Sterol Constituents from Seven Mushrooms[1)]

Yasunori YAOITA, Makiko ENDO, Yoshino TANI, Kaori MACHIDA, Keiko AMEMIYA, Katsuyuki FURUMURA, and Masao KIKUCHI*

Tohoku Pharmaceutical University, 4–1 Komatsushima 4-chome, Aoba-ku, Sendai, Miyagi 981–8558, Japan.
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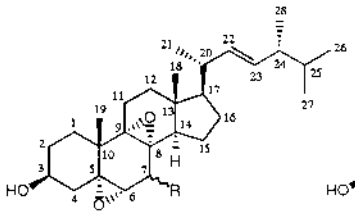
Six new sterols, 5α,6α;8α,9α-diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7α-diol (1), 5α,6α;8α,9α-diepoxy-(22*E*, 24*R*)-ergost-22-ene-3β,7β-diol (2), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (3), (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β-triol (4), (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β,9α-tetrol (5) and (24*S*)-ergost-7-ene-3β,5α,6β,9α-tetrol (6), have been isolated from seven mushrooms, *Amanita pantherina*, *Amanita virgineoides*, *Lactarius piperatus*, *Lyophyllum shimeji*, *Tricholoma portentosum*, *Hypsizigus marmoreus* and *Lentinula edodes* together with eighteen known ones (7—24). The structures of these new compounds were elucidated on the basis of their spectral data.

Key words sterol; mushroom; structure elucidation

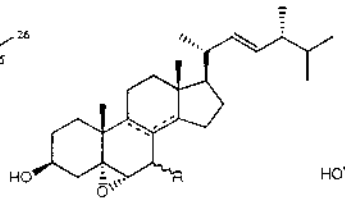
Recently we reported the isolation and structural elucidation of sterols from six edible mushrooms.[2—4)] In a continuation of our investigation of the sterol constituents of mushrooms, we describe here the isolation and structural elucidation of six new sterols, 5α,6α;8α,9α-diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7α-diol (**1**), 5α,6α;8α,9α-diepoxy-(22*E*, 24*R*)-ergost-22-ene-3β,7β-diol (**2**), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**3**), (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β-triol (**4**), (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β,9α-tetrol (**5**) and (24*S*)-ergost-7-ene-3β,5α,6β,9α-tetrol (**6**), as well as eighteen known ones, 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (**7**),[2)] 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (**8**),[2)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (**9**),[2)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (**10**),[4)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (**11**),[2)] (24*S*)-ergost-7-ene-3β,5α,6β-triol (**12**),[4)] ergosta-7,24(28)-diene-3β,5α,6β-triol (**13**),[4)] (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β, 5α,6β-triol (**14**),[2)] (22*E*,24*R*)-ergosta-8(14),22-diene-3β,5α, 6β,7α-tetrol (**15**),[2)] 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**16**),[2)] 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**17**),[2)] 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (**18**),[4)] 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**19**),[4)] 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (**20**),[2)] 5α,8α-epidioxy-(24*S*)-ergost-6-en-3β-ol (**21**),[2)] 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,9(11), 22-trien-3β-ol (**22**),[2)] (22*E*,24*R*)-ergosta-5,7,9(11),22-tetraen-3β-ol (**23**)[3)] and (22*E*,24*R*)-ergosta-7,22-dien-3β-ol (**24**)[3)] from seven mushrooms, *Amanita pantherina* (DC.: FR.) KROMBH. (Tengutake in Japanese, Amanitaceae, compounds **1**, **9**, **10**, **11**, **12**, **13**, **14**, **20**, **21**, **22**, **23** and **24**), *Amanita virgineoides* BAS (Shiroonitake in Japanese, Amanitaceae, compounds **1**, **6**, **9**, **10**, **11**, **12**, **15** and **17**), *Lactarius piperatus* (SCOP.: FR.) S. F. GRAY (Tsuchikaburi in Japanese, Russulaceae, compounds **1**, **2**, **9**, **10**, **11**, **16** and **17**), *Lyophyllum shimeji* (KAWAM.) HONGO (Honshimeji in Japanese, Tricholomataceae, compounds **1**, **9**, **17**, **18** and **19**), *Tricholoma portentosum* (FR.) QUEL. (Shimofurishimeji in Japanese, Tricholomataceae, compounds **1**, **3**, **7**, **8**, **9**, **10**, **11**, **12**, **17**, **19** and **20**), *Hypsizigus marmoreus* (PECK) BIGELOW (Bunashimeji in Japanese, Tricholomataceae, compounds **1** and **2**) and *Lentinula edodes* (BERK.) SING. (Shiitake in Japanese, Pleurotaceae, compounds **1**, **4**, **5** and **12**). This is the first time that compounds **7**—**24** have been isolated from these mushrooms. Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ −53.6°. The molecular formula was determined to be $C_{28}H_{44}O_4$ by high-resolution (HR)-MS. The IR spectrum showed the presence of hydroxyl groups (3420 cm^{-1}). The ^{1}H-NMR spectrum (Table 1), obtained with the aid of a ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum, showed signals due to two tertiary methyl groups [δ_H 0.67 (3H, H_3-18), 1.33 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.81 (3H, H_3-26), 0.83 (3H, H_3-27), 0.91 (3H, H_3-28), 0.99 (3H, H_3-21)], suggesting that **1** should be an ergostane-type sterol.[2—4)] Other signals in the ^{1}H-NMR spectrum indicated a trisubstituted epoxide-bearing methine hydrogen [δ_H 3.13 (1H, H-6)],[2)] two hydroxy-bearing methine hydrogens [δ_H 3.98 (1H, H-3), 4.03 (1H, H-7)][2)] and two disubstituted olefinic hydrogens [δ_H 5.14 (1H, H-22), 5.20 (1H, H-23)]. The ^{13}C-NMR spectrum (see Experimental), obtained with the aid of a ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum, contained 28 signals that included three oxygenated quaternary carbons [δ_C 65.0 (C-8), 67.1 (C-5), 70.2 (C-9)]. The double-bond equivalents for the molecular formula of **1** is seven, ascribed to one disubstituted double bond, two epoxide rings and the steroid nucleus. Detailed analysis of the ^{1}H–^{1}H COSY spectrum of **1** implied connectivities for H-6–H7 and H-7–OH-7 (Fig. 1). In the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectra, the C-H long-range correlations observed are shown in Fig. 1. From the above data, the planar structure of **1** was deduced to be 5,6;8,9-diepoxyergost-22-ene-3,7-diol. The stereostructure was determined as follows. In the nuclear Overhauser effect (NOE) difference spectra, an NOE was detected between H_3-19 and H-7β. This NOE implies that the B-ring of **1** adopts a boat-type conformation, as a result of incorporation of the 5α,6α-epoxide moiety and the hydroxyl group at C-7 has an α-configuration (Fig. 2). Furthermore, an NOE was detected between H_3-19 and H_3-18. A Dreiding model showed that an 8α,9α-epoxide ring was the only possible structure which could account for this NOE (Fig. 2). The configuration of the hydroxyl group at C-

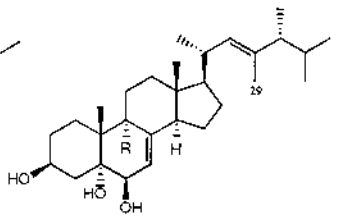
* To whom correspondence should be addressed.



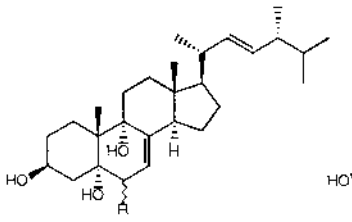
1 R = α - OH
2 R = β - OH



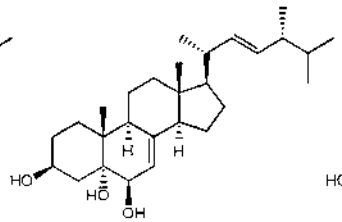
3 Δ^8, R = β - OH
7 Δ^8, R = α - OH
8 $\Delta^{8(14)}$, R = α - OH



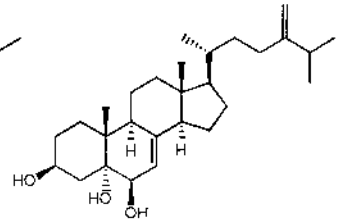
4 R = H
5 R = OH



6 R = β - OH, 22,23-dihydro
9 R = β - OH
10 R = α - OH



11
12 22,23-dihydro



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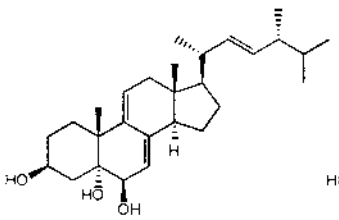

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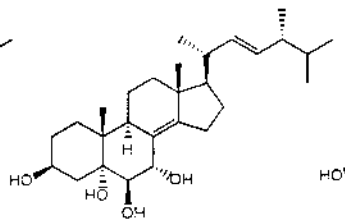

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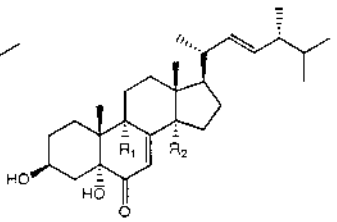
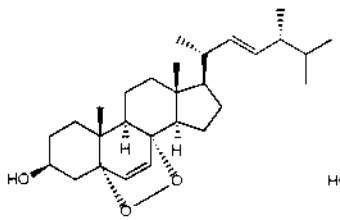
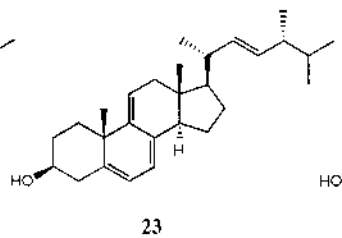


16 R_1 = H, R_2 = H
17 R_1 = OH, R_2 = H
18 R_1 = OH, R_2 = H, 22,23-dihydro
19 R_1 = OH, R_2 = OH



20
21 22,23-dihydro
22 $\Delta^{9(11)}$



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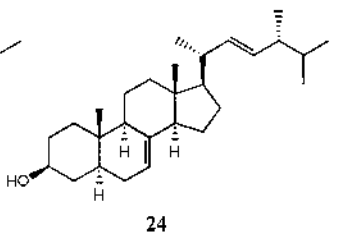

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Chart 1

3 was determined to be β by comparing the ^{1}H- and ^{13}C-NMR data of the C-3 hydroxyl-bearing methine of **1** with those of 5α,6α-epoxy-3β-hydroxy A/B *trans* sterols.[4] The stereochemistry at C-22 and C-24 in the side chain was determined to be *E* and *R*, respectively, by comparison of the ^{1}H- and ^{13}C-NMR data with those of authentic ergosterol. Based on this evidence, the structure of **1** was determined to be 5α,6α;8α,9α-diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7α-diol.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ −17.2°. The molecular formula was determined to be $C_{28}H_{44}O_4$ by HR-MS. The IR spectrum showed the presence of hydroxyl groups (3396 cm^{-1}). The ^{1}H-NMR spectrum of **2** was virtually identical to that of **1** except at the C-7 position. In the ^{1}H-NMR spectrum, the chemical shifts of the H_3-18 and H_3-19 methyl groups in pyridine-d_5 were shifted downfield by the pyridine-induced deshielding effect[5] ($\delta_{C_5D_5N} - \delta_{CDCl_3}$; $\Delta\delta$, H_3-18, +0.24 ppm, H_3-19, +0.25 ppm). This deshielding effect implies that the hydroxyl group at C-7 has a β configuration (Fig. 2). Thus, **2** was the 7-epimer of **1**. From the above data, the structure of **2** was determined to be 5α,6α;8α,9α-diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7β-diol.

Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**—**6** (600 MHz, $CDCl_3$)

Hydrogen	**1**	**2**	**3**[a)]	**4**	**5**	**6**[a)]
3	3.98 m	3.97 m	3.93 m	4.08 m	4.14 m	4.14 m
4β	2.20 dd (12.8, 11.4)	2.20 dd (12.8, 11.4)	2.20 dd (12.9, 11.5)	2.15 dd (12.8, 12.1)	2.19 dd (12.8, 11.0)	2.20 dd (12.0, 11.2)
6	3.13 d (2.6)	2.94 d (2.9)	3.14 d (2.9)	3.63 m	3.73 m	3.73 m
7	4.03 dd (9.5, 2.6)	4.52 dd (6.2, 2.9)	4.39 br s	5.36 br dd (5.5, 2.6)	5.42 dd (5.5, 2.6)	5.43 dd (5.6, 2.4)
14					2.46 m	2.46 m
18	0.67 s	0.73 s	0.63 s	0.62 s	0.63 s	0.61 s
19	1.33 s	1.43 s	1.28 s	1.09 s	1.15 s	1.15 s
20				2.36 m	2.36 m	
21	0.99 d (6.6)	0.99 d (6.6)	1.02 d (6.6)	0.95 d (6.6)	0.95 d (5.5)	0.93 d (5.9)
22	5.14 dd (15.4, 8.1)	5.13 dd (15.4, 8.4)	5.16 dd (15.1, 7.8)	4.90 dd (9.5, 1.5)	4.91 dd (9.5, 1.1)	
23	5.20 dd (15.4, 7.7)	5.21 dd (15.4, 7.7)	5.23 dd (15.1, 7.3)			
26	0.81 d (7.0)	0.81 d (6.6)	0.82 d (6.3)	0.85 d (6.6)	0.85 d (6.6)	0.78 d (6.8)[b)]
27	0.83 d (7.0)	0.83 d (6.6)	0.84 d (6.3)	0.79 d (6.6)	0.79 d (6.6)	0.86 d (6.8)
28	0.91 d (7.0)	0.91 d (7.0)	0.91 d (6.8)	0.94 d (7.0)	0.94 d (7.0)	0.79 d (6.8)[b)]
29				1.51 d (1.5)	1.51 d (1.1)	
7-OH	2.28 br d (9.5)	1.65 d (6.2)				

Coupling constants (J in Hz) are given in parentheses. *a*) Measured at 400 MHz. *b*) Assignments may be interchangeable.

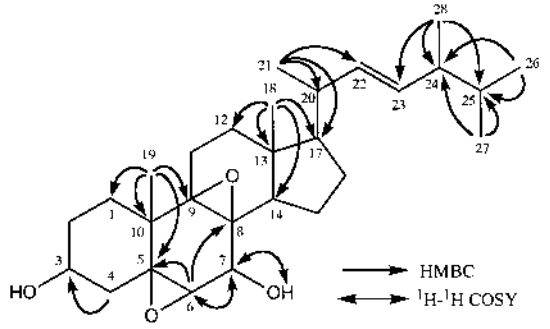

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**

5α,6α;8α,9α-Diepoxy-3β,7α-dihydroxy and 5α,6α;8α,9α-diepoxy-3β,7β-dihydroxy moieties are unprecedented in the natural sterols previously known.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −37.0°. The molecular formula was determined to be $C_{28}H_{44}O_3$ by HR-MS. The IR spectrum showed the presence of hydroxyl groups (3414 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.63 (3H, H_3-18), 1.28 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.91 (3H, H_3-28), 1.02 (3H, H_3-21)], suggesting that **3** should also be an ergostane-type sterol. Other signals in the ^{1}H-NMR spectrum indicated a trisubstituted epoxide-bearing methine hydrogen [δ_H 3.14 (1H, H-6)],[2)] two hydroxy-bearing methine hydrogens [δ_H 3.93 (1H, H-3), 4.39 (1H, H-7)][2)] and two disubstituted olefinic hydrogens [δ_H 5.16 (1H, H-22), 5.23 (1H, H-23)]. The ^{13}C-NMR spectrum contained 28 signals that included a fully substituted double bond [δ_C 126.5 (C-8), 137.0 (C-9)]. Comparison of these data with the spectral data for **7**[2)] revealed that they were identical except at C-7. In the ^{1}H-NMR spectrum of **3**, the chemical shifts of H_3-18 and H_3-19 methyl groups in pyridine-d_5 were shifted downfield[5)] ($\delta_{C_5D_5N}-\delta_{CDCl_3}$; $\Delta\delta$, H_3-18, +0.22 ppm, H_3-19, +0.27 ppm), indicating that the hydroxyl group at C-7 has a β configuration (Fig. 2). From the above data, the structure of **3** was determined to be 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol. Compound **3** is the first example of a naturally occurring 5α,6α-epoxy-3β,7β-dihydroxy-Δ^8-sterol.

Compound **4** was isolated as an amorphous powder, $[\alpha]_D$ −15.9°. The molecular formula was determined to be $C_{29}H_{48}O_3$ by HR-MS [*m/z* 426 (M^+-H_2O)] and ^{13}C-NMR data. The IR spectrum showed the presence of hydroxyl groups (3454 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.62 (3H, H_3-18), 1.09 (3H, H_3-19)], four secondary methyl groups [δ_H 0.79 (3H, H_3-27), 0.85 (3H, H_3-26), 0.94 (3H, H_3-28), 0.95 (3H, H_3-21)], an olefinic methyl group [δ_H 1.51 (3H, H_3-29)], two hydroxyl-bearing methine hydrogens [δ_H 3.63 (1H, H-6), 4.08 (1H, H-3)][2)] and two trisubstituted olefinic hydrogens [δ_H 4.90 (1H, H-22), 5.36 (1H, H-7)]. Inspection of the spectral data revealed that **4** was identical to **11** except for the side chain. The structure of the side chain was determined by an HMBC spectrum, in which long-range C–H correlations were observed from the H_3-21 methyl group to C-20 and C-22; the H_3-26 and H_3-27 methyl groups to C-24 and C-25; the H_3-28 methyl group to C-23, C-24 and C-25; and the H_3-29 methyl group to C-22, C-23 and C-24 confirmed the presence of a 23,24-dimethyl-Δ^{22}-sterol side chain. The stereochemistry at C-22 and C-24 was determined to be *E* and *R*, respectively, by comparison of the ^{1}H-NMR data reported for 5α,8α-epidioxy-(22*E*,24*R*)-23-methylergosta-6,22-dien-3β-ol[4)] and 3β,5α,9α-trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one.[4)] Based on this evidence, the structure of **4** was determined to be (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β-triol. The isolation of sterols with this side chain from terrestrial sources is rare.[4)]

Compound **5** was isolated as an amorphous powder.[6)] The molecular formula was determined to be $C_{29}H_{48}O_4$ by HR-MS [*m/z* 442 (M^+-H_2O)]. The IR spectrum showed the presence of hydroxyl groups (3488 cm^{-1}). The electron ionization (EI)-MS gave fragment ion peaks at *m/z* 388 (M^+-4H_2O), 249 (M^+-4H_2O-side chain) and 207 (M^+-4H_2O and ring D fission), indicating that **5** has four hydroxyl groups and a mono-unsaturated C_{10}-side chain. The ^{1}H-NMR spectrum of **5** was similar to that of **9** except for the side chain. The structure of the side-chain was determined to be (22*E*,24*R*)-23,24-dimethyl-Δ^{22}-sterol side chain by comparison of the ^{1}H-NMR data of compound **4**. From the above

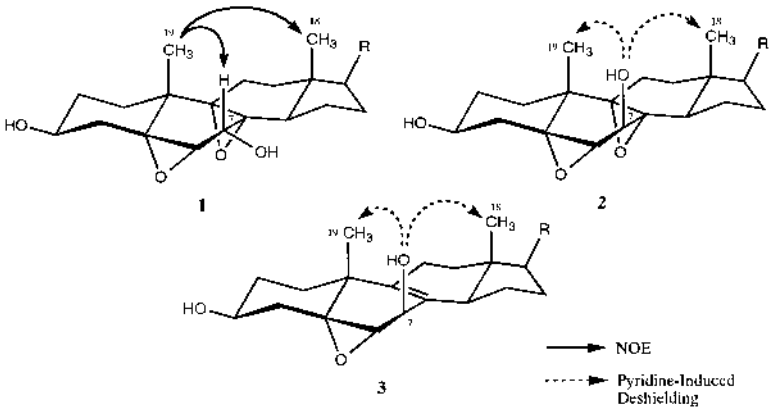

Fig. 2. NOEs and Pyridine-Induced Deshieldings for **1**—**3**

data, the structure of **5** was determined to be (22*E*,24*R*)-23-methylergosta-7,22-diene-3β,5α,6β,9α-tetrol.

Compound **6** was isolated as an amorphous powder.[6] The molecular formula was determined to be $C_{28}H_{48}O_4$ by HR-MS [*m/z* 430 (M^+-H_2O)]. The IR spectrum showed the presence of hydroxyl groups (3464 cm^{-1}). The EI-MS gave fragment ion peaks at *m/z* 376 (M^+-4H_2O), 249 (M^+-4H_2O-side chain) and 207 (M^+-4H_2O and ring D fission), indicating that **6** has four hydroxyl groups and a conventional saturated steroidal C_9-side chain. The ^{1}H-NMR spectrum of **6** was virtually identical to that of **5** except the side chain. The structure of the side chain was determined to be (24*S*)-24-methylsterol side chain by comparison of the ^{1}H-NMR data of compounds **12**, **18** and **21**. From the above data, the structure of **6** was determined to be (24*S*)-ergost-7-ene-3β,5α,6β,9α-tetrol.

Experimental

General Procedures Optical rotations were determined with JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; dd, double doublet; br, broad; m, multiplet). The EI- and HR-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, RI-8010) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh).

Material The fresh fruit bodies of *Amanita pantherina* were collected at Sendai City in Miyagi Prefecture, Japan, in September 1997. The fresh fruit bodies of *Amanita virgineoides* were collected at Sendai City in Miyagi Prefecture, Japan, in July 1997. The fresh fruit bodies of *Lactarius piperatus* were collected at Sendai City in Miyagi Prefecture, Japan, in July 1997. *Lyophyllum shimeji* (from Morioka City in Iwate Prefecture, Japan, in October, 1997), *Tricholoma portentosum* (from Morioka City in Iwate Prefecture, Japan, in October, 1997), *Hypsizigus marmoreus* (from Nagano City in Nagano Prefecture, Japan) and *Lentinula edodes* (from Sendai City in Miyagi Prefecture, Japan) were purchased in a food market.

Extraction and Isolation 1) *A. pantherina*: The fresh fruit bodies of *A. pantherina* (0.6 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (7.3 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 21 fractions (frs. 1—21). Fraction 4 was purified by preparative HPLC [mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C] to give **23** (0.3 mg) and **24** (0.2 mg). Fraction 6 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **20** (0.8 mg), **21** (0.2 mg) and **22** (0.2 mg). Fraction 14 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give a mixture of **10**, **13** and **14**, **11** (0.5 mg) and **12** (0.6 mg). The mixture of **10**, **13** and **14** was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1); flow rate, 1.5 ml/min; column temperature, 40 °C] to give **10** (0.1 mg) and inseparable mixture of **13** and **14** (0.2 mg). Fraction 16 was purified by preparative HPLC [mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C] to give a mixture of **1** and **9**. The mixture of **1** and **9** was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.6 mg) and **9** (0.6 mg).

2) *A. virgineoides*: The fresh fruit bodies of *A. virgineoides* (0.4 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.5 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 22 fractions (frs. 1—22). Fraction 12 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **17** (0.4 mg). Fraction 18 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **10** (0.4 mg), **11** (7.2 mg) and **12** (0.8 mg). Fraction 20 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.5 mg), **6** (0.1 mg), **9** (1.7 mg) and **15** (0.5 mg).

3) *L. piperatus*: The fresh fruit bodies of *L. piperatus* (0.1 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.7 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 24 fractions (frs. 1—24). Fraction 12 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **16** (0.9 mg). Fraction 14 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **17** (0.2 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.8 mg), **2** (0.4 mg), **9** (0.4 mg), **10** (0.5 mg) and **11** (1.3 mg).

4) *L. shimeji*: The fresh fruit bodies of *L. shimeji* (0.6 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (2.3 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 26 fractions (frs. 1—26). Fraction 15 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **17** (2.2 mg), **18** (0.3 mg) and **19** (0.5 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.3 mg) and **9** (0.4 mg).

5) *T. portentosum*: The fresh fruit bodies of *T. portentosum* (0.5 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (2.7 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 28 fractions (frs. 1—28). Fraction 7 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **20** (1.0 mg). Fraction 13 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **8** (0.4 mg), **17** (1.4 mg) and **19** (0.5 mg). Fraction 16 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **3** (0.5 mg) and **7** (1.2 mg). Fraction 19 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.4 mg), **9** (1.7 mg),

10 (0.3 mg), **11** (1.9 mg) and **12** (0.2 mg).

6) *H. marmoreus*: The fresh fruit bodies of *H. marmoreus* (4.3 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (10.5 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 19 fractions (frs. 1—19). Fraction 13 was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1); flow rate, 1.5 ml/min; column temperature, 40 °C] to give **1** (0.6 mg) and **2** (0.6 mg).

7) *L. edodes*: The fresh fruit bodies of *L. edodes* (4.7 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (7.1 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 25 fractions (frs. 1—25). Fraction 17 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **4** (0.6 mg) and **12** (0.2 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1); flow rate, 1.5 ml/min; column temperature, 40 °C] to give **1** (0.3 mg) and **5** (0.2 mg).

All known compounds (**7**—**24**) were identified by comparison of its chromatographic behaviour, and MS and ^{1}H-NMR data with those of the authentic samples.

5α,6α;8α,9α-Diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7α-diol (**1**): Amorphous powder. $[\alpha]_D^{20}$ −53.6° (*c*=0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3420. HR-MS *m/z*: 444.3217 (M^+, Calcd for $C_{28}H_{44}O_4$; 444.3240). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 12.4 (C-18), 17.7 (C-28), 19.6 (C-26), 20.0 (C-27), 21.2 (C-21), 21.7 (C-19), 22.2 (C-11), 23.2 (C-15), 27.9 (C-1), 28.0 (C-16), 30.3 (C-2), 32.5 (C-12), 33.1 (C-25), 35.4 (C-10), 40.3 (C-20), 40.6 (C-4, C-13), 42.8 (C-24), 52.3 (C-17), 52.9 (C-14), 60.7 (C-6), 65.0 (C-8), 66.4 (C-7), 67.1 (C-5), 68.4 (C-3), 70.2 (C-9), 132.3 (C-23), 135.2 (C-22).

5α,6α;8α,9α-Diepoxy-(22*E*,24*R*)-ergost-22-ene-3β,7β-diol (**2**): Amorphous powder. $[\alpha]_D^{20}$ −17.2° (*c*=0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3396. HR-MS *m/z*: 444.3261 (M^+, Calcd for $C_{28}H_{44}O_4$; 444.3240). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. (600 MHz, C_5D_5N) δ: 0.84 (6H, d, *J*= 7.0 Hz, H_3-26, 27), 0.93 (3H, d, *J*=7.0 Hz, H_3-28), 0.97 (3H, s, H_3-18), 1.05 (3H, d, *J*=6.6 Hz, H_3-21), 1.68 (3H, s, H_3-19), 2.61 (1H, d, *J*=12.8, 11.4 Hz, H-4β), 3.28 (1H, d, *J*=2.9 Hz, H-6), 4.42 (1H, m, H-3), 4.79 (1H, dd, *J*=6.2, 2.9 Hz, H-7), 5.22 (2H, m, H-22, 23). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 12.3 (C-18), 17.6 (C-28), 19.6 (C-26), 20.0 (C-27), 21.1 (C-21), 21.3 (C-11, C-19), 21.6 (C-15), 28.5 (C-1, C-16), 30.3 (C-2), 33.1 (C-25), 33.3 (C-12), 35.7 (C-10), 40.4 (C-13, C-20), 40.5 (C-4), 42.8 (C-24), 53.0 (C-17), 54.5 (C-14), 59.3 (C-6), 61.3 (C-8), 62.5 (C-5), 65.6 (C-7), 68.4 (C-3), 66.9 (C-9), 132.3 (C-23), 135.3 (C-22).

5α,6α-Epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**3**): Amorphous powder. $[\alpha]_D^{26}$ −37.0° (*c*=0.05, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3414. HR-MS *m/z*: 428.3315 (M^+, Calcd for $C_{28}H_{44}O_3$; 428.3290). ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1. (400 MHz, C_5D_5N) δ: 0.85 (3H, s, H_3-18), 0.85 (6H, d, *J*=6.8 Hz, H_3-26, 27), 0.94 (3H, d, *J*=6.9 Hz, H_3-28), 1.08 (3H, d, *J*=6.8 Hz, H_3-21), 1.55 (3H, s, H_3-19), 2.60 (1H, d, *J*=12.5, 11.5 Hz, H-4β), 3.46 (1H, d, *J*=2.8 Hz, H-6), 4.38 (1H, m, H-3), 4.74 (1H, m, H-7), 5.23 (2H, m, H-22, 23). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 11.5 (C-18), 17.6 (C-28), 19.6 (C-26), 19.9 (C-27), 21.0 (C-21), 22.9 (C-19), 23.0 (C-11), 23.6 (C-15), 29.2 (C-16), 29.7 (C-2), 30.8 (C-1), 33.1 (C-25), 36.2 (C-12), 37.9 (C-10), 39.0 (C-4), 40.5 (C-20), 41.9 (C-13), 42.8 (C-24), 51.1 (C-14), 54.3 (C-17), 60.1 (C-6), 63.2 (C-5), 67.0 (C-7), 68.6 (C-3), 126.5 (C-8), 132.2 (C-23), 135.5 (C-22), 137.0 (C-9).

(22*E*,24*R*)-23-Methylergosta-7,22-diene-3β,5α,6β-triol (**4**): Amorphous powder. $[\alpha]_D^{23}$ −15.9° (*c*=0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3454. HR-MS *m/z*: 426.3470 (M^+-H_2O, Calcd for $C_{29}H_{46}O_2$; 426.3498). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 12.4 (C-18), 13.2 (C-29), 17.0 (C-28), 18.9 (C-19), 20.1 (C-21), 20.8 (C-26), 21.7 (C-27), 22.1 (C-15), 22.9 (C-11), 27.3 (C-16), 30.7 (C-2), 30.9 (C-25), 33.0 (C-1), 34.8 (C-20), 37.2 (C-10), 39.3 (C-4), 39.5 (C-12), 43.5 (C-13), 43.7 (C-9), 50.2 (C-24), 54.7 (C-14), 56.8 (C-17), 67.7 (C-3), 73.7 (C-6), 76.0 (C-5), 131.2 (C-22), 135.7 (C-23), 144.1 (C-8), 117.5 (C-7).

(22*E*,24*R*)-23-Methylergosta-7,22-diene-3β,5α,6β,9α-tetrol (**5**): Amorphous powder. IR ν_{max} ($CHCl_3$) cm^{-1}: 3488. HR-MS *m/z*: 442.3422 (M^+-H_2O, Calcd for $C_{29}H_{46}O_3$; 442.3447). EI-MS *m/z*: 442 (M^+-H_2O), 424 (M^+-2H_2O), 406 (M^+-3H_2O), 388 (M^+-4H_2O), 249 (M^+-4H_2O-side chain), 207 (M^+-4H_2O and ring D fission). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1.

(24*S*)-Ergost-7-ene-3β,5α,6β,9α-tetrol (**6**): Amorphous powder. IR ν_{max} ($CHCl_3$) cm^{-1}: 3464. HR-MS *m/z*: 430.3420 (M^+-H_2O, Calcd for $C_{28}H_{46}O_3$; 430.3447). EI-MS *m/z*: 430 (M^+-H_2O), 412 (M^+-2H_2O), 394 (M^+-3H_2O), 376 (M^+-4H_2O), 249 (M^+-4H_2O-side chain), 207 (M^+-4H_2O and ring D fission). ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1.

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References and Notes

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6) The size of the available sample did not allow us to obtain the optical rotation and ^{13}C-NMR spectrum.