Phytochemistry, Vol. 20, No. 6, pp. 1349–1351, 1981.
Printed in Great Britain.

0031-9422/81/061349-03 $02.00/0
Pergamon Press Ltd.

THE STEROL COMPOSITION OF MUSHROOMS

H. YOKOKAWA and T. MITSUHASHI

Tachikawa College of Tokyo, 3-6-33, Azuma-cho, Akishima-shi, Tokyo, 196 Japan and Department of Chemistry, Tokyo Gakugei University, 4-1-1, Nukuikita-machi, Koganei-shi, Tokyo, 180 Japan

(*Received* 4 *September* 1980)

Key Word Index—Basidiomycetes; mushrooms; sterols; ergosterol; 5α-ergost-7-en-3β-ol; ergosta-5,8,22-trien-3β-ol.

Abstract—Sterols were isolated from ten mushrooms, *Hygrocybe punicea*, *Lampteromyces japonicus*, *Leucopaxillus giganteus*, *Lentinus edodes*, *Flammulina velutipes*, *Amanita caesarea*, *Coprinus atramentarius*, *Russula foetens*, *R. nigricans* and *R. senecis*. The compositions of the sterol fractions were determined by GLC, combined GC/MS, and ^{1}H NMR. Ergosterol was present in all the mushrooms. Other sterols found were 5α-cholest-7-en-3β-ol and ergosta-5,7-dien-3β-ol. Ergosta-5,8,22-trien-3β-ol was isolated from *F. velutipes*.

INTRODUCTION

We have made a systematic study of the fatty acid and sterol composition of Basidiomycetes. In the present investigation we examined the sterol composition of the unsaponifiable materials in ten mushrooms. The composition of the fatty acids of these mushrooms has been reported previously [1].

Ergosterol in *Lentinus edodes* has been reported by Sumi [2] and Ono *et al.* [3], but the presence of ergost-7-en-3β-ol in this mushroom has not been mentioned. The presence of the latter has, however, been ascertained in *Amanita caesarea* [4] and *Ganoderma applanatum* [5].

The presence of ergosta-5,8,22-trien-3β-ol has been ascertained in *Xanthoria parietina* [6], *Ustilago longissima* [7], a mutant of *Neurospora crassa* [8] and *Saccharomyces cerevistiae* [9], but not in mushrooms.

RESULTS AND DISCUSSION

The percentages of total lipids and unsaponifiable materials, and the percentages of the $\Delta^{5,7}$-sterols in the unsaponifiable materials are shown in Table 1. The percentages of total lipids were high in *Lampteromyces japonicus* and *Amamita caesarea*, compared to the other samples. The percentages of unsaponifiable materials and of the $\Delta^{5,7}$-sterol contained in the unsaponifiable materials varied with different samples. The percentages of unsaponifiable materials were high in *L. japonicus*, *Leucopaxillus giganteus*, *Lentinus edodes* and *Russula senecis*, and $\Delta^{5,7}$-sterols in the unsaponifiable materials were 50% or more in *L. giganteus*, *Coprinus atramentarius* and *Russula nigricans*.

The results of the GLC analyses of the sterols are shown in Table 2. Ergosterol was contained in all ten mushrooms and comprised 80% or more of the sterols in *L. giganteus*, *L. edodes*, *A. caesarea*, *R. nigricans* and *R. senecis*. The content of ergosta-5,7-dien-3β-ol was higher than that of ergosterol in *L. japonicus* and *C. attramentarius*. Ergost-7-en-3β-ol was present in *L. edodes*, *A. caesarea* and *R. senecis*. Ergosta-5,8,22-trien-3β-ol was present only in *Flammulina velutipes*, and 5α-cholest-7-en-3β-ol only in *L. giganteus*.

The sterol fraction from *L. edodes* obtained by preparative TLC was further fractionated after

Table 1. Total lipid compositions of mushrooms

	Total lipid (% of dry material)	Unsaponifiable materials (% of ether extract)	$\Delta^{5,7}$-sterol (% of unsaponifiables)
Hygrocybe punicea	3.6	11.2	31.1
Lampteromyces japonicus	15.6	24.7	14.3
Leucopaxillus giganteus	2.9	22.5	54.9
Lentinus edodes	5.3	26.9	36.1
Flammulina velutipes	5.0	15.5	25.5
Amanita caesarea	11.0	7.0	42.7
Coprinus atramentarius	2.5	18.1	55.5
Russula foetens	0.6*	10.7	12.2
R. nigricans	7.4	8.9	50.4
R. senecis	5.8	26.5	11.5

*Per cent of wet material.

Table 2. Compositions of sterol fractions (%)

	I*	II	III	IV	V	VI
Hygrocybe punicea			78.8		21.2	
Lampteromyces japonicus			45.6		54.4	
Leucopaxillus giganteus	6.8		85.9		7.3	
Lentinus edodes			83.6		16.4	
Flammulina velutipes		30.1	45.3	14.1	10.5	
Amanita caesarea			82.5		17.5	
Coprinus atramentarius			24.5		75.5	
Russula foetens			70.9		29.1	
R. nigricans			83.8		16.2	
R. senecis			84.5			15.5

*I: Cholest-7-en-3β-ol, II: ergosta-5,8,22-trien-3β-ol, III: ergosterol, IV: ergosta-7,22-dien-3β-ol, V: ergosta-5,7-dien-3β-ol, VI: ergost-7-en-3β-ol.

acetylation. The acetate was chromatographed with hexane–C_6H_6 (3:2) three times on 20% $AgNO_3$ preparative TLC plates, and two fractions were obtained. Fraction 1 (most polar) was identified as ergosteryl acetate on the basis of the GLC results, mp and UV. Fraction 2 (least polar) was recrystallized from MeOH–Me_2CO (1:1). It had a RR_t on GLC of 1.52, mp of 150.0°, and gave colourless crystals (RR_t of ergost-7-en-3β-yl acetate, 1.52 [10], mp 150° [11]. The mass spectrum of this substance showed a molecular ion at m/z 442, as well as fragment ions at m/z 427, 382, 367, 315, 273, 256, 255, 299 and 219. The peak at m/z 255 is characteristic of the fragment ions in sterols with a Δ^7-unsaturated structure [12]. This fragmentation pattern was similar to that of ergost-7-en-3β-ol [12].

The ^{1}H NMR spectrum of this compound had signals with δ values of 0.53 (3 H, C-18), 0.77 (3 H, C-27), 0.80 (3 H, C-19), 0.77 (3 H, C-28), 0.85 (3 H, C-26), 0.91 (3 H, C-21), 2.03 (3 H, C-3, OAc) and 4.63 (1 H, C-3). The UV spectrum did not show $\Delta^{5,7}$-sterol absorption. From the results of GLC, mp, UV, MS and ^{1}H NMR, fraction 2 was identified as ergost-7-en-3β-yl acetate.

The GLC analysis of the sterols of *L. japonicus* showed two components; one with a RR_t of 1.31 (A) and the other with a RR_t of 1.51 (B). The GC/MS of compound A showed a molecular ion at m/z 396, and fragment ions at m/z 378, 363, 337, 271, and 253 (base peak). The fragment ion at m/z 337 is characteristic of $\Delta^{5,7}$-sterols [13] and the fragmentation pattern was in good agreement with that of ergosterol [13]. Thus, compound A was identified as ergosterol.

The molecular ion of compound B was at m/z 398, and fragment ions at m/z 383, 380, 365, 339 and 253 were also observed. This pattern of fragmentation was similar to that of ergosterol, except for the molecular ion. It was in good agreement with that of ergosta-5,7-dien-3β-ol [14]. Compound B was identified as 5,7-ergostadienol which has been reported previously to occur in *Agaricus campestris* [15].

The sterol fraction from *F. velutipes* was obtained by preparative TLC and acetylated. The steryl acetates were chromatographed on $AgNO_3$–Si gel with hexane–C_6H_6 (3:2), developed three times. The band at R_f 0.35 (ergosteryl acetate R_f 0.22) was scraped off and extracted with Et_2O to yield colourless crystals which were recrystallized in MeOH–Me_2CO (1:1). The purity, determined by GLC (RR_t 1.21), was 98% and the mp was 125.8° (the reported mp of ergosta-5,8,22-trien-3β-yl acetate is 123–126° [6, 7]. The mass spectrum revealed a molecular ion at m/z 438, and other fragment ions at m/z 380, 379, 378, 363, 337, 313, 253 and 211. The fragment ion at m/z 337 suggested a ring B diene [15].

The ^{1}H NMR spectrum showed signals at δ 0.67 (3 H, *s*, C-18), 1.20 (3 H, *s*, C-19), 1.02 (3 H, *d*, J = 6 Hz, C-21), 0.84 (3 H, *d*, J = 7 Hz, C-26), 0.85 (3 H, *d*, J = 7 Hz, C-27), 0.93 (3 H, *d*, J = 7 Hz, C-28), 5.19 (2 H, *m*, C-22, C-23, *trans*-olefinic protons) and 5.45 (1 H, *m*, C-6).

The MS and ^{1}H NMR spectra were in agreement with those cited in the literature [6, 7] for ergosta-5,8,22-trien-3β-ol. The IR spectrum showed absorption for a carbonyl at 1730 and 1268 cm^{-1}, and for a *trans*-disubstituted olefin at 968 cm^{-1}. A clear absorption pattern was not obtained in the UV, which indicated that a conjugated double bond was not present. From these results, this material was identified as the acetate of ergosta-5,8,22-trien-3β-ol (lichesterol).

According to Safe *et al.* [7] lichesterol is a precursor of ergosterol. It is present in mutant strains of *N. crassa* and *S. cerevisiae*, but not in the wild types and has not been reported previously in other fungi. It has been found so far only in lichens. However, in the present study we found that lichesterol is present also in the mushroom *F. velutipes*.

EXPERIMENTAL

The mps were determined on a Mettler FP-1 (Shiber Kikai Co., Ltd., Tokyo) automatic analyser, and values were not corrected. IR spectra were recorded in KBr pellets. UV spectra were measured in EtOH. Operating conditions for GLC were: glass column (2 m × 3 mm i.d.); packing material, 3% OV-17 Chromosorb W AW; detector temp., 270°; N_2 gas flow rate, 50 ml/min; relative retention time (RR_t) for cholesterol 1.00. GC/MS spectra were performed on an OV-17 column (1.5%) and the operating conditions were: column temp., 246°; He carrier gas, 30 ml/min; molecular separator, 290°; ion source, 310°; ionizing voltage, 70 eV; accelerating high voltage, 3.5 kV. MS were obtained with a direct inlet system, ionizing voltage, 70 eV; ion source, 180°; sample temp., 110°, accelerating high voltage, 4.6 V. ^{1}H NMR spectra were measured on a 100 MHz instrument in $CDCl_3$ with TMS as internal reference.

Materials were collected in Tokyo and Saitama Pref. The material was dried in an electric drying oven at 60°, powdered, and the lipids were extracted with Et_2O. The Et_2O extract of the lipids was treated with 3 vols of Me_2CO, the neutral lipid fraction (easily soluble in Me_2CO) was refluxed for 1 hr in 10% ethanolic

KOH soln, and the unsaponifiable material was extracted with Et_2O. The content of $\Delta^{5,7}$-sterols in the unsaponifiable material was determined by UV using the Glover–Morton formula [16]. About 30 mg of the unsaponifiable material was solubilized in $CHCl_3$, spotted onto a 20 × 20 cm plate coated with a 0.5 mm layer of Wako gel B-10 (Wako Pure Chemical Industries), and developed 3× with hexane–Et_2O (4:1). After development, the plate was sprayed with a 0.01% Rhodamine-6G soln in EtOH and observed under UV light (3600 Å). The sterol zone was scraped off and eluted with Et_2O. It was then recrystallized with MeOH–Me_2CO (1:1) for subsequent GLC analysis. 20% $AgNO_3$–Si gel plates (0.5 mm layer), developed 3× with hexane–C_6H_6 (3:2), were used for further fractionation of the sterol mixtures in the form of their acetates. The acetates were prepared in Ac_2O–pyridine (1:1) overnight.

Acknowledgements—We would like to express our sincere thanks to Professor T. Mastumoto of the Faculty of Science and Technology, Nihon University, for his many helpful suggestions; to Dr. T. Itoh and T. Takido for conducting MS, GC/MS and ^1H NMR measurements; and to Mrs. S. Ogura for her gift of the sterol of *L. japonicus*.

REFERENCES

1. Yokokawa, H., Hino, N. and Mizukoshi, H. (1979) *J. Tachikawa Coll. Tokyo* **12,** 21.
2. Sumi, M. (1928) *P.I.A.* **4,** 116.
3. Ono, T., Sugiura, W., Matsuoka, K. and Arimoto, H. (1973) *J. Jpn. Soc. Food Nutr.* **26,** 547.
4. Wieland, H. and Coutella, G. (1941) *Ann. Chem.* **548,** 270.
5. Strigina, L. I., Elkin, Yu. N. and Elyakov, G. B. (1971) *Phytochemistry* **10,** 2361.
6. Lenton, J. R., Goad, L. J. and Goodwin, T. W. (1973) *Phytochemistry* **12,** 1135.
7. Safe, S., Safe, L. M. and Maass, W. S. G. (1975) *Phytochemistry* **14,** 1821.
8. Morris, D. C., Safe, S. and Subden, R. (1974) *Biochem. Genet.* **12,** 459.
9. Barton, D. H. R., Corrie, J. E. T., Wieddowson, D. A., Bard, M. and Woods, R. A. (1974) *J. Chem. Soc. Perkin Trans.* **1,** 1326.
10. Yokokawa, H., Ishizima, E., Ishii, I., Kanayama, Y. and Endo, S. (1978) *Yukagaku* **27,** 847.
11. Yukagaku-Kyokai edi. (1971) *Yushikagaku Binran,* p. 51. Maruzen, Tokyo.
12. Morisaki, N. and Ikekawa, N. (1973) *J. Synth. Org. Chem. (Jpn)* **31,** 573.
13. Jeong, T. M., Itoh, T., Tamura, T. and Matsumoto, T. (1975) *Steroids* **25,** 741.
14. Goulston, G., Mercer, E. L. and Goad, L. J. (1975) *Phytochemistry* **14,** 457.
15. Brooks, C. J., Horning, E. C. and Young, J. S. (1968) *Lipids* **3,** 391.
16. Glover, M. and Morton, R. A. (1952) *Biochem. J.* **51,** 1.