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Sterol Constituents from the Sclerotium of *Polyporus umbellatus* Fries

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From the sclerotium of *Polyporus umbellatus* Fries (Polyporaceae), 9α-hydroxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone (**1**) and ergosta-7,22-diene-3β,5α,6β-triol (**2**) were isolated. This is the first report on the natural occurrence of **1**. The structures of these compounds were elucidated by spectroscopy.

Keywords——*Polyporus umbellatus*; Polyporaceae; sclerotium; sterol

The sclerotium of *Polyporus umbellatus* Fries (Cho-rei in Japanese, Polyporaceae) is used as a diuretic in Chinese medicine,[1] in which ergosterol,[2] ergosterol peroxide,[2] ergosta-7,22-dien-3-ol,[2] ergosta-7,22-dien-3-one,[2] ergosta-4,6,8(14),22-tetraen-3-one,[3] 2-hydroxytetracosanoic acid[4] and glucan[5] have been identified. In this paper, we report the isolation and identification of two compounds, 9α-hydroxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone (**1**) and ergosta-7,22-diene-3β,5α,6β-triol (**2**) from the material.

1 was isolated as colorless oil, $[\alpha]_D$ +100.8°. The molecular formula was determined to be $C_{21}H_{32}O_3$ by high-resolution (HR)-MS. The IR spectrum suggested the presence of hydroxyl group (3567 cm^{-1}) and α, β-unsaturated-γ-lactone (1752 cm^{-1}). The UV spectrum also suggested the presence of α,β-unsaturated-γ-lactone (λ_{max}=222 nm). The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a tertiary methyl group [δ_H 0.61 (s, H-18), δ_C 11.8 (C-18)], a (24*R*)-methyl-Δ^{22}-sterol side chain [δ_H 0.83 (d, J=6.6 Hz, H-26), 0.84 (d, J=6.6 Hz, H-27), 0.92 (d, J=6.6 Hz, H-28), 1.04 (d, J=6.6 Hz, H-21), 5.16 (dd, J=15.2, 7.9 Hz, H-22), 5.26 (dd, J=15.2, 7.3 Hz, H-23), δ_C 17.6 (C-28), 19.7 (C-27), 20.0 (C-26), 21.0 (C-21), 33.1 (C-25), 40.1 (C-20), 42.9 (C-24), 132.9 (C-23), 134.6 (C-22)],[6] a methylene [δ_H 2.28 (ddd, J=13.2, 4.0, 2.3 Hz, Heq-11), δ_C 35.1 (C-11)], a methine [δ_H 2.64 (ddd, J=9.6, 6.6, 1.7 Hz, H-14), δ_C 50.3 (C-14)], an olefin [δ_H 5.64 (d, J=1.7 Hz, H-7), δ_C 112.4 (C-7), 170.4 (C-8)], a hemi-ketal carbon [δ_C 104.6 (C-9)] and a carbonyl carbon [δ_C 170.4 (C-6)]. The electron ionization (EI)-MS gave fragment ion peaks at m/z 314 (M^+-H_2O), 271 (M^+-H_2O−isopropyl) and 187 (M^+-H_2O−side chain−H_2). These spectral data and molecular formula suggested that **1** has most likely the structure illustrated in Fig. 2. The structure of **1** was further confirmed by the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum. The olefinic proton (H-7) showed connectivity to C-6, C-8 and C-9, the tertiary methyl protons (H-18) to C-12, C-13, C-14 and C-17, and the secondary methyl protons (H-21) to C-17, C-20 and C-22 (Fig. 1). The results of the optical rotation and mass and ^{1}H- and ^{13}C-NMR spectra were in accord with those of 9α-hydroxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone. This compound was recently synthesized as an intermediate in the synthesis of 9α-methoxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone (**3**), which was isolated from the sponge *Dictyonella incisa*,[6a] by Riccardis *et al.*[7] Accordingly, **1** was determined to be as shown in Fig. 2. Riccardis *et al.*[7] pointed out that **1** should be the true natural product in the sponge and that **3** should be an artifact produced during the extraction with methanol. However, no paper has reported about isolation of **1** from natural sources before.

2 was isolated as amorphous powder, $[\alpha]_D$ −28.6°. The molecular formula was determined to be $C_{28}H_{46}O_3$ by HR-MS [m/z 412 (M^+-H_2O)]. The IR spectrum suggested the presence of hydroxyl group (3417 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra were in accord with those of ergosta-7,22-diene-3β,5α,6β-triol.[8] Thus, **2** was determined to be as shown in Fig. 2. This is the first report of the isolation of **2** from *Polyporus umbellatus*. Further studies on other constituents of *P. umbellatus* are under way.

EXPERIMENTAL

General procedures Optical rotations were determined with a JASCO DIP-360 digital polarimeter, IR spectra 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 a JEOL JNM-EX-270 (270 and 67.8 MHz, respectively) spectrometer. Chemical shifts were

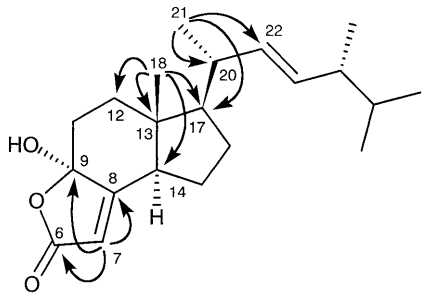

Fig. 1. Long-Range Correlations Detected by HMBC of **1**

given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; br s, broad singlet; d, doublet; dd, double doublet; ddd, double 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) and preparative HPLC on a Tosoh HPLC system (pump, CCPM; detector, UV-8000) using a TSK gel silica-60 column (Tosoh).

Extraction and isolation The dried sclerotium of *Polyporus umbellatus* used for this study was purchased from Uchida Wakanyaku Co., Ltd. in 1992. The dried sclerotium of *P. umbellatus* (3.0 kg) was extracted with MeOH (×3) under reflux for 8 h. The MeOH extract was concentrated under reduced pressure and the residue was extracted, successively, with *n*-hexane, $CHCl_3$, Et_2O, AcOEt, EtOH and H_2O. The *n*-hexane-soluble fraction was concentrated under reduced pressure to gave a residue (3.2 g). This residue was subjected to silica-gel column chromatography using benzene-AcOEt (7 : 3) to give 23 fractions (frs. 1-23). Fraction 3 was rechromatographed on a silica-gel column by using *n*-hexane-AcOEt (8 : 2) to give 27 fractions (frs. 1′-27′). Fraction 27′ was purified by preparative HPLC (column, TSK gel silica-60, 7.8 mm i.d.×30 cm; mobile phase, *n*-hexane-acetone (6 : 1); flow rate, 1.0 ml/min; UV detector, 226 nm) to give **1** (1.2 mg). Fraction 10 (fr. 10) gave **2** (1.1 mg).

9α-Hydroxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone (1) Colorless oil. $[\alpha]_D^{22}$ +100.8° (c =0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3567, 1752. UV λ_{max}^{MeOH} nm (log ε): 222 (3.9). HR-MS m/z: 332.2359 (M^+, Calcd for $C_{21}H_{32}O_3$; 332.2351). EI-MS m/z: 332 (M^+), 314 (M^+-H_2O), 271 (M^+-H_2O−isopropyl), 187 (M^+-H_2O−side chain−H_2). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.61 (3H, s, H-18), 0.83 (3H, d, J=6.6 Hz, H-26), 0.84 (3H, d, J=6.6 Hz, H-27), 0.92 (3H, d, J=6.6 Hz, H-28), 1.04 (3H, d, J=6.6 Hz, H-21), 2.28 (1H, ddd, J=13.2, 4.0, 2.3 Hz, Heq-11), 2.64 (1H, ddd, J=9.6, 6.6, 1.7 Hz, H-14), 5.16 (1H, dd, J=15.2, 7.9 Hz, H-22), 5.26 (1H, dd, J=15.2, 7.3 Hz, H-23), 5.64 (1H, d, J=1.7 Hz, H-7). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ: 11.8 (C-18), 17.6 (C-28), 19.7 (C-27), 20.0 (C-26), 21.0 (C-21), 21.4 (C-15), 28.8 (C-16), 33.1 (C-25), 35.1 (C-11), 35.3 (C-12), 40.1 (C-20), 42.9 (C-24), 48.8 (C-13), 50.3 (C-14), 55.4 (C-17), 104.6 (C-9), 112.4 (C-7), 132.9 (C-23), 134.6 (C-22), 170.4 (C-6, C-8).

Ergosta-7,22-diene-3β,5α,6β-triol (2) Amorphous powder. $[\alpha]_D^{21}$ −28.6° (c=0.1, pyridine). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3417. HR-MS m/z: 412.3318 (M^+-H_2O, Calcd for $C_{28}H_{44}O_2$; 412.3342). EI-MS m/z: 412 (M^+-H_2O), 394 (M^+-2H_2O), 376 (M^+-3H_2O), 361 ($M^+-3H_2O-CH_3$), 251 (M^+-3H_2O−side chain), 209 (M^+-3H_2O and ring D fission). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.60 (3H, s, H-18), 0.82 (3H, d, J=6.6 Hz, H-26), 0.84 (3H, d, J=5.6 Hz, H-27), 0.92 (3H, d, J=6.6 Hz, H-28), 1.03 (3H, d, J=6.6 Hz, H-21), 1.09 (3H, s, H-19), 3.63 (1H, br s, H-6), 4.08 (1H, m, H-3), 5.16 (1H, dd, J=15.2, 7.9 Hz, H-22), 5.23 (1H, dd, J=15.2, 7.3 Hz, H-23), 5.37 (1H, m, H-7). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ: 12.3 (C-18), 17.6 (C-28), 18.9 (C-19), 19.7 (C-27), 20.0 (C-26), 21.1 (C-21), 22.1 (C-15), 22.9 (C-11), 27.9 (C-16), 30.9 (C-1), 33.0 (C-2), 33.1 (C-25), 37.2 (C-10), 39.2 (C-4), 39.5 (C-12), 40.4 (C-20), 42.8 (C-24), 43.5 (C-13), 43.8 (C-9), 54.8 (C-14), 56.0 (C-17), 67.7 (C-3), 73.7 (C-6), 76.0 (C-5), 117.6 (C-7), 132.2 (C-23), 135.4 (C-22), 144.0 (C-8).

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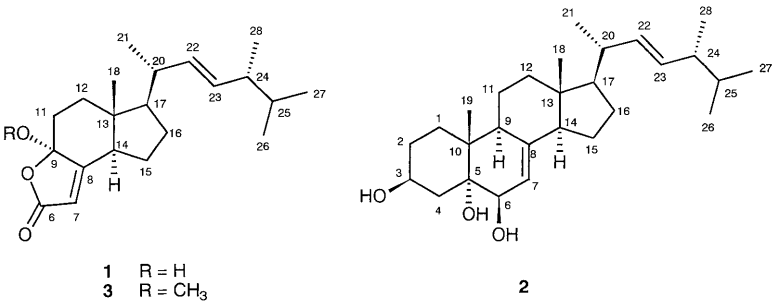

1 R = H
3 R = CH_3

2

Fig. 2.

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