bd)[a]	0.68	1.02	0.93 (d)[b]	1.72 (bs)	0.94, 0.89	1.10	2.08	
IIc	3.20 (m)	3.60 (m)		4.10 (bt)[a]	5.12 (bd)[a]	0.68	1.03	0.91 (d)[b]	1.72 (bs)	0.82, 1.03	1.06		
IId	4.50 (m)	3.60 (m)		4.13 (bt)[a]	5.15 (bd)[a]	0.68	1.03	0.91 (d)[b]	1.72 (bs)	0.92	1.05	2.05	
IIe		3.60 (m)		4.12 (bt)[a]	5.15 (bd)[a]	0.70	1.18	0.92 (d)[b]	1.72 (bs)	1.11	1.13		
III		3.98 [g]	6.09 (bs)		5.44 (bs)	0.72	1.12	0.92 (d)[d]	1.74 (bs)	1.09	1.13		

a) J=9 Hz, *b*) J=6 Hz, *c*) J=8 Hz, *d*) J=7 Hz, *e*) Obscured by other peaks.
f) broadend triplet of J=*ca* 4 Hz, *g*) sextet J=9.7, 9.7, 4.5 Hz

From *Echinodontium tinctorium* Ellis et Ev., a fungus saprophytic to conifers in Pacific coast of North America, Bond, *et al.*[5] isolated a triterpene designated echinodol and elucidated the structure (Ia) from the physical data and a correlation with lanosta-8,24-diene. The comparison of the physical data of our compounds with those of echinodol and the derivatives suggested that they contain the same nucleus. Indeed, the direct comparison of the sodium borohydride reduction product (Ia) of Ib with an authentic sample of echinodol showed their identity. The physical data of Ib, Id, Ie, and If also coincided well with those reported in the literature.[5] Non-identity of Ig and Ih with Ia and Id respectively and the chemical shifts and the coupling patterns[6] of C-3-protons of Ig, Ih, and Ii suggest that Ig and Ih are C-3-epimers of Ia and Id respectively. Thus the structures of the new triterpenoids are established as Ib, Ie, Ig, and Ih in Chart 1 and they are respectively designated as echinodone, deacetylechinodone, 3-epiechinodol, and deacetyl-3-epiechinodol.

The structure of the tetrahydropyran part of echinodol, including the stereochemistry of C-20, C-22, and C-23, suggested chiefly by the NMR spectra,[5] was again neatly supported by the NMR spectra of all the compounds as shown in Table I. The ambiguity existed in the absolute configuration at C-16 was excluded by the coupling pattern of the C-16 proton (δ 3.98 (sextet, J=9.7, 9.7, 4.5 Hz)) of the triene[5] (III) prepared by the dehydration of Ie.

5) F.T. Bond, D.S. Fullerton, L.A. Sciuchetti, and P. Catalfomo, *J. Am. Chem. Soc.*, **88**, 3882 (1966).
6) H.K. Adam, T.A. Bryce, I.M. Campbell, N.J. McCorkindale, A. Gaudemer, R. Gmelin, and J. Polonsky, *Tetrahedron Letters*, **1967**, 1461.

Table II. Mass Spectra of the Triterpenoids (*m/e* (Relative Intensity in %))

	a	b (c-H_2O)	c (d-H_2O)	d (e-H_2O)	M+-CH_3 -CH_3COOH (-H_2O)	M+- CH_3COOH	M+-CH_3 (-H_2O)	M+
Ib		271 (10)	297 (55)	313 (8)	421 (16) 403 (2)	436 (100)	481 (2)	496 (3)
Ie		271 (8)	297 (47)	313 (100)			439 (8) 421 (5)	454 (17)
IIe	109 (100)	271 (5)	297 (12)	313 (3)			423 (49) 405 (3)	438 (40)
IIa	109 (75)	273 (4) 255 (5)	299 (7) 281 (6)	315 (4) 297 (3)			425 (100) 407 (33)	440 (75)
IIc	109 (33)	273 (4) 255 (8)	299 (7) 281 (3)	315 (2) 297 (4)			425 (100) 407 (33)	440 (73)
Ia		273 (6) 255 (11)	299 (47) 281 (14)	315 (6) 297 (11)	423 (25) 405 (18)	438 (100)	483 (8)	498 (8)

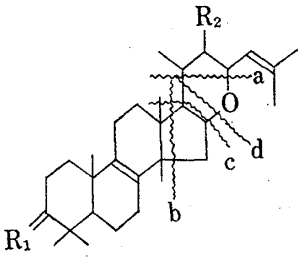

Chart 2

The mass spectra of the compounds are shown in Table II and the assignments of the fragmentations (Chart 2) also support the structures.[7]

The other three triterpenes, IIa, mp 194—195°, $C_{30}H_{48}O_2$, IIc, mp 194—195°, $C_{30}H_{48}O_2$, and IIe, mp 182—183°, $C_{30}H_{46}O_2$, are also closely related each other. The compounds IIa and IIc are monohydroxy compounds (IIa, ν_{max} 3450 cm^{-1}, IIc, ν_{max} 3450 cm^{-1}), and formed monoacetates (IIb and IId). The oxidation of IIa and IIc with chromium trioxide-pyridine afforded the same ketone ($\nu_{C=O}$ 1710 cm^{-1}), which was proved to be identical with the natural product (IIe). Reduction of IIe with sodium borohydride gave back IIc. The comparison of the mass spectra of IIa or IIc and IIe with those of Ia and Ib or Ie respectively showed entirely the same fragments except those containing the side chain (Table II). In the NMR spectra (Table I) the signal corresponding to the proton attached to C-22 in Ia—Ii disappeared and the triplet signal due to the proton at C-23 was now broadend by further coupling, while other signals including the methin proton at C-16 and those in a isopropylidene group appeared as in the case of Ia—Ii. These facts lead to the conclusion that the compounds IIa—IIe lack oxygen function at C-22 in the same nucleous as Ia—Ii. The comparison of the chemical shifts and the coupling patterns of the carbinyl protons at C-3[6] indicated that IIa bears an axial hydroxyl, while IIc, equatorial. Thus the structures of IIa, IIc, and IIe, corresponding to deacetoxy-3-epiechinodol, deacetoxyechinodol, and deacetoxyechinodone respectively, were proposed.

Besides these triterpenoids, a compound (IVa), mp 148—149°, $C_{31}H_{42}O_7$, was isolated by the chromatographic separation of the same fraction. The UV (λ_{max} 236.5, 274 (log ε 4.1, 3.0)), IR (ν_{max} 1748, 1725, 1600, 1265, 1225 cm^{-1}), NMR (δ 1.90 (3H, s), 2.11 (3H, s), 7.4—8.1 (5H)), and hydrolysis to a diterpene (IVb), $C_{20}H_{34}O_4$, mp 188—190°, suggested that IVa is monobenzoate diacetate of IVb but, due to the scarcity of the sample, further work was abandond.

The comparison of the ether extract of *E. tsugicola* with that of the sample of *E. tinctorium*,[8] from which echinodol (Ia) was isolated,[5] by TLC and GLC showed that the former is devoid of Ia while the latter contains Ia as the major triterpenoid and shows the

7) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structural Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, San Francisco, 1964.
8) The samples were supplied by Dr. K. Aoshima, Government Forest Experiment Station, Tokyo.

presence of the closely related compounds as the minor constituents. *Echinodontium spp.* had been classified in Hydnaceae but now is classified in a new family, Echinodontiacea. Some closely situated fungi such as *Stereum taxodii* LENTZ & Mc KAY and *Laurilia sulcatum* (BURT) GROSS[8] were examined for the presence of the related compounds by TLC but there was no sign of the presence of the triterpenoids, though they were proved to contain sterols as the common constituents of Basidiomycetes.

Experimental

Extraction of Triterpenoids from *Echinodontium tsugicola*——Fruit bodies of the fungus (300 g), collected at Marunuma, Gumma Prefecture, in October 1969, were air-dried, crushed, and extracted twice with ether (7.5 liter) at room temperature. The combined extracts (13.8 g) were treated with hot benzene to remove orange-yellow amorphous powder (0.4 g). The benzene extract (12.5 g) was chromatographed on a column of alumina (Woelm neutral, 500 g) and eluted successively with (i) benzene (2.8 liter), (ii) benzene–ether (99:1, 2.3 liter), (iii) benzene–ether (98.5:1.5, 11.4 liter), (iv) ether (3 liter), and (v) MeOH (3 liter). The fraction (iii) (6.3 g) was rechromatographed under the similar conditions and each fraction was examined by TLC and GLC.

The fractions eluted with benzene containing IIe and Ib were combined and further separated by preparative layer chromatography (Silica gel HF_{254}). Deacetoxyechinodone (IIe) was recrystallized from MeOH to colorless needles (5 mg), mp 182—183°, $[\alpha]_D^{24}$ +84.1° (c=0.82, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_2$: C, 82.1; H, 10.6. Found: C, 81.4; H, 10.5. Mass Spectrum (Chart 2 and Table II): M^+ 438.346 *m/e* (Calcd. 438.350). IR ν_{max}^{KBr} cm^{-1}: 1710. NMR (Table I). Echinodone (Ib) was recrystallized from MeOH to colorless needles (26 mg), mp 225—227° (lit.[5] mp 227—229°), $[\alpha]_D^{22}$ +67° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{48}O_4$: C, 77.3; H, 9.7. Found: C, 76.9, H, 9.7. Mass Spectrum (Chart 2 and Table II): M^+ 496.346 *m/e* (Calcd. 496.352). UV λ_{max}^{EtOH} mμ (log ε): 278 (1.8). IR ν_{max}^{KBr} cm^{-1}: 1748, 1705, 1225. NMR (Table I).

The next fraction containing the diterpene ester (IVa) was recrystallized from MeOH to colorless prisms of mp 148—149° (115 mg), $[\alpha]_D^{22}$ +81° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{42}O_7$: C, 70.7; H, 8.0. Found: C, 70.6; H, 8.1. Mass Spectrum: M^+ 526. 293 *m/e* (Calcd. for $C_{31}H_{42}O_7$, 526. 295). UV λ_{max}^{EtOH} mμ (log ε): 236.5, 274, 281 (4.1, 3.0, 3.0). IR ν_{max}^{KBr} cm^{-1}: 3580, 1748, 1725, 1265, 1225. NMR δ ($CDCl_3$): 0.7—2.6 (28H), 1.90 (3H, s), 2.11 (3H, s), 5.1—5.5 (3H), 7.4—8.1 (5H).

The fractions eluted with benzene–ether (98:2) containing IIa and IIc were combined and further purified by preparative layer chromatography. Deacetoxy-3-epiechinodol (IIa), recrystallized from MeOH, colorless needles (10 mg), showed mp 194—195°, $[\alpha]_D^{19}$ +32.5° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_2$: C, 81.8, H, 11.0). Found: C, 81.3; H, 10.9. Mass Spectrum (Chart 2 and Table II): M^+ 440.366 *m/e* (Calcd. for $C_{30}H_{48}O_2$, 440.365). IR ν_{max}^{KBr} cm^{-1}: 3450. NMR (Table I). Deacetoxyechinodol (IIc) was obtained as colorless needles (10 mg) of mp 194—195° (from MeOH), $[\alpha]_D^{18}$ +58.3° (c=0.96, $CHCl_3$). Mass Spectrum (Chart 2 and Table II): M^+ 440.366 *m/e* (Calcd. for $C_{30}H_{48}O_2$, 440.365). IR ν_{max}^{KBr} cm^{-1}: 3450. NMR (Table I).

The benzene–ether (95:5) fractions were collected and again separated by preparative layer chromatography into three components (Ig, Ie, and Ih). 3-Epiechinodol (Ig) was obtained as colorless needles (290 mg) of mp 230—231° from MeOH, $[\alpha]_D^{19}$ +45° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{50}O_4$: C, 77.1; H, 10.1. Found: C, 76.8; H, 10.0. Mass Spectrum (Chart 2 and Table II). IR ν_{max}^{KBr} cm^{-1}: 3480, 1750, 1228. NMR (Table I). Deacetylechinodol (Ie), recrystallized from MeOH (120 mg), showed mp 186—187° (lit.[5] mp 186—187°), $[\alpha]_D^{20.5}$ +54.2° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_3$: C, 79.2; H, 10.2. Found: C, 78.9; H, 10.2. Mass Spectrum (Chart 3 and Table II): M^+ 454.342 *m/e* (Calcd. for $C_{30}H_{46}O_3$, 454.345). UV ν_{max}^{EtOH} mμ (log ε): 278 (1.8). IR ν_{max}^{KBr} cm^{-1}: 3440, 1710. NMR (Table I). Deacetyl-3-epiechinodol (Ih), recrystallized from MeOH, colorless needles (120 mg), showed mp 232—233°, $[\alpha]_D^{17}$ +45° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.9; H, 10.6. Found: C, 78.6; H, 10.5. IR ν_{max}^{KBr} cm^{-1}: 3480. NMR (Table I).

Acetylation of Ie——The compound (Ie) was acetylated by Ac_2O–pyridine by the conventional method to give colorless needles of mp 220—222° (from MeOH), which was identified with Ib (mixed mp, TLC, GLC, and IR).

Hydrolysis of Ib to Ie——Ib (40 mg) in 2.5% KOH–EtOH (6 ml) was allowed to stand at room temperature overnight. Recrystallization from MeOH afforded colorless needles (25 mg) of mp 182—183°, which was identified with Ie.

Reduction of Ib with $LiAlH_4$ to Id——Ib (54 mg) in ether (anhyd., 20 ml) was added dropwise with stirring at room temperature in $LiAlH_4$ (200 mg) suspended in ether (anhyd., 20 ml) and the mixture was refluxed for 1 hr. The excess amount of $LiAlH_4$ was destroyed by wet ether. After acidification the reaction product was extracted with ether, washed, and dried. Recrystallization from MeOH gave Id as colorless needles (36 mg) of mp 226—228°, $[\alpha]_D^{22}$ +43.6 (c=0.3, $CHCl_3$) (lit.[5] mp 228—229°, $[\alpha]_D$ +47°). IR ν_{max} cm^{-1}: 3420. NMR (Table I).

Reduction of Ib with $NaBH_4$ to Ia——Ib (33 mg) in dioxane (3 ml) was added with stirring at room temperature to $NaBH_4$ (10 mg) in water (1 ml) and dioxane (1 ml). After 30 min the excess amount of $NaBH_4$ was decomposed by the addition of AcOH and the reaction product was extracted with $CHCl_3$ and purified by preparative layer chromatography to give Ia as colorless needles (21 mg) of mp 230—231° (from MeOH) $[\alpha]_D^{28}$ +42° (c=0.9, $CHCl_3$) (lit.[5] mp 236—238°, $[\alpha]_D$ +48°). IR ν_{max} cm^{-1}: 3520, 1735, 1230. NMR (Table I). The identification with the authentic sample of echinodol (Ia) has been carried out by mixed mp, TLC, GLC, IR and NMR.

Acetylation of Ig and Ih——The acetate (Ii) of Ig, prepared by the ordinary method, showed mp 191—192° (from MeOH), $[\alpha]_D^{19}$ +16.2° (c=0.65, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{52}O_5$: C, 75.5; H, 9.7. Found: C, 75.0; H, 9.6. IR ν_{max}^{KBr} cm^{-1}: 1748, 1730, 1238. NMR (Table I).

The same acetate (Ii), mp 192—193°, was obtained from Ih and identified.

Oxidation of Ig to Ib——Ig (24 mg) was dissolved in pyridine (1 ml) and allowed to stand with CrO_3–pyridine (200 mg in 2 ml) at room temperature overnight. The reaction mixture was extracted with $CHCl_3$ and purified by preparative layer chromatography to give Ib as colorless needles (18 mg) of mp 222—223° (from MeOH). The identity with the natural product (Ib) was confirmed by the ordinary methods.

Hydrolysis of Ig to Ih——Ig (21 mg) was hydrolysed with 2.5% KOH–EtOH (4 ml) at room temperature overnight. Recrystallization from MeOH afforded Ih (13 mg), mp 230—232°, and the identification with the natural product (Ih) was carried out.

Oxidation of Ih to Ie——Ih (215 mg) in pyridine (5 ml) was treated with CrO_3–pyridine (300 mg in 3 ml) at room temperature overnight. The reaction mixture was extracted with $CHCl_3$ and separated by TLC into two fractions. One of them was recrystallized from MeOH to give colorless needles (125 mg) of mp 182—183° and identified with Ie. The other was proved to be the starting material (30 mg).

Oxidation of Ih and Ie to If——Ih (439 mg) in acetone (30 ml) was added with Jones' reagent[9] (1.6 ml). After standing at room temperature for 2.5 hr, the reaction products were extracted with ether, washed, and separated by preparative layer chromatography to give the main product, which was recrystallized from MeOH to the ketone (If), colorless needles (124 mg) of mp 171—172° (lit.[5] mp 168—171°), $[\alpha]_D^{20}$ +68° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1710. NMR (Table I). By the same procedure Ie (58 mg) gave If (23 mg), mp 170—171°.

Hydrogenation of Ib to Ic——Ib (18 mg) in EtOH (5 ml) was hydrogenated in the presence of Pt-catalyst (5 mg). Recrystallization from MeOH gave colorless needles of the dihydro derivative (Ic) (13 mg) of mp 154—155°, IR ν_{max}^{KBr} cm^{-1}: 1745, 1707, 1383, 1228. NMR (Table I).

Dehydration of Ie to the Diene (III)——Ie (50 mg) in pyridine (2 ml) was added with $POCl_3$ (0.2 ml) and the reaction mixture was allowed to stand at room temperature overngiht. After working up usual the reaction products were separated by preparative layer chromatography to afford colorless needles (23 mg) of mp 188—189° (lit.[5] mp 189—190°), $[\alpha]_D^{20}$ +72.9° (c=0.62, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 235 (4.1). IR ν_{max}^{KBr} cm^{-1}: 1700, 1610, 1138, NMR (Table I).

Acetylation of IIa——IIa gave the acetate (IIb) by the conventional method, colorless needles (from MeOH), mp 197—198°, $[\alpha]_D^{20}$ −17° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1735, 1235. NMR (Table I).

Acetylation of IIc——IIc was acetylated to give the acetate (IId), mp 186—188° (from MeOH), $[\alpha]_D^{19}$ +48° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1735, 1238. NMR (Table I).

Oxidation of IIa and IIc to IIe——IIa (30 mg) in pyridine (1 ml) was allowed to stand with CrO_3–pyridine (200 mg in 2 ml) at room temperature overnight. After extraction with $CHCl_3$, the reaction product was purified by preparative layer chromatography to give IIe as colorless needles (20 mg), mp 182—183°. The same compound was also obtained from IIc by the same procedure. The product was identified with the natural product (IIe) by every means of identification.

Reduction of IIe with $NaBH_4$ to IIc——IIe (2.4 mg) in dioxane (1 ml) was added with stirring at room temperature to $NaBH_4$ (2.5 mg) in dioxane–water (0.5 ml). After 30 min the excess amount of the reagent was decomposed by the addition of AcOH and the product was extracted with $CHCl_3$. Chromatography and recrystallization from MeOH gave colorless needles (1.8 mg) of mp 190—192°. The product was identified with IIc by the ordinary methods.

Hydrolysis of the Diterpene Ester (IVa)——The ester (IVa) (201 mg) was hydrolyzed with 5% KOH–EtOH (10 ml) for 3 hr and the hydrolysate was extracted with ether. The extract was recrystallized from acetone to give colorless needles (IVb) (103 mg) of mp 188—190°, $[\alpha]_D^{21}$ +38° (c=0.75, $CHCl_3$). Mass Spectrum, M^+ 338.247 m/e (calcd. for $C_{20}H_{34}O_4$, 338.245). IR ν_{max}^{KBr} cm^{-1}: 3340, 1053, 812.

The aqueous layer after extraction with ether was acidified and extracted with ether. Recrystallization from water gave colorless leaflet (37 mg) of mp 122°, IR ν_{max}^{Nujol} cm^{-1}: 1700, 1605, 1585, which was identified with benzoic acid.

The diterpene (IVb) was acetylated by Ac_2O–pyridine to give colorless leaflet of mp 203—204°, $[\alpha]_D^{21}$ +64.9° (c=0.9, $CHCl_3$). Mass Spectrum, M^+ 422.264 m/e (calcd. for $C_{24}H_{38}O_6$, 422.269). IR ν_{max}^{KBr} cm^{-1}:

9) A. Bowers, T.G. Halsall, E.R.H. Jones, and A.J. Lemin, *J. Chem. Soc.*, **1953**, 2548.

3360, 1750, 1250, 1225, 810. NMR δ ($CDCl_3$): 0.65—2.5 (29 H), 2.02 (6H, s), 3.92 (1H, bd, J=7 Hz), 5.09 (2H).

Thin-Layer Chromatography——For thin-layer chromatography Silica gel H or HF_{254} was used. Hexane–AcOEt (7:3) was employed for the solvent and the detection was carried out by heating on a hot plate after spraying 20% vanillin–phosphoric acid.

Gas Chromatography——Gas chromatography was carried out on 1.5% OV-1 on Chromosorb W column at 250° on a Hitachi F6-D Gas Chromatograph.

Acknowledgement The authors thank Dr. K. Aoshima, Government Forest Experiment Station, for the advice and the supply of the specimens of fungi and Dr. F.T. Bond, University of California, San Diego, for the donation of the sample of echinodol. The authors indebted to Professor T. Takahashi, Tokyo University, for the informations on mass spectral data and to Professor M. Tomoeda, Kanazawa University, for his help in physical determinations.