New Sterols from Two Edible Mushrooms, *Pleurotus eryngii* and *Panellus serotinus*[1)]

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Two edible mushrooms, *Pleurotus eryngii* and *Panellus serotinus*, have been investigated chemically. Two new sterols, 5α,9α-epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol (1) and 3β,5α-dihydroxyergost-7-en-6-one (2), have been isolated from *P. eryngii*, together with six known ones (3—8). Compound 1 was also isolated from *P. serotinus*. The structures of the new compounds were elucidated on the basis of their spectral data.

Key words sterol; mushroom; *Pleurotus eryngii*; *Panellus serotinus*

Recently we reported the isolation and structural elucidation of sterols,[2)] sesquiterpenoids,[3,4)] triterpenoids,[2i,4)] and ceramides[5)] from 17 mushrooms. It has been reported that ergosterol and ergosterol peroxide from the edible mushroom *Hypsizigus marmoreus* can inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear edema and tumor promotion during two-stage carcinogenesis with 7,12-dimethylbenz[*a*]anthracene (DMBA) and TPA in mice.[6)] Therefore, these sterols may possibly prove useful for producing cancer chemopreventive agents.[6)] In a continuation of our investigation of the sterol constituents of edible mushrooms, we describe here the isolation and structural elucidation of two new sterols, 5α,9α-epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol (**1**) and 3β,5α-dihydroxyergost-7-en-6-one (**2**), as well as six known ones, 6β-acetoxy-(22*E*)-ergosta-7,22-diene-3β,5α-diol (**3**),[7)] 3β,5α-dihydroxy-(22*E*)-ergosta-7,22-dien-6-one (**4**),[2a)] ergosterol peroxide (**5**),[2a,8)] 5α,9α-epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol (**6**),[2i)] 5α,9α-epidioxy-3β-hydroxy-(22*E*)-ergosta-7,22-dien-6-one (**7**)[2b)] and 3β,5α,9α-trihydroxy-(22*E*)-ergosta-7,22-dien-6-one (**8**),[2a,9)] from two edible mushrooms, *Pleurotus eryngii* (DC.: FR.) QUÉL. (eringi in Japanese, Pleurotaceae, compounds **1**—**8**) and *Panellus serotinus* (PERS.: FR.) KÜHN. (mukitake in Japanese, Tricholomataceae, compound **1**). This is the first time that compounds **3**—**8** have been isolated from *P. eryngii*. Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as a colorless amorphous solid, $[\alpha]_D$ −33.9°. The molecular formula was determined to be $C_{28}H_{42}O_4$ by high-resolution mass spectroscopy (HR-MS). The ^{1}H-NMR spectrum (*vide* Experimental) showed signals due to two tertiary methyl groups [δ_H 0.93 (3H, H_3-18), 1.17 (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), 1.00 (3H, H_3-21)], suggesting that **1** was a sterol belonging to the ergostane series.[2)] Other signals in the ^{1}H-NMR spectrum indicated an oxygenated methine proton [δ_H 4.02 (1H, H-3)] and four disubstituted olefinic protons [δ_H 5.17 (1H, H-22), 5.24 (1H, H-23), 5.56 (1H, H-7), 5.89 (1H, H-6)]. The ^{13}C-NMR spectrum (*vide* Experimental), obtained with the aid of a distortionless enhancement by polarization transfer (DEPT) spectrum, revealed 28 carbon signals that included an oxygenated methine carbon [δ_C 66.1 (C-3)], four oxygenated quaternary carbons [δ_C 63.8 (C-8), 75.2 (C-14), 85.8 (C-5),

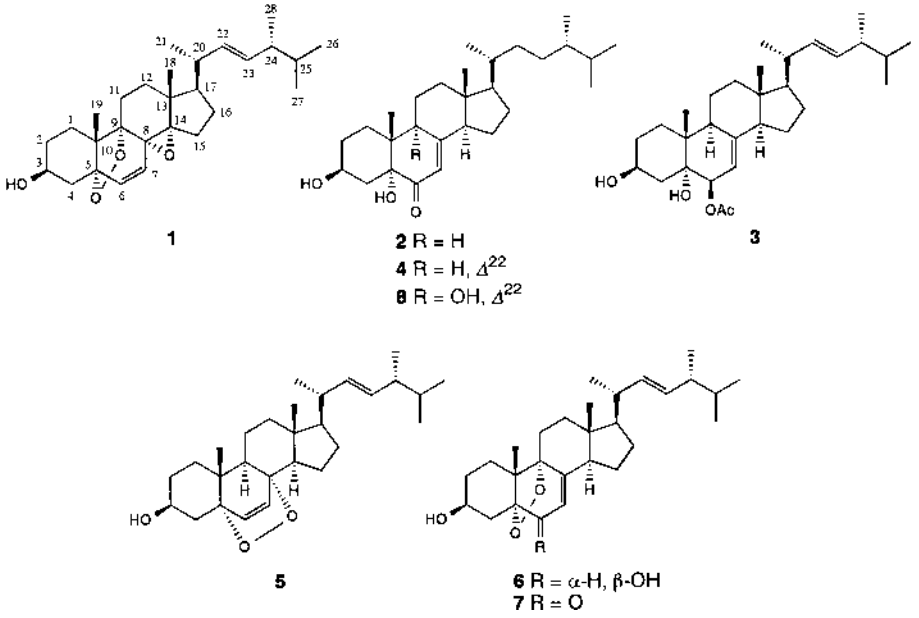

Chart 1

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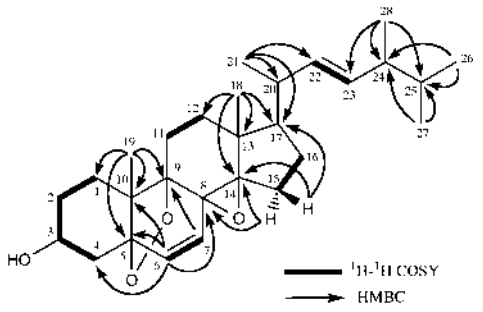

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**

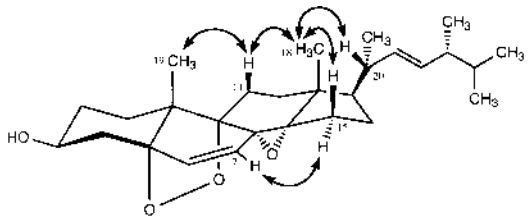

Fig. 2. NOEs Detected for **1**

86.9 (C-9)], and four olefinic methine carbons [δ_C 128.7 (C-7), 132.8 (C-23), 134.7 (C-22), 135.6 (C-6)]. The IR absorption (3340 cm^{-1}) and the chemical shift value of an oxygenated methine [δ_H 4.02 (1H); δ_C 66.1 (CH)] indicated the presence of a secondary hydroxyl group.[2] The chemical shift values of two oxygenated quaternary carbons at δ_C 85.8 and 86.9 indicated the presence of an epidioxy group.[2i] There were eight degrees of unsaturation in the molecule according to the molecular formula. One epidioxy group, two disubstituted double bonds, and the steroid nucleus accounted for seven of those. The remaining degree of unsaturation was assumed to be an epoxy group on the basis of the ^{13}C-NMR data [δ_C 63.8 (C-8), 75.2 (C-14)].[10] The ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum of **1** implied connectivities for H_2-1–H_2-2, H_2-2–H-3, H-3–H_2-4, H-6–H-7, H_2-11–H_2-12, H_2-15–H_2-16 and H-22–H-23 (Fig. 1). Interpretation of the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum revealed correlations from H-6 to C-4, C-5, C-8, and C-10; H-7 to C-9; H-15α to C-8 and C-14; H-15β to C-14 and C-17; 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-epidioxy-8,14-epoxyergosta-6,22-dien-3-ol. The chemical shift value and the multiplicity of the hydroxy-bearing methine proton at C-3 [δ_H 4.02 (1H, m, $W_{1/2}$ 21 Hz)] were those normally seen for 3β-hydroxy-5α-oxygenated A/B *trans* sterols.[2,11] These data indicate that the epidioxy group was assigned to be 5α and 9α. In the nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum, cross-peaks were observed between the H-7 and H-15α; H-11β and H_3-18; H-11β and H_3-19; and H-15β and H_3-18. A Dreiding model showed that an 8α,14α-epoxy group was the only possible structure that could account for these NOEs (Fig. 2). The 17β-orientation of the side chain was disclosed by NOESY cross-peak H_3-18/H-20β (Fig. 2). The geometry of the Δ^6-double bond was deduced to be *Z* from the ^{1}H–^{1}H coupling constant (*J*=9.5 Hz) between H-6 and H-7. The *E* configuration of the Δ^{22}-double bond was also shown by the ^{1}H–^{1}H coupling constant (*J*=15.4 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 the ^{1}H- and ^{13}C-NMR data with those of ergosterol.[2f,12] Thus the structure of **1** was determined to be 5α,9α-epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol.

Compound **2** was isolated as a colorless amorphous solid, $[\alpha]_D$ +28.6°. The molecular formula was determined to be $C_{28}H_{46}O_3$ by HR-MS, indicating six degrees of unsaturation. The IR spectrum showed the presence of a hydroxyl group (3600 cm^{-1}) and α,β-unsaturated ketone (1671 cm^{-1}). The UV spectrum also suggested the presence of α,β-unsaturated ketone (λ_{max}=246 nm). The electron ionization (EI)-MS gave fragment ion peaks at *m/z* 394 (M^+-2H_2O), 267 (M^+-2H_2O−side chain) and 225 (M^+-2H_2O and ring D fission). This suggested that **2** was a C_{28} sterol containing two hydroxyl groups, a conventional saturated steroidal C_9-side chain, and a di-unsaturated skeleton. The ^{1}H-NMR spectrum of **2** was virtually identical to that of **4** except for the side chain. The structure of the side chain was determined to be a (24*S*)-24-methylsterol side chain by comparison of the ^{1}H-NMR data with that of 22-dihydroergosterol.[2f,12] From the above data, the structure of **2** was determined to be 3β,5α-dihydroxyergost-7-en-6-one.

In conclusion, we described here the isolation and structural elucidation of two new sterols, 5α,9α-epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol (**1**) and 3β,5α-dihydroxyergost-7-en-6-one (**2**), from the two edible mushrooms *P. eryngii* (compounds **1** and **2**) and *P. serotinus* (compound **1**). Compound **1** is the first example of a naturally occurring 5α,9α-epidioxy-8α,14α-epoxy-3β-hydroxy-6-ene sterol. Compound **2** has a 3β,5α-dihydroxy-7-en-6-one steroid nucleus. The isolation of a sterol with the 3β,5α-dihydroxy-7-en-6-one moiety from an edible mushroom is rather unusual, and only one sterol, 3β,5α-dihydroxy-(22*E*)-ergosta-7,22-dien-6-one (**4**), has so far been reported from *Grifola frondosa*,[2a] *Pleurotus ostreatus*,[2b] *Lactarius piperatus*,[2e] and *Tricholoma matsutake*.[2g]

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. ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM-LA 600 (600 and 150 MHz, respectively) spectrometer. 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, CCPS; detector, RI-8020) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh). HPLC conditions: mobile phase, MeOH–H_2O (9 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C.

Materials *P. eryngii* (cultivated fruit bodies from Sendai City, Miyagi Prefecture, Japan) and *P. serotinus* (natural fruit bodies from Morioka City, Iwate Prefecture, Japan) were purchased in a food market.

Extraction and Isolation *P. eryngii*: The fresh fruit bodies of *P. eryngii* (2.0 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (4.7 g) was chromatographed on a silica-gel column using *n*-hexane–EtOAc (7 : 3—1 : 7), EtOAc, and MeOH to afford 37 fractions (frs. 1—37). Fraction 7 was purified by preparative HPLC to give **1**

(1.4 mg), **5** (0.2 mg), and **7** (4.4 mg). Fraction 15 was purified by preparative HPLC to give **2** (0.4 mg), **3** (0.3 mg), **4** (2.6 mg), and **6** (1.7 mg). Fraction 18 was purified by preparative HPLC to give **8** (3.9 mg).

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 11 was purified by preparative HPLC to give **1** (0.6 mg).

All known compounds (**3**—**8**) were identified by comparison of their chromatographic behavior, and their MS and ^{1}H-NMR data with those of the authentic samples (**4**—**8**) or by comparison of their physical data with reported values (**3**).

5α,9α-Epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol (**1**): Colorless amorphous solid. $[\alpha]_D^{19}$ −33.9° (*c*=0.06, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3340. HR-MS *m*/*z*: 442.3061 (M^+, Calcd for $C_{28}H_{42}O_4$: 442.3083). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 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*=7.0 Hz, H_3-28), 0.93 (3H, s, H_3-18), 1.00 (3H, d, *J*=6.6 Hz, H_3-21), 1.17 (3H, s, H_3-19), 1.38 (1H, ddd, *J*=13.9, 3.7, 3.3 Hz, H-1β), 1.45—1.60 (8H, m, H-2a, H-11α, H_2-12, H_2-16, H-17, H-25), 1.67 (1H, dd, *J*=14.3, 11.4 Hz, H-4β), 1.68 (1H, ddd, *J*=15.0, 15.0, 4.8 Hz, H-15β), 1.73 (1H, m, H-11β), 1.86 (1H, m, H-24), 1.91 (1H, ddd, *J*=13.9, 13.9, 3.3 Hz, H-1α), 1.96 (1H, m, H-2b), 2.01 (1H, ddd, *J*=15.0, 9.2, 9.2 Hz, H-15α), 2.13 (1H, m, H-20β), 2.23 (1H, ddd, *J*=14.3, 4.8, 1.8 Hz, H-4α), 4.02 (1H, m, H-3), 5.17 (1H, dd, *J*=15.4, 8.4 Hz, H-22), 5.24 (1H, dd, *J*=15.4, 7.7 Hz, H-23), 5.56 (1H, d, *J*=9.5 Hz, H-7), 5.89 (1H, d, *J*=9.5 Hz, H-6). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.5 (C-18 or C-19), 15.6 (C-18 or C-19), 17.6 (C-28), 19.7 (C-26), 19.8 (C-11), 20.0 (C-27), 21.0 (C-21), 26.5 (C-16), 27.2 (C-15), 27.6 (C-1), 30.8 (C-2), 33.1 (C-25), 33.3 (C-12), 35.6 (C-4), 39.2 (C-20), 40.3 (C-13), 42.9 (C-24), 50.5 (C-10), 55.7 (C-17), 63.8 (C-8), 66.1 (C-3), 75.2 (C-14), 85.8 (C-5), 86.9 (C-9), 128.7 (C-7), 132.8 (C-23), 134.7 (C-22), 135.6 (C-6).

3β,5α-Dihydroxyergost-7-en-6-one (**2**): Colorless amorphous solid. $[\alpha]_D^{30}$ +28.6° (*c*=0.04, MeOH). IR ν_{max} $CHCl_3$ cm^{-1}: 3600, 1671. UV λ_{max} MeOH nm (log ε): 246 (3.7). HR-MS *m*/*z*: 430.3476 (M^+, Calcd for $C_{28}H_{46}O_3$: 430.3447). EI-MS *m*/*z* (%): 430 (M^+, 17), 394 (100), 267 (6), 225 (5). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.60 (3H, s, H_3-18), 0.78 (3H, d, *J*=7.0 Hz, H_3-28), 0.79 (3H, d, *J*=6.6 Hz, H_3-27), 0.86 (3H, d, *J*=7.0 Hz, H_3-26), 0.94 (3H, d, *J*=6.2 Hz, H_3-21), 0.95 (3H, s, H_3-19), 2.51 (1H, m, H-14), 4.03 (1H, m, H-3), 5.66 (1H, br s, H-7).

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References and Notes

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