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Triterpenoids of Lanostane Group from Fruit Bodies of Nine Basidiomycetous Species

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Eight species of Aphyllophorales and one species of Agaricales of Basidiomycetes were examined for the triterpenoidal constituents and six compounds (I, II, III, IVa, VIIIa, IXa) were isolated and identified (Table I). The new acid from *Melanoporia rosea* (as the methyl ester) (IVa) was suggested to be 12β-hydroxycarbomethoxyacetylquercinic acid methyl ester.

More than thirty tetracyclic triterpenes of lanostane group have so far been obtained from fruit bodies or mycelia of Polyporaceae, Basidiomycetes.[2] Most of these compounds possess a carboxyl group at C_{20} with or without one carbon unit at C_{24}.

We have examined on the fruit bodies of 97 species of wood-rotting fungi, especially of Polyporaceae and related families for the distribution of triterpenes and sterols. The studies on the constituents of *Poria cocos* (FR.) WOLF and *Echinodontium tsugicola* (P. HENN.) IMAZ. have been reported in the previous papers.[3,4]

In this paper, isolation of the triterpenoids from nine species as shown in Table I will be reported.

Fresh, ripe fruit bodies of each species were air-dried, crushed and extracted with ether for one week at room temperature. In order to facilitate the identification and the separation of the triterpenes the hexane insoluble part of the extracts was methylated with diazo-

TABLE I. Triterpenoids of the Basidiomycetes

Species (Japanese name)	Family	Compound identified
Daedalea tanakae (MURR.) AOSHIMA (Himeshiroamitake)	Polyporaceae	I
Melanoporia rosea (ALB. et SCHW. ex FR.) AOSHIMA (Barairosarunokoshikake)	Polyporaceae	I, II, III, IVa
Melanoporia juniperina AOSHIMA (Nikuamitake)	Polyporaceae	I, II, III
Gloeophyllum abietinum (BULL. ex FR.) KARST. (Kogeirokaigaratake)	Polyporaceae	VIIIa, IXa
G. sepiarium (WULF. ex FR.) KARST. (Kikaigaratake)	Polyporaceae	VIIIa, IXa
G. striatum (SWARTZ. ex FR.) MURR. (Hirohanokikaigaratake)	Polyporaceae	VIIIa, IXa
Lentinus lepideus FR. (Matsuofuji)	Tricholomataceae	VIIIa, IXa
Spongiporus appendiculatus (BERK. et BR.) AOSHIMA (Shirokaimentake)	Polyporaceae	II, VIIIa, IXa
Veluticeps angularis (LLOYD) AOSHIMA et FURUKAWA (Chizugatasarunokoshikake)	Veluticepsaceae	IXa

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[1] Location: *Kamiyoga-1-chome, Setagayaku, Tokyo.*

[2] G. Ourisson, P. Crabbé, and O.R. Rodig, "Tetracyclic Triterpenes," Hermann, Paris, 1964.

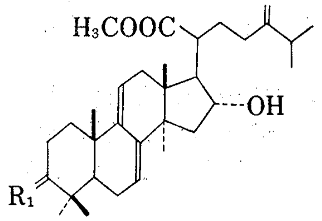
[3] A. Kanematsu and S. Natori, *Yakugaku Zasshi*, **90**, 475 (1970); *idem*, *Chem. Pharm. Bull.* (Tokyo), **18**, 779 (1970).

[4] A. Kanematsu and S. Natori, *Chem. Pharm. Bull.* (Tokyo), **20**, 1993 (1972).

methane and separated by column and preparative thin-layer chromatographies (TLC) to give each compound as the methyl ester as shown in Table I. Sterol fraction from the hexane soluble part will be reported in a forth-coming paper.

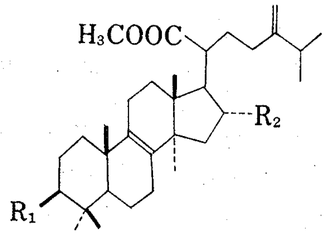
From the white, small fruit bodies of *Daedalea tanakae* grown on conifers and broad-leaved trees, methyl polyporenate C (I) was isolated. The isolation of the acid from *D. dickinsii* had been reported.[5]

In the case of two species of *Melanoporia*, producing perennial fruit bodies and growing on conifers and broad-leaved trees, the same constituent (I) was isolated together with methyl tumulosate (II) contaminated with the corresponding 7,9(11)-diene (III). In addition, a new triterpene was obtained from *M. rosea*. The methyl ester of the new acid (IVa), $C_{36}H_{56}O_8$, $[\alpha]_D+4.66°$, showed one spot in TLC, gave an oily monoacetate (IVb), $C_{38}H_{58}O_9$, $[\alpha]_D-5.26°$, and was converted into a diol monomethyl ester (Va), $C_{32}H_{52}O_5$, $[\alpha]_D+19.0°$, by the hydrolysis with methanolic potassium hydroxide, followed by the methylation. The diol (Va) was further derived to the diacetate (Vb) and the ketone (VII). Thus the methyl ester (IVa) was proved to be an acyl ester of the compound (Va) having two secondary hydroxyls and one

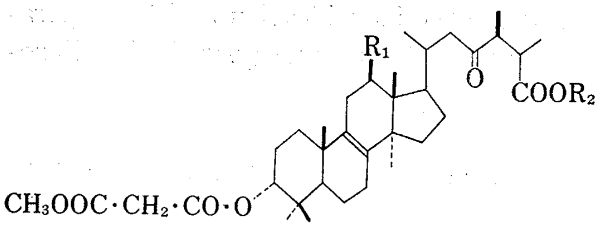


I : R=O methyl polyporenate C

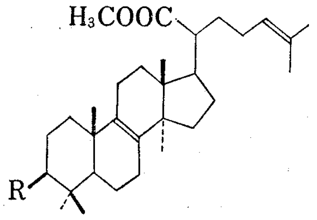
III : $R=\,^{OH}_{H}$ methyl dehydrotumulosate



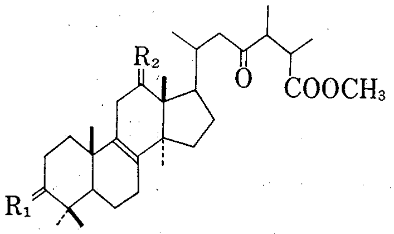
II : $R_1=OH$, $R_2=OH$ methyl tumulosate
VIIIa : $R_1=OH$, $R_2=H$ methyl eburicoate
VIIIb : $R_1=OAc$, $R_2=H$
VIIIc : $R_1=OTMS$, $R_2=H$



IVa : $R_1=OH$, $R_2=CH_3$
IVb : $R_1=OAc$, $R_2=CH_3$
VIa : $R_1=H$, $R_2=H$
VIb : $R_1=H$, $R_2=CH_3$



IXa : R=OH methyl trametenolate
IXb : R=OAc
IXc : R=OTMS



Va : $R_1=\,^{OH}_{H}$, $R_2=\,^{OH}_{H}$

Vb : $R_1=\,^{OAc}_{H}$, $R_2=\,^{OAc}_{H}$

VII : $R_1=\ =O$, $R_2=\ =O$

Chart 1

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5) H. Inouye, K. Tokura, and T. Hayashi, *Yakugaku Zasshi*, **92**, 62 (1972); H. Inouye and K. Tokura, *ibid.*, **92**, 859 (1972).

methoxycarbonyl group. Since there exist no olefinic proton observed in the nuclear magnetic resonance (NMR) spectra, one double bond was accomodated at the 8-position as in the case of most of the fungal triterpenes. From the molecular formulae and infrared (IR) spectra the remaining oxygen function was assumed to be a carbonyl. The acyl group was suggested to be methoxycarbonylacetyl by the mass spectrum of IVa, *m/e* 498 ($M^+-C_4H_6O_4$), 483 ($M^+-CH_3-C_4H_6O_4$), 465 ($M^+-CH_3-H_2O-C_4H_6O_4$), the loss of $C_4H_4O_3$ by the hydrolysis, and by the NMR spectrum (δ 3.70 (3H), 3.38 (2H)).[6] As the malonate conjugate of fungal triterpene the structure of carboxyacetylquercinic acid (VIa) was elucidated.[6] The spectral data of IVa and the derivatives showed close similarities with those of the methyl ester (VIb) of VIa, except the presence of an additional secondary hydroxyl group in IVa (δ, 4.10). Especially the fragments caused by the loss of 158 mass units in the mass spectra (Chart 2) and the presence of two secondary methyl groups appearing in rather lower fields (δ 1.12, 1.03) in the NMR spectra suggested the presence of the same side chain.

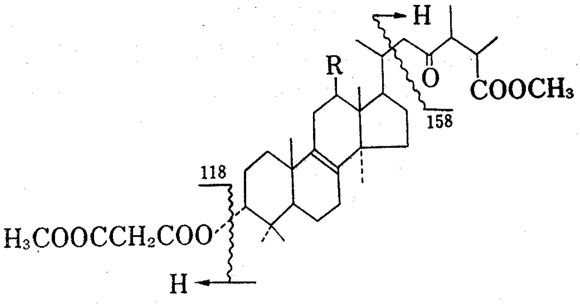

Chart 2

These results showed that the methyl ester of the new acid (IVa) correspond to a monohydroxy derivative of carboxyacetylquercinic acid dimethyl ester (VIb).

Table II. NMR Spectra of the Triterpenoids (δ in ppm in $CDCl_3$ solution)

	CH_3					H		OCH_3	$COCH_2$-CO	$OCOCH_3$	
	18	19	30, 31	32	27, 28	3	12			3	12
IVa	0.72	1.01	0.92	0.87	1.03(d)[a] 1.12(d)[a]	4.68(m)	4.10(m)	3.62 3.70	3.38		
IVb	0.80	0.98	0.98, 0.92	0.87	1.04(d)[a] 1.12(d)[a]	4.66(m)	5.20(t)[b]	3.60 3.68	3.36		2.02
Va	0.72	0.98	0.95, 0.90	0.87	1.03(d)[a] 1.12(d)[a]	3.38(m)	4.05(t)[b]	3.58			
Vb	0.82	0.99	0.99, 0.92	0.85	1.05(d)[a] 1.13(d)[a]	4.60(m)	5.18(t)[b]	3.60		2.03	2.03
VIb	0.74	1.00	0.92	0.88	1.04(d)[a] 1.12(d)[a]	4.66(m)		3.61 3.68	3.38		
VII	1.09—1.18	1.09—1.18	1.09—1.18	0.79	1.09—1.18			3.62			

a) $J=6$ Hz, *b*) $J=7$ Hz.
In IVa—VIb the C_{21}-methyls appears at 0.9—1.0 ppm overlapping with other methyl signals.

Although there exists no decisive evidence for the location of the hydroxyl group, 12β-position was assumed to be most preferable from the following evidences. As shown in Table II the additional hydroxyl group does not effect the chemical shifts of any of the angular methyl groups.[7-10] The ketone (VII) does not show the properties of 1,2-diketone and α,β-

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[6] H.K. Adam, T.A. Bryce, I.M. Campbell, N.J. McCorkindale, A. Gaudemer, R. Gmelin, and J. Polonsky, *Tetrahedron Letters*, **1967**, 1461.

[7] F. Hemmert, B. Lacoume, J. Levisalles, and G.R. Pettit, *Bull. Soc. Chim. France*, **1966**, 976; F. Hemmert, A. Lablache-Combier, B. Lacoume, and J. Levisalles, *Bull. Soc. Chim. France*, **1966**, 982.

[8] A.I. Cohen, D. Rosenthal, G.W. Krakower, and J. Fried, *Tetrahedron*, **21**, 3171 (1965).

[9] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 338 (1962).

[10] M. Kotake (Ed.), "Jikken Kagaku Kōza, Suppl.," 12, Maruzen, Tokyo, 1967, p. 333.

unsaturated carbonyl. These facts favour 1β-, 6α- and 12β-positions. By the acetylation (IVa→IVb, Va→Vb), the highest methyl signals (assigned as C_{18}-methyls) were deshielded while, by the oxidation (Va→VII), one of the methyl groups (assigned as C_{32}-methyl) was shielded (Table II). Therefore we propose the structure, 12β-hydroxycarbomethoxyacetyl quercinic acid methyl ester (IVa) for the methyl ester of the new compound as a more preferable formulation. Lack of the material prevented further examination.

Table III. The GLC of the Methyl Esters of the Triterpene Acids

Compound or origin	Retention time (min)	
IXa	15.6	
VIIIa		17.0
Gloeophyllum abietinum	15.5	16.9
G. sepiarium	15.6	17.1
G. striatum	15.6	17.1
Lentinus lepideus	15.6	17.1
Veluticeps angularis	15.6	

1.5% OV-1, column temp. 260°, N_2 flow 50 ml/min

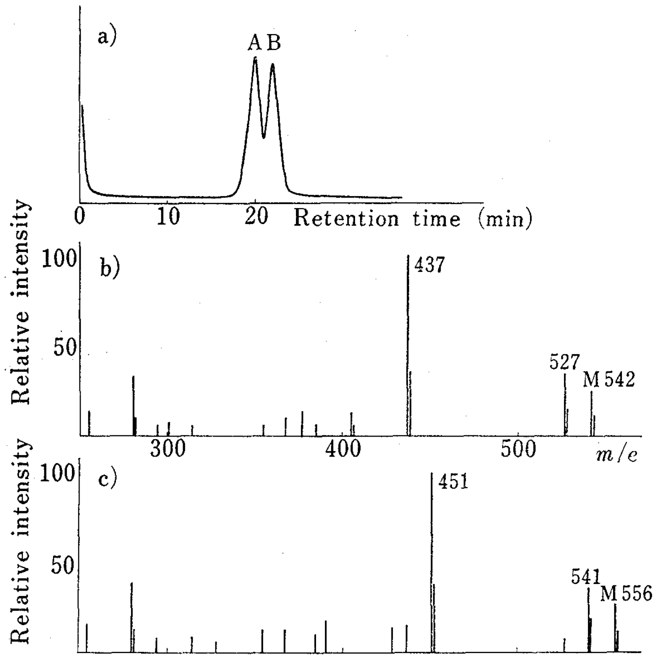

Fig. 1. a) Gas Chromatogram of the Trimethylsilyl Ethers of Triterpenes from *Lentinus lepideus*, b) Mass Spectrum of Fraction A in a), c) Mass Spectrum of Fraction B in a)

LKB gas chromatograph-mass spectrometer; column, 1% OV-1 on chromosorb W, 2 m, 3 mm; detector, total ion current; F. H. temp., 300°; column temp., 270°; sep. temp., 280°

The three species of *Gloeophyllum*, grown on conifers, afforded a mixture of triterpenes as the methyl esters, which were proved to be methyl eburicoate (VIIIa) and methyl trametenolate (IXa) by gas chromatography (GLC) (Table III, Fig. 1). Although taxonomically situated rather different, *Lentinus lepideus* (Tricholomataceae in Agaricales) grown on conifers, afforded the same mixture. In this occassion further identification was carried out by gas chromatograph–mass spectrometory (GC-MS) (Fig. 1). Namely, trimethylsilyl ethers of the mixture from *L. lepideus* showed two peaks in GLC and each component revealed the fragment peaks, *m/e* 542, 527, 437 and *m/e* 566, 541, 451, respectively, agreeing with M^+, M^+-CH_3, M^+-CH_3-ROH of methyl trametenolate trimethylsilyl ether (IXc), $C_{34}H_{58}O_3Si$, and methyl eburicoate trimethylsilyl ether (VIIIc), $C_{35}H_{60}O_3Si$. The NMR spectrum of the triterpene mixture also supported the result. The signals at δ 5.03 and at δ 4.71, 4.63 were assigned as the C_{24} vinylic proton of IXa and the C_{28} vinylic protons of VIIIa respectively and the ratio of the intensities was about 1:2. The isolation of the same mixture was reported from *Gloeophyllum trabeum* (*Lenzites trabeum*, *Daedalea trabea*)[11,12] and the separation as the methyl esters was reported to be practically

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11) V.R. Villanueva, *Phytochemistry*, 10, 427 (1971).

12) I.L. Batey, J.T. Pinkey, B.J. Ralph, and J.J.H. Simes, *Aust. J. Chem.*, 25, 2511 (1972).

hard. The fractional crystallization of methyl ester acetates of the mixture has been found to be effective for the separation. Recently the separation by the dihydro derivatives was reported.[12] As for the metabolites of *Lentinus lepideus*, some phenolics were reported[13,14] and eburicoic acid (VIIIa) has been listed up,[15] but there has been no original report concerning about the triterpene constituents. The same result was also obtained from the fungus mycelia of the same species cultured in liquid media.

Spongiporus appendiculatus, grown mostly on oak trees, produces annual, large fruit bodies, reddish yellow or orange, becoming paler with age. From the fungus methyl eburicoate (VIIIa) contaminated with methyl trametenolate (IXa) and methyl tumulosate (II) were isolated.

Veluticeps angularis grows on ceder and cypress trees and forms perennial, woody and dark brown fruit bodies. Methyl trametenolate (IXa) was isolated from the fungus. In this case methyl eburicoate (VIIIa) was not accompanied.

Two general types of decay of woods by wood-rotting fungi are known; the white-rots and the brown-rots. All the nine species reported here for the presence of triterpenes belong to the brown-rotting fungi which attack cellulose and hemicellulose but leave lignin.

This point will be precisely discussed in a forth-coming paper.

Experimental

***Daedalea tanakae* (Murr.) Aoshima**——Fruit bodies of the fungus (6 g), collected at Minakami, Gumma Prefecture, were air-dried, crushed, and extracted with ether for one week at room temperature. The hexane insoluble part (0.10 g) of the ethereal extract (0.12 g) was treated with CH_2N_2 and separated by preparative layer chromatography to afford methyl polyporenate C (I), mp 178—180° (MeOH), 4.45 mg. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 237 (4.08), 244 (4.14), 252 (3.98), IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 1735, 1687, 895. NMR ($CDCl_3$) δ: 3.66 (3H, s), 4.08 (1H, m), 4.68 (1H, m), 4.72 (1H, m), *ca.* 5.4 (2H, m). Identified with the authentic sample by TLC, GLC, IR, and NMR.

***Melanoporia rosea* (Alb. et Schw. ex Fr.) Aoshima**——Fruit bodies of the fungus (15 g), collected at Sōunkyo, Hokkaido, gave the ethereal extract (6.27 g). The methylated products obtained by the same procedure were separated by preparative layer chromatography to afford the following compounds:

i) Methyl polyporenate C (I), mp 198—198.5° (MeOH), 250.8 mg. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 236 (4.20), 244 (4.24), 252 (4.11). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 1735, 1710(sh), 1685, 890. NMR ($CDCl_3$) δ: 3.65 (3H, s), 4.10 (1H, m), 4.63 (1H, m), 4.70 (1H, m), 5.2 (1H, m), 5.35 (1H, m). Identified with the authentic sample by TLC, GLC, IR, and NMR.

ii) The mixture of methyl tumulosate (II) and methyl dehydrotumulosate (III), mp 135—137° (MeOH), 169.5 mg. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 236 (3.91), 244 (3.92), 252 (3.73). (The ratio of II: III, 52: 48). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3440, 1715, 1648, 890. NMR ($CDCl_3$) δ: 3.66 (3H, s), 3.22 (1H, m), 4.06 (1H, m), 4.65 (1H, m), *ca.* 5.30 (1H, m). Identified with the authentic sample by TLC, GLC, IR, and NMR.

iii) 12β-Hydroxycarbomethoxyacetylquercinic acid methyl ester (IVa), mp 116—117° (MeOH), 72.7 mg, $[\alpha]_D^{21}$ $+4.66°$ ($CHCl_3$, $c=0.97$). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 1750, 1735, 1708, 1700. Mass Spectrum *m/e*: M^+, 616.409 (calcd, for $C_{36}H_{56}O_8$: 616.398), 598 (M^+-H_2O), 583 (M^+-CH_3-H_2O), 498 (M^+-$C_4H_6O_4$), 483 (M^+-CH_3-$C_4H_6O_4$), 465 (M^+-CH_3-H_2O-$C_4H_6O_4$), 325 (M^+-CH_3-$C_4H_6O_4$-$C_8H_{14}O_3$), 307 (M^+-CH_3-H_2O-$C_4H_6O_4$-$C_8H_{14}O_3$). NMR (Table II).

IVa gave the acetate (IVb) by the conventional method, an oily substance, $[\alpha]_D^{19}$ $-5.26°$ ($CHCl_3$, $c=0.76$). Mass Spectrum *m/e*: 658 (M^+, $C_{38}H_{58}O_9$), 643 (M^+-CH_3), 598 (M^+-$C_2H_4O_2$), 583 (M^+-CH_3-$C_2H_4O_2$), 480 (M^+-$C_2H_4O_2$-$C_4H_6O_4$), 465 (M^+-CH_3-$C_2H_4O_2$-$C_4H_6O_4$), 440 (M^+-$C_2H_4O_2$-$C_8H_{14}O_3$), 307 (M^+-CH_3-H_2O-$C_4H_6O_4$-$C_8H_{14}O_3$). NMR (Table II). IVa (50.19 mg) was hydrolysed with boiling 5% KOH–MeOH (10 ml) for 1 hr. After working up usual and methylation with CH_2N_2, the reaction product was purified by preparative layer chromatography to give the diol (Va) as colorless powder (36.88 mg), mp 53—55° (MeOH), $[\alpha]_D^{21}$ $+19.0°$ ($CHCl_3$, $c=1.0$). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3480, 1740, 1720. $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1723, 1708. Mass Spectrum *m/e*: M^+ 516.379 (calcd. for $C_{32}H_{52}O_5$: 516.381), 498 (M^+-H_2O) 483 (M^+-CH_3-H_2O), 465 (M^+-CH_3-$2H_2O$), 325 (M^+-CH_3-H_2O-$C_8H_{14}O_3$), 307 (M^+-CH_3-$2H_2O$-$C_8H_{14}O_3$). NMR (Table II). The diol (Va) (49.41 mg) was acetylated by Ac_2O-pyridine by the conventional method to give the diacetate (Vb) as colorless powder

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13) J.H. Birkinshaw and W.P.K. Findlay, *Biochem. J.*, **34**, 82 (1940).

14) H. Shimazono, *Arch. Biochem. Biophys.*, **83**, 206 (1959).

15) S. Shibata, S. Natori, and S. Udagawa, "List of Fungal Product," University of Tokyo Press, 1964, p. 119; S. Natori, unpublished data.

(43.70 mg), mp 56—57° (MeOH). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1740, 1720, 1240. NMR (Table II). The diol (Va) (36.88 mg) in acetone (3 ml) was added with Jones' reagent[16] (0.1 ml). After standing at room temperature for 10 min, the reaction products were extracted with ether, washed, and separated by preparative layer chromatography to give the ketone (VII), an oily substance (10.60 mg), IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 1725(sh), 1715(sh), 1705, 1700, NMR (Table II), and the minor product, an oily substance (4.4 mg), IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 1722(sh), 1715(sh), 1705, 1700.

***M. juniperina* Aoshima**——Fruit bodies (15 g), collected at Kiso, Nagano Prefecture, gave the ethereal extract (0.1 g). The preparative layer chromatography of the methylated products give two compounds; methyl polyporenate C (I), 1.27 mg, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3440, 1735, 1687, 895, and methyl tumulosate (II), 1.41 mg, UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ε): 243 (3.62), 252 (3.53) (contaminated with methyl dehydrotumulosate (III) about 25%), IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3440, 1735, 1645, 890. Identified with the authentic sample by TLC, GLC, and IR, respectively.

***Gloeophyllum* spp.**——Fruit bodies of *Gloeophyllum abietinum* (Bull. ex Fr.) Karst. (14 g), collected at Yoga, Tokyo, *G. sepiarium* (Wulf. ex Fr.) Karst. (10 g), collected at the foot of Mt. Teshio, Hokkaido, and *G. striatum* (Swartz. ex Fr.) Murr. (7.2 g), collected at Marunuma, Gumma Prefecture, were used for the study. The ethereal extracts (0.27 g, 0.30 g, and 0.27 g, respectively) were treated with the same procedure as before and separated by preparative layer chromatography to give the mixture of methylated products (27.87 mg, 21.50 mg and 8.34 mg), which were shown to be the same mixture of methyl trametenolate (IXa) and methyl eburicoate (VIIIa) by GLC (Table III) and by comparison with IR and NMR. The ratio (VIIIa: IXa) of the two compounds determined by gas chromatograms are 73: 27, 78: 22 and 77: 23 respectively.

***Lentinus lepideus* Fr.**——Fruit bodies (78 g), collected at Aobayama, Sendai, gave the ethereal extract (1.0 g). The hexane insoluble part (0.95 g), methylated with CH_2N_2, was separated by preparative layer chromatography to give a mixture of methyl trametenolate (IXa) and methyl eburicoate (VIIIa) (147.0 mg). According to GLC, the mixed ratio (IXa: VIIIa) is 41: 59. The identification was carried out by GC-MS (Table III, Fig. 1). NMR (CDCl_3) δ: 3.22 (1H, m), 3.62 (3H, s), 4.63, 4.71 (each 1/2H, m), 5.03 (1/2H, m).

***Spongiporus appendiculatus* (Berk. et Br.) Aoshima**——Fruit bodies (32.5 g), collected at Nayoro, Hokkaido, gave the ethereal extract (0.32 g). The hexane insoluble part (0.21 g), methylated with CH_2N_2, was chromatographed on a column of alumina (Woelm, neutral, 200 g) and eluted successively with benzene, 2% ether–benzene, ether and MeOH. The 2% ether–benzene fraction was further separated by preparative layer chromatography into two components. i) Methyl eburicoate (VIIIa), mp 110—113° (MeOH), 8.33 mg, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3460, 1735, 1645, 890. Identified with the authentic sample by TLC, GLC, and IR. The mother liquor showed the presence of methyl trametenolate (IXa) by GLC. ii) Methyl tumulosate (II), mp 138—140° (MeOH), 11.49 mg, UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ε): 237 (3.77), 244 (3.83), 252 (3.67). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3440, 1735, 1645, 890.

***Veluticeps angularis* (Lloyd) Aoshima et Furukawa**——Fruit bodies (20 g), collected at Kiso, Nagano Prefecture, gave the ethereal extract (2.95 g). The hexane insoluble part (2.38 g), methylated with CH_2N_2, was separated by column chromatography of alumina (Woelm, neutral, 100 g) and eluted successively benzene, 2% ether–benzene, 5% ether–benzene, ether and MeOH. The 2% ether–benzene fraction was further purified by preparative layer chromatography. Methyl trametenolate (IXa), mp 132—135° (MeOH), 110.1 mg. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3360, 1735. NMR (CDCl_3) δ: 1.57 (3H, m), 1.66 (3H, m), 3.62 (3H, s), 3.20 (1H, m), 5.05 (1H, m). Identified with the authentic sample by TLC, GLC, IR, and NMR. The acetate (IXb), colorless needles of mp 140—142° (MeOH), IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1738, 1245, NMR (CDCl_3) δ: 2.02 (3H, s), 4.50 (1H, m), was identified with methyl acetyltrametenolate (IXb) (mixed mp, TLC, GLC, and IR).

The Culture of *Lentinus lepideus* Fr.——The fungus culture[17] was grown in potato-dextrose medium in Roux bottles for 45 days. The dried mycelium (29.5 g) was extracted successively with hexane and ether. The ethereal extract (1.25 g) treated with CH_2N_2 was separated by preparative layer chromatography to give the same mixture as the fruit bodies. The fraction was acetylated by Ac_2O-pyridine to give colorless needles of mp 120—130° showing 2 peaks by GLC. Fractional crystallization from MeOH afforded colorless needles, mp 152—153°, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1730, 1245, 890. NMR (CDCl_3) δ: 2.05 (3H, s), 4.05 (1H, m), 4.75, 4.67 (each 1H, m), which was identified with methyl acetyleburicoate (VIIIb) by the ordinary methods. By the further fractional crystallization checked by GLC, another colorless needles, mp 145—147° (MeOH), was obtained from the mother liquor. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1730, 1245. NMR (CDCl_3) δ: 1.60 (3H, m), 1.70 (3H, m), 2.05 (3H, s), 4.52 (1H, m), 5.12 (1H, m). Identified with methyl acetyltrametenolate (IXb) by the ordinary methods.

Thin-Layer Chromatography——For thin-layer chromatography Silicagel H or HF_{254} were used. Hexane–AcOEt (7: 3) and CHCl_3: MeOH (9: 1) were employed for the solvent and the detection was carried out by heating on a hot plate after spraying 20% vanillin–phosphoric acid.

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[16] A. Bowers, T.G. Halsall, E.R.H. Jones, and A.J. Lemin, *J. Chem. Soc.*, **1953**, 2548.

[17] Identified and provided by Dr. K. Aoshima, Government Forest Experiment Station.

Gas Chromatography——Gas chromatography was carried out on 1.5% OV-1 on Shimalite W and 1.5% QF-1 on Chromosorb W on a Hitachi F6-D Gas Chromatograph.

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