Sterol Constituents from the Fruit Bodies of *Grifola frondosa* (FR.) S. F. GRAY

Takaaki ISHIZUKA, Yasunori YAOITA, and Masao KIKUCHI*

Tohoku College of Pharmacy, 4–4–1 Komatsushima, Aoba-ku, Sendai 981, Japan.
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Four new sterols, 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7β-diol (1), (22*E*,24*R*)-ergosta-8,22-diene-3β,5α,6β,7α-tetrol (2), (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6β-triol (3) and 3β,5α,6β-trihydroxy-(22*E*,24*R*)-ergost-22-en-7-one (4), have been isolated from the fruit bodies of *Grifola frondosa* (FR.) S. F. GRAY (Polyporaceae) together with fourteen known ones (5—18), of which two (5 and 6) are reported for the first time from a natural source. The structures of these compounds were elucidated on the basis of spectral data.

Key words *Grifola frondosa*; Polyporaceae; sterol

The fruit bodies of *Grifola* (*G.*) *frondosa* (FR.) S. F. GRAY (Maitake in Japanese, Polyporaceae), an edible mushroom, contain ergosterol,[1] fungisterol,[1] 4α-methyl-8, 24(28)-ergostadienol,[1] lanosterol,[1] lanost-8-en-3β-ol,[1] triacylglycerols,[2] cerebrosides,[3] lectin[4] and an antitumor-active glucan.[5] In a continuation of our investigation of the sterol constituents in this edible mushroom, we describe herein the isolation and structural elucidation of four new sterols, 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7β-diol (**1**), (22*E*,24*R*)-ergosta-8,22-diene-3β,5α,6β,7α-tetrol (**2**), (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6β-triol (**3**) and 3β,5α,6β-trihydroxy-(22*E*,24*R*)-ergost-22-en-7-one (**4**), as well as fourteen known ones, 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**5**), 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**6**), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (**7**),[6] (22*E*, 24*R*)-ergosta-8(14),22-diene-3β,5α,6β,7α-tetrol (**8**),[7] 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (**9**),[6] (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6α-triol (**10**),[8] 3β-hydroxy-(22*E*,24*R*)-ergosta-5,22-dien-7-one (**11**),[9] 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**12**),[10] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α-triol (**13**),[8] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (**14**),[11] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (**15**),[12] 5α,8α-epidioxy-(24*S*)-ergost-6-en-3β-ol (**16**),[13] 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (**17**)[14] and 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,9(11),22-trien-3β-ol (**18**)[13] from the fruit bodies of *G. frondosa*. This paper presents a full account of the isolation and structure elucidation of these constituents.[15]

The fresh fruit bodies of *G. frondosa* were extracted with Et_2O. The Et_2O extract was subjected to silica gel column chromatography and preparative HPLC to furnish eighteen sterols (**1**—**18**). This is the first time that compounds **5** and **6** have been isolated from a natural source and that compounds **7**—**18** have been isolated from the fruit bodies of *G. frondosa*.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ −52.4°. The molecular formula was determined to be $C_{28}H_{44}O_3$ by high-resolution (HR)-MS. The IR spectrum showed a hydroxyl absorption (3611 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.90 (3H, H_3-18), 1.02 (3H, H_3-19)], a 24-methyl-Δ^{22}-sterol side chain [δ_H 0.82 (3H,

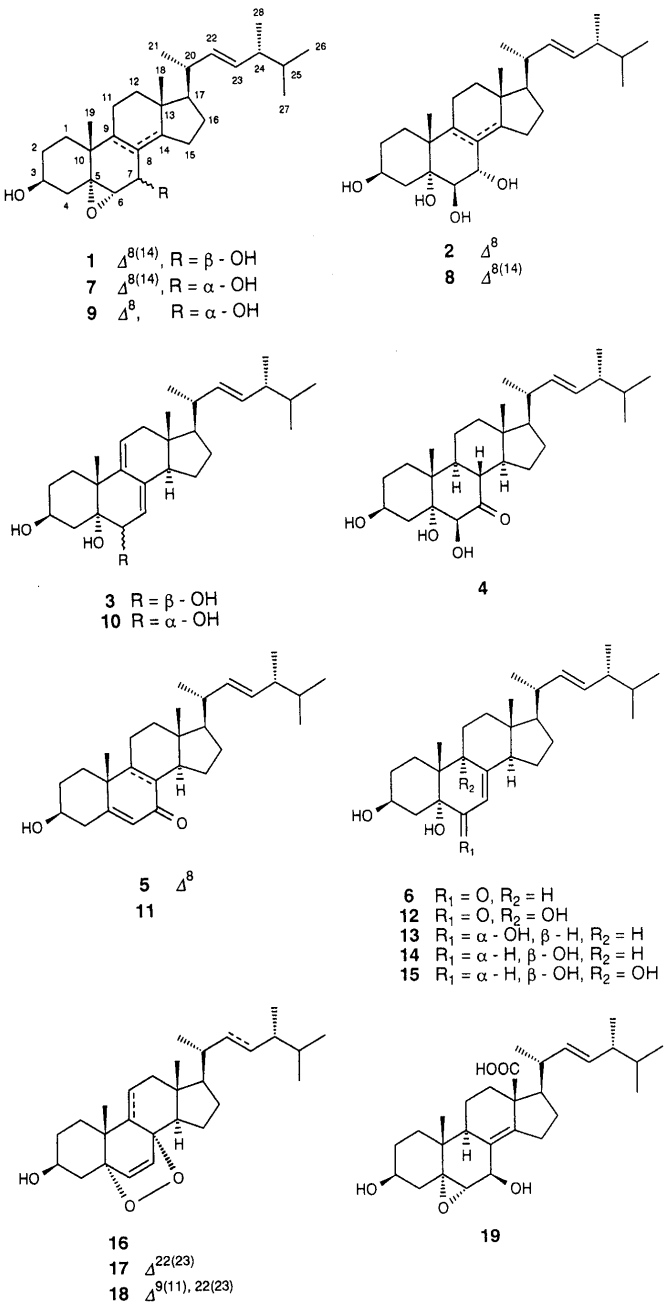

Chart 1

* To whom correspondence should be addressed.

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

Proton	1	2	3	4	5[a]	6[b]
3	3.89 m	4.16 m	4.12 m	4.10 m	3.67 m	4.04 m
4		β 2.03 dd (12.7, 11.1)	β 2.07 dd (12.8, 11.4)	β 2.22 dd (12.7, 12.0)		
6	3.00 d (2.5)	3.75 br s	3.82 br s	3.51 s	6.04 d (1.5)	
7	4.52 br s	3.96 br s	5.45 br d (5.6)			5.66 br s
8				2.93 dd (11.2, 11.1)		
11			5.75 br d (6.3)			
18	0.90 s	0.66 s	0.60 s	0.70 s	0.65 s	0.61 s
19	1.02 s	1.30 s	1.28 s	1.37 s	1.35 s	0.96 s
21	1.02 d (6.6)	1.04 d (6.8)	1.02 d (6.6)	1.01 d (6.6)	1.05 d (7.0)	1.04 d (6.6)
22	5.20 m	5.16 dd (15.2, 7.8)	5.16 dd (15.2, 7.8)	5.19 m	5.19 dd (15.4, 6.2)	5.15 dd (15.2, 7.6)
23	5.20 m	5.24 dd (15.2, 5.9)	5.26 dd (15.2, 7.1)	5.19 m	5.24 dd (15.4, 7.0)	5.25 dd (15.2, 7.1)
26	0.82 d (6.8)	0.82 d (6.6)	0.83 d (6.8)	0.82 d (6.8)	0.83 d (6.2)	0.82 d (6.8)
27	0.84 d (6.8)	0.84 d (6.8)	0.84 d (6.6)	0.83 d (6.8)	0.84 d (6.6)	0.84 d (6.8)
28	0.92 d (6.9)	0.92 d (6.8)	0.92 d (6.8)	0.91 d (6.9)	0.92 d (7.0)	0.92 d (6.8)

Coupling constants (J in Hz) are given in parentheses. *a*) Measured at 400 MHz. *b*) Measured at 270 MHz.

H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.02 (3H, H_3-21), 5.20 (2H, H-22 and H-23)], a trisubstituted epoxide bearing a methine proton [δ_H 3.00 (1H, H-6)] and two methine protons on carbons bearing a hydroxyl group [δ_H 3.89 (1H, H-3), 4.52 (1H, H-7)]. The ^{13}C-NMR spectrum contained twenty-eight signals that included a fully substituted double bond [δ_C 126.8 (C-8), 151.6 (C-14)]. Comparison of these data with the spectral data for compound **7** revealed that they were identical except at C-7. In the ^{1}H-NMR spectrum, the chemical shift of the H_3-19 methyl group in pyridine-d_5 was shifted downfield by the pyridine-induced deshielding effect[16] ($\delta_{C_5D_5N} - \delta_{CDCl_3}$; $\Delta\delta$, H_3-19, +0.26 ppm). As the hydroxyl group forms a hydrogen bond with the nitrogen atom of pyridine, pyridine-induced shifts are expected to be observed around it.[16a] This deshielding effect implies that the B-ring of **1** adopts a boat-type conformation and that the hydroxyl group at C-7 has β-configuration (Chart 2). The stereochemistry of the side chain was determined by comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of the (22*E*,24*R*)-methyl-Δ^{22}-sterol side chain.[17] Thus, **1** was the 7-epimer of **7**. Based on this evidence, the structure of **1** was determined to be 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7β-diol. The natural occurrence of 3β,7β-dihydroxy-5α,6α-epoxy-$\Delta^{8(14)}$-sterols is extremely rare, and only one such sterol, *viz.*, ergokonin B (**19**), has so far been reported, in the mycelium of *Trichoderma koningii*.[18] Compound **1** may be a biosynthetic precursor of **19**.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ +50.0°. The molecular formula was determined to be $C_{28}H_{46}O_4$ by HR-MS [m/z 428 ($M^+ - H_2O$)] and ^{13}C-NMR data. The IR spectrum showed hydroxyl absorptions (3612, 3398 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.66 (3H, H_3-18), 1.30 (3H, H_3-19)], a 24-methyl-Δ^{22}-sterol side chain [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.04 (3H, H_3-21), 5.16 (1H, H-22), 5.24 (1H, H-23)], one of the methylene protons [δ_H 2.03 (1H, H-4)] and three methine protons on hydroxyl-bearing carbons [δ_H 3.75 (1H, H-6), 3.96 (1H, H-7), 4.16 (1H, H-3)]. The ^{13}C-NMR spectrum contained twenty-eight signals that included four oxygenated carbons [δ_C 67.0 (C-3), 72.7 (C-7), 75.3 (C-5), 79.3 (C-6)] and a fully substituted double bond [δ_C 127.8 (C-8), 137.6 (C-9)]. The electron ionization (EI)-MS gave fragment ion peaks at m/z 374 ($M^+ - 4H_2O$), 249 ($M^+ - 4H_2O$ − side chain) and 207 ($M^+ - 4H_2O$ and ring D fission), indicating that **2** has four hydroxyl groups and a mono-unsaturated C_9 side chain. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **8** revealed that **2** is the Δ^8 isomer of **8**. The calculated chemical shifts[19] of the H_3-18 and H_3-19 methyl groups of the 3β,5α,6β,7α-tetrahydroxy-Δ^8-steroid are very close to those found in **2**. In the ^{1}H-NMR spectrum of **2**, the chemical shifts of H-3α, H-4β and the H_3-19 methyl group in pyridine-d_5 were shifted downfield by the pyridine-induced deshielding effect,[16] indicating the presence of a 3β,5α,6β-trihydroxyl grouping in the sterol skeleton (Chart 2). The configuration of the hydroxyl group at C-7 was determined to be a by comparing the ^{1}H-NMR data of the C-7 methine proton of **2** with that of 3β,5α,6β,7α-tetrahydroxycholest-8-en-11-one.[20] The stereochemistry of the side chain was determined by comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1**. From the above data, the structure of **2** was determined to be (22*E*,24*R*)-ergosta-8,22-diene-3β,5α,6β,7α-tetrol. The natural occurrence of 3β,5α,6β,7α-tetrahydroxy-Δ^8-sterols is also rare, and only one such sterol, *viz.*, (24*S*)-24-ethylcholest-8-ene-3β,5α,6β,7α-tetrol, has so far been reported, in the marine sponge *Neofibularia*

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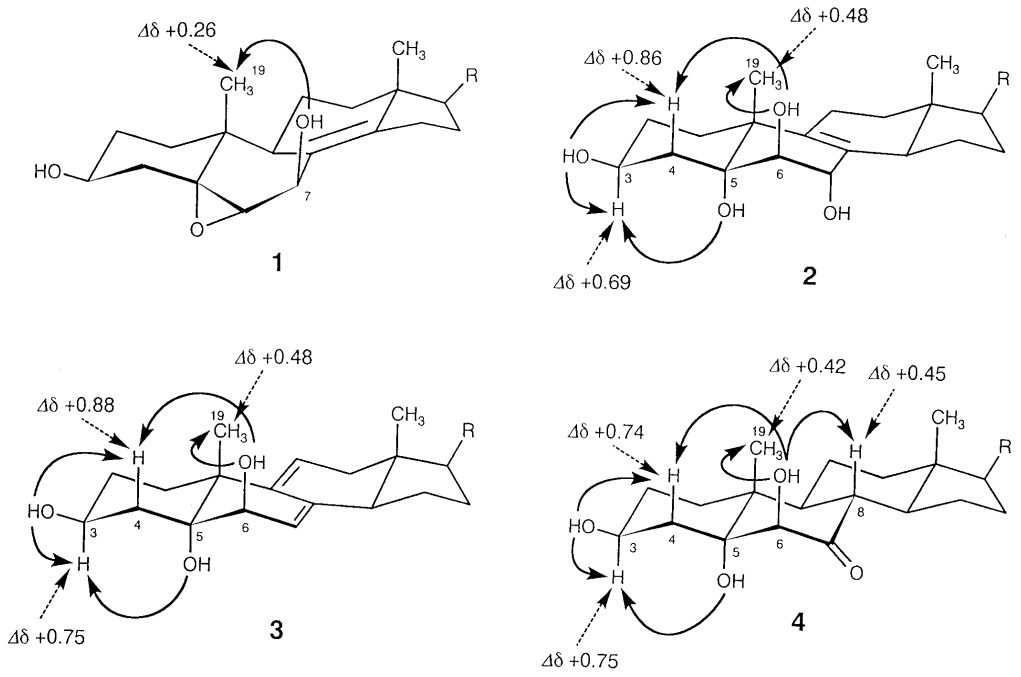

Chart 2. Pyridine-Induced Deshieldings (⟶) in **1**—**4**

nolitangere.[21]

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −3.7°. The molecular formula was determined to be $C_{28}H_{44}O_3$ by HR-MS. The IR spectrum showed hydroxyl absorptions (3612, 3414 cm^{-1}). The UV spectrum suggested the presence of a conjugated diene system (λ_{max} = 244 nm). The ^{1}H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.60 (3H, H_3-18), 1.28 (3H, H_3-19)], a 24-methyl-Δ^{22}-sterol side chain [δ_H 0.83 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.02 (3H, H_3-21), 5.16 (1H, H-22), 5.26 (1H, H-23)], one of the methylene protons [δ_H 2.07 (1H, H-4)], two methine protons on hydroxy-bearing carbons [δ_H 3.82 (1H, H-6), 4.12 (1H, H-3)] and two olefinic protons [δ_H 5.45 (1H, H-7), 5.75 (1H, H-11)]. The EI-MS gave fragment ion peaks at *m/z* 374 ($M^+ - 3H_2O$), 249 ($M^+ - 3H_2O$ − side chain) and 207 ($M^+ - 3H_2O$ − ring D fission), indicating that **3** has three hydroxyl groups, one conjugated double bond and a mono-unsaturated C_9 side chain. Comparison of these data with those for compound **10** revealed that they were identical except at C-6. The 3β,5α,6β-trihydroxy system was determined by the pyridine-induced deshielding effect (Chart 2).[16] The stereochemistry of the side chain was determined by comparison of the ^{1}H- and ^{13}C-NMR signals of **3** with those of **1** and **2**. Thus, **3** is the 6-epimer of **10**. From the above data, the structure of **3** was determined to be (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6β-triol.

Compound **4** was isolated as an amorphous powder, $[\alpha]_D$ −54.1°. The molecular formula was determined to be $C_{28}H_{46}O_4$ by HR-MS. The IR spectrum showed hydroxyl absorptions (3614, 3410 cm^{-1}) and a six-membered ring ketone (1708 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.70 (3H, H_3-18), 1.37 (3H, H_3-19)], a 24-methyl-Δ^{22}-sterol side chain [δ_H 0.82 (3H, H_3-26), 0.83 (3H, H_3-27), 0.91 (3H, H_3-28), 1.01 (3H, H_3-21), 5.19 (2H, H-22 and H-23)], one of the methylene protons [δ_H 2.22 (1H, H-4)], a methine proton [δ_H 2.93 (1H, H-8)] and two methine protons on hydroxyl-bearing carbons [δ_H 3.51 (1H, H-6), 4.10 (1H, H-3)]. The presence of the 7-keto function led to the appearance of the H-8β signal further downfield at δ 2.93 in the ^{1}H-NMR spectrum.[22] The EI-MS gave fragment ion peaks at *m/z* 392 ($M^+ - 3H_2O$), 267 ($M^+ - 3H_2O$ − side chain) and 225 ($M^+ - 3H_2O$ and ring D fission), indicating that **4** has three hydroxyl groups and a mono-unsaturated C_9 side chain. The 3β,5α,6β-trihydroxy system was determined by the pyridine-induced deshielding effect (Chart 2).[16] The stereochemistry of the side chain was determined by comparison of the ^{1}H-NMR spectrum of **4** with that of (22*E*,24*R*)-methyl-Δ^{22}-sterol side chain[17a] and **1**, **2** and **3**. Based on this evidence, the structure of **4** was determined to be 3β,5α,6β-trihydroxy-(22*E*,24*R*)-ergost-22-en-7-one. Compounds **3** and **4** are the first examples of a naturally occurring 3β,5α,6β-trihydroxy-$\Delta^{7,9(11)}$-sterol and a 3β,5α,6β-trihydroxy-7-keto sterol, respectively.

Compound **5** was isolated as an amorphous powder, $[\alpha]_D$ −28.3°. The molecular formula was determined to be $C_{28}H_{42}O_2$ by HR-MS. The IR spectrum showed hydroxyl (3457 cm^{-1}) and cross-conjugated dienone (1662, 1626, 1593 cm^{-1}) absorptions. The UV spectrum also suggested the presence of a cross-conjugated dienone system (λ_{max} = 246 nm).[23] The ^{1}H- (Table 1) and ^{13}C-NMR spectra of **5**, obtained with the aid of ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), as well as the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectra, indicated the compound to be 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one. Compound **5** has been synthesized by Burke *et al.*,[24] but its isolation from natural sources has not previously been reported.

Compound **6** was isolated as an amorphous solid, $[\alpha]_D$

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+9.1°. The molecular formula was determined to be $C_{28}H_{44}O_3$ by HR-MS. The IR spectrum showed hydroxyl (3457 cm^{-1}) and α,β-unsaturated ketone (1667 cm^{-1}) absorptions. The UV spectrum also suggested the presence of α,β-unsaturated ketone (λ_{max} = 244 nm). The ^{1}H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.61 (3H, H_3-18), 0.96 (3H, H_3-19)], a 24-methyl-Δ^{22}-sterol side chain [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.04 (3H, H_3-21), 5.15 (1H, H-22), 5.25 (1H, H-23)], a methine proton on a hydroxyl-bearing carbon [δ_H 4.04 (1H, H-3)] and a trisubstituted olefinic proton [δ_H 5.66 (1H, H-7)]. These spectral data indicated the compound to be 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one. The structure of **6** was confirmed by chemical transformation of ergosterol acetate into **6** according to a literature method.[25] Treatment of ergosterol acetate with $Na_2Cr_2O_7$ afforded 3β-acetoxy-5α-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**6′**). Deprotection of the 3-acetoxyl group of **6′** furnished **6**. This is the first isolation of **6** from a natural source, although **6** has already been synthesized by Valisolalao *et al.*[12a)]

Experimental

General Procedures Optical rotations were determined with a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL LA 600 (600 and 150 MHz, respectively), JEOL JNM-GSX 400 (400 and 100 MHz, respectively), and JEOL JNM-EX 270 (270 and 67.8 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; br s, broad singlet; d, doublet; br d, broad doublet; dd, double doublet; m, multiplet). The EI-MS 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, CCPS; detector, RI-8020) using a TSK gel ODS-120T (10 μm, 7.8 mm i.d. × 30 cm) column (Tosoh). HPLC conditions: mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min.

Extraction and Isolation The fresh fruit bodies of *G. frondosa* used for this study were purchased from Mogamimachi Maitake Seisan Kumiai (Yamagata, Japan) in 1996. The fresh fruit bodies of *G. frondosa* (20 kg) were extracted three times with Et_2O at room tempertaure for 10 d. The Et_2O extract (21 g) was chromatographed on a silica-gel column using *n*-hexane–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 9 fractions (frs. 1—9). Fraction 2 was purified by preparative HPLC to give **16** (0.5 mg), **17** (20.0 mg) and **18** (2.0 mg). Fraction 3 was purified by preparative HPLC to give **11** (0.4 mg). Fraction 4 was purified by preparative HPLC to give **5** (1.1 mg). Fraction 5 was purified by preparative HPLC to give **1** (1.9 mg) and **9** (15.0 mg). Fraction 6 was **7** (1.4 mg). Fraction 7 was purified by preparative HPLC to give **3** (2.7 mg), **4** (1.9 mg), **10** (1.5 mg) and **13** (1.0 mg). Fraction 8 was purified by preparative HPLC to give **6** (1.1 mg), **12** (4.3 mg), **14** (8.2 mg) and **15** (4.7 mg). Fraction 9 was purified by preparative HPLC to give **2** (1.0 mg) and **8** (2.4 mg). All known compounds (**7**—**18**) were identified by comparison of their physical data with reported values (**7**—**10**, **12**—**18**) or by direct comparison with an authentic sample (**11**).

5α,6α-Epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7β-diol (1) Amorphous powder. $[\alpha]_D^{18}$ −52.4° (*c* = 0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3611. HR-MS *m/z*: 428.3276 (M^+, Calcd for $C_{28}H_{44}O_3$; 428.3291). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. (600 MHz, C_5D_5N) δ: 0.85 (6H, d, *J* = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, d, *J* = 6.9 Hz, H_3-28), 0.96 (3H, s, H_3-18), 1.07 (3H, d, *J* = 6.6 Hz, H_3-21), 1.28 (3H, s, H_3-19), 2.55 (1H, dd, *J* = 13.2, 11.7 Hz, H-4β), 3.37 (1H, d, *J* = 2.5 Hz, H-6β), 4.31 (1H, m, H-3α), 4.88 (1H, br d, *J* = 6.6 Hz, H-7α), 5.27 (2H, m, H-22, H-23). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 16.9 (C-19), 17.7 (C-18, C-28), 19.3 (C-11), 19.7 (C-26), 20.0 (C-27), 21.1 (C-21), 25.9 (C-15), 28.0 (C-16), 31.2 (C-2), 33.0 (C-1), 33.1 (C-25), 34.9 (C-10), 37.1 (C-12), 39.3 (C-9), 39.6 (C-4), 40.5 (C-20), 42.9 (C-24), 43.7 (C-13), 55.4 (C-17), 60.3 (C-6), 63.6 (C-7), 64.9 (C-5), 68.7 (C-3), 126.8 (C-8), 132.4 (C-23), 135.2 (C-22), 151.6 (C-14).

(22*E*,24*R*)-Ergosta-8,22-diene-3β,5α,6β,7α-tetrol (2) Amorphous powder. $[\alpha]_D^{20}$ +50.0° (*c* = 0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3612, 3398. HR-MS *m/z*: 428.3284 ($M^+ - H_2O$, Calcd for $C_{28}H_{44}O_3$; 428.3291). EI-MS *m/z*: 374 ($M^+ - 4H_2O$), 249 ($M^+ - 4H_2O$ − side chain), 207 ($M^+ - 4H_2O$ and ring D fission). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. (600 MHz, C_5D_5N) δ: 0.83 (6H, d, *J* = 5.4 Hz, H_3-26, H_3-27), 0.84 (3H, s, H_3-18), 0.92 (3H, d, *J* = 6.9 Hz, H_3-28), 1.08 (3H, d, *J* = 6.6 Hz, H_3-21), 1.78 (3H, s, H_3-19), 2.73 (1H, *J* = 13.2, 7.3 Hz, H-14α), 2.89 (1H, dd, *J* = 12.0, 11.7 Hz, H-4β), 4.46 (1H, br s, H-6α), 4.60 (1H, br s, H-7β), 4.85 (1H, m, H-3α). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 11.3 (C-18), 17.7 (C-28), 19.6 (C-26), 20.0 (C-27), 21.0 (C-21), 23.1 (C-19), 23.4 (C-11), 23.7 (C-15), 28.9 (C-16), 31.9 (C-2), 33.1 (C-1, C-25), 36.1 (C-12), 40.5 (C-4, C-20), 40.7 (C-10), 42.0 (C-13), 42.8 (C-24), 49.4 (C-14), 54.4 (C-17), 67.0 (C-3), 72.7 (C-7), 75.3 (C-5), 79.3 (C-6), 127.8 (C-8), 132.1 (C-23), 135.5 (C-22), 137.6 (C-9).

(22*E*,24*R*)-Ergosta-7,9(11),22-triene-3β,5α,6β-triol (3) Amorphous powder. $[\alpha]_D^{20}$ −3.7° (*c* = 0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3612, 3414. UV λ_{max}^{MeOH} nm (log ε): 244 (4.0). HR-MS *m/z*: 428.3317 (M^+, Calcd for $C_{28}H_{44}O_3$; 428.3290). EI-MS *m/z*: 428 (M^+), 374 ($M^+ - 3H_2O$), 249 ($M^+ - 3H_2O$ − side chain), 207 ($M^+ - 3H_2O$ and ring D fission). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. (600 MHz, C_5D_5N) δ: 0.71 (3H, s, H_3-18), 0.83 (3H, d, *J* = 6.8 Hz, H_3-26), 0.84 (3H, d, *J* = 6.8 Hz, H_3-27), 0.93 (3H, d, *J* = 6.8 Hz, H_3-28), 1.02 (3H, d, *J* = 6.6 Hz, H_3-21), 1.76 (3H, s, H_3-19), 2.95 (1H, dd, *J* = 12.7, 11.5 Hz, H-4β), 4.46 (1H, br s, H-6α), 4.87 (1H, m, H-3α), 5.14 (1H, dd, *J* = 15.2, 7.8 Hz, H-22), 5.24 (1H, dd, *J* = 15.2, 7.1 Hz, H-23), 5.79 (1H, br s, H-7 or H-11), 6.06 (1H, br s, H-7 or H-11). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 11.5 (C-18), 17.6 (C-28), 19.6 (C-26), 19.9 (C-27), 20.7 (C-21), 23.1 (C-15), 26.3 (C-19), 28.7 (C-16), 29.7 (C-1), 30.8 (C-2), 33.1 (C-25), 40.3 (C-20), 40.7 (C-10), 42.2 (C-4), 42.5 (C-12, C-13), 42.8 (C-24), 51.4 (C-14), 56.0 (C-17), 67.7 (C-3), 74.0 (C-6), 75.3 (C-5), 118.2 (C-7), 126.4 (C-11), 132.3 (C-23), 135.2 (C-22), 138.9 (C-8), 140.0 (C-9).

3β,5α,6β-Trihydroxy-(22*E*,24*R*)-ergost-22-en-7-one (4) Amorphous powder. $[\alpha]_D^{20}$ −54.1° (*c* = 0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3614, 3410, 1708. HR-MS *m/z*: 446.3378 (M^+, Calcd for $C_{28}H_{46}O_4$; 446.3396). EI-MS *m/z*: 446 (M^+), 392 ($M^+ - 3H_2O$), 267 ($M^+ - 3H_2O$ − side chain), 225 ($M^+ - 3H_2O$ and ring D fission). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. (600 MHz, C_5D_5N) δ: 0.74 (3H, s, H_3-18), 0.83 (3H, d, *J* = 6.6 Hz, H_3-26), 0.84 (3H, d, *J* = 6.6 Hz, H_3-27), 0.92 (3H, d, *J* = 6.9 Hz, H_3-28), 1.05 (3H, d, *J* = 6.6 Hz, H_3-21), 1.79 (3H, s, H_3-19), 2.96 (1H, dd, *J* = 13.2, 11.4 Hz, H-4β), 3.38 (1H, dd, *J* = 11.4, 11.2 Hz, H-8β), 4.31 (1H, d, *J* = 4.3 Hz, H-6α), 4.85 (1H, m, H-3α), 7.85 (1H, d, *J* = 4.3 Hz, 6-OH).

3β-Hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (5) Amorphous powder. $[\alpha]_D^{24}$ −28.3° (*c* = 0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3457, 1662, 1626, 1593. UV λ_{max}^{MeOH} nm (log ε): 246 (4.0). HR-MS *m/z*: 410.3158 (M^+, Calcd for $C_{28}H_{42}O_2$; 410.3185). ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 12.0 (C-18), 17.7 (C-28), 19.7 (C-26), 20.0 (C-27), 21.1 (C-21), 23.7 (C-19), 24.6 (C-11), 24.7 (C-15), 29.5 (C-16), 30.7 (C-2), 33.1 (C-25), 34.7 (C-1), 35.6 (C-12), 40.3 (C-20), 41.8 (C-4), 41.9 (C-10), 42.3 (C-13), 42.9 (C-24), 48.4 (C-14), 53.4 (C-17), 71.9 (C-3), 126.8 (C-6), 132.1 (C-23), 134.0 (C-8), 135.5 (C-22), 160.9 (C-9), 161.5 (C-5), 186.3 (C-7).

3β,5α-Dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (6) Amorphous solid. $[\alpha]_D^{25}$ +9.1° (*c* = 0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3457, 1667. UV λ_{max}^{MeOH} nm (log ε): 248 (3.9). HR-MS *m/z* : 428.3307 (M^+, Calcd for $C_{28}H_{44}O_3$; 428.3290). ^{1}H-NMR (270 MHz, $CDCl_3$): Table 1.

Oxidation of Ergosterol Acetate by $Na_2Cr_2O_7$ A solution of $Na_2Cr_2O_7 \cdot 2H_2O$ (28 mg) in acetic acid (2 ml) was added to a solution of ergosterol acetate (93.5 mg) in benzene (5 ml) at 0 °C. The reaction mixture was stirred for 22 h at room temperature, water was added, and the mixture was extracted with $CHCl_3$. After work-up, the product was purified by preparative HPLC (column, TSK gel ODS-120T 7.8 mm i.d. × 30 cm; mobile phase, MeOH; column temperature, 40 °C; flow rate, 1.8 ml/min; RI detector) to give 3β-acetoxy-5α-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**6′**) (13.5 mg). Amorphous powder. $[\alpha]_D^{25}$ −2.92° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3585, 3442, 1719, 1670, 1619. UV λ_{max}^{MeOH} nm (log ε): 249 (4.2). HR-MS *m/z*: 470.3414 (M^+, Calcd for $C_{30}H_{46}O_4$; 470.3396). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.60 (3H, s, H_3-18), 0.82 (3H, d, *J* = 6.8 Hz, H_3-26), 0.84 (3H, d, *J* = 6.6 Hz, H_3-27), 0.92 (3H, d, *J* = 6.8 Hz, H_3-28), 0.96 (3H, s, H_3-19), 1.03 (3H, d, *J* = 6.6 Hz, H_3-21), 2.03 (3H, s, 3-OAc), 5.10 (1H, m, H-3α), 5.21 (2H, m, H-22,

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H-23), 5.65 (1H, dd, $J=2.1$, 2.1 Hz, H-7).

Deprotection of 6′ A solution of **6′** (10 mg) in MeOH (2 ml) was treated with Na_2CO_3 (15 mg), and the mixture was stirred for 6 h at room temperature. Water was added and the mixture was extracted with $CHCl_3$. After work-up, the product was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (10 : 1); column temperature, 40 °C; flow rate, 1.5 ml/min; UV detector, 248 nm) to give **6** (4.5 mg).

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