Sterol Constituents from Seven Mushrooms

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Six new sterols, 5α,6α,8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7α-diol (1), 5α,6α,8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7β-diol (2), 5α,6α-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7α-diol (3), (22E,24R)-23-methyl-ergosta-7,22-diene-3β,5α,αβ-triol (4), (22E,24R)-23-methyl-ergosta-7,22-diene-3β,5α,αβ,9α-tetrol (5) and (24S)-ergost-7-ene-3β,5α,6β,9α-tetrol (6), as well as eighteen known ones, 5α,6α-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7α-diol (7), 5α,6α-epoxy-(22E,24R)-ergosta-8-(14),22-diene-3β,7α-diol (8), (22E,24R)-ergost-7,22-diene-3β,5α,αβ,9α-tetrol (9), (22E,24R)-ergost-7,22-diene-3β,5α,αβ,9α-tetrol (10), (22E,24R)-ergost-7-ene-3β,5α,6β,9α-tetrol (11), (24S)-ergost-7-ene-3β,5α,6β,9α-tetrol (12), ergosta-7,24(28)-diene-3β,5α,6β,9α-tetrol (13), (22E,24R)-ergost-7,9(11),22-triene-3β,5α,6β,9α-tetrol (14), (22E,24R)-ergosta-8-(14),22-diene-3β,5α,αβ,9α-tetrol (15), 3β,5α-dihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (16), 3β,5α-trihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (17), 3β,5α,9α-trihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (18), 3β,5α,9α,14α-tetrahydroxy-(22E,24R)-ergosta-7,22-dien-6-one (19), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergosta-6,22-dien-3β-ol (20), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergost-6-en-3β-ol (21), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergosta-6,9(11),22-triene-3β-ol (22), (22E,24R)-ergost-5,9(11),22-tetraen-3β-ol (23) and (22E,24R)-ergosta-7,22-dien-3β-ol (24) from seven mushrooms, Amanita pantherina, Amanita virgineoides, Lactarius piperatus, Lyophyllum shimeji, Tricholoma portentosum, Hypsizigus marmoreus and Lentinula edodes together with eighteen known ones (7–24). The structures of these new compounds were elucidated on the basis of their spectral data.

Key words: sterol; mushroom; structure elucidation.

Recently we reported the isolation and structural elucidation of sterols from six edible mushrooms. In a continuation of our investigation of the sterol constituents of mushrooms, we describe here the isolation and structural elucidation of six new sterols, 5α,6α,8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7α-diol (1), 5α,6α,8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7β-diol (2), 5α,6α-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7α-diol (3), (22E,24R)-23-methyl-ergosta-7,22-diene-3β,5α,αβ-triol (4), (22E,24R)-23-methyl-ergosta-7,22-diene-3β,5α,αβ,9α-tetrol (5) and (24S)-ergost-7-ene-3β,5α,6β,9α-tetrol (6), as well as eighteen known ones, 5α,6α-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7α-diol (7), 5α,6α-epoxy-(22E,24R)-ergosta-8-(14),22-diene-3β,7α-diol (8), (22E,24R)-ergost-7,22-diene-3β,5α,αβ,9α-tetrol (9), (22E,24R)-ergost-7,22-diene-3β,5α,αβ,9α-tetrol (10), (22E,24R)-ergost-7-ene-3β,5α,6β,9α-tetrol (11), (24S)-ergost-7-ene-3β,5α,6β,9α-tetrol (12), ergosta-7,24(28)-diene-3β,5α,6β,9α-tetrol (13), (22E,24R)-ergost-7,9(11),22-triene-3β,5α,6β,9α-tetrol (14), (22E,24R)-ergosta-8-(14),22-diene-3β,5α,αβ,9α-tetrol (15), 3β,5α-dihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (16), 3β,5α-trihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (17), 3β,5α,9α-trihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (18), 3β,5α,9α,14α-tetrahydroxy-(22E,24R)-ergosta-7,22-dien-6-one (19), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergosta-6,22-dien-3β-ol (20), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergost-6-en-3β-ol (21), 5α,8α-epoxy-22,24-epoxy-(22E,24R)-ergosta-6,9(11),22-triene-3β-ol (22), (22E,24R)-ergost-5,9(11),22-tetraen-3β-ol (23) and (22E,24R)-ergosta-7,22-dien-3β-ol (24) from seven mushrooms, Amanita pantherina, Amanita virgineoides, Lactarius piperatus, Lyophyllum shimeji, Tricholoma portentosum, Hypsizigus marmoreus and Lentinula edodes together with eighteen known ones (7–24). This is the first time that compounds 7–24 have been isolated from these mushrooms. Extraction and isolation were carried out as described in the Experimental section.

Compound 1 was isolated as an amorphous powder, [α]D

−53.6°. The molecular formula was determined to be C32H44O3 by high-resolution (HR)-MS. The IR spectrum showed the presence of hydroxyl groups (3420 cm

−1). The 1H-NMR spectrum (Table 1), obtained with the aid of a 1H–1H shift correlation spectroscopy (1H–1H COSY) spectrum, showed signals due to two tertiary methyl groups [δH

6.73 (3H, H-18), 1.33 (3H, H-19)] and four secondary methyl groups [δH

8.01 (3H, H-26), 0.83 (3H, H-27), 0.91 (3H, H-28), 0.90 (3H, H-21)], suggesting that 1 should be an ergostane-type sterol. Other signals in the 1H-NMR spectrum indicated a trisubstituted epoxide-bearing methine hydrogen [δH

3.13 (1H, H-6), 2] two hydroxy-bearing methine hydrogens [δH

3.98 (1H, H-3), 4.03 (1H, H-7)] and two disubstituted olefinic hydrogens [δH

5.14 (1H, H-22), 5.20 (1H, H-23)]. The 13C-NMR spectrum (see Experimental), obtained with the aid of a 1H-detected heteronuclear multiple quantum coherence (HMQC) spectrum, contained 28 signals that included three oxygenated quaternary carbons [δC

65.0 (C-8), 67.1 (C-5), 70.2 (C-9)]. The double-bond equivalents for the molecular formula of 1 is seven, ascribed to one dissubstituted double bond, two epoxide rings and the steroid nucleus. Detailed analysis of the 1H–1H COSY spectrum of 1 implied connectivities for H-6–H7 and H-7–OH-7 (Fig. 1). In the 1H-detected heteronuclear multiple bond connectivity (HMBC) spectra, the C-H long-range correlations observed are shown in Fig. 1. From the above data, the planar structure of 1 was deduced to be 5,6,8,9-diepoxyergost-22-ene-3,7-diol. The stereostructure was determined as follows. In the nuclear Overhauser effect (NOE) difference spectra, an NOE was detected between H-19 and H-7β. This NOE implies that the B-ring of 1 adopts a boat-type conformation, as a result of incorporation of the 5α,6α-epoxide moiety and the hydroxyl group at C-7 has an α-configuration (Fig. 2). Furthermore, an NOE was detected between H-19 and H-21. A Dreiding model showed that an 8α,9α-epoxide ring was the only possible structure which could account for this NOE (Fig. 2). The configuration of the hydroxyl group at C-
3 was determined to be β by comparing the \(^1\)H- and \(^1\)C-NMR data of the C-3 hydroxyl-bearing methine of 1 with those of 5α,6α-epoxy-3β-hydroxy A/B trans sterols.\(^4\) The stereochemistry at C-22 and C-24 in the side chain was determined to be E and R, respectively, by comparison of the \(^1\)H- and \(^1\)C-NMR data with those of authentic ergosterol. Based on this evidence, the structure of 1 was determined to be 5α,6α;8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7α-diol.

Compound 2 was isolated as an amorphous powder, \([\alpha]_D^{17} -17.2^\circ\). The molecular formula was determined to be C\(_{28}\)H\(_{44}\)O\(_4\) by HR-MS. The IR spectrum showed the presence of hydroxyl groups (3396 cm\(^{-1}\)). The \(^1\)H-NMR spectrum of 2 was virtually identical to that of 1 except at the C-7 position. In the \(^1\)H-NMR spectrum, the chemical shifts of the H\(_{1}-18\) and H\(_{1}-19\) methyl groups in pyridine-\(d_5\) were shifted downfield by the pyridine-induced deshielding effect\(^5\) (\(\delta_{C,D,N} - \delta_{\text{CDCl}_3}\); \(\Delta\delta\), H\(_{1}-18\), +0.24 ppm, H\(_{1}-19\), +0.25 ppm). This deshielding effect implies that the hydroxyl group at C-7 has a β configuration (Fig. 2). Thus, 2 was the 7-epimer of 1. From the above data, the structure of 2 was determined to be 5α,6α;8α,9α-diepoxy-(22E,24R)-ergost-22-ene-3β,7β-diol.
5α,6α,8α,9α-Diepoxy-3β,7α-dihydroxy and 5α,6α,8α,9α-Diepoxy-3β,7β-dihydroxy moieties are unprecedented in the natural sterols previously known.

Compound 3 was isolated as an amorphous powder, $\delta_0$, $-37.0^\circ$. The molecular formula was determined to be $C_{29}H_{56}O_3$ by HR-MS. The IR spectrum showed the presence of hydroxyl groups (3414 cm$^{-1}$). The 1H-NMR spectrum showed signals due to two tertiary methyl groups [$\delta_\text{H}$, 0.63 (3H, H-18), 1.28 (3H, H-19)] and four secondary methyl groups [$\delta_\text{H}$, 0.82 (3H, H-26), 0.84 (3H, H-27), 0.91 (3H, H-28), 1.02 (3H, H-21)], suggesting that 3 should also be an ergostane-type sterol. Other signals in the 1H-NMR spectrum indicated a trisubstituted epoxide-bearing methine hydro- 

den [$\delta_\text{H}$, 3.14 (1H, H-6)], two hydroxy-bearing methine hydrogen atoms [$\delta_\text{H}$, 3.93 (1H, H-3), 4.39 (1H, H-7)] and two disubstituted olefinic hydrogens [$\delta_\text{H}$, 5.16 (1H, H-22), 5.23 (1H, H-23)]. The 1C-NMR spectrum contained 28 signals that included a fully substituted double bond [$\delta_\text{C}$, 126.5 (C-8), 137.0 (C-9)]. Comparison of these data with the spectral data for 5 revealed that they were identical except at C-7. In the 1H-NMR spectrum of 3, the chemical shifts of H-18 and H-19 methyl groups in pyridine-$d_5$ were shifted downfield ($\delta_\text{C,OD} \sim \delta_\text{CDCl}_3$; $\Delta\delta$, H-18, +0.22 ppm, H-19, +0.27 ppm), indicating that the hydroxyl group at C-7 has a $\beta$ configuration (Fig. 2). From the above data, the structure of 3 was determined to be $5\alpha,6\alpha$-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7β-diol. Compound 3 is the first example of a naturally occurring $5\alpha,6\alpha$-epoxy-3β,7β-dihydroxy-Δ$^8$-sterol.

Compound 4 was isolated as an amorphous powder, $\delta_0$, $-15.9^\circ$. The molecular formula was determined to be $C_{29}H_{56}O_3$ by HR-MS ($m/z$ 426 (M$^+ - H_2O$)) and 13C-NMR data. The IR spectrum showed the presence of hydroxyl groups (3454 cm$^{-1}$). The 1H-NMR spectrum showed signals due to two tertiary methyl groups [$\delta_\text{H}$, 0.62 (3H, H-18), 1.09 (3H, H-19)], four secondary methyl groups [$\delta_\text{H}$, 0.79 (3H, H-27), 0.85 (3H, H-26), 0.94 (3H, H-28), 0.95 (3H, H-21)], an olefinic methyl group [$\delta_\text{H}$, 1.51 (3H, H-29)], two hydroxyl-bearing methine hydrogens [$\delta_\text{H}$, 3.63 (1H, H-6), 4.08 (1H, H-3)] and two trisubstituted olefinic hydrogens [$\delta_\text{H}$, 4.90 (1H, H-22), 5.36 (1H, H-7)]. Inspection of the spectral data revealed that 4 was identical to 11 except for the side chain. The structure of the side chain was determined by an HMBC spectrum, in which long-range C-H correlations were observed from the H-21 methyl group to C-20 and C-22; the H-26 and H-27 methyl groups to C-24 and C-25; the H-28 methyl group to C-23, C-24 and C-25; and the H-29 methyl group to C-22, C-23 and C-24 confirmed the presence of a 23,24-dimethyl-Δ$^2$-sterol side chain. The stereochemistry at C-22 and C-24 was determined to be $E$ and $R$, respectively, by comparison of the 1H-NMR data reported for $5\alpha,8\alpha$-epidioxy-(22E,24R)-23-methylergosta-6,22-dien-3β-ol$^{14}$ and $3\beta,5\alpha,9\alpha$-trihydroxy-(22E,24R)-23-methylergosta-7,22-dien-6-one.$^{14}$ Based on this evidence, the structure of 4 was determined to be (22E,24R)-23-methylergosta-7,22-dien-3β,5α,6β-triol. The isolation of sterols with this side chain from terrestrial sources is rare.$^{14}$

Compound 5 was isolated as an amorphous powder.$^6$ The molecular formula was determined to be $C_{29}H_{56}O_3$ by HR-MS ($m/z$ 442 (M$^+ - H_2O$)). The IR spectrum showed the presence of hydroxyl groups (3488 cm$^{-1}$). The electron ionization (EI)-MS gave fragment ion peaks at $m/z$ 388 (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 $C_{19}$-side chain. The 1H-NMR spectrum of 5 was similar to that of 9 except for the side chain. The structure of the side-chain was determined to be (22E,24R)-23,24-dimethyl-Δ$^2$-sterol side chain by comparison of the 1H-NMR data of compound 4. From the above
data, the structure of 5 was determined to be (22E,24R)-23-methylergosta-7,22-diene-3β,5α,6β,9α-tetrol.

Compound 6 was isolated as an amorphous powder. The molecular formula was determined to be C_{29}H_{44}O_{3} by HR-MS [m/z 430 (M^+ - H2O)]. The IR spectrum showed the presence of hydroxyl groups (3464 cm\(^{-1}\)). The EI-MS gave fragment ion peaks at m/z 376 (M^+ - H2O), 249 (M^+ - 4H2O-side chain) and 207 (M^+ - 4H2O and ring D), indicating that 6 has four hydroxyl groups and a conventional saturated steroidal C_{24} side chain. The 1H-NMR spectrum of 6 was virtually identical to that of 5 except the side chain. The structure of the side chain was determined to be (24S)-24-methylsterol side chain by comparison of the 1H-NMR data of compounds 12, 18 and 21. From the above data, the structure of 6 was determined to be (24S)-ergost-7-ene-3β,5α,6β,9α-tetrol.

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 spectrophotometer. 1H- and 13C-NMR spectra were recorded with 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; br, broad; m, multiplet). The EI- and HR-MS were recorded on a JOEL 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. x 30 cm) column (Tosoh).

Material: The fresh fruit bodies of Amanita pantherina were collected at Sendai City in Miyagi Prefecture, Japan, in September 1997. The fresh fruit bodies of Amanita virgineoides were collected at Sendai City in Miyagi Prefecture, Japan, in July 1997. The fresh fruit bodies of Lactarius piperatus were collected at Sendai City in Miyagi Prefecture, Japan, in July 1997. Lycophyllum shimeji (from Morioka City in Iwate Prefecture, Japan, in October, 1997), Tricholoma portentosum (from Morioka City in Iwate Prefecture, Japan, in October, 1997), Hypsizygus marmoreus (from Nagano City in Nagano Prefecture, Japan) and Lentinula edodes (from Sendai City in Miyagi Prefecture, Japan) were purchased in a food store.

Extraction and Isolation: 1) A. pantherina: The fresh fruit bodies of A. pantherina (0.6 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (7.5 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3−1:7), EtOAc and MeOH, to afford 21 fractions (frs. 1−21). Fraction 4 was purified by preparative HPLC [mobile phase, MeOH; flow rate, 1.0 mL/min; column temperature, 40°C] to give 23 (0.3 mg) and 24 (0.2 mg). Fraction 6 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 20 (0.8 mg), 21 (0.2 mg) and 22 (0.2 mg). Fraction 14 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give a mixture of 10, 13 and 14, 11 (0.5 mg) and 12 (0.6 mg). The mixture of 10, 13 and 14 was purified by preparative HPLC [mobile phase, MeOH-H2O (4:1); flow rate, 1.5 mL/min; column temperature, 40°C] to give 10 (0.1 mg) and inseparable mixture of 13 and 14 (0.2 mg). Fraction 16 was purified by preparative HPLC [mobile phase, MeOH; flow rate, 1.0 mL/min; column temperature, 40°C] to give a mixture of 1 and 9. The mixture of 1 and 9 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 1 (0.6 mg) and 9 (0.6 mg).

2) A. virgineoides: The fresh fruit bodies of A. virgineoides (0.4 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (1.5 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3−1:7), EtOAc and MeOH, to afford 22 fractions (frs. 1−22). Fraction 12 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 17 (0.4 mg). Fraction 18 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 10 (0.4 mg), 11 (7.2 mg) and 12 (0.8 mg). Fraction 20 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 1 (0.5 mg), 6 (0.1 mg), 9 (1.7 mg) and 15 (0.5 mg).

3) L. piperatus: The fresh fruit bodies of L. piperatus (0.1 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (1.7 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3−1:7), EtOAc and MeOH, to afford 24 fractions (frs. 1−24). Fraction 12 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 16 (0.9 mg). Fraction 14 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 17 (0.2 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 1 (0.8 mg), 2 (0.4 mg), 9 (0.4 mg), 10 (0.5 mg) and 11 (1.3 mg).

4) L. shimeji: The fresh fruit bodies of L. shimeji (0.6 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (2.3 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3−1:7), EtOAc and MeOH, to afford 26 fractions (frs. 1−26). Fraction 15 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 17 (2.2 mg), 18 (0.3 mg) and 19 (0.5 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 1 (0.3 mg) and 9 (0.4 mg).

5) T. portentosum: The fresh fruit bodies of T. portentosum (0.5 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (2.7 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3−1:7), EtOAc and MeOH, to afford 28 fractions (frs. 1−28). Fraction 7 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 20 (1.0 mg). Fraction 13 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 8 (0.4 mg), 17 (1.4 mg) and 19 (0.5 mg). Fraction 16 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 3 (0.5 mg) and 7 (1.2 mg). Fraction 19 was purified by preparative HPLC [mobile phase, MeOH-H2O (9:1); flow rate, 1.0 mL/min; column temperature, 40°C] to give 1 (0.4 mg), 9 (1.7 mg),...
10 (0.3 mg), 11 (1.9 mg) and 12 (0.2 mg).

6) *H. marmoratus*: The fresh fruit bodies of *H. marmoratus* (4.3 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (10.5 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3:1-7), EtOAc and MeOH, to afford 19 fractions (frs. 1—19). Fraction 13 was purified by preparative HPLC [mobile phase, MeOH-H₂O (4:1); flow rate, 1.5 ml/min; column temperature, 40 °C] to give 1 (0.6 mg) and 2 (0.6 mg).

7) *L. edodes*: The fresh fruit bodies of *L. edodes* (4.7 kg) were extracted three times with EtOH at room temperature for 2 weeks. The EtOH extract (7.1 g) was chromatographed on a silica-gel column using n-hexane-EtOAc (7:3:1-7), EtOAc and MeOH, to afford 25 fractions (frs. 1—25). Fraction 17 was purified by preparative HPLC [mobile phase, MeOH-H₂O (9:1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give 4 (0.6 mg) and 12 (0.2 mg). Fraction 22 was purified by preparative HPLC [mobile phase, MeOH-H₂O (4:1); flow rate, 1.5 ml/min; column temperature, 40 °C] to give 1 (0.3 mg) and 5 (0.2 mg).

All known compounds (7—24) were identified by comparison of their chromatographic behaviour, and MS and 1H-NMR data with those of the authentic samples.

5a,6a,5α,6α-Diepoxy-(22E,24R)-ergost-22-ene-3β,5α,6β-triol (1): Amorphous powder. [α]D20° = −53.6° (c = 0.06, CHCl₃). IR νmax (CHCl₃) cm⁻¹: 3420. HR-MS m/z: 442.3217 (M⁺, Calc. for C₂₃H₃₆O₄; 442.3240). 1H-NMR (600 MHz, CDCl₃): see Table 1. 13C-NMR (150 MHz, CDCl₃) δ: 12.4 (C-18), 17.7 (C-28), 19.6 (C-26), 20.0 (C-20), 21.2 (C-21), 21.7 (C-19), 22.2 (C-11), 23.2 (C-15), 27.9 (C-1), 28.0 (C-16), 30.3 (C-3), 32.5 (C-12), 33.1 (C-25), 35.4 (C-10), 40.3 (C-20), 40.6 (C-4, C-13), 42.8 (C-24), 52.3 (C-17), 52.9 (C-14), 60.7 (C-6), 65.0 (C-8), 66.4 (C-7), 67.1 (C-5), 68.4 (C-3), 70.2 (C-9), 132.2 (C-23), 135.2 (C-22).

5a,6a,5α,6α-Diepoxy-(22E,24R)-ergost-22-ene-3β,7β-diol (2): Amorphous powder. [α]D20° = −17.2° (c = 0.06, CHCl₃). IR νmax (CHCl₃) cm⁻¹: 3369. HR-MS m/z: 444.3261 (M⁺, Calc. for C₂₃H₃₆O₄; 444.3240). 1H-NMR (600 MHz, CDCl₃): see Table 1. (600 MHz, CDCl₃) δ: 0.84 (6H, d, J = 7.0 Hz, H₂-26, 27), 0.92 (3H, d, J = 7.0 Hz, H₂-28), 0.97 (3H, s, H₁-18), 1.05 (3H, d, J = 6.6 Hz, H₂-21), 1.68 (3H, s, H₁-19), 2.61 (1H, d, J = 12.8, 11.4 Hz, H-4β), 3.28 (1H, d, J = 2.9 Hz, H-6), 4.42 (1H, m, H-3), 4.79 (1H, dd, J = 6.2, 2.9 Hz, H-7), 5.22 (2H, m, H₂-22, 23). 13C-NMR (150 MHz, CDCl₃) δ: 12.3 (C-18), 17.6 (C-28), 19.6 (C-26), 20.0 (C-20), 21.1 (C-21), 21.3 (C-11, C-19), 21.6 (C-15), 28.5 (C-1, C-16), 30.3 (C-2), 33.1 (C-25), 33.3 (C-12), 35.7 (C-10), 40.4 (C-13, C-20), 40.5 (C-4), 42.8 (C-24), 53.0 (C-17), 54.5 (C-14), 59.3 (C-6), 61.3 (C-8), 62.5 (C-5), 65.6 (C-7), 68.4 (C-3), 66.9 (C-9), 132.3 (C-23), 135.3 (C-22).

5α,6α-Epoxy-(22E,24R)-ergost-8,22-diene-3β,7β-diol (3): Amorphous powder. [α]D20° = −37.0° (c = 0.05, CHCl₃). IR νmax (CHCl₃) cm⁻¹: 3414. HR-MS m/z: 428.3315 (M⁺, Calc. for C₂₃H₃₆O₄; 428.3290). 1H-NMR (400 MHz, CDCl₃): see Table 1. (400 MHz, CDCl₃) δ: 0.85 (3H, s, H₁-18), 0.85 (6H, d, J = 6.8 Hz, H₂-26, 27), 0.94 (3H, d, J = 6.9 Hz, H₂-28), 1.08 (3H, d, J = 6.8 Hz, H₂-21), 1.55 (3H, s, H₁-19), 2.60 (1H, d, J = 12.5, 11.5 Hz, H-4β), 3.46 (1H, d, J = 2.8 Hz, H-6), 4.38 (1H, m, H-3), 4.74 (1H, m, H-7), 5.23 (2H, m, H₂-22, 23). 13C-NMR (100 MHz, CDCl₃) δ: 11.5 (C-18), 17.6 (C-28), 19.6 (C-26), 19.9 (C-27), 21.0 (C-21), 22.9 (C-19), 23.0 (C-11), 23.6 (C-15), 29.2 (C-16), 29.7 (C-2), 30.8 (C-1), 33.1 (C-25), 36.2 (C-12), 37.9 (C-10), 39.0 (C-4), 40.5 (C-20), 41.9 (C-13), 42.8 (C-24), 51.1 (C-14), 54.3 (C-17), 60.1 (C-6), 63.2 (C-5), 67.0 (C-7), 68.6 (C-3), 126.5 (C-8), 132.2 (C-23), 135.5 (C-22), 137.0 (C-9).

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References and Notes
6) The size of the available sample did not allow us to obtain the optical rotation and 13C-NMR spectrum.