Sterol Constituents from Five Edible Mushrooms[1)]

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Eight new sterols, 5α,8α-epidioxy-(22*E*,24*R*)-23-methylergosta-6,22-dien-3β-ol (1), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one (2), 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (3), 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (4), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (5), 5α,9α-epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (6), 5α,9α-epidioxy-3β-hydroxy-(24*S*)-ergost-7-en-6-one (7) and 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β,14α-triol (8), have been isolated from five edible mushrooms, *Lentinus edodes*, *Flammulina velutipes*, *Hypsizigus marmoreus*, *Pleurotus ostreatus* and *Pholiota nameko* together with fifteen known ones (9—23), of which two (16 and 17) are reported for the first time from a fungal source. The structures of these new compounds were elucidated on the basis of their spectral data.

Key words sterol; *Lentinus edodes*; *Flammulina velutipes*; *Hypsizigus marmoreus*; *Pleurotus ostreatus*; *Pholiota nameko*

Recently we reported the isolation and structural elucidation of 23 sterols from the fruit bodies of *Grifola frondosa* (FR.) S. F. GRAY (Maitake in Japanese).[1,2)] In a continuation of our investigation of the sterol constituents of mushrooms, we describe here the isolation and structural elucidation of eight new sterols, 5α,8α-epidioxy-(22*E*,24*R*)-23-methylergosta-6,22-dien-3β-ol (**1**), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one (**2**), 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (**3**), 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**4**), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (**5**), 5α,9α-epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**6**), 5α,9α-epidioxy-3β-hydroxy-(24*S*)-ergost-7-en-6-one (**7**) and 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β,14α-triol (**8**), as well as fifteen known ones, 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (**9**),[2)] 5α,8α-epidioxy-(24*S*)-ergost-6-en-3β-ol (**10**),[2)] 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**11**),[2)] 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**12**),[2)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (**13**),[2)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α-triol (**14**),[2)] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (**15**),[2)] (24*S*)-ergost-7-ene-3β,5α,6β-triol (**16**),[3b,c)] ergosta-7,24(28)-diene-3β,5α,6β-triol (**17**),[3)] (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6α-triol (**18**),[2)] (22*E*,24*R*)-ergosta-5,7,9(11),22-tetraen-3β-ol (**19**),[1,4)] (24*S*)-ergosta-5,7-dien-3β-ol (**20**),[1,5)] (22*E*,24*R*)-ergosta-5,8,22-trien-3β-ol (**21**),[1,6)] (22*E*,24*R*)-ergosta-7,22-dien-3β-ol (**22**)[1,7)] and (24*S*)-ergost-7-en-3β-ol (**23**)[1,5)] from five edible mushrooms, *Lentinus edodes* (BERK.) SING. (Shiitake in Japanese, compounds **1**, **2**, **3**, **4**, **5**, **8**, **9**, **10**, **11**, **13**, **14**, **15** and **18**), *Flammulina velutipes* (CURT. : FR.) SING. (Enokitake in Japanese, compounds **5**, **9**, **10**, **15**, **16**, **19** and **23**), *Hypsizigus marmoreus* (PECK) BIGELOW (Bunashimeji in Japanese, compounds **3**, **4**, **5**, **6**, **7**, **10**, **11**, **15**, **16**, **17**, **20** and **23**), *Pleurotus ostreatus* (JACQ.: FR.) KUMMER (Hiratake in Japanese, compounds **3**, **4**, **5**, **6**, **11**, **12**, **15** and **16**) and *Pholiota nameko* (T. ITO) S. ITO et IMAI in IMAI. (Nameko in Japanese, compounds **3**, **4**, **5**, **11**, **16**, **19**, **20**, **21**, **22** and **23**). Extraction and isolation were carried out as described in the Experimental section. Although the sterol composition of the above five edible mushrooms has been reported,[8)] this is the first time that compounds **16** and **17** have been isolated from a fungal source and compounds **9**—**15**, **18**—**23** have been isolated from these edible mushrooms.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ −37.0°. The molecular formula was determined to be $C_{29}H_{46}O_3$ from high-resolution (HR)-MS and ^{13}C-NMR data. The IR spectrum showed a hydroxyl absorption (3588 cm^{-1}). The ^1H-NMR spectrum (Table 1) showed signals due to two tertiary methyl groups [δ_H 0.65 (3H, H_3-18), 0.67 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.90 (3H, H_3-27), 0.91 (3H, H_3-26), 0.97 (3H, H_3-28), 1.01 (3H, H_3-21)], which suggested an ergostane skeleton.[2)] Other signals in the ^1H-NMR spectrum indicated an olefinic methyl group [δ_H 1.51 (3H, H_3-29)], an oxygenated methine proton [δ_H 3.91 (1H, H-3)], a trisubstituted olefinic proton [δ_H 4.92 (1H, H-22)] and two disubstituted olefinic protons [δ_H 5.95 (1H, H-6), 6.30 (1H, H-7)]. The chemical shift of the oxygenated methine proton at δ 3.91 suggested a hydroxy-bearing methine proton by comparison with the ^1H-NMR data of 3β-hydroxy-5α-oxygenated A/B *trans* sterols.[2)] The ^{13}C-NMR spectrum (Table 2) contained 29 signals that included two oxygenated quaternary carbons [δ_C 78.9 (C-8), 81.7 (C-5)]. The chemical shifts of two oxygenated quaternary carbons at δ 78.9 and 81.7 suggested the presence of a peroxy group.[9)] Inspection of the spectral data revealed that **1** was identical to **9** except for the side-chain. The structure of the side-chain was determined by electron ionization (EI)-MS and long-range C–H correlations from the ^1H-detected heteronuclear multiple bond connectivity (HMBC) spectrum. The fragment ion was observed at *m/z* 303 due to the loss of a $C_{10}H_{19}$ from *m/z* 442 (M^+) and thus suggested a mono-unsaturated C_{10}-side chain. The long-range C–H correlations observed from the H_3-21 methyl group to C-20 and C-22; the H_3-26 and 27 methyl groups to C-24; 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 assigned as *E* and *R*, respectively, by comparison with the ^1H-NMR data reported for

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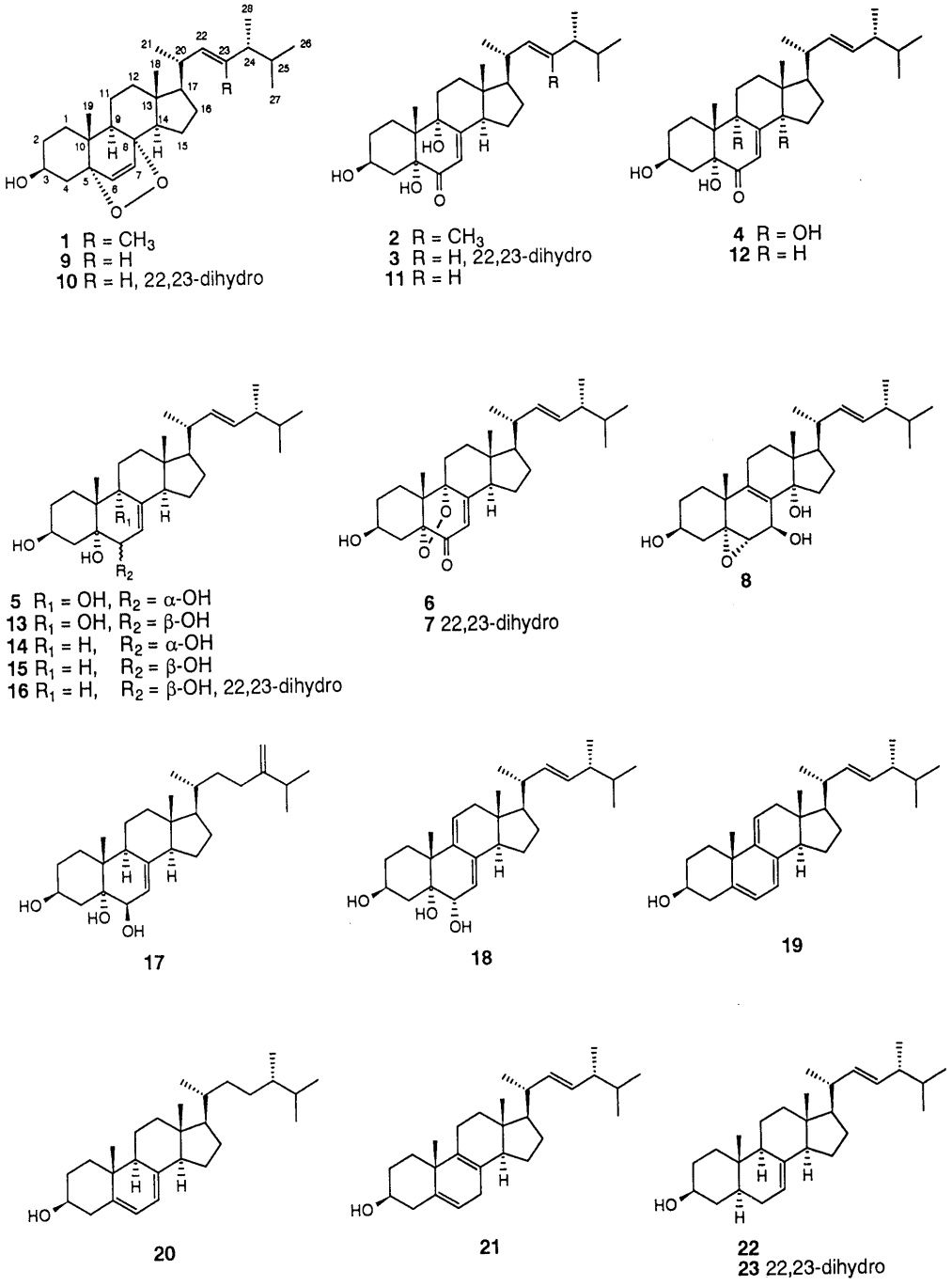

Chart 1

(22*E*,24*R*)-23,24-dimethylcholesta-5,22-dien-3β-ol.[10d] Based on this evidence, the structure of **1** was determined to be 5α,8α-epidioxy-(22*E*,24*R*)-23-methylergosta-6,22-dien-3β-ol. Although a sterol with the (22*E*,24*R*)-23,24-dimethyl-Δ^{22}-side-chain has been detected in marine organisms,[10] this is the first case of the isolation of a sterol with this side-chain in terrestrial sources.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ $-40.0°$. The molecular formula was determined to be $C_{29}H_{46}O_4$ from HR-MS and ^{13}C-NMR data. The IR spectrum showed hydroxyl (3588, 3413 cm^{-1}) and α,β-unsaturated ketone (1676, 1626 cm^{-1}) absorptions. The UV spectrum also suggested the presence of an α,β-unsaturated ketone (λ_{max} = 237 nm). The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.68 (3H, H_3-18), 1.15 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.82 (3H, H_3-27), 0.85 (3H, H_3-26), 0.96 (3H, H_3-28), 1.00 (3H, H_3-21)], which suggested an ergostane skeleton.[2] Other signals in the ^{1}H-NMR spectrum indicated an olefinic methyl group [δ_H 1.51 (3H, H_3-29)], a hydroxy-bearing methine proton [δ_H 4.65 (1H, H-3)], a trisubstituted olefinic proton [δ_H 5.95 (1H, H-7)] and three hydroxyls [δ_H 6.32 (1H, 9-OH), 6.34 (1H, 3-OH), 8.63 (1H, 5-OH)]. The ^{13}C-NMR spectrum contained 29 signals that included three oxygenated carbons [δ_C 66.8 (C-3), 75.1 (C-9), 79.8 (C-5)], a ketone carbonyl [δ_C 199.2 (C-6)] and olefinic signals [δ_C 120.3 (C-7), 131.6 (C-22), 135.6 (C-23), 164.2 (C-8)]. Comparison of these data with the spectral data for compound **11** revealed that they were identical except

Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**—**8** (600 MHz)

Proton	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[c,d]	**7**[c,d]	**8**[c,e]
1		α 2.83 m	α 2.84 m	α 2.70 ddd (14.0, 14.0, 3.8)	α 2.25 ddd (13.6, 13.6, 4.0)			
2					α 1.95 br d (12.1)			
3	3.91 m	4.65 m	4.65 m	4.61 m	4.03 m	3.98 m	3.98 m	3.93 m
4		α 2.83 m β 2.35 dd (14.3, 11.4)	α 2.84 m β 2.35 dd (13.9, 11.4)	α 2.82 dd (12.0, 5.3)		α 2.29 ddd (14.8, 4.8, 2.1) β 1.68 dd (14.8, 11.2)	α 2.29 ddd (14.8, 4.5, 2.3) β 1.68 dd (14.8, 11.1)	β 2.22 dd (12.7, 12.0)
6	5.95 d (8.4)				3.96 br s			3.22 d (2.9)
7	6.30 d (8.4)	5.95 d (1.8)	5.93 d (1.8)	6.25 s	5.06 dd (1.8, 1.8)	5.94 d (2.6)	5.94 d (2.6)	4.76 d (2.9)
14		3.00 m	2.97 m		2.48 m	2.50 m	2.49 m	
18	0.65 s	0.68 s	0.61 s	0.73 s	0.58 s	0.68 s	0.66 s	0.91 s
19	0.67 s	1.15 s	1.15 s	1.13 s	1.05 s	1.10 s	1.09 s	1.30 s
20				2.13 m	2.02 m			2.23 m
21	1.01 d (6.6)	1.00 d (6.6)	0.94 d (5.9)	1.09 d (6.6)	1.02 d (6.6)	1.03 d (6.6)	0.94 d (5.9)	1.02 d (6.6)
22	4.92 d (10.3)			5.26 dd (15.4, 8.1)	5.17 dd (15.4, 8.1)	5.16 dd (15.2, 7.6)		5.25 dd (15.1, 8.3)
23				5.31 dd (15.4, 7.3)	5.22 dd (15.4, 7.7)	5.26 dd (15.2, 7.1)		5.33 dd (15.1, 7.3)
26	0.91 d (6.6)	0.85 d (6.2)	0.81[f] d (6.6)	0.85 d (7.0)	0.82 d (7.0)	0.82 d (6.8)	0.79[f] d (6.8)	0.82 d (7.3)
27	0.90 d (6.6)	0.82 d (6.6)	0.87 d (6.6)	0.86 d (6.6)	0.84 d (6.6)	0.84 d (6.8)	0.86 d (6.8)	0.84 d (7.1)
28	0.97 d (6.6)	0.96 d (7.3)	0.80[f] d (6.2)	0.94 d (7.0)	0.92 d (7.0)	0.92 d (6.9)	0.78[f] d (6.8)	0.92 d (7.1)
29	1.51 d (1.5)	1.51 s						
3-OH		6.34 d (4.8)	6.33 d (4.8)	6.36 d (4.8)				
5-OH		8.63 s	8.62 s	8.45 s	3.62 s			
9-OH		6.32 br s	6.30 s	6.81 s	3.49 s			

Coupling constants (J in Hz) are given in parentheses. *a*) Measurement in C_6D_6. *b*) Measurement in C_5D_5N. *c*) Measurement in $CDCl_3$. *d*) Measured at 270 MHz. *e*) Measured at 400 MHz. *f*) Assignments may be interchangeable.

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**—**6** and **8** (150 MHz)

Carbon	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[a]	**8**[c,d]
1	34.4	26.4	26.5	25.7	26.5	29.0	30.1
2	30.6	31.5	31.5	31.3	30.3	30.8	29.7
3	66.3	66.8	66.8	66.6	67.3	66.5	68.7
4	37.5	38.2	38.2	37.8	40.2	31.1	39.0
5	81.7	79.8	79.8	79.6	77.1	91.0	62.5
6	135.8	199.2	199.2	199.3	70.3	195.0	59.8
7	130.8	120.3	120.3	122.1	120.3	125.1	65.8
8	78.9	164.2	164.2	158.9	142.6	160.4	128.9
9	51.9	75.1	75.0	77.2	74.5	85.6	137.3
10	37.2	42.3	42.2	42.8	41.0	56.8	38.3
11	20.8	29.0	29.0	31.0	28.0	22.7	21.9
12	39.7	35.5	35.5	28.5	35.1	36.3	30.7
13	44.5	45.4	45.5	47.3	43.8	42.3	45.7
14	52.0	52.0	51.9	86.2	50.5	53.1	85.3
15	23.7	22.7	22.8	27.4	22.8	23.0	31.9
16	28.3	27.8	28.0	28.0	28.1	28.1	24.0
17	57.1	57.0	56.3	50.5	55.8	55.4	49.0
18	13.0	12.5	12.2	16.6	11.7	12.1	16.2
19	18.1	20.4	20.4	20.2	20.3	15.6	22.6
20	35.2	35.0	36.7	40.4	40.4	40.5	38.9
21	20.3	20.3	19.2	21.5	21.1	21.2	21.3
22	131.7	131.6	33.8	135.2	135.4	135.4	133.6
23	135.8	135.6	31.0	132.4	132.2	132.7	135.0
24	50.6	50.4	39.3	43.1	42.8	43.3	43.1
25	31.1	31.0	31.7	33.3	33.1	33.4	33.1
26	21.1	21.0	17.7	19.9	19.6	19.9	19.7
27	22.0	22.0	20.7	20.2	20.0	20.2	20.0
28	17.2	17.2	15.6	17.8	17.6	18.0	17.6
29	13.3	13.3					

a) Measurement in C_6D_6. *b*) Measurement in C_5D_5N. *c*) Measurement in $CDCl_3$. *d*) Measured at 100 MHz.

for the side-chain. The structure of the side-chain was determined to be a (22*E*,24*R*)-23,24-dimethyl-Δ^{22}-sterol by comparison of the MS and ^{1}H-NMR data of **1** and (22*E*,24*R*)-23,24-dimethylcholesta-5,22-dien-3β-ol.[10d] From the above data, the structure of **2** was determined to be 3β,5α,9α-trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −21.5°. 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 (3600, 3427 cm^{-1}) and α,β-unsaturated ketone (1675, 1625 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR spectra due to the steroid nucleus of **3** were identical with those of **2**, and those of the C-17 side-chain were different. The structure of the side-chain was determined from EI-MS and the HMBC spectrum. The fragment ion was observed at m/z 301 due to the loss of a C_9H_{19} from the fragment ion at m/z 428 ($M^+ - H_2O$) and thus suggested a conventional saturated steroidal C_9-side chain. The long-range C–H correlations observed from the H_3-21 methyl group to C-20 and C-22; the H_3-26 and 27 methyl groups to C-24 and C-25; and the H_3-28 methyl group to C-23, C-24 and C-25 confirmed the presence of a 24-methylsterol side-chain. The stereochemistry at C-24 was determined to be *S* by comparison of ^{1}H- and ^{13}C-NMR data with those of dihydrobrassicasterol.[11] From the above data, the structure of **3** was determined to be 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one.

Compound **4** was isolated as an amorphous powder, $[\alpha]_D$ −22.7°. The molecular formula was determined to

be $C_{28}H_{44}O_5$ by HR-MS [*m/z* 442 ($M^+ - H_2O$)] and ^{13}C-NMR data. The IR spectrum showed hydroxyl group (3354 cm^{-1}) and α,β-unsaturated ketone (1687 cm^{-1}) absorptions. In comparing the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **11**, **4** contained one more hydroxyl group, the position and configuration of which was determined as follows. In the HMBC spectrum, a cross peak was observed between the H_3-18 methyl group at δ 0.73 and the C-14 at δ 86.2, so that this hydroxyl group is attached at C-14. In the nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum, a cross peak was observed between the H_3-18 methyl group and H-20, so that the stereochemistry at C-17 and C-20 was assigned as β and *R*, respectively. In the ^{13}C-NMR spectrum of **4**, the C-17 signal appeared at δ 50.5, 5.6 ppm higher than that of **11** [δ 56.1 (C-17)].[2] This is due to the γ-gauche effect of the 14-hydroxyl group, so that the hydroxyl group at C-14 has an α configuration. Based on this evidence, the structure of **4** was determined to be 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one. Compound **4** is the first example of a naturally occurring 3β,5α,9α,14α-tetrahydroxy-7-en-6-one sterol.

Compound **5** was isolated as an amorphous powder, $[\alpha]_D$ −28.8°. The molecular formula was determined to be $C_{28}H_{46}O_4$ from HR-MS [*m/z* 428 ($M^+ - H_2O$)] and ^{13}C-NMR data. The IR spectrum showed hydroxyl (3608, 3443 cm^{-1}) absorptions. The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.58 (3H, H_3-18), 1.05 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.02 (3H, H_3-21)], which suggested an ergostane skeleton.[2] Other signals in the ^{1}H-NMR spectrum indicated to two oxygenated methine protons [δ_H 3.96 (1H, H-6), 4.03 (1H, H-3)], a trisubstituted olefinic proton [δ_H 5.06 (1H, H-7)] and two disubstituted olefinic protons [δ_H 5.17 (1H, H-22), 5.22 (1H, H-23)]. The chemical shifts of two oxygenated methine protons at δ 3.96 and 4.03 indicated hydroxy-bearing methine protons, by comparison of ^{1}H-NMR data with that of known sterols.[2] The 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 **5** has four hydroxyl groups and a mono-unsaturated C_9 side-chain. Comparison of these data with the spectral data for **13** revealed that they were identical except at C-6. In the NOESY spectrum, a cross peak was observed between the H_3-19 methyl group and H-6β, and the configuration of the hydroxyl group at C-6 was determined to be α. Thus, **5** was the 6-epimer of **13**. From the above data, the structure of **5** was determined to be (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol.

Compound **6** was isolated as a very unstable amorphous powder.[12] The molecular formula was determined to be $C_{28}H_{42}O_4$ from HR-MS and ^{13}C-NMR data. The IR spectrum showed hydroxyl group (3606, 3386 cm^{-1}) and α,β-unsaturated ketone (1685, 1643 cm^{-1}) absorptions. The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.68 (3H, H_3-18), 1.10 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.03 (3H, H_3-21)], which suggested an ergostane skeleton.[2] Other signals in the ^{1}H-NMR spectrum indicated methylene protons [δ_H 1.68 (1H, H-4β), 2.29 (1H, H-4α)], a methine proton [δ_H 2.50 (1H, H-14)], an oxygenated methine proton [δ_H 3.91 (1H, H-3)], two disubstituted olefinic protons [δ_H 5.16 (1H, H-22), 5.26 (1H, H-23)] and a trisubstituted olefinic proton [δ_H 5.94 (1H, H-7)]. The chemical shifts of the oxygenated methine proton at δ 3.91 indicated a hydroxy-bearing methine proton by comparison of ^{1}H-NMR data with that of known sterols.[2] The ^{13}C-NMR spectrum contained 28 signals that included two oxygenated quaternary carbons [δ_C 85.6 (C-9), 91.0 (C-5)], a ketone carbonyl [δ_C 195.0 (C-6)] and olefinic signals [δ_C 125.1 (C-7), 132.7 (C-23), 135.4 (C-22), 160.4 (C-8)]. The chemical shifts of two oxygenated quaternary carbons at δ 85.6 and 91.0 suggested the presence of a peroxy group.[13] From the HMBC spectrum, the planar structure of **6** was deduced to be 5,9-epidioxy-3-hydroxyergosta-7,22-dien-6-one (Fig. 1). The complexity and chemical shift of the hydroxy-bearing methine proton at C-3 were those normaly seen for 3β-hydroxy-5α-oxygenated A/B *trans* sterols.[2,14] This data indicated that the configuration of the epidioxide ring was 5α and 9α. The stereochemistry at C-22 and C-24 in the side-chain was determined to be *E* and *R*, respectively, by comparison of ^{1}H- and ^{13}C-NMR data with those of authentic ergosterol. Based on this evidence, the structure of **6** was determined to be 5α,9α-epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one.

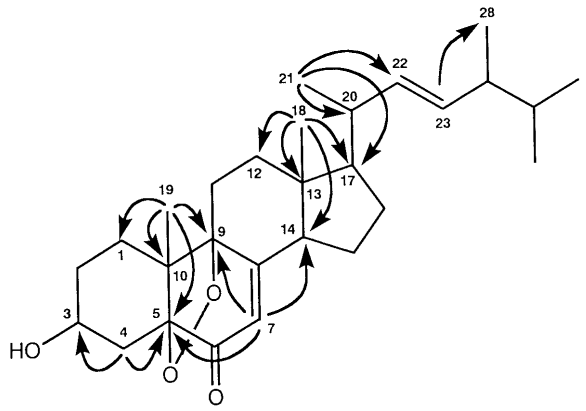

Fig. 1. HMBC Correlations (→) for **6**

Compound **7** was also isolated as a very unstable amorphous powder.[15] The molecular formula was determined to be $C_{28}H_{44}O_4$ by HR-MS and chemical ionization (CI)-MS [*m/z* 445 $(M+H)^+$] . The IR spectrum showed hydroxyl (3605, 3386 cm^{-1}) and α,β-unsaturated ketone (1685, 1641 cm^{-1}) absorptions. The ^{1}H-NMR spectrum of **7** was virtually identical to that of **6** except the side-chain. The structure of the side-chain was determined to be (24*S*)-24-methylsterol by comparison of the ^{1}H-NMR data of dihydrobrassicasterol.[11] From the above data, the structure of **7** was determined to be 5α,9α-epidioxy-3β-hydroxy-(24*S*)-ergost-7-en-6-one. Although 5α,8α-epidioxy sterols have been found to occur in fungal[2] and marine sources,[16] compounds **6** and **7** are the first example of a naturally occurring 5α,9α-epidioxy sterol.

Compound **8** was isolated as an amorphous powder,

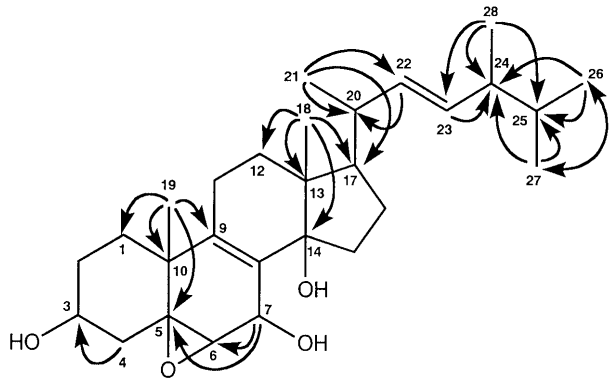

Fig. 2. HMBC Correlations (→) for **8**

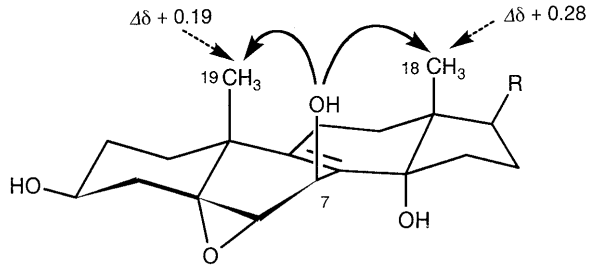

Fig. 3. Pyridine-Induced Deshieldings (→) in **8**

$[\alpha]_D$ −19.6°. The molecular formula was determined to be $C_{28}H_{44}O_4$ from HR-MS [*m/z* 426 ($M^+ - H_2O$)] and ^{13}C-NMR data. The IR spectrum showed a hydroxyl group (3506 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.91 (3H, H_3-18), 1.30 (3H, H_3-19)] and four secondary methyl groups [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.02 (3H, H_3-21)], which suggested an ergostane skeleton.[2] Other signals in the ^{1}H-NMR spectrum indicated three oxygenated methine protons [δ_H 3.22 (1H, H-6), 3.93 (1H, H-3), 4.76 (1H, H-7)] and two disubstituted olefinic protons [δ_H 5.25 (1H, H-22), 5.33 (1H, H-23)]. The chemical shifts of three oxygenated methine protons at δ 3.22, 3.93 and 4.76 indicated a trisubstituted epoxide-bearing methine, a hydroxy-bearing methine and hydroxy-bearing methine protons, respectively, by comparison of the ^{1}H-NMR data with known sterols.[2,17] The ^{13}C-NMR spectrum contained 28 signals that included a fully substituted double bond [δ_C 128.9 (C-8), 137.3 (C-9)]. From the HMBC spectrum, the planar structure of **8** was deduced to be 5,6-epoxyergosta-8,22-diene-3,7,14-triol (Fig. 2). 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[18] ($\delta_{C_5D_5N}$–δ_{CDCl_3}; $\Delta\delta$, H_3-18, +0.28 ppm, H_3-19, +0.19 ppm). This deshielding effect implies that the B-ring of **8** adopts a boat-type conformation, as a result of incorporation of the 5α,6α-epoxide moiety and the hydroxyl group at C-7 has a β configuration (Fig. 3). The configuration of the hydroxyl group at C-3 was determined to be β by comparing the ^{1}H- and ^{13}C-NMR data of the C-3 hydroxy-bearing methine of **8** with those of 5α,6α-epoxy-3β-hydroxy A/B *trans* sterols.[2,17] The configuration of the 14-hydroxyl group was determined as follows. In the NOESY spectrum, a cross peak was observed between the H_3-18 methyl group and H-20, so that the stereochemistry at C-17 and C-20 was assigned as β and *R*, respectively. In the ^{13}C-NMR spectrum, the C-17 signal of **8** appeared at δ 49.0, 4.7 ppm higher than that of 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol [δ 53.7 (C-17)].[2] This is due to the γ-gauche effect of the 14-hydroxyl group, so that the hydroxyl group at C-14 has an α configuration. The stereochemistry at C-22 and C-24 in the side-chain was determined to be *E* and *R*, respectively, by comparison of ^{1}H- and ^{13}C-NMR data with those of authentic ergosterol. Based on this evidence, the structure of **8** was determined to be 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β,14α-triol. Compound **8** is the first example of a naturally occurring 5α,6α-epoxy-3β,7β,14α-trihydroxy-Δ^8-sterol.

Experimental

General Procedures Optical rotations were determined on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR 1725X infrared spectrophotometer and UV spectra on a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM-LA 600 (600 and 150 MHz, respectively), JEOL JNM-LA 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; ddd, double double doublet; m, multiplet). The EI-MS, CI-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, CCPD; detector, RI-8010, RI-8020, UV-8011, UV-8020).

Materials *Lentinus edodes* (from Sendai), *Flammulina velutipes* (from Sendai, cultivated as unripe fruit bodies), *Hypsizigus marmoreus* (from Nagano), *Pleurotus ostreatus* (from Sendai) and *Pholiota nameko* (from Yamagata) were purchased in a food market.

Extraction and Isolation 1) *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–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 26 fractions (frs. 1—26). Fraction 6 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **1** (1.0 mg), **9** (4.0 mg) and **10** (0.5 mg). Fraction 13 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **2** (0.5 mg), **3** (0.9 mg), **4** (1.0 mg) and **11** (17.1 mg). Fraction 16 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **5** (1.3 mg), **8** (0.5 mg), **14** (0.6 mg) and **18** (0.6 mg). Fraction 19 was **15** (3.3 mg). Fraction 22 was **13** (6.2 mg).

2) *F. velutipes*: The fresh fruit bodies of *F. velutipes* (4.1 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (2.5 g) was chromatographed on a silica-gel column using *n*-hexane–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 21 fractions (frs. 1—21). Fraction 4 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, 1.5 ml/min; RI detector) to give **19** (0.9 mg) and **23** (0.7 mg). Fraction 7 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **9** (2.2 mg) and **10** (0.9 mg). Fraction 17 was separated by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH; column temperature, 40 °C; flow rate, 1.5 ml/min; RI detector) to give a mixture of **5**, **15** and **16**. This mixture was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; UV detector, 205 nm) to give **5** (0.2 mg), **15** (0.5 mg) and **16** (0.2 mg).

3) *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–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 19 fractions (frs. 1—19). Fraction 4 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, 1.0 ml/min; RI detector) to give **20** (0.6 mg) and **23** (0.8 mg). Fraction 7 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **10** (0.4 mg). Fraction 8 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **6** (8.4 mg) and **7** (4.2 mg). Fraction 12 was separated by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; UV detector, 240 nm) to give **3** (1.0 mg), **4** (1.4 mg) and **11** (3.6 mg). Fraction 13 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give a mixture of **5** and **17**, **15** (38.0 mg) and **16** (12.8 mg). The mixture of **5** and **17** was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (4 : 1); column temperature, 40 °C; flow rate, 1.5 ml/min; UV detector, 205 nm) to give **5** (1.4 mg) and **17** (0.3 mg).

4) *P. ostreatus*: The fresh fruit bodies of *P. ostreatus* (2.7 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (5.7 g) was chromatographed on a silica-gel column using *n*-hexane–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 16 fractions (frs. 1—16). Fraction 6 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **6** (1.9 mg). Fraction 13 was purified by preparative HPLC (column, TSK gel ODS-80Tm, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; UV detector, 240 nm) to give **3** (0.6 mg), **4** (0.4 mg) and **11** (3.5 mg). Fraction 15 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **5** (0.4 mg), **12** (0.2 mg), **15** (13.4 mg) and **16** (0.2 mg).

5) *P. nameko*: The fresh fruit bodies of *P. nameko* (3.0 kg) were extracted three times with MeOH at room temperature for 1 month. The MeOH extract (120 g) was extracted with Et_2O to afford the Et_2O extract (5.4 g). The Et_2O extract was chromatographed on a silica-gel column using *n*-hexane–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 18 fractions (frs. 1—18). Fraction 4 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, 1.0 ml/min; RI detector) to give **19** (0.2 mg), **20** (1.3 mg), **21** (0.04 mg), **22** (0.9 mg), **23** (0.9 mg). Fraction 11 was purified by preparative HPLC (column, TSK gel ODS-80Tm, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; UV detector, 240 nm) to give **3** (1.0 mg), **4** (0.7 mg) and **11** (3.0 mg). Fraction 14 was purified by preparative HPLC (column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (9 : 1); column temperature, 40 °C; flow rate, 1.0 ml/min; RI detector) to give **5** (0.6 mg) and **16** (0.2 mg).

All known compounds (**9**—**23**) were identified by comparison of their physical data with reported values.

5α,8α-Epidioxy-(22*E*,24*R*)-23-methylergosta-6,22-dien-3β-ol (1) Amorphous powder. $[\alpha]_D^{16}$ −37.0° (*c* = 0.05, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3588. HR-MS *m/z* : 442.3417 (M^+, Calcd for $C_{29}H_{46}O_3$; 442.3447). EI-MS *m/z*: 442 (M^+), 424 ($M^+ - H_2O$), 408 ($M^+ - H_2O_2$), 390 ($M^+ - H_2O - H_2O_2$), 303 (M^+ − side-chain), 251 ($M^+ - H_2O - H_2O_2$ − side-chain), 207 ($M^+ - H_2O - H_2O_2$ and ring D fission). ^{1}H-NMR (600 MHz, C_6D_6): see Table 1. (270 MHz, $CDCl_3$) δ: 0.78 (3H, d, *J* = 6.6 Hz, H_3-27), 0.84 (3H, d, *J* = 6.4 Hz, H_3-26), 0.84 (3H, s, H_3-18), 0.89 (3H, s, H_3-19), 0.93 (6H, d, *J* = 6.9 Hz, H_3-21, H_3-28), 1.50 (3H, d, *J* = 1.3 Hz, H_3-29), 3.96 (1H, m, H-3), 4.88 (1H, d, *J* = 10.4 Hz, H-22), 6.24 (1H, d, *J* = 8.6 Hz, H-6), 6.51 (1H, d, *J* = 8.6 Hz, H-7). ^{13}C-NMR (150 MHz, C_6D_6): see Table 2.

3β,5α,9α-Trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one (2) Amorphous powder. $[\alpha]_D^{18}$ −40.0° (*c* = 0.05, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3588, 3413, 1676, 1626. UV λ_{max}^{MeOH} nm (log ε): 237 (4.0). HR-MS *m/z*: 458.3384 (M^+, Calcd for $C_{29}H_{46}O_4$; 458.3396). EI-MS *m/z*: 404 ($M^+ - 3H_2O$), 265 ($M^+ - 3H_2O$ − side-chain). ^{1}H-NMR (600 MHz, C_5D_5N): see Table 1. (270 MHz, $CDCl_3$) δ: 0.64 (3H, s, H_3-18), 0.79 (3H, d, *J* = 6.4 Hz, H_3-27), 0.85 (3H, d, *J* = 6.4 Hz, H_3-26), 0.94 (3H, d, *J* = 6.8 Hz, H_3-28), 0.96 (3H, d, *J* = 6.6 Hz, H_3-21), 1.03 (3H, s, H_3-19), 1.514 (3H, d, *J* = 1.3 Hz, H_3-29), 2.74 (1H, m, H-14), 3.03 (1H, br s, OH), 4.02 (1H, m, H-3), 4.02 (1H, br s, OH), 4.91 (1H, d, *J* = 9.9 Hz, H-22), 5.67 (1H, d, *J* = 2.1 Hz, H-7). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 2.

3β,5α,9α-Trihydroxy-(24*S*)-ergost-7-en-6-one (3) Amorphous powder. $[\alpha]_D^{28}$ −21.5° (*c* = 0.09, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3427, 1675, 1625. UV λ_{max}^{MeOH} nm (log ε): 236 (3.8). HR-MS *m/z*: 428.3288 ($M^+ - H_2O$, Calcd for $C_{28}H_{44}O_3$; 428.3290). EI-MS *m/z*: 428 ($M^+ - H_2O$), 301 ($M^+ - H_2O$ − side-chain), 265 ($M^+ - 3H_2O$ − side-chain). ^{1}H-NMR (600 MHz, C_5D_5N): see Table 1. (270 MHz, $CDCl_3$) δ: 0.61 (3H, s, H_3-18), 0.78 (3H, d, *J* = 6.8 Hz, H_3-28 or H_3-26), 0.79 (3H, d, *J* = 6.4 Hz, H_3-26 or H_3-28), 0.86 (3H, d, *J* = 6.8 Hz, H_3-27), 0.94 (3H, d, *J* = 5.8 Hz, H_3-21), 1.02 (3H, s, H_3-19), 2.74 (1H, m, H-14), 4.02 (1H, br s, OH), 4.06 (1H, m, H-3), 5.67 (1H, d, *J* = 2.0 Hz, H-7). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 2.

3β,5α,9α,14α-Tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (4) Amorphous powder. $[\alpha]_D^{19}$ −22.7° (*c* = 0.04, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3354, 1687. UV λ_{max}^{MeOH} nm (log ε): 225 (3.9). HR-MS *m/z*: 442.3095 ($M^+ - H_2O$, Calcd for $C_{28}H_{42}O_4$; 442.3083). EI-MS *m/z*: 388 ($M^+ - 4H_2O$), 263 ($M^+ - 4H_2O$ − side-chain). ^{1}H-NMR (600 MHz, C_5D_5N): see Table 1. ^{13}C-NMR (150 MHz, C_5D_5N): see Table 2.

(22*E*,24*R*)-Ergosta-7,22-diene-3β,5α,6α,9α-tetrol (5) Amorphous powder. $[\alpha]_D^{24}$ −28.8° (*c* = 0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3608, 3443. HR-MS *m/z*: 428.3260 ($M^+ - H_2O$, Calcd for $C_{28}H_{44}O_3$; 428.3290). 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. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

5α,9α-Epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (6) Very unstable amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3606, 3386, 1685, 1643. HR-MS *m/z*: 442.3066 (M^+, Calcd for $C_{28}H_{42}O_4$; 442.3083). CI-MS *m/z*: 443 $[M+H]^+$. EI-MS *m/z*: 424 ($M^+ - H_2O$), 408 ($M^+ - H_2O_2$), 299 ($M^+ - H_2O$ − side-chain), 257 ($M^+ - H_2O$ and ring D fission). ^{1}H-NMR (270 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, C_6D_6): see Table 2.

5α,9α-Epidioxy-3β-hydroxy-(24*S*)-ergost-7-en-6-one (7) Very unstable amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3386, 1685, 1641. HR-MS *m/z*: 444.3239 (M^+, Calcd for $C_{28}H_{44}O_4$; 442.3239). CI-MS *m/z*: 445 $[M+H]^+$. EI-MS *m/z*: 426 ($M^+ - H_2O$), 410 ($M^+ - H_2O_2$), 299 ($M^+ - H_2O$ − side-chain), 257 ($M^+ - H_2O$ and ring D fission). ^{1}H-NMR (270 MHz, $CDCl_3$): see Table 1.

5α,6α-Epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β,14α-triol (8) Amorphous powder. $[\alpha]_D^{21}$ −19.6° (*c* = 0.05, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3506. HR-MS *m/z*: 426.3123 ($M^+ - H_2O$, Calcd for $C_{28}H_{42}O_3$; 426.3134). ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1. (400 MHz, C_5D_5N) δ: 0.82 (3H, d, *J* = 6.8 Hz, H_3-26), 0.83 (3H, d, *J* = 6.8 Hz, H_3-27), 0.90 (3H, d, *J* = 6.8 Hz, H_3-28), 1.12 (3H, d, *J* = 6.8 Hz, H_3-21), 1.19 (3H, s, H_3-18), 1.49 (3H, s, H_3-19), 2.57 (1H, dd, *J* = 13.0, 11.1 Hz, H-4β), 3.58 (1H, d, *J* = 4.6 Hz, H-7α), 5.24 (2H, m, H-22, H-23), 6.04 (1H, br s, OH), 6.36 (1H, d, *J* = 4.3 Hz, OH). ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2.

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