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FATTY ACID AND STEROL COMPOSITIONS IN MUSHROOMS OF TEN SPECIES OF POLYPORACEAE*

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Abstract—The simple lipids present in ten species of Polyporaceae (*Piptororus betulinus*, *Coriolus pargamenus*, *C. versicolor*, *C. heteromorphus*, *Formitopsis cytisina*, *F. pinicola*, *Microporus flabelliformis*, *Gloephyllum saepiarium*, *Crytoderma citrinum* and *Grifola frondosa*) were investigated. The fatty acids that these species had in common were C_{16}-saturated acids (except in *P. betulinus*) and C_{18}-unsaturated acids. Ergosterol and ergosta-7,22-dien-3β-ol were isolated from these mushrooms. Lupeol was obtained from *G. saepiarium*. Ergost-7-en-3β-ol, lanosterol and 24-methylene-24,25-dihydrolanosterol were tentatively identified.

INTRODUCTION

The present report deals with the composition of the fatty acids and sterols present in ten species of Polyporaceae (Table 1). Previously, ergosta-7,22-dien-3β-ol was isolated from *P. yucatensis* [1]. A triterpenoid (polyporenic acid A) has been reported to be present in *P. betulinus* [2], and the fatty acid composition has also been studied in *M. fabelliformis* [3] and *G. frondosa* [4]. However, nothing is known about any of the minor sterol components of these three mushrooms. There are many reports about the sterols of the Polyporaceae [1–5], but none on the triterpene monols except for 24-methylene-24,25-dihydrolanosterol in *Polyporus sulfereus* [6].

RESULTS AND DISCUSSION

Table 1 shows the place and date of collection of the mushrooms examined. Table 2 lists the contents of lipids and of unsaponifiable matter (see Experimental). The yields of ether extract and unsaponifiable matter in *P. betulinus* was much greater than for the other species, probably because it contained triterpenoids.

Part of the fatty acids was converted into methyl esters and these were analysed by GLC (Table 3). In *P. betulinus*, the main saturated fatty acid was $C_{18:0}$, in other species it was $C_{16:0}$. The main unsaturated fatty acid was $C_{18:2}$ except in *G. frondosa* where it was a $C_{18:1}$ acid. *F. cytisina* had a larger number of fatty acid components compared to the other species and it was also characterized by a larger amount of $C_{17:0}$ and $C_{18:3}$ acids.

The $\Delta^{5,7}$-sterol contents in the unsaponifiable matter were calculated using the Glover–Morton empirical formula [7] from the UV absorption spectra. In Table 2, the result is shown as a percentage of the unsaponifiable matter.

The unsaponifiable material was separated by preparative TLC into four fractions: less polar compounds

Table 1. Species of Polyporaceae analysed

	Place of collection	Date of collection
Piptororus betulinus	Daibosatsu-pass, Yamanashi Pref.	November 1973
Coriolus pargamenus	Uenohara-shi, Yamanashi Pref.	November 1977
C. versicolor	Tokorozawa-shi, Saitama Pref.	September 1978
C. heteromorphus	Sayama-shi, Saitama Pref.	October 1977
Formitopsis cytisina	Tokorozawa-shi, Saitama Pref.	September 1977
F. pinicola	Sendai-shi, Miyagi Pref.	October 1977
Microporus flabelliformis	Tokorozawa-shi, Saitama Pref.	November 1977
Gloephyllum saepiarium	Tokorozawa-shi, Saitama Pref.	October 1978
Cryptoderma citrinum	Sayama-shi, Saitama Pref.	November 1978
Grifola frondosa	Nakano-ku, Tokyo	October 1978

* Part II in this series. For Part I see Yokokawa, H., Ishizima, E., Ishii, K., Kanayama, Y. and Endo, S. (1978) *Yukagaku* **27**, 847.

Table 2. Total lipid compositions of Polyporaceae species

	Samples (dry wt/g)	Ether extract (% of dry wt)	Unsaponifiable matter (% of ether extract)	$\Delta^{5,7}$-sterol (% of unsaponifiables)
Piptororus betulinus	415.0	17.6	74.8	13.9
Coriolus pargamenus	307.0	0.7	25.0	26.9
C. versicolor	126.0	0.5	50.0	
C. heteromorphus	327.0	2.1	17.2	5.4
Formitopsis cytisina	100.0	1.0	40.0	31.6
F. pinicola	519.0	2.1	16.4	49.5
Microporus flabelliformis	106.5	1.5	12.5	30.1
Gloephyllum saepiarium	988.0	0.3	51.4	14.0
Cryptoderma citrinum	64.5	1.5	10.0	15.0
Grifola frondosa	40.0	1.5	50.0	43.3

Table 3. Fatty acid composition (%)

	Carbon number										
	10:0	12:0	14:0	14:1	16:0	16:1	17:0	18:0	18:1	18:2	18:3
Piptororus betulinus			2.3	0.8	4.8	3.1		26.7	25.0	34.3	2.9
Coriolus pargamenus		tr*	tr	0.5	9.3	4.1		tr	32.9	53.2	
C. versicolor		tr	0.3		16.9	2.4	0.7	0.6	9.1	67.1	2.9
C. heteromorphus			tr		24.0			tr	24.9	51.1	tr.
Formitopsis cytisina		0.2	0.5	4.5	24.4	2.3	3.8	4.2	17.1	34.5	4.3
F. pinicola			4.2	0.9	19.9	2.1		6.9	25.2	40.8	tr
Microporus flabelliformis			0.7		12.9	1.1		0.4	21.9	59.2	3.8
Gloephyllum saepiarium	0.7	0.9	1.7		10.4	1.1	tr	tr	17.4	65.2	2.6
Cryptoderma citrinum	0.3	0.4	1.6		23.7	0.7		1.6	7.0	64.7	
Grifola frondosa		0.5	0.9		17.3	3.1		0.9	43.8	31.1	2.4

*tr: trace, less than 0.1%.

(hydrocarbons, aliphatic alcohols, etc.) (fraction 1), 4,4-dimethylsterols (fraction 2), 4-monomethylsterols (fraction 3) and 4-demethylsterols (fraction 4). Table 4 shows the percentage yield of the four fractions from the unsaponifiable matter. The sterol fractions were acetylated and examined by GLC and UV absorption (Table 5). The sterol of *F. cytisina* was acetylated and subsequently separated into two fractions by preparative TLC. Fraction 1 had a RR_f of 1.31 on GLC and mp of 179.5° consistent for ergosteryl acetate (lit. RR_f 1.31 [1], mp 181° [8]). The UV spectrum (λ_{max} at 262, 272, 282, 293 nm) was characteristic of $\Delta^{5,7}$-sterols [9]. The IR spectrum showed a peak at 975 cm^{-1}, characteristic of a *trans*-Δ^{22}-double bond [10]. MS showed a molecular ion at *m/e* 438 and other fragment ions at *m/e* 423 ($M^+ - Me$), 394 ($M^+ - 44$), 379 ($M^+ - 59$), 363 ($M^+ - 75$), 341 ($M^+ - 97$), 313 ($M^+ - SC$) and 253 [$M^+ - (SC + 60)$] in accordance with that of ergosteryl acetate [11].

Recrystallization of fraction 2 from MeOH Me_2CO (1:1), produced colourless crystals, mp 181° and RR_f 1.32 on GLC (lit. ergosta-7,22-dien-3β-yl acetate RR_f 1.32 [1],

Table 4. Yield of four fractions from the unsaponifiable matter

	Fraction*			
	1	2	3	4
Piptororus betulinus	9.1	4.9	2.5	83.5
Coriolus pargamenus	3.9	8.6	1.6	85.9
C. versicolor	7.3	7.6	5.8	79.3
C. heteromorphus	35.1	16.5	2.8	45.6
Formitopsis cytisina	8.5	9.3	4.1	78.1
F. pinicola	29.6	10.9	3.9	55.6
Microporus flabelliformis	7.9	7.9	3.5	80.7
Gloephyllum saepiarium	28.9	15.1	8.1	47.9
Cryptoderma citrinum	16.9	17.3	9.6	56.2
Grifola frondosa	10.1	6.1	2.5	81.3

* 1: Less polar (hydrocarbons, aliphatic alcohols, etc.), 2: 4,4-dimethylsterols, 3:4-monomethylsterols, 4:4-demethylsterols.

Table 5. 4-Demethylsterol composition (%)

	I*	II	III	IV
Piptororus betulinus		73.4	26.6	
Coriolus pargamenus	59.3			40.7
C. versicolor	49.0	31.6		20.0
C. heteromorphus		70.1	29.9	
Formitopsis cytisina	73.3	26.7		
F. pinicola	70.8			29.2
Microporus flabelliformis	68.4		31.6	
Gloephyllum saepiarium	45.4	34.2		20.4
Cryptoderma citrinum	57.5	22.1		20.4
Grifola frondosa	80.3			19.7

*I: Ergosterol, II: ergosta-7,22-dien-3β-ol, III: ergosta-5,7-dien-3β-ol, IV: ergost-7-en-3β-ol.

mp 180.8° [13]. IR peaks are at cm^{-1}: 840, 790 $\left(\begin{matrix}R_1\\R_2\end{matrix}\!\!>\!C\!=\!C\!<\!\!\begin{matrix}R_3\\H\end{matrix}\right)$; 963 $\left(\begin{matrix}R_1\\H\end{matrix}\!\!>\!C\!=\!C\!<\!\!\begin{matrix}H\\R_2\end{matrix}\right)$ and 975 (*trans*-Δ^{22}) [11]. The MS showed a molecular ion at *m/e* 440 and other fragment ions at *m/e* 425 ($M^+ - 15$), 396 ($M^+ - 44$), 381 ($M^+ - 59$), 343 ($M^+ - 97$), 315 ($M^+ - SC$), 313 (base peak) and 255 [$M^+ - (SC + 60)$]. This fragmentation pattern was characteristic of a Δ-steryl acetate. The ^1H NMR spectrum, which included signals at δ 0.54 (3H, C-18), 0.79 (3H, C-27), 0.84 (3H, C-26), 0.88 (3H, C-28), 1.02 (3H, C-21), 2.04 (3β-*O*-acetyl protons), gave results also consistent with this identification. There was no absorption in the UV spectrum characteristic of a $\Delta^{5,7}$-sterol. These results were consistent with its identification as ergosta-7,22-dien-3β-yl acetate [1, 12].

Table 6 shows the 4,4-dimethylsterol compositions determined on GLC by comparison of *RR*$_t$ values with those reported for authentic samples [13].

The 4,4-dimethylsterol fraction of *G. saepiarium* was separated into three components by preparative argentation TLC of its acetate. The main methylsterol zone was eluted with ether. The colourless crystalline compound obtained had a *RR*$_t$ on GLC which was identical to that of lupeyl acetate. Crystallization of the compound gave crystals with mp 216.8° (lit. [14] 213–215°). The presence of a carbonyl group was indicated by IR peaks at 1740 and 1250 cm^{-1}, and a methylene group by a peak at 1640 cm^{-1}. The ^1H NMR spectrum, which included signals at δ 0.78 (3H, *s*, C-28), 0.84 (9H, *s*, C-23, C-24, C-25), 0.94 (3H, *s*, C-27), 1.03 (3H, *s*, C-26), 1.68 (3H, *s*, C-30), 2.04 (3H, *s*, C-3β-OAc), 4.48 (1H, *m*, $W_{1/2}$ = 18 Hz), and 4.57 and 4.67 (both 1H and *br. s*, C-29) [15], was also consistent with the identification of lupeyl acetate. The MS showed a molecular ion at *m/e* 468 and other fragment ions at *m/e* 453 ($M^+ - Me$), 393 ($M^+ - (Me + HOAc)$), 386, 218 and 189. This fragmentation pattern agrees with that of lupeyl acetate. Lupeol has been isolated from many higher plants [16–18], but it has not been reported previously in mushrooms (Polyporaceae).

Table 6. 4,4-Dimethylsterol composition (%)

	I*	II	III	IV
Coriolus pargamenus	31.5	68.5		
C. heteromorphus	38.5	61.5		
Formitopsis cytisina		72.2		27.8
F. pinicola	52.8	47.8		
Microporus flabelliformis	35.2	38.0		26.8
Gloephyllum saepiarium			90.0	10.0
Cryptoderma citrinum	86.6	13.4		
Grifola frondosa	55.5	44.5		

*I: Lanosterol, II: 24-methylene-24,25-dihydrolanosterol, III: lupeol, IV: others.

EXPERIMENTAL

General methods. Recrystallization was from MeOH–Me_2CO. TLC and CC were employed as described previously [1]. IR spectra were recorded in KBr and UV spectra in EtOH soln. GLC was carried out on a 2 m × 3 mm glass column (3% OV-17 chromosorb W AW, 80–100 mesh, 260°). FID (sterol), 20% DEGS chromosorb W AW 60–80 mesh, 200° FID (fatty acid). The *RR*$_t$ on GLC for the acetates of sterols and methylsterols were taken as 1.0 for cholesteryl acetate. ^1H NMR (spectra) were obtained at 100 MHz in $CDCl_3$ with TMS as int. standard.

Extraction procedure. Each sample was cut into small pieces, dried in an oven at a temp. below 60°, and subsequently extracted with Et_2O. The Et_2O extracts of *P. betulinus* and *F. pinicola* were treated with hexane and the hexane-insoluble parts were removed by filtration. The total lipids were treated with Me_2CO and separated into Me_2CO-soluble neutral lipids and insoluble phospholipids. The neutral lipids (Me_2CO-soluble part) were saponified by refluxing with a 10% KOH–EtOH soln for 1 hr and the unsaponifiable matter was obtained by Et_2O extraction. The H_2O-soluble part was acidified by H_2SO_4, and fatty acids were obtained by Et_2O extraction.

Part of the fatty acids was refluxed with 1% *p*-toluene sulfonic acid in MeOH [19] for 1.5 hr to yield their methyl esters. These were subsequently dissolved in hexane, and passed through a Si gel column which removed the brown colour and any free fatty acids. The methyl esters of the fatty acids were analysed by GLC.

Fractionation of the unsaponifiable matter by prep. TLC (0.5 mm) on Si gel using hexane–C_6H_6 (4:1) was performed as described previously [1]. The sterol and methylsterol fractions were acetylated by Ac_2O–pyridine (1:1) and subsequently determined by GLC. The sterol and methylsterol fractions were fractionated by 20% $AgNO_3$ Si gel (1:4) prep. TLC (0.5 mm) developing 3 × with hexane–C_6H_6 (4:1).

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REFERENCES

1. Yokokawa, H., Ishizima, E., Ishii, K., Kanayama, Y. and Endo, S. (1978) *Yukagaku* **27**, 847.
2. Cross, L. C., Eliot, C. G., Heilbron, I. M. and Jones, E. R. H. (1940) *J. Chem. Soc.* 632.
3. Mitsuhashi, T., Toyama, A. and Endo, S. (1973) *Bull. Tokyo Gakugei Univ.* **25**, 80.

4. Endo, S., Mitsuhashi, T., Enomoto, T., Yonezawa, Y., Koyatsu, R., Katagiri, U. and Sugiyama, N. (1978) *Bull. Tokyo Gakugei Univ.* **30**, 85.
5. Stringina, L. I., Elkin, Y. N. and Elyakov, G. B. (1971) *Phytochemistry* **10**, 2361.
6. Barton, D. H. R., Harrison, D. M. and Moss, G. P. (1966) *Chem. Commun.* 595.
7. Glover, M., Glover, J. and Morton, R. T. (1952) *Biochem. J.* **51**, 1.
8. Yukagaku-kyokai edi. (1971) *Yushikagaku Binran*, p. 51. Maruzen, Tokyo.
9. Fieser, L. F. and Fieser, M. (1959) *Steroids*, p. 93. Reinhold, New York.
10. Johnson, J. C., Grostic, M. F. and Jensen, A. O. (1957) *Analyt. Chem.* **29**, 468.
11. Jeong, Y. M., Itho, T., Tamura, T. and Matsumoto, T. (1975) *Steroids* **25**, 741.
12. Ruyle, W. V., Chamberlin, E. M., Chemerda, J. M., Sita, G. E., Aliminosa, L. M. and Erickson, R. L. (1952) *J. Am. Chem. Soc.* **74**, 5929.
13. Jeong, T. M., Itho, T., Tamura, T. and Matsumoto, T. (1975) *Lipids* **10**, 634.
14. Lindgren, B. O. and Svahn, C. M. (1966) *Acta Chem. Scand.* **20**, 1720.
15. Shingu, T., Yokoi, T., Niwa, M. and Kikuchi, T. (1973) *Chem. Pharm. Bull.* **21**, 2252.
16. Palmer, M. A. and Bowden, B. N. (1975) *Phytochemistry* **14**, 1813.
17. Jeong, T. M., Yang, M. S. and Matsumoto, T. (1977) *J. Korean Chem. Soc.* **21**, 193.
18. Van, D. M., Daenens, P. and Laruelle, P. (1975) *Rev. Fr. Corps Gras* **22**, 619.
19. Usui, H. and Yoshitomi, K. (1962) *Yukagaku* **11**, 551.