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DISTRIBUTION OF TETRACYCLIC TRITERPENOIDS OF LANOSTANE GROUP AND STEROLS IN THE HIGHER FUNGI ESPECIALLY OF THE POLYPORACEAE AND RELATED FAMILIES

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Key Word Index—Basidiomycetes; Aphyllophorales; Polyporaceae; white-rot fungi; brown-rot fungi; triterpenoids; lanostanes; sterols.

Abstract—The fruit bodies of 97 species of wood-rotting fungi, mainly of Polyporaceae and related families, were examined for the distribution of triterpenes and sterols. Triterpene acids of lanostane group were detected exclusively from the fungi causing brown-rot of woods, while sterols were found to occur commonly in both brown-rot and white-rot fungi. The most abundant sterol was found to be ergosta-7,22-dien-3β-ol. The presence and absence of the triterpene acids is discussed from the point of view of fungal phylogeny.

INTRODUCTION

Fungi belonging to the Polyporaceae of Basidiomycetes are known as the source of tetracyclic triterpenes of the lanostane group [1, 2] and more than thirty compounds, especially of C_{30} or C_{31} carboxylic acid such as trametenolic acid (**1**) and eburicoic acid (**2**), have been isolated. Although lanosterol (**3**) and cycloartenol (**4**), have been shown to be the intermediates for the biosynthesis of sterols from squalene in animals and fungi and in higher plants respectively [3, 4], the occurrence of other triterpenoids of the lanostane and the related groups are known only from a few sources [5, 6]. The wide occurrence of the triterpene acids of this type in Basidiomycetes is noteworthy and contrasts to the presence of a limited number of fusidane and protostane derivatives in a few Ascomycetes [2, 7].

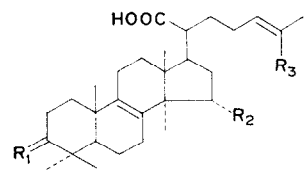
In previous papers we reported the isolation and characterization of the triterpenoids from *Poria cocos* [8, 9], *Echinodontium tsugicola* [10], and nine other species [11]. In this paper, the distribution of tetracyclic triterpenes of the lanostane group and of sterols in about one hundred species of wood-rotting fungi of Polyporaceae and related families is reported and the relationship of the two types of wood-rotting fungi, brown-rot and white-rot, is discussed.

RESULTS

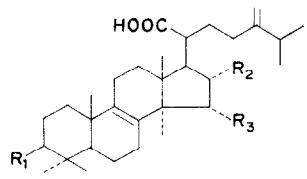
Fruit bodies of 97 species of wood-rotting fungi, mostly collected in Japan, were examined in this study. The ethereal extracts were examined initially for the presence of sterols and triterpenoids by TLC and GLC. When the presence of the triterpene carboxylic acids was apparent, the extracts were divided into hexane-soluble and hexane-insoluble fractions. The ether extract or the hexane-soluble fraction was used for the analysis of sterols and the hexane-insoluble fraction for that of the triterpene acids.

Steroids were identified by GLC, MS and, occasionally, combined gas–liquid chromatography–mass spectroscopy (GC–MS) of their trimethylsilyl ethers as in Fig. 1 and Table 1.

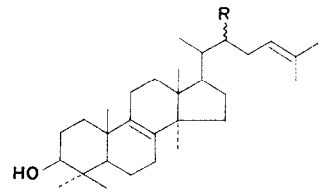
The major sterol of higher fungi has been assumed to be ergosterol (**5**). However, reliable identification by modern methods has been carried out in limited cases [1, 2, 12]. Our survey revealed that the major sterols widely occurring in the



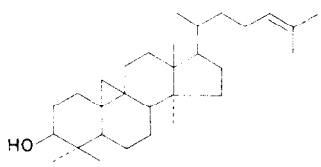
1 R_1: βOH R_2: H R_3: Me
Trametenolic acid
8 R_1: αOAc R_2: H R_3: Me
34 R_1: = O R_2: H R_3: Me
37 R_1: βOAc R_2: H R_3: Me
38 R_1: βOH R_2: OH R_3: Me
39 R_1: βOH R_2: OH R_3: COOH



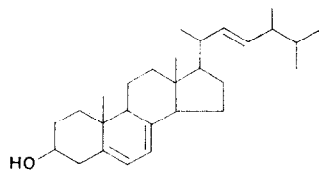
2 R_1: OH R_2: R_3: H
Eburicoic acid
9 R_1: R_2: OH R_3: H
Tumulosic acid
11 R_1: OAc R_2: OH R_3: H
Pachymic acid
40 R_1: R_3: OH R_2: H
Sulphurenic acid



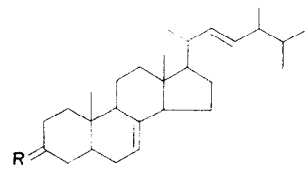
3 R: H Lanosterol
14 R: OH Inotodiol



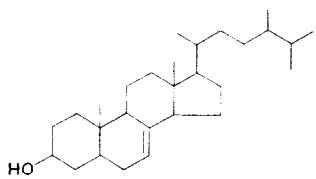
4 R: Cycloartenol



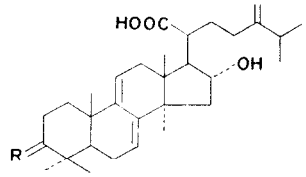
5 Ergosterol
27 (22,23-dihydro)



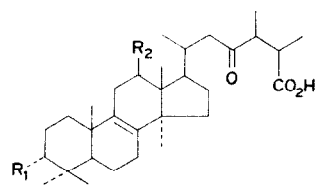
6 R: β-OH Ergosta-7,22-dien-3β-ol
28 R: =O
30 R: β-stearate



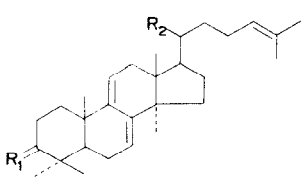
7 Ergost-7-en-3β-ol



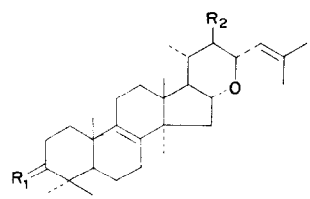
10 R: βOH
12 R: = O
Polyporenic acid C



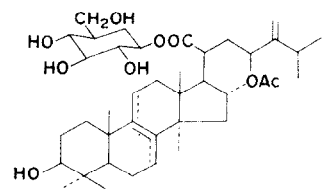
13 R_1: $OCOCH_2COOH$ R_2: H
Carboxyacetylquercinic acid
23 R_1: $OCOCH_2CO_2H$ R_2: OH
24 R_1: OH R_2: H
Quercinic acid
25 R_1: $OCOCH_2CO_2Me$ R_2: H



15 R_1: βOH R_2: CO_2H
32 R_1: βOH R_2: CH_2OH
33 R_1: = O R_2: CH_2OH
44 R_1: βOH R_2: Me



16 R_1: = O R_2: OAc Echinodone
17 R_1: = O R_2: OH
18 R_1: αOH R_2: OAc
19 R_1: αOH R_2: OH
20 R_1: αOH R_2: H
21 R_1: βOH R_2: H
22 R_1: = O R_2: H
31 R_1: βOH R_2: OAc Echinodol



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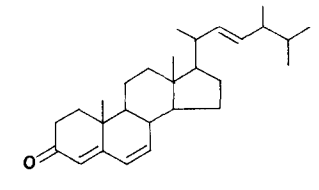

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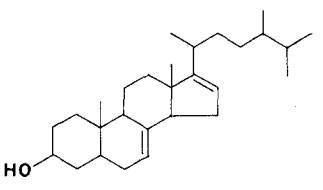
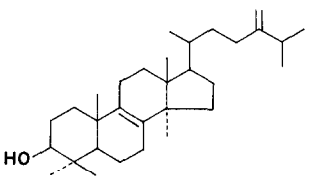


35 (16,17-dihydro)
36



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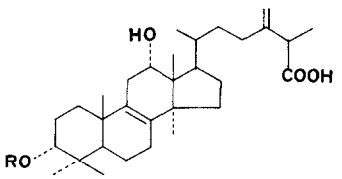
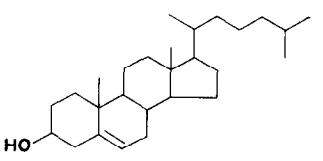
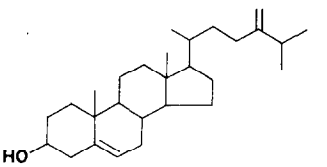


42 R: H Polyporenic acid A
43 R: –Ac, $-OC(CH_2)_4Me$
$-OCCH_2COOH$, $-OCCH_2CO_2Me$
$-OCCH_2C(OH)MeCH_2CO_2H$
$-OCCH_2C(OH)MeCH_2CO_2Me$



45 Cholesterol



46 24-Methylenecholesterol

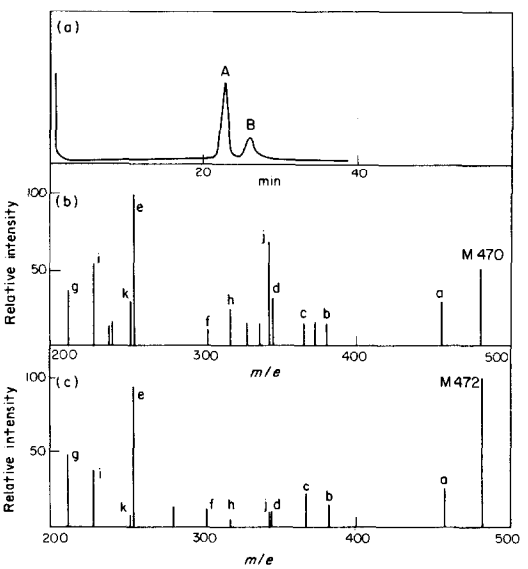


Fig. 1. (a) Gas chromatogram of the trimethylsilyl ethers of sterols from *Trametes palisoti*: (b) MS of Fraction A in (a): (c) MS of Fraction B in (a). LKB gas chromatograph–mass spectrometer; column, 1% OV-1 on chromosorb W, 2 m, 3 mm; detecter, total ion current; F. H. temp., 300°; column temp., 250°; sep. temp., 280°.

higher fungi are the tri-, di-, or mono-ene of a C_{28}-sterol (M^+ at *m/e* 468, 470, 472, respectively in the MS). The C_{28}-triene was identified with ergosterol (**5**) by the characteristic UV spectra [13] and by GLC comparison with an authentic sample. The C_{28}-diene, the major sterol in most of fungi, was assumed to be ergosta-7,22-dien-3β-ol (5,6-dihydroergosterol) (**6**) from the mass spectral characteristics [14]; i.e. the fragment ions at *m/e* 345 (fragment d in Table 1) and *m/e* 255 (fragment e) indicated the presence of one of double bond both in the nucleus and in the side chain: the characteristic ions corresponding to Δ^5 and $\Delta^{24(28)}$ sterols were absent, and the ions at *m/e* 318 (fragment h) and *m/e* 229 (fragment i) suggested the presence of the nuclear double bond at 7-position [14–16]. The C_{28}-monoene was the minor component and was assumed to be ergost-7-en-3β-ol (**7**) from the fragmentation pattern. Comparison of GLC and MS with authentic samples established the identification of these compounds.

Ergosta-7,22-dien-3β-ol (**6**) is the major sterol in the Polyporaceae and related families. Ergosterol (**5**) is usually as a minor constituent along with ergost-7-en-3β-ol (fungisterol) (**7**), but is the major sterol in *Formitopsis pinicola*, *Grifola umbellata*, *Lentinus lepideus* and *Poria cocos* (Table 2).

The hexane-insoluble fraction, mainly consisting of triterpene carboxylic acids was treated with diazomethane and analysed by TLC and GLC (Table 3). In some cases GC–MS spectroscopy and isolation followed by identification by spectral methods was carried out. The details have been given in a previous paper [11]. Using these methods we have identified eight triterpene carboxylic acids (**1**, **2**, **8**–**13**) and one trimethylsterol (**14**) as known compounds and 3β-hydroxylanosta-7,9(11),24-trien-21-oic acid [9] (**15**), seven echinodol derivatives [10] (**16**–**22**) and 12β-hydroxycarboxyacetylquercinic acid [11] (**23**) as new compounds. The

Table 1. Relative retention times in gas chromatography and

Sterol trimethylsilyl ether	Relative retention times* Gas chromatographic system A	B	C	M+	a	b
ergosterol (**5**)	2·61 (2·22)†	2·67 (3·45)	2·68 (4·08)	468 (40)	453 (2)	378 (24)
ergosta-7,22-dien-3β-ol (**6**)	2·69 (2·28)	2·70 (3·51)	2·65 (4·15)	470 (44)	455 (28)	380 (22)
ergost-7-en-3β-ol (**7**)	3·12 (2·61)	3·09 (3·97)	3·15 (4·95)	472 (100)	457 (38)	382 (31)
cholesterol (**45**)	2·28 (1·88)					
24-methylenecholesterol§ (**46**)	2·65 (2·25)					380 (79)
lanosterol (**3**)	3·25 (2·95)	3·25 (4·49)	3·42 (5·16)			
Sterols of						
Grifola umbellata	2·61 (2·22)	2·67 (3·46)	2·68 (4·08)	468		378
Fomitopsis pinicola	2·62 (2·22)	2·68 (3·45)	2·69 (4·08)	468 (11)		378 (31)
Trametes palisoti	2·70, 3·12 (2·28, 2·62)	2·72, 3·09	2·65, 3·15 (4·15, 4·96)	470 472	455 457	380
Phellinus igniarius	2·70, 3·12 (2·28, 2·61)	2·72, 3·10	2·65, 3·15 (4·15, 4·97)	470 472		
Daedaleopsis confragosa	2·70, 3·12 (2·28, 2·61)			470 472	455 457	380
Trametes palisoti-A				470 (52)	455 (30)	380 (15)
-B				472 (100)	457 (26)	382 (15)

* Relative to cholestane: A: 1·5 m × 0·3 cm glass column, OV-1 1·5% at 250°, N_2 45 ml/min, cholestane 4·05 min; B: 1·5 m × 0·3 cm glass column, OV-17 1·5% at 260°, N_2 60 ml/min, cholestane 5·60 min; C: 1·5 m × 0·3 cm glass column, QF-1 1·5% at 210°, N_2 80 ml/min, cholestane 3·65 min.

† Relative retention time of the free sterol.

occurrence of triterpenes is limited to about 20 species but the amounts of these compounds are generally rather high compared with those of the sterols (Table 2). The procedures used may miss triterpenoids occurring as glycosides but, as far as we are aware only one such compound has so far been reported [34]. Recently, acid conjugates of the triterpenoids have been isolated [34, 54] and we have detected two of these (**13**, **23**) using our methods.

DISCUSSION

The metabolites of Basidiomycetes have been investigated widely and the chemistry of the fungal triterpenes has been well established [5]. Recently, the chemotaxonomy of Basidiomycetes using metabolites such as amino acids, pigments, urea, and amines [17–19] and the fungal phylogeny from chemical, metabolic and enzymatic points of view [20] have also been discussed. Since triterpene carboxylic acids with lanostane skeleton occur rather widely in the Polyporaceae and related higher fungi but are rare in other plants, they might be considered as chemical markers in the chemotaxonomy of the higher fungi.

Almost all species in Polyporaceae and related families of Aphyllophorales (Basidiomycetes) are wood-rotting fungi, which are classified into two types; white-rot fungi (lignin decomposers) and brown-rot fungi (cellulose decomposers); their characteristics are shown in Table 4 [21]. In the species examined in this study, 76 are white-rot fungi and 21 species are brown-rot types. In the latter, 19 species contained triterpene acids, the only exceptions being *Phaeolus schweinitzii* and *Pycnoporellus flugens*. Both these species are taxonomically closely related and rather unique among the Polyporaceae causing brown-rot. On the other hand, all of the 76 species of white-rot fungi contained none of the acids in detectable amounts. However, three species (*Echinodontium tsugicola* [10], *E. tinctorium* [22] and *Fuscoporia obliqua* 123, 24])

fragment ions in MS of sterol trimethylsilyl ethers

Mass spectrum (*m/e*)‡ (relative intensity, %)									
c	d	e	f	g	h	i	j	k	l
363 (100)		253 (33)		211 (31)		227 (9)		251 (12)	
365 (22)	345 (33)	255 (89)	303 (16)	213 (36)	318 (28)	229 (59)	343 (64)	253 (28)	
367 (38)	345 (27)	255 (92)	303 (12)	213 (54)	318 (7)	229 (38)	343 (23)	253 (15)	
365 (20)		255 (20)		213 (29)				253 (29)	296 (59)
363		253		211		227		251	
363 (100)	343 (6)	253 (60)		211 (42)		227 (24)		251 (40)	
365 367	345	255	303	213	318	229	343	253	
365 367		255		213		229		253	
365 367		255	303	213	318	229		253	
365 (16) 367 (22)	345 (32) 345 (11)	255 (100) 255 (93)	303 (10) 303 (11)	213 (36) 213 (47)	318 (24) 318 (5)	229 (54) 229 (38)	343 (69) 343 (11)	253 (30) 253 (8)	

‡ M^+: Molecular ion, a: M-15, b: M-ROH, c: M-15-ROH, d: M-side chain, e: M-side chain-ROH, f: M-side chain-42, g: M-side chain-42-ROH, h: M-side chain-27, i: M-side chain-27-RO, j: M-side chain-2H, k: M-side chain-2H-ROH, l: M-84-ROH (R = trimethylsilyl or acetyl).

§ Acetate for the mass spectrum data.

‖ GC-MS data in Fig. 1.

belonging to the white-rot fungi produce triterpenoids lacking the carboxyl group. Twenty-five species reported in the literature as sources of triterpene acids [1, 2, 5, 25] belong to the brown-rot fungi, while four species *Polyporus hispidus* [26] (*Inonotus hispidus*), *Polyporus benzoinus* [27] (*Ishnoderma resinosum*), *Fomes hartigii* [28] (*Phellinus hartigii*) and *Polyporus sanguineus* [29] (*Pycnoporus sanguineus*) belong to the white-rot fungi. Our results showed, however, that none of these fungi collected in Japan contained triterpene acids.

Nobles [30] proposed that the production of an extracellular oxidase which is characteristic for white-rot fungi, should be taken as an important key in the taxonomy of Polyporaceae since the brown-rot fungi do not produce the enzyme. At this stage we cannot explain the meaning of the presence of the triterpene acids on biogenetic grounds but our findings add one more example of the difference in metabolism of the two types of wood-rotting fungi of the Aphyllophorales.

EXPERIMENTAL

Materials. The fruit-bodies (in some cases sclerotia) of fungi were collected mostly in Japan as shown in Table 2 and identified by one of us (K. A.).

Extraction and separation. Fruit-bodies including context and trama or sclerotia (5–20 g) were air-dried, crushed and extracted with Et_2O for a week at room temp. The extracts were examined first for the presence of triterpenes and steroids by TLC and GLC. When they showed the presence of the triterpene acids, the extracts were divided into hexane-soluble and -insoluble parts. For sterols and trimethylsterols, the ethereal extract or the hexane-soluble part of the extract was separated by preparative TLC and the sterol fraction was collected and used for the analyses by TLC and GLC. For triterpene acids, the hexane-insoluble part of the extract was treated with ethereal-CH_2N_2, chromatographed on alumina or silica-gel columns, or silica-gel TLC plates, and used for further identification. In some cases, full isolation in the crystalline state was carried out. Further details and modification of the separation procedure of the acids were given in the preceding paper [11].

Trimethylsilylation. Triterpene or sterol in tetrahydrofuran was treated with BSTFA (bis-trimethyl silyl trifluoroacetamide) and allowed to stand for 10 min at room temp. For GLC and GC–MS analyses, the reaction mixtures was used, while for MS analysis, the trimethylsilyl ether was purified by preparative TLC before application.

Table 2. Distribution of triterpenes and steroids

Species (synonym)*	Japanese name	Family†	Type‡	Place and date of collection	Et_2O ext. (%)	Compounds identified	Method of identification	Compound known in literature
Abortiporus biennis (Bull. ex Fr.) Sing.	Niku-uchiwatake	P	W	Toikanbetsu, Hokkaidō	3·10			
Amauroderma mollis (Sommerf.) Karst.	Shikatake	P	W	Izu, Shizuoka Dec. 1968	2·73	**6**, **7**	GLC	
Bierkandera adusta (Willd. ex Fr.) Karst.	Yakeirotake	P	W	Hokkaido July 1970	0·44	**6**, **7**	GLC	
B. fumosa (Pers. ex Fr.) Karst.	Himemogusatake	P	W	Sendai, Miyagi May 1968	0·50	**6**, **7**	GLC	
Coriolus brevis (Berk.) Aoshima	Niku-usubatake	P	W	Tanzawa, Kanagawa Sept. 1968	0·83	**6**, **7**	GLC	
C. flabelliformis (Klotz) Aoshima	Uchiwatake	P	W	Tanegashima, Kagoshima Sept. 1968	0·70	**6**, **7**	GLC	
C. hirsutus (Wulf. ex Fr.) Quél. [31]	Aragekawaratake	P	W	Hiraniwa, Iwate July 1969	0·90	**6**, **7**	GLC	**5** [31]
C. meyenii (Klotz) Aoshima		P	W	New Guinea	0·68	**6**	GLC	
C. pubescens (Schum. ex Fr.) Quél.	Yakifutake	P	W	Okutama, Tokyo Nov. 1969	0·97	**6**	GLC, MS	
C. sabluteus Aoshima	Shimauchiwatake	P	W	Tanegashima, Kagoshima Sept. 1968	0·53	**6**, **7**	GLC	
C. unicolor (Bull. ex Fr.) Pat.	Midareamitake	P	W	Tomakomai, Hokkaido July 1970	0·54	**6**, **7**	GLC	
C. versicolor (L. ex Fr.) Quél. [31] (*Polystictus versicolor* Fr. [32, 33])	Kawaratake	P	W	Onejime, Kagoshima Sept. 1968	0·97	**6**, **7**	GLC	**5** [31, 33]
Cryptoporus volvatus (Peck) Hubbard	Hitokuchitake	P	W	Izu, Shizuoka April 1970	11·41	**6**, **7**	GLC	
Daedalea dickinsii Yasuda (*Trametes dickinsii* Berk. [34])	Horokutake	P	B	Hiraniwa, Iwate July 1969	4·38	**6**, **7** **9**, **10**, **12**, **13**	GLC GLC, IR, UV, NMR	**10**, **12**, **13**, **24**, **25**, **26** [34]
D. tanakae (Murr.) Aoshima [11]	Himeshiroamitake	P	B	Minakami, Gunma Feb. 1969	2·33	**6**, **7** **12**	GLC IR, NMR	
Daedaleopsis confragosa (Bolt. ex Fr.) Schroet. [35]	Chamidareamitake	P	W	Aizu, Fukushima July 1969	4·39	**6**, **7**	GLC, MS	**5**, **27** [35]
D. tata (Berk.) Aoshima	Senbeitake	P	W	Tanegashima, Kagoshima Sept. 1968	1·02	**6**, **7**	GLC	
D. purpurea (Cooke) Imaz. et Aosh.	Mi-iroamitake	P	W	Hachijojima, Tokyo March 1970	0·82	**6**, **7**	GLC	
D. styracina (P. Henn. et Shirai) Imaz.	Egonokitake	P	W	Kiyosumi Chiba Nov. 1969	1·70			
D. tricolor (Bull. ex Fr.) Bond. et Sing. [35]	Chakaigaratake	P	W	Kiyosumi, Chiba Nov. 1969	0·31	**6**	GLC	**5**, **27** [35]
Dentipellis echinospora Furukawa	Hanaricharitakemodoki	Hyd	W	Marunuma, Gunma May 1970	1·60	**6**, **7**	GLC	
Echinodontium tsugicola (P. Henn.) Imaz. [10]	Man-nenharitake	P	W	Marunuma, Gunma Oct. 1969	3·12	**16**–**22**	IR, NMR, MS	
E. tinctorium Ellis et Ev. [22]		P	W	U.S.A.	0·66	**31**	TLC, GLC	**31** [22]
Favolus alveolarius (Bosc. ex Fr.) Quél.	Hachinosutake	P	W	Kiyosumi, Chiba Nov. 1969	1·15	**6**, **7**	GLC	
F. arcularius (Batsch ex Fr.) Ames [31]	Amisugitake	P	W	Hiraniwa, Iwate July 1969	1·15	**6**, **7**	GLC	**5** [31]
F. rarius (Pers. ex Fr.) Imaz.	Kiashigurotake	P	W	Okutama, Tokyo Nov. 1969	1·70			
Fistulina hepatica Fr.	Kanzotake	F	W	Kyoto June 1972	1·50			
Fomes fomentarius (L. ex Fr.) Kickx [31, 36–39]	Tsuriganetake	P	W	Nayoro, Hokkaido June 1969	0·88	**6**	GLC	**5** [31, 37, 38], **6** [39], **28** [36, 39], **29** [38], **30** [39]
Fomitella cytisina (Berk.) Aoshima	Bekkotake	P	W	Tanzawa, Kanagawa Sept. 1968	1·73	**6**, **7**	GLC GC, MS	
F. latissima (Bres.) Aoshima	Kashisaruno-koshikakemodoki	P	W	Hetsuka Kagoshima	0·46	**6**	GLC	
F. rhodophaea (Lév.) Aoshima	Osurumetake	P	W	Shinjuku, Tokyo Sept. 1969	1·85	**6**, **7**	GLC	

Table 2 (*contd.*)

Species (synonym)*	Japanese name	Family†	Type‡	Place and date of collection	Et_2O ext. (%)	Compounds identified	Method of identification	Compound known in literature
Fomitopsis pinicola (Swartz ex Fr.) Karst. [33] (*Polyporus pinicola* Fr. [28, 40, 45] *Fomes pinicola* [42])	Tsugasaruno-koshikake	P	B	Hiraniwa, Iwate July 1969	10·86	**5** **8**, **(12)**	GLC, MS IR, NMR	**5** [33, 42] **6**, **7**, **32**, **33** [40, 45], **8**, **34** [28, 40, 45], **2** [42]
Fuscoporia obliqua (Pers. ex Fr.) Aoshima§ (*Inonotus obliquus* (Pers.) Pil. [24], *Poria obliqua* Bres [23])	Kaba-anatake	P	W	Nayoro, Hokkaidō July 1970	0·52	**14**	IR, NMR, MS	**3**, **14** [23, 24]
Ganoderma applanatum (Pers.) Pat. [43] (*Fomes applanatus* [44], *Elfeingia applanata* (Blum. et Nees ex Fr.) Kuntze [31])	Kofukisaruno-koshikake	P	W	Yoga, Toyko May 1967	0·98	**6**, **7**	GLC	**5** [31], **35**, **36** [43], **6**, **28** [44]
G. fornicatum (Fr.) Pat.‖	Kuroganeman-nentake	P	W	Palau	1·67			
Gloeophyllum abietinum (Bull. ex Fr.) Karst. [11]	Kogeirokaigaratake	P	B	Yōga, Tokyo May 1970	1·18	**1**, **2** **6**	GLC, IR GLC	
G. odoratum (Wulf. ex Fr.) Imaz. (*Trametes odorata* (Wulf.) Fr. [41, 46]	Nioiamitake	P	B	Hirogawara, Yamanashi Sept. 1969	9·11	**1** **6**, **7**	IR, NMR GLC	**1** [41, 46], **5**, **37**, **38**, **39** [46]
G. sepiarium (Wulf. ex Fr.) Karst. [11]	Kikaigaratake	P	B	Teshio, Hokkaidō July 1970	3·02	**1**, **2** **6**, **7**	GLC, IR GLC	
G. striatum (Swartz ex Fr.) Murr. [11]	Hirohanokikaigara-take	P	B	Marunuma, Gunma Oct. 1969	3·73	**1**, **2** **6**, **7**	GLC, IR GLC	
G. trabeum (Pers. ex Fr.) Murr. (*Lenzites trabea* [47, 48], *Daedalea trabea* [49])	Kichirimentake	P	B	Marunuma, Gunma May 1970	2·47	**1**, **2** **6**, **7**	GLC, IR GLC, MS	**1**, **38** [47, 49], **5**, **40** [48, 49], **6**, **41** [48]
Glocopours amorphus (Fr.) Clem. et Shear	Urabenitake	P	W	Yōga, Tokyo Oct. 1967	5·0	**6**	GLC	
Grifola umbellata (Pers. ex Fr.) Pilát§	Choreimaitake	P	W	Sōunkyō, Hokkaidō	1·09	**5**	GLC, MS	
Hapalopilus croceus (Pers. ex Fr.) Donk ‖	Ōkabochatake	P	W	Ōdaigahara, Nara Aug. 1971	4·75			
Heterobasidion insularis (Murr.) Aoshima ‖	Rengatake	P	W	Kinkasan, Miyagi Nov. 1969	5·28			
H. sensitivum (Yasuda) Aoshima ‖	Akazometake	P	W	Asakawa, Tokyo	22·84			
Hirschioporus abietinus (Dicks. ex Fr.) Donk ‖ (*Polyporus abietinus* [56])	Shihaitake	P	W	Kinkasan, Miyagi Nov. 1969	0·38			**5** [56]
H. durus (Jungh.) Aoshima	Yosooisaruno-koshikake	P	W	Hetsuka, Kagoshima	0·70	**6**	GLC	
H. fusco-violaceus (Fr.) Donk	Usubashihaitake	P	W	Hirogawara, Yamanashi Sept. 1969	0·59	**6**, **7**	GLC	
Hirschioporus sp. ‖		P	W	New Guinea	0·76			
Inonotus hispidus (Bull. ex Fr.) Karst. ‖ (*Polyporus hispidus* Fr. [26])	Yakekogetake	Hym	W	Masutomi, Yamanashi Aug. 1965	2·19			**2** [26]
I. mikadoi (Lloyd) Bond.‖	Kawausotake	Hym	W	Kiyosumi, Chiba Nov. 1969	0·70			
I. sciurinus Imaz.‖	Rakkotake	Hym	W	Okutama, Tokyo Nov. 1969	0·31			
Ishnoderma resinosum (Schrad. ex Fr.) Karst. (*Polyporus benzoinus* (Wahl.) Fr. [27])	Yanitake	P	W	Daisen, Tottori Oct. 1971	0·35	**6**, **7**	GLC	**12** [27]
Laetiporus sulphureus (Bull. ex Fr.) Bond. et Sing. *Polyporus sulphureus* (Bull) Fr. [45, 50–52], *Laetiporus sulphureus* (Bull. ex Fr.) Bond. et Sing. var. *miniatus* (Jungh.) Imaz. [31])	Masutake	P	B	Okutama, Tokyo May 1969	0·35	**1**, **2** **6**	GLC, IR, NMR, MS GLC	**1** [52], **2** [50–52]
Laurilia sulcatum (Burt) Kotl. et Pouz.‖	Kasaurokotake-modoki	S	W	North-East China	2·54			
Lentinus lepideus Fr. [11]	Matsuōji	T	B	Sendai, Miyagi Sept. 1972	1·28	**5** **1**, **2**	GLC, MS GLC, IR NMR, MS GC–MS	**5** [31, 51], **6** [45], **40** [51]

Table 2 (*contd.*)

Species (synonym)*	Japanese name	Family†	Type‡	Place and date of collection	Et_2O ext. (%)	Compounds identified	Method of identification	Compound known in literature
Lenzites betulina (L.) Fr.	Kaigaratake	P	W	Kiyosumi, Chiba Oct. 1968	0·91	**6**, **7**	GLC	
Leucofomes ulmarius (Sow. ex Fr.) Pouz.	Ōshirosaruno-koshikake	P	W	Hetsuka, Kagoshima	0·71	**6**	GLC	
Melanoporia caianderi (Karst.) Aoshima	Kenikuamitake	P	B	Sounkyo Hokkaido	17·5	**6** (**9**), (**12**)	GLC TLC, IR	
M. junipertina Aoshima [11]	Nikuamitake	P	B	Kiso, Nagano	0·67	**6**, **7** **12**, (**9**)	GLC TLC, IR	
M. nigra (Berk. et Curt.) Murr.	Kurosaruno-koshikake	P	B	New Guinea	8·55	**6**, **7** (**9**), (**12**), (**23**)	GLC TLC, IR	
M. purpuracea Aoshima	Murasakisaruno-koshikake	P	B	Kyoto	0·70	**6**, **7** (**9**), (**12**)	GLC TLC, IR	
M. rosea (Alb. et Schw. ex Fr.) Aoshima [11]	Barairosaruno-koshikake	P	B	Sounkyo Hokkaido	41·8	**9**, **12**, **23**	IR, NMR, MS	
Merulius tremellosus Schrad. ex Fr.	Shiwatake	M	W	Okutama, Tokyo Nov. 1969	3·06			
Oxyporus populinus (Schum. ex Fr.) Donk	Shirosaruno-koshikake	P	W	Tomakomai, Hokkaido July 1970	0·56	**6**, **7**	GLC	
Perenniporia ochroleuca (Berk.) Donk	Uzuratake	P	W	Yoga, Tokyo Oct. 1969	12·89	**6**, **7**	GLC	
P. tephlopora (Mont.) Aoshima	Shinosaruno-koshikake	P	W	Tokyo	2·12	**6**, **7**	GLC	
Phaeolus schweinitzii (Fr.) Pat.	Kaimentake	P	B	Mt. Fuji Sept. 1970	3·00	**6**, **7**	GLC, MS, GC–MS	
Phellinus gilvus (Schw. ex Fr.) Pat.	Nendotake	Hym	W	Kinkasan, Miyagi Nov. 1969	0·70			
P. hartigii (Allesch. et Schnabl) Imaz. (*Fomes hartigii* Allescher (Fr.) [28])	Momisaruno-koshikake	Hym	W	Nayoro, Hokkaido Sept. 1969	1·25	**6**	GLC	**1** [28]
P. igniarius (L. ex Fr.) Quel.	Nisehokuchitake	Hym	W	Nayoro, Hokkaido Sept. 1969	0·31	**6**, **7**	GLC, MS	
P. linteus (Berk. et Curt) Aoshima (*Fomes rimosus* Berk.) [53]	Meshimakobu	Hym	W	Hachijojima, Tokyo March 1970	0·45	**6**, **7**	GLC	**5** [53]
P. pachyphloeum Pat.	Kurosujisaruno-koshikake	Hym	W	New Guinea	0·59	**6**	GLC	
P. robustus (Karst.) Bourd. et Galz.	Kobusaruno-koshikake	Hym	W	Tanegashima, Kagoshima Sept. 1968	4·50	**6**, **7**	GLC	
Piptoporus betulinus (Bull. ex Fr.) Karst. [54] (*Polyporus betulinus* Fr. [55, 56])	Kanbatake	P	B	Hirogawara, Yamanashi Sept. 1969	9·24	**6**, **7** **12**	GLC IR, NMR	**5** [54, 56], **9**, **10**, **12** [55], **42** [54, 55], **44** [54]
Poria cocos (Fr.) Wolf [8, 9, 26, 31, 56–59]§¶ (*Pachyma Hoelen* Rumph. [60])	Matsuhodo	P	B	Mobara, Chiba May 1968	0·57	**5** **7**, **9**, **10**, **11**, **12** **15**		**2** [26, 59], **5** [34, 56, 60], **11** [57, 58]
P. nigrescens Bres.	Hadairoanatake-modoki	P	W	Marunuma, Gunma May 1970	0·65	**6**, **7**	GLC	
P. subacida (Peck) Sacc. [56]	Kin-iroanatake	P	W	Okutama, Tokyo Nov. 1969	1·62	**6**, **7**	GLC	**5** [56]
Porodaedalea abietis (Karst.) Aoshima	Matsunokatawatake	Hym	W	Mt. Fuji	0·47	**6**	GLC	
P. pini (Thore ex Fr.) Murr. (*Cryptoderma pini* (Thore ex Fr.) [31], *Fomes pini* [39])	Ezosarunokoshikake	Hym	W	Shikotsuko, Hokkaido	0·52	**6**	GLC	**3**, **44** [39], **5** [31]
P. sanfordii (Lloyd) Aoshima	Chōjitake	Hym	W	Kinkasan, Miyagi Nov. 1969	0·29	**6**, **7**	GLC	
Porodisculus pendulus (Schw.) Murr.	Nurudetake	P	W	Minakami, Gunma Feb. 1969	4·39			
Pycnoporellus flugens (Rostk.) Aoshima	Kabochatake	P	B	Nikkō, Gunma	5·14			
Pycnoporus coccineus (Fr.) Karst.	Hi-irotake	P	W	Sayama, Tokyo Sept. 1969	1·40	**6**, **7**	GLC, MS	
Roscofomes cesatianus (P. Henn.) Aoshima	Kurobudōtake	P	W	Hetsuka, Kagoshima	3·70	**6**	GLC	

Table 2 (*contd.*)

Species (synonym)*	Japanese name	Family†	Type‡	Place and date of collection	Et_2O ext (%)	Compounds identified	Method of identification	Compound known in literature
Stereum fasciatum (Schw.) Fr. [31]	Chaurokotake	S	W	Izu. Shizuoka May 1970	0·52	**6**	GLC	**5** [31]
S. hirsutum (Willd.) Fr.	Kiurokotake	S	W	Hokkaido July 1970	1·37	**6**	GLC	
S. princeps (Jungh.) Lev.	Ourokotake	S	W	Hetsuka, Kagoshima	2·19			
S. taxodii Lentz et McKay	Kasaurokotake	S	W	Otoyo, Kochi	1·56			
Spongiporus appendiculatus (Berk. et Br.) Aoshima [11]	Shirokaimentake	P	B	Nayoro, Hokkaido Dec. 1967	0·98	**6**, **7** (**1**), **2**, **9**	GLC GLC, IR	
Trametes gibbosa Fr.	Ochirimentake	P	W	Aizu, Fukushima July 1969	0·63	**6**, **7**	GLC	
T. orientalis (Yas.) Imaz. [37]	Kujiratake	P	W	Kiyosumi, Chiba Oct. 1968	1·00	**6**, **7**	GLC	**5** [37]
T. palisoti (Fr.) Imaz.	Chirimentake	P	W	Aizu, Fukushima July 1969	0·70	**6**, **7**	GLC, MS, GC–MS	
T. suaveolens (L. ex Fr.) Fr.	Shiroamitake	P	W	Marunuma, Gunma Oct. 1969	0·64	**6**	GLC	
Tyromyces albellus (Peck) Bond. et Sing.		P	W	Marunuma, Gunma Oct. 1969	4·79	**6**	GLC	
T. tangerianus Aoshima		P	W	New Guinea	51·36			
Veluticeps angularis (Lloyd) Aoshima et Furukawa [11]	Chizugata-sarunokoshikake	V	B	Kiso, Nagano	14·75	**1**	GLC, IR, NMR	

* Synonym used in the previous work.

† P: Polyporaceae, Hyd: Hydnaceae, F: Fistulinaceae, Hym: Hymenochaetaceae, S: Stereaceae, T: Tricholomataceae, M: Meruliaceae, V: Veluticepsaceae.

‡ W: white-rot, B: brown-rot.

§ Sclerotia.

‖ The absence of triterpene carboxylic acid was confirmed. However, analysis of the ethereal fraction has not been carried out due to the scarcity of the material or the complexity of the extracts.

• The taxonomical situation of the species is doubtful.

Table 3. R_f Values and relative retention times of triterpene acid methyl esters

Methyl ester	R_f*	Relative retention time†
Pinicolic acid A (**34**)	0·85	4·50
Pachymic acid (**11**)	0·70	11·22
Carbomethoxyacetylquercinic acid (**25**)	0·67	9·61
Polyporenic acid C (**12**)	0·63	6·44
Quercinic acid (**24**)	0·62	8·53
Eburicoic acid (**2**)	0·62	4·89
Trametenolic acid (**1**)	0·60	4·42
3α-Acetoxylanosta-8,24-dien-21-oic acid (**8**)	0·60	4·78
3β-Hydroxylanosta-7,9(11),24-trien-21-oic acid (**15**)	0·58	
Tumulosic acid (**9**)	0·36	6·92
Sulphrenic acid (**40**)	0·35	8·78
12β-Hydroxycarboxyacetylquercinic acid (**23**)	0·27	
Ergosterol (**5**)	0·52	

* On silica gel H, solvent system, hexane–AcOEt (4:1), detected by vanillin–phosphoric acid.

† Relative to cholestane, 1·5 m × 0·3 cm glass column, OV-1 1·5% at 260°, N_2 50 ml/min, cholestane 3·60 min.

Table 4. Characters of brown-rot and white-rot fungi [21]

Type	Morphology	Sexual compatibility	Phenol oxidase	Other metabolic systems
Brown-rot	Mono- or di-mitic hyphal system Basidiospore mostly smooth surface	Bipolar	Tyrosinase (extracellular oxidase not present)	
White-rot	Di- or tri-mitic hyphal system	Tetrapolar	Tyrosinase Laccase (extracellular oxidase present)	Oxalic acid metabolism
	Ornamentation of spore surface			Amyloidity of basidiospore sometimes present

Thin layer chromatography. Silica-gel H or HF_{254} was used with hexane–AcOEt (4–1:1) and $CHCl_3$–MeOH (5–1:1) as solvents; detection was by heating after spraying vanillin-phosphoric acid (Table 3).

Gas chromatography was carried out using 1·5% OV-1 on Shimalite W, 1·5% OV-17 on Shimalite W and 1·5% QF-1 on Chromosorb W columns at 210–260° on a Hitachi F6-D gas chromatograph.

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