New Sterols and Triterpenoids from Four Edible Mushrooms[1)]

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Four edible mushrooms, *Panellus serotinus*, *Lepista nuda*, *Tricholoma matsutake* and *Naematoloma sublateritium*, have been investigated chemically. Two new sterols, 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6α-diol (1) and 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol (2), have been isolated from *Panellus serotinus.* Compound 2 was also isolated from *Lepista nuda.* A new sterol, 3β,5α,9α,14β-tetrahydroxy-(22*E*)-ergosta-7,22-dien-6-one (3), and compound 2 have been isolated from *Tricholoma matsutake.* Three new triterpenoids, sublateriols A—C (4—6), have been isolated from *Naematoloma sublateritium.* The structures of the new compounds were elucidated on the basis of their spectral data.

Key words sterol; triterpenoid; mushroom

Recently we reported the isolation and structural elucidation of sterols,[2)] sesquiterpenoids,[1,3)] triterpenoids,[1)] and ceramides[4)] from fourteen mushrooms. In a continuation of our investigation of the chemical constituents from mushrooms, we describe here the isolation and structural elucidation of three new sterols, 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6α-diol (**1**), 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol (**2**) and 3β,5α,9α,14β-tetrahydroxy-(22*E*)-ergosta-7,22-dien-6-one (**3**), and three new triterpenoids, sublateriols A—C (**4**—**6**), as well as eighteen known compounds, (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-ol (**7**),[2c,5)] ergosterol (**8**),[2f,6)] (22*E*)-ergosta-5,8,22-trien-3β-ol (**9**),[2c,7)] ergosta-5,7-dien-3β-ol (**10**),[2c,6)] (22*E*)-ergosta-7,22-dien-3β-ol (**11**),[2c,8)] ergost-7-en-3β-ol (**12**),[2c,6)] 5α,8α-epidioxy-(22*E*)-ergosta-6,22-dien-3β-ol (**13**),[2a,9)] 5α,6α:8α,9α-diepoxy-(22*E*)-ergost-22-ene-3β,7α-diol (**14**),[2e)] 5α,9α-epidioxy-3β-hydroxy-(22*E*)-ergosta-7,22-dien-6-one (**15**),[2b)] 3β,5α,9α,14α-tetrahydroxy-(22*E*)-ergosta-7,22-dien-6-one (**16**),[2b)] 3β,5α,9α-trihydroxy-(22*E*)-ergosta-7,22-dien-6-one (**17**),[2a,10)] 3β,5α,9α-tetrahydroxyergost-7-en-6-one (**18**),[2b)] (22*E*)-ergosta-7,22-diene-3β,5α,6β-triol (**19**),[2a,11)] ergost-7-ene-3β,5α,6β-triol (**20**),[2b,12)] (22*E*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (**21**),[2b)] (22*E*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (**22**),[2a,10)] fasciculol B (**23**)[13,14)] and fasciculol C (**24**)[14,15)] from four edible mushrooms, *Panellus* (*P.*) *serotinus* (PERS.: FR.) KÜHN. (Mukitake in Japanese, Tricholomataceae, compounds **1**, **2**, **7**—**12**, **19**—**21**), *Lepista* (*L.*) *nuda* (BULL.: FR.) COOKE (Murasakishimeji in Japanese, Tricholomataceae, compounds **2**, **13**—**22**), *Tricholoma* (*T.*) *matsutake* (S. ITO et IMAI) SING. (Matsutake in Japanese, Tricholomataceae, compounds **2**, **3**) and *Naematoloma* (*N.*) *sublateritium* (FR.) KARST. (Kuritake in Japanese, Strophariaceae, compounds **4**—**6**, **23**, **24**). This is the first time that compounds **7**—**12**, **19**—**21**, and compounds **13**—**22**, have been isolated from *P. serotinus* and *L. nuda*, respectively. Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ +8.8°. The molecular formula was determined to be $C_{28}H_{44}O_4$ by high-resolution (HR)-MS, indicating seven degrees of unsaturation. The electron ionization (EI)-MS gave fragment ion peaks at *m/z* 317 [M^+−side chain (s.c.)−2H], 299 (317−H_2O), 283 (M^+−s.c.−2H_2O) and 257 [299−42 (ring D)]. The ^{1}H-NMR spectrum (*vide* Experimental), 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 0.59 (3H, H_3-18), 1.20 (3H, H_3-19)], four secondary methyl groups [δ_H 0.82 (3H, H_3-26), 0.84 (3H, H_3-27), 0.92 (3H, H_3-28), 1.01 (3H, H_3-21)], two oxygenated methine protons [δ_H 3.89 (1H, H-3), 3.98 (1H, H-6)], two disubstituted olefinic protons [δ_H 5.15 (1H, H-22), 5.23 (1H, H-23)] and a trisubstituted olefinic proton [δ_H 5.31 (1H, H-7)]. The ^{13}C-NMR spectrum (Table 1), obtained with the aid of a ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and a distortionless enhancement by polarization transfer (DEPT) spectra, revealed 28 carbon signals that included two oxygenated methine carbons [δ_C 66.6 (C-3), 73.0 (C-6)], two oxygenated quaternary carbons [δ_C 84.3 (C-9), 88.3 (C-5)] and four olefinic carbons [δ_C 123.2 (C-7), 132.4 (C-23), 135.2 (C-22), 141.6 (C-8)]. The IR absorption (3422 cm^{-1}) and the chemical shift values of two oxygenated methines [δ_H 3.89 (1H), 3.98 (1H); δ_C 66.6 (CH), 73.0 (CH)] indicated the presence of two secondary hydroxyl groups. The chemical shift values of two oxygenated quaternary carbons at δ_C 84.3 and 88.3 and the unsaturation degree indicated the presence of an epidioxy group. Based on these spectral data, **1** was suggested to be an ergostane-type sterol with two hydroxyl groups, one epidioxy group, one disubstituted double bond and one trisubstituted double bond. The ^{1}H–^{1}H COSY spectrum of **1** implied connectivities for H-3 to H_2-4 and H-6 to H-7 (Fig. 1). Interpretation of the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum revealed correlations between H-4α to C-2, C-3, C-5 and C-10; H-7 to C-5, C-9 and C-14; H_3-18 to C-12, C-13, C-14 and C-17; H_3-19 to C-1, C-5, C-9 and C-10; H_3-21 to C-17, C-20 and C-22; H_3-26 and H_3-27 to C-24 and C-25; and H_3-28 to C-23, C-24 and C-25 (Fig. 1). Therefore, the planar structure of **1** was deduced to be 5,9-epidioxyergosta-7,22-diene-3,6-diol. The chemical shift value and the multiplicity of the hydroxy-bearing methine proton at C-3 [δ_H 3.89 (1H, m, $W_{1/2}$ 22 Hz)] were those normally seen for 3β-hydroxy-5α-oxygenated A/B *trans* sterols.[2,16)] This data indicated that the epidioxy group was assigned to be 5α and 9α. In the nuclear Overhauser effect correlation spectroscopy (NOESY) sprctrum, a cross peak was observed between the

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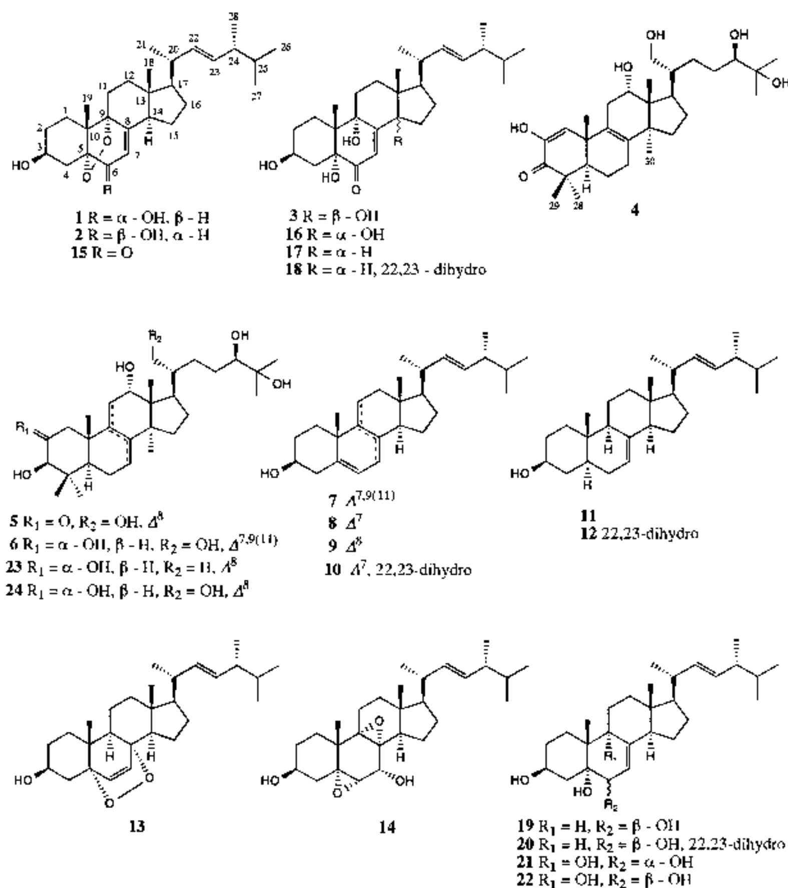

Chart 1

H_3-19 and H-6β, and the configuration of the hydroxyl group at C-6 was determined to be α (Fig. 2). The geometry of the Δ^{22}-double bond was deduced to be *E* from the ^1H–^1H coupling constant ($J=15.1$ Hz) between H-22 and H-23. The stereochemistry at C-20 and C-24 was determined to be *R* and *R*, respectively, by comparison of ^1H- and ^{13}C-NMR data with those of ergosterol (**8**) (Table 2).[17] The co-occurence of **1** with **8** in the same mushroom indicates that the absolute structure of **1** is probably the same as **8**. Thus, the structure of **1** was determined to be 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6α-diol.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ $-24.4°$. The molecular formula was determined to be $C_{28}H_{44}O_4$ by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3410 cm^{-1}). The ^{13}C-NMR spectrum of **2** was quite similar to that of **1**. The ^1H-NMR spectrum of **2** also showed a similar pattern to that of **1**, except for the chemical shifts of signals due to the hydroxy-bearing methine proton at C-6 and the trisubstituted olefinic proton at C-7. These data indicated that compound **2** was an epimer of **1** at C-6. In the ^1H-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[18] ($\delta_{C_5D_5N}-\delta_{CDCl_3}$; $\Delta\delta$, H_3-19, $+0.32$ ppm). This deshielding effect implies that the hydroxyl group at C-6 has a β configuration (Fig. 2). From the above data, the structure of **2** was determined to be 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol. Compounds **1** and **2** have 5α,9α-epidioxy-3β,6α-dihydroxy-7-ene and 5α,9α-epidioxy-3β,6β-dihydroxy-7-ene moieties, respectively, which are unprecedented in the natural sterols previously known.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ $-73.7°$. The molecular formula was determined to be $C_{28}H_{44}O_5$ by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3460 cm^{-1}) and a carbonyl group (1677 cm^{-1}). The ^1H-NMR spectrum showed signals due to two tertiary methyl groups [δ_H 0.99 (3H, H_3-19), 1.00 (3H, H_3-18)], four secondary methyl groups [δ_H 0.83 (3H, H_3-26), 0.85 (3H, H_3-27), 0.95 (3H, H_3-28), 1.00 (3H, H_3-21)], a hydroxy-bearing methine proton [δ_H 4.06 (1H, H-3)], two disubstituted olefinic protons [δ_H 5.34 (1H, H-23), 5.45 (1H, H-22)] and a trisubstituted olefinic proton [δ_H 6.50 (1H, H-7)]. The ^{13}C-NMR spectrum revealed 28 carbon signals that included three oxygenated quaternary carbons [δ_C 75.9 (C-9), 79.3 (C-5), 84.6 (C-14)] and four olefinic carbons [δ_C 122.8 (C-7), 132.9 (C-23), 135.3 (C-22), 166.9 (C-8)]. The NMR data of **3** closely resembled those of **16** except for some signals surrounding C-14. The ^1H–^1H COSY, HMQC and HMBC data provided evidence of the same planar structure for **3** as that of **16**. The difference between **3** and **16** was traced to differences in the stereochemistry of the hydroxyl group at C-14. In the ^1H-NMR spectrum, the chemical shift of the H_3-18 methyl group in pyridine-d_5 was shifted downfield ($\delta_{C_5D_5N}-\delta_{CDCl_3}$; $\Delta\delta$, H_3-18, $+0.33$ ppm),[18] indicating that the hydroxyl group at C-14 has a β configuration (Fig. 2). This was evident from the NOESY spectrum in pyridine-d_5, in which a cross-peak was seen between OH-14 and H_3-18 (Fig. 2). Thus, **3** was the epimer of **16** at C-14. Based on

Table 1. ^{13}C-NMR Chemical Shifts of Compounds **1**—**6**

Position	**1**[a,d]	**2**[a,d]	**3**[b,e]	**4**[b,e]	**5**[b,d]	**6**[c,d]
1	28.9	28.7	26.0	126.5	50.7	45.1
2	30.8	31.7	31.7	146.9	211.5	69.5
3	66.6	66.8	66.7	200.9	83.3	84.0
4	34.1	34.8	38.1	44.5	45.2	40.2
5	88.3	86.6	79.3	48.4	50.0	50.6
6	73.0	72.2	199.4	18.8	18.8	24.2
7	123.2	122.5	122.8	25.9	26.5	124.6
8	141.6	141.8	166.9	136.2	136.1	143.6
9	84.3	84.7	75.9	131.2	132.2	148.9
10	54.2	51.0	43.3	39.1	43.3	39.8
11	22.8	23.1	28.0	34.4	33.0	118.0
12	36.6	36.6	37.4	73.1	73.0	76.1
13	42.1	41.9	49.8	50.9	50.4[f]	48.5
14	52.1	52.1	84.6	50.5	50.4[f]	51.1
15	23.1	23.2	41.9	32.2	32.3	33.7
16	28.2	28.1	28.6	28.2	28.3[g]	28.5
17	55.5	55.5	56.1	38.7	38.6	40.0
18	11.6	11.7	17.5	17.2	16.9	17.6
19	16.2	17.3	22.9	24.4	20.0	24.1
20	40.3	40.4	39.5	44.1	44.0	44.3
21	21.1	21.1	20.4	61.2	61.2	62.1
22	135.2	135.2	135.3	28.3	28.3[g]	28.1
23	132.4	132.3	132.9	29.8	29.8	29.3[h]
24	42.8	42.8	43.3	79.1	79.0	79.4
25	33.1	33.1	33.4	72.8	72.7	73.9
26	19.6	19.7	19.9	26.1	26.1	25.7
27	20.0	20.0	20.2	26.0	25.9	24.9
28	17.6	17.6	17.9	26.8	29.1	29.3[h]
29				21.8	17.1	17.7
30				23.9	24.0	28.7

a) Measurement in $CDCl_3$. *b*) Measurement in C_5D_5N. *c*) Measurement in CD_3OD. *d*) Measured at 100 MHz. *e*) Measured at 150 MHz. *f*—*h*) Signals overlapped.

this evidence, the structure of **3** was determined to be $3\beta,5\alpha,9\alpha,14\beta$-tetrahydroxy-(22*E*)-ergosta-7,22-dien-6-one. Compound **3** is the first example of a naturally occurring $3\beta,5\alpha,9\alpha,14\beta$-tetrahydroxy-7-en-6-one sterol.

Compound **4**, named sublateriol A, was isolated as an amorphous powder, $[\alpha]_D$ +82.4°. The molecular formula was determined to be $C_{30}H_{48}O_6$ by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3425 cm^{-1}) and a carbonyl group (1672 cm^{-1}). The UV absorption maximum at 267 nm suggested a monoenolized α-diketone.[19] The ^{1}H-NMR spectrum showed signals due to seven tertiary methyl groups [δ_H 0.73 (3H, H_3-18), 1.14 (3H, H_3-29), 1.23 (3H, H_3-19), 1.25 (3H, H_3-28), 1.35 (3H, H_3-30), 1.50 (3H, H_3-27), 1.53 (3H, H_3-26)], two oxygenated methine protons [δ_H 3.86 (1H, H-24), 4.33 (1H, H-12)], an oxygenated methylene proton [δ_H 4.14 (2H, H-21)] and a trisubstituted olefinic proton [δ_H 6.81 (1H, H-1)]. The ^{13}C-NMR spectrum, obtained with the aid of HMQC and DEPT spectra, indicated the pres-

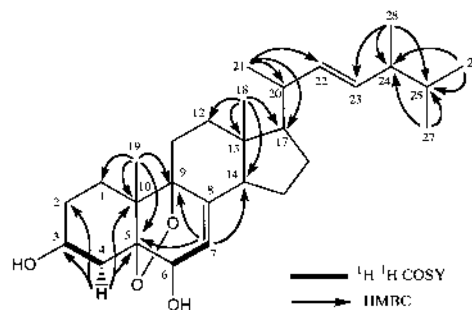

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**

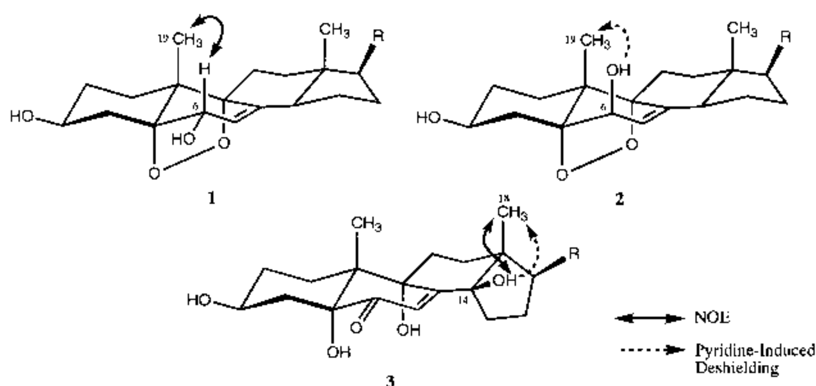

Fig. 2. NOEs and Pyridine-Induced Deshieldings for **1**—**3**

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts of the Side Chain of Compounds **1**, **4**, **8** and **24**[a]

Position	**1**[b]		**8**[b]		**4**[c]		**24**[c]	
	^{1}H	^{13}C	^{1}H[d]	^{13}C[e]	^{1}H	^{13}C	^{1}H[d]	^{13}C[e]
20		40.3		40.4		44.1		44.1
21	1.01 (d, 6.6)	21.1	1.04 (d, 6.6)	21.1	4.14 (br s)	61.2	4.12 (br s)	61.2
22	5.15 (dd, 15.1, 7.8)	135.2	5.16 (dd, 15.2, 7.4)	135.6	1.83 (m), 2.36 (m)	28.3	1.82 (m), 2.35 (m)	28.4
23	5.23 (dd, 15.1, 7.3)	132.4	5.23 (dd, 15.2, 6.9)	132.0	1.95 (2H, m)	29.8	1.94 (2H, m)	29.8
24		42.8		42.8	3.86 (br d, 9.5)	79.1	3.85 (dd, 9.9, 2.2)	79.1
25		33.1		33.1		72.8		72.8
26	0.82 (d, 6.6)	19.6	0.82 (d, 6.8)	19.7	1.53 (s)	26.1	1.52 (s)	26.1
27	0.84 (d, 6.8)	20.0	0.84 (d, 6.8)	20.0	1.50 (s)	26.0	1.49 (s)	26.0
28	0.92 (d, 6.8)	17.6	0.92 (d, 6.9)	17.6				

a) Figures in parentheses on ^{1}H-NMR denote *J* values (Hz). *b*) Measurement in $CDCl_3$. *c*) Measurement in C_5D_5N. *d*) Measured at 600 MHz. *e*) Measured at 150 MHz.

ence of seven methyl, eight methylene, six methine and nine quarternary carbons. The IR absorption (3425 cm^{-1}) and the chemical shift values of two oxygenated methine [δ_H 3.86 (1H), 4.33 (1H); δ_C 73.1 (CH), 79.1 (CH)] and an oxygenated methylene [δ_H 4.14 (2H); δ_C 61.2 (CH_2)] indicated the presence of two secondary hydroxyl groups and a primary hydroxyl group. Detailed analysis of the ^1H–^1H COSY spectrum of **4** implied connectivities for H-5 to H_2-7; H_2-11 to H-12; H_2-15 to H-17; H-17 to H-20; and H_2-21 to H-24 (Fig. 3). Interpretation of the HMBC spectrum revealed correlations from H-1 to C-2; H_3-18 to C-12, C-13, C-14 and C-17; H_3-19 to C-1, C-5, C-9 and C-10; H_3-26 and H_3-27 to C-24 and C-25; H_3-28 and H_3-29 to C-3, C-4 and C-5; and H_3-30 to C-8, C-13, C-14 and C-15 (Fig. 3). Therefore, the planar structure of **4** was deduced to be as shown in Fig. 3. The stereostructure was ascertained by NOE interactions observed in a NOESY spectrum (Fig. 4). The *trans*-junctions of rings A/B and rings C/D were based on NOESY correlations of H_3-28/H-5α, H_3-28/H_3-29, H_3-29/H_3-19, H_3-19/H_3-18, H_3-18/H-15β, H_3-18/H-16β, and H_3-30/H-17α. The hydroxyl group at C-12 has an α configuration, which was supported by the NOESY cross-peak between H-12β and H_3-18. The stereochemistry at C-24 was determined to be *R* by comparison of ^1H- and ^{13}C-NMR data with those of fasciculol C (**24**) (Table 2). The absolute structure of fasciculol C (**24**) has been established by Ikeda *et al.*[15] Fasciculols having the *R* configuration of the hydroxyl group at C-24 have been isolated from mushrooms belonging to the genus *Naematoloma.*[13—15,20] The *R* and *S* configuration of the hydroxyl group at C-24 of the side chain can be distinguished by NMR spectroscopy mainly by the chemical shift of the hydroxy-bearing methine at C-24.[21] Based on this evidence, the structure of **4** was determined to be (24*R*)-2,12α,21,24,25-pentahydroxylanosta-1,8-dien-3-one.

Compound **5**, named sublateriol B, was isolated as an amorphous powder, $[\alpha]_D$ +89.7°. The molecular formula was determined to be $C_{30}H_{50}O_6$ by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3389 cm^{-1}) and a carbonyl group (1712 cm^{-1}). The ^1H- and ^{13}C-NMR data for **5** were quite similar to those of **4** except for the signals ascribed to ring A. The signals due to a methylene group [δ_H 2.44 (1H, H-1α), 2.63 (1H, H-1β); δ_C 50.7 (C-1)], an oxygenated methine group [δ_H 4.22 (1H, H-3); δ_C 83.3 (C-3)] and a carbonyl carbon [δ_C 211.5 (C-2)] were observed in the ^1H- and ^{13}C-NMR spectra of **5** instead of signals of the trisubstituted double bond between C-1 and C-2, and the carbonyl group at C-3 in **4**. The IR absorption (3389 cm^{-1}) and the chemical shift value of an oxygenated methine [δ_H 4.22 (1H); δ_C 83.3 (CH)] indicated the presence of a secondary hydroxyl group. In the HMBC spectrum, long-range ^1H–^{13}C correlation of H_2-1 to C-2, as well as H_3-28 and H_3-29 to C-3, revealed that a carbonyl group and a hydroxyl group were attached to C-2 and C-3, respectively. The hydroxyl group at C-3 has a β configuration, which was supported by the NOESY cross-peaks between H-1α and H-3α; H-3α and H-5α; and H-3α and H_3-28. Accordingly, the structure of **5** was determined to be (24*R*)-3β,12α,21,24,25-pentahydroxylanost-8-en-2-one.

Compound **6**, named sublateriol C, was isolated as an amorphous powder, $[\alpha]_D$ +63.4°. The molecular formula was determined to be $C_{30}H_{50}O_6$ by HR-MS [*m/z* 488 (M^+− H_2O)] and ^{13}C-NMR data. The IR spectrum showed the presence of a hydroxyl group (3430 cm^{-1}). The ^1H- and ^{13}C-NMR spectra of **6** were quite similar to those of **24**, except that **6** possesses signals of two trisubstituted double bonds [δ_H 5.55 (1H, H-11), 5.66 (1H, H-7); δ_C 118.0 (C-11), 124.6 (C-7), 143.6 (C-8), 148.9 (C-9)] instead of the signal of the fully substituted double bond between C-8 and C-9 in **24**. The HMBC correlations of H-11 to C-8, H-12β to C-11, H_3-19 to C-9, and H_3-30 to C-8 implied the presence of a $\Delta^{7,9(11)}$-diene system in **6**. This was evident from the UV absorption maxima at 237 (sh), 245 and 254 (sh) nm.[19] Therefore, the structure of **6** was determined to be (24*R*)-lanosta-7,9(11)-diene-2α,3β,12α,21,24,25-hexaol.

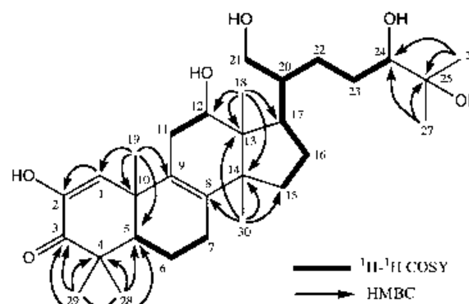

Fig. 3. ^1H–^1H COSY and HMBC Correlations for **4**

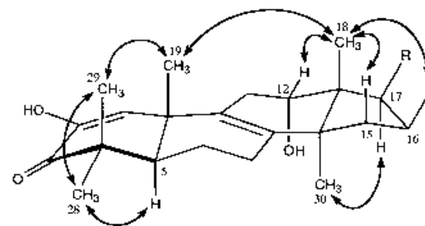

Fig. 4. NOEs Detected for **4**

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer and UV spectra on a Beckman DU-64 spectrophotometer. ^1H- and ^{13}C-NMR spectra were recorded using 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; ddd, double 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). HPLC conditions: flow rate, 1.0 ml/min; column temperature, 40 °C.

Material *Panellus serotinus* (from Morioka City in Iwate Prefecture, Japan), *Lepista nuda* (from Morioka City in Iwate Prefecture, Japan), *Tricholoma matsutake* (from Korea) and *Naematoloma sublateritium* (from Morioka City in Iwate Prefecture, Japan) were purchased in a food market.

Extraction and Isolation *P. serotinus*: The fresh fruit bodies of *P. serotinus* (1.1 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (2.2 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 40 fractions (frs. 1—40). Fraction 6 was purified by preparative HPLC (mobile phase, MeOH) to give **7** (50.8 mg), **8** (587.0 mg), **9** (95.9 mg), **10** (39.5 mg), **11** (33.9 mg) and **12** (16.9 mg). Fraction 17 was purified by preparative HPLC (mobile phase, MeOH) to give **1** (1.1 mg). Fraction 20 was purified by preparative HPLC (mobile phase, MeOH) to give **2** (0.8 mg). Fraction 31 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to

give **19** (2.1 mg), **20** (0.4 mg) and **21** (0.3 mg).

L. nuda: The fresh fruit bodies of *L. nuda* (0.3 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.4 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 29 fractions (frs. 1—29). Fraction 10 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **13** (0.9 mg) and **15** (0.8 mg). Fraction 18 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **2** (0.1 mg), **16** (0.1 mg), **17** (1.0 mg) and **18** (0.1 mg). Fraction 24 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **19** (1.1 mg), **20** (0.2 mg) and **21** (0.2 mg). Fraction 25 was separated by preparative HPLC (mobile phase, MeOH) to give a mixture of **14** and **22**. This mixture was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **14** (0.1 mg) and **22** (0.7 mg).

T. matsutake: The fresh fruit bodies of *T. matsutake* (4.7 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (8.7 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 31 fractions (frs. 1—31) . Fraction 15 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **2** (0.9 mg). Fraction 20 was purified by preparative HPLC [mobile phase, MeOH–H_2O (9 : 1)] to give **3** (1.0 mg).

N. sublateritium: The fresh fruit bodies of *N. sublateritium* (1.1 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (5.9 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 60 fractions (frs. 1—60). Fraction 33 was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1)] to give **5** (1.5 mg). Fraction 37 was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1)] to give **23** (2.7 mg). Fraction 41 was purified by preparative HPLC [mobile phase, MeOH–H_2O (4 : 1)] to give **4** (0.9 mg). Fraction 50 was purified by preparative HPLC [mobile phase, MeOH–H_2O (7 : 2)] to give **6** (0.7 mg) and **24** (11.2 mg).

All known compounds (**7**—**24**) were identified by comparison of their chromatographic behavior, and their MS and ^{1}H-NMR data with those of the authentic samples (**7**—**22**) or by comparison of their physical data with reported values (**23** and **24**).

5α,9α-Epidioxy-(22*E*)-ergosta-7,22-diene-3β,6α-diol (1) Amorphous powder. $[\alpha]_D^{23}$ +8.8° (c=0.1, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3422. HR-MS *m/z*: 444.3242 (M^+, Calcd for $C_{28}H_{44}O_4$: 444.3240). EI-MS *m/z*: (%): 444 (M^+, 6), 317 (78), 299 (21), 283 (9), 257 (11). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.59 (3H, s, H_3-18), 0.82 (3H, d, J=6.6 Hz, H_3-26), 0.84 (3H, d, J=6.8 Hz, H_3-27), 0.92 (3H, d, J=6.8 Hz, H_3-28), 1.01 (3H, d, J=6.6 Hz, H_3-21), 1.20 (3H, s, H_3-19), 1.36 (1H, dd, J=14.4, 11.0 Hz, H-4β), 1.94 (1H, d, J=8.5 Hz, OH-6), 2.02 (1H, m, H-20), 2.21 (1H, m, H-14), 2.60 (1H, ddd, J=14.4, 4.6, 2.2 Hz, H-4α), 3.89 (1H, m, H-3), 3.98 (1H, dd, J=8.5, 2.4 Hz, H-6), 5.15 (1H, dd, J=15.1, 7.8 Hz, H-22), 5.23 (1H, dd, J=15.1, 7.3 Hz, H-23), 5.31 (1H, dd, J=2.4, 2.4 Hz, H-7). ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1.

5α,9α-Epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol (2) Amorphous powder. $[\alpha]_D^{19}$ −24.4° (c=0.08, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3410. HR-MS *m/z*: 444.3237 (M^+, Calcd for $C_{28}H_{44}O_4$: 444.3240). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.63 (3H, s, H_3-18), 0.82 (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=6.8 Hz, H_3-28), 1.01 (3H, d, J=6.8 Hz, H_3-21), 1.26 (3H, s, H_3-19), 1.66 (1H, d, J=6.6 Hz, OH-6), 2.24 (1H, m, H-14), 3.77 (1H, ddd, J=6.6, 4.4, 3.2 Hz, H-6), 4.03 (1H, m, H-3), 5.15 (1H, dd, J=15.1, 7.8 Hz, H-22), 5.23 (1H, dd, J=15.1, 7.3 Hz, H-23), 5.50 (1H, dd, J=4.4, 2.7 Hz, H-7); (400 MHz, C_5D_5N) δ: 0.68 (3H, s, H_3-18), 0.85 (3H, d, J=6.6 Hz, H_3-26), 0.86 (3H, d, J=6.8 Hz, H_3-27), 0.95 (3H, d, J=6.8 Hz, H_3-28), 1.03 (3H, d, J=6.6 Hz, H_3-21), 1.58 (3H, s, H_3-19), 2.52 (1H, ddd, J=14.1, 4.4, 1.7 Hz, H-4α), 2.82 (1H, dd, J=14.1, 11.2 Hz, H-4β), 4.26 (1H, m, H-6), 4.49 (1H, m, H-3), 5.17 (1H, dd, J=15.4, 8.3 Hz, H-22), 5.25 (1H, dd, J=15.4, 7.6 Hz, H-23), 5.82 (1H, dd, J=4.4, 2.9 Hz, H-7), 6.36 (1H, d, J=4.6 Hz, OH-3), 7.07 (1H, d, J=5.9 Hz, OH-6). ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1.

3β,5α,9α,14β-Tetrahydroxy-(22*E*)-ergosta-7,22-dien-6-one (3) Amorphous powder. $[\alpha]_D^{18}$ −73.7° (c=0.1, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3460, 1677. UV λ_{max} MeOH nm (log ε): 236 (3.7). HR-MS *m/z*: 460.3177 (M^+, Calcd for $C_{28}H_{44}O_5$: 460.3189). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.83 (3H, d, J=6.6 Hz, H_3-26), 0.85 (3H, d, J=6.6 Hz, H_3-27), 0.95 (3H, d, J=6.6 Hz, H_3-28), 0.99 (3H, s, H_3-19), 1.00 (3H, s, H_3-18), 1.00 (3H, d, J=6.6 Hz, H_3-21), 2.33 (1H, ddd, J=13.9, 13.9, 4.0 Hz, H-1α), 2.38 (1H, m, H-20), 2.80 (1H, m, H-15α), 4.06 (1H, m, H-3), 5.34 (1H, dd, J=15.4, 8.1 Hz, H-23), 5.45 (1H, dd, J=15.4, 8.8 Hz, H-22), 6.50 (1H, s, H-7); (600 MHz, C_5D_5N) δ: 0.81 (3H, d, J=6.6 Hz, H_3-26), 0.82 (3H, d, J=6.6 Hz, H_3-27), 0.92 (3H, d, J=7.0 Hz, H_3-28), 1.12 (3H, d, J=6.6 Hz, H_3-21), 1.13 (3H, s, H_3-19), 1.33 (3H, s, H_3-18), 2.55 (1H, m, H-20), 2.84 (2H, m, H-1α, H-4α), 3.15 (1H, ddd, J=13.2, 13.2, 8.4 Hz, H-15α), 4.64 (1H, m, H-3), 5.30 (1H, dd, J=15.4, 8.1 Hz, H-23), 5.61 (1H, dd, J=15.4, 8.1 Hz, H-22), 5.83 (1H, s, OH-14), 6.28 (1H, br s, OH-3), 6.52 (1H, s, OH-9), 7.14 (1H, s, H-7), 8.85 (1H, s, OH-5). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 1.

Sublateriol A (4) Amorphous powder. $[\alpha]_D^{30}$ +82.4° (c=0.09, MeOH). IR ν_{max} $CHCl_3$ cm^{-1}: 3425, 1672, 1656. UV λ_{max} MeOH nm (log ε): 267 (3.7). HR-MS *m/z*: 504.3440 (M^+, Calcd for $C_{30}H_{48}O_6$: 504.3451). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.73 (3H, s, H_3-18), 1.14 (3H, s, H_3-29), 1.23 (3H, s, H_3-19), 1.24 (1H, m, H-15α), 1.25 (3H, s, H_3-28), 1.35 (3H, s, H_3-30), 1.50 (3H, s, H_3-27), 1.52 (1H, m, H-16β), 1.53 (3H, s, H_3-26), 1.63 (3H, m, H_2-6, H-20), 1.76 (1H, m, H-15β), 1.83 (1H, m, H-22a), 1.95 (2H, m, H_2-23), 2.04 (1H, dd, J=11.4, 4.0 Hz, H-5), 2.10 (1H, m, H_2-7), 2.16 (1H, m, H-16α), 2.36 (1H, m, H-22b), 2.62 (1H, br d, J=18.3 Hz, H-11α), 2.90 (1H, m, H-17), 3.00 (1H, dd, J=18.3, 8.8 Hz, H-11β), 3.86 (1H, br d, J=9.5 Hz, H-24), 4.14 (2H, br s, H_2-21), 4.33 (1H, br d, J=8.8 Hz, H-12), 5.54 (1H, br s, OH-25), 5.94 (1H, br s, OH-24), 6.05 (1H, d, J=2.6 Hz, OH-12), 6.81 (1H, s, H-1), 6.94 (1H, t, J=4.8 Hz, OH-21), 10.1 (1H, br s, OH-2). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 1.

Sublateriol B (5) Amorphous powder. $[\alpha]_D^{32}$ +89.7° (c=0.2, MeOH). IR ν_{max} KBr cm^{-1}: 3389, 1712. HR-MS *m/z*: 506.3626 (M^+, Calcd for $C_{30}H_{50}O_6$: 506.3607). ^{1}H-NMR (400 MHz, C_5D_5N) δ: 0.70 (3H, s, H_3-18), 0.92 (3H, s, H_3-29), 1.01 (3H, s, H_3-19), 1.33 (3H, s, H_3-28), 1.41 (3H, s, H_3-30), 1.49 (3H, s, H_3-27), 1.52 (3H, s, H_3-26), 1.87 (1H, dd, J=12.7, 2.0 Hz, H-5), 2.28 (1H, d, J=18.5 Hz, H-11α), 2.44 (1H, d, J=12.0 Hz, H-1α), 2.63 (1H, d, J=12.0 Hz, H-1β), 2.73 (1H, dd, J=18.5, 8.5 Hz, H-11β), 2.91 (1H, m, H-17), 3.85 (1H, br d, J=9.0 Hz, H-24), 4.13 (1H, br s, H_2-21), 4.22 (1H, d, J=3.7 Hz, H-3), 4.28 (1H, br d, J=9.3 Hz, H-12), 5.50 (1H, br s, OH-25), 5.89 (1H, br s, OH-24), 5.91 (1H, d, J=3.7 Hz, OH-3), 6.00 (1H, d, J=2.4 Hz, OH-12), 6.92 (1H, br s, OH-21). ^{13}C-NMR (100 MHz, C_5D_5N): see Table 1.

Sublateriol C (6) Amorphous powder. $[\alpha]_D^{28}$ +63.4° (c=0.07, MeOH). IR ν_{max} $CHCl_3$ cm^{-1}: 3430. UV λ_{max} MeOH nm (log ε): 237 sh (4.0), 245 (4.1), 254 sh (4.0). HR-MS *m/z*: 488.3532 ($M^+ - H_2O$, Calcd for $C_{30}H_{48}O_5$: 488.3502). ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.62 (3H, s, H_3-18), 0.91 (3H, s, H_3-29), 1.03 (3H, s, H_3-28), 1.06 (3H, s, H_3-19), 1.13 (3H, s, H_3-30), 1.14 (3H, s, H_3-27), 1.17 (3H, s, H_3-26), 1.21 (1H, dd, J=11.2, 4.6 Hz, H-5), 1.43 (1H, dd, J=12.7, 12.2 Hz, H-1α), 1.45 (3H, m, H-20, H-22, H-23), 1.50 (1H, m, H-15), 1.57 (1H, m, H-23), 1.75 (2H, m, H-15, H-16), 2.13 (3H, m, H_2-6, H-16), 2.33 (1H, dd, J=12.7, 4.4 Hz, H-1β), 2.48 (1H, m, H-17), 2.94 (1H, d, J=9.5 Hz, H-3), 3.27 (1H, d, J=10.0 Hz, H-24), 3.70 (1H, m, H-2), 3.71 (1H, d, J=11.7 Hz, H-21a), 3.78 (1H, dd, J=11.7, 2.0 Hz, H-21b), 4.16 (1H, d, J=5.9 Hz, H-12), 5.55 (1H, br d, J=5.4 Hz, H-11), 5.66 (1H, br d, J=5.9 Hz, H-7). ^{13}C-NMR (100 MHz, CD_3OD): see Table 1.

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References and Notes

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