Photochemical Reaction of Ergosta-4,6,8(14),22-tetraen-3-one

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Chemical properties of ergosta-4,6,8(14),22-tetraen-3-one (1) were investigated. Though 1 is rather stable to acids or bases, it reacts easily with two molecules of oxygen on irradiation with UV light to give 6x,9x-epipodroxy-14x-hydroperoxyergosta-4,7,22-trien-3-one (2), which is transformed successively to 6x,7x;8x,9x-diepoxo-14x-hydroperoxyergosta-4,22-dien-3-one (3) and 14x-hydroperoxy-9x-hydroxyergosta-4,7,22-triene-3,6-dione (4) under these reaction conditions.

Key words ergosta-4,6,8(14),22-tetraen-3-one; photooxidation; fungal metabolite; 6x,9x-epipodroxy-14x-hydroperoxyergosta-4,7,22-trien-3-one; 6x,7x;8x,9x-diepoxy-14x-hydroperoxyergosta-4,22-dien-3-one; 14x-hydroperoxy-9x-hydroxyergosta-4,7,22-triene-3,6-dione

Ergosta-4,6,8(14),22-tetraen-3-one (1) is a fungal metabolite derived from ergosterol.1) It shows blue fluorescence on irradiation with long-wavelength UV light, so it can be identified easily by TLC. We found that all the fruit bodies of more than 100 fungal species showed the presence of 1 on TLC (unpublished data). Thus, 1 is a common component of fungi, and, if 1 is found in a higher plant, the possibility of fungal infection must be considered. Recently, 1 was identified from a sponge, Dysidea herbacea, together with other 3-oxo-4,6,8(14)-trisaturated sterols.2) In this paper, we describe the chemical properties of 1 as a basis for future studies on its physiological role.

Though ergosta-4,6,8(14),22-tetraen-3-one (1) has a conjugated ketone, it is rather stable under acid or alkaline conditions, such as 1% p-toluenesulfonic acid in CHCl₃, 1% H₂SO₄ in MeOH and 1% MeONa in MeOH. On the other hand, when 1 was exposed to UV light, even sunlight, it decomposed to a considerable extent. Therefore, our attention was focused on the photochemical reaction of 1.

A solution of 1 (200 mg) in CHCl₃ (50 ml) was stirred at room temperature under irradiation with UV light (100 W high-pressure mercury arc) through Pyrex for 5 h.3) The reaction was monitored by HPLC. Time courses of the starting compound, 1, and main products, 2, 3 and 4, are shown in Fig. 1. It seems that the first product was compound 2, which was then degraded to 3 and 4. To determine the structures, the products, 2 (46 mg), 3 (16 mg) and 4 (13 mg), were isolated by column chromatography on silica gel.

Compound 2, colorless needles from n-hexane, mp 179-183℃, [x]D +126° (c=0.5, CHCl₃), was formulated as C₂₃H₄₁O₇ from the high-resolution electron impact mass spectrum (HR-EI-MS). The UV spectrum showed the absorption maximum at 245 nm (log ε 4.12), indicating the presence of an αβ-unsaturated ketone. The 13C-NMR spectrum showed the presence of the same side chain as in 1, an αβ-unsaturated ketone [198.7 (C), 124.1 (CH), 161.5 (C)], one more double bond [130.7 (CH), 141.9 (C)], and three oxygenated carbons [76.7 (CH), 84.2 (C), 95.6 (C)]. By 13C-1H, 1H-1H and long-range 13C-1H correlation spectroscopy (COSY), the plane structure was deduced to be as shown in Fig. 2. Considering the molecular formula, the possible structures of 2 were limited to 2a and 2b in Fig. 2. Judging from the fact that an intense cross peak was observed between the methyl proton signal of C-18 and that of C-19 in nuclear Overhauser effect correlation spectroscopy (NOESY), only the structure 2a where the peroxide ring is linked from the z-side is acceptable (Fig. 3). Therefore, the position of the hydroperoxy group was also determined to be at C-14, with the configuration remaining to be confirmed. For this purpose, the 1H-NMR spectrum was measured in C₆H₁₂N and compared with that in CDCl₃. As the hydroperoxy group forms a hydrogen bond with the nitrogen atom of pyridine, pyridine-induced shifts are expected to be observed around it.4) In fact, the signals

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of H-12\(\alpha\), H-15\(\alpha\) and H-17\(\alpha\) showed downfield shifts of 0.19, 0.42 and 0.23 ppm, respectively, indicating a \(\alpha\)-orientation of the hydroperoxy group, while H-12\(\beta\) and H-15\(\beta\), of which the configuration was confirmed by NOE correlation with the signals of H\(\alpha\)-19 and H\(\alpha\)-18, respectively, showed shifts of 0.02 and 0.07 ppm. Thus, the structure of 2 was determined as 6\(\alpha\),9\(\alpha\)-epidioxy-14\(\alpha\)-hydroperoxyergosta-4,7,22-trien-3-one.

Compound 3, colorless needles, mp 196—199 °C, [\(\alpha\)]\(D\) +66° (c=0.5, THF), was formulated as C\(_{28}\)H\(_{46}\)O\(_5\) by elemental analysis and a signal count in the \(^{13}\)C-NMR spectrum (6CH\(_3\)+6CH\(_2\)+9CH+7C). The UV spectrum \(\lambda_{max}\) (MeOH): 230 nm (log \(\varepsilon\) 4.12) showed the absorption of an \(\alpha\beta\)-unsaturated ketone. The \(^{13}\)C-NMR spectrum showed the presence of the same side chain as in 2 and another \(\alpha\beta\)-unsaturated ketone [196.7 (C), 133.5 (CH), 156.7 (C)]. Among the remaining signals, those at \(\delta\) 93.0 (C), 67.9 (C), 59.6 (C), 49.1 (CH) and 46.5 (CH) were assigned to oxygenated carbons. Though the signals at \(\delta\) 49.1 and 46.5 appear at rather higher field than those of usual oxygenated carbons, the chemical shifts of the correlated proton signals [\(\delta\) 3.50 (1H, dd, \(J=3.7, 0.6\) Hz) and 3.59 (1H, d, \(J=3.7\) Hz), respectively] supported the presence of the oxygen functions. Based on \(^{13}\)C-\(^{1}H\), \(^{13}\)C-\(^{13}\)C-COSY and long-range \(^{13}\)C-\(^{13}\)C-COSY, the structure was considered to be a 6,7,8,9,14-tetraoxynated 4-en-3-one. Considering the molecular formula and the chemical shifts, two epoxy rings at C-6 to C-7 and C-8 to C-9 and a hydroxyperoxy group at C-14 were deduced. As the signals of H-6 (\(\delta\) 5.20) and H-7 (\(\delta\) 3.59) were correlated with the methyl signal of C-19 (\(\delta\) 1.37) and C-18 (\(\delta\) 0.87), respectively, in the NOESY spectrum, the \(\alpha\)-configuration of the epoxy ring at C-6 and C-7 was determined. The correlation of the H-12\(\beta\) signal (\(\delta\) 1.95) with the H-19 signal (\(\delta\) 1.37) in the NOESY spectrum indicated the \(\alpha\)-configuration of the other epoxy ring. As shown in Fig. 1, 3 was formed from 2, and so the hydroperoxy group was expected to exist in \(\alpha\)-configuration. Thus, the structure of 3 was determined as 6\(\alpha\),7\(\alpha\),8\(\alpha\),9\(\alpha\)-diepoxide-14\(\alpha\)-hydroperoxyergosta-4,22-dien-3-one.

Compound 4, colorless needles from benzene, mp 182—185 °C, [\(\alpha\)]\(D\) -112° (c=0.25, EtOH), was formulated as C\(_{28}\)H\(_{46}\)O\(_5\) from the HR-ESI-MS. The \(^{13}\)C-NMR spectrum showed the presence of the same side chain as in 2, two \(\alpha\beta\)-unsaturated ketones [\(\delta\) 199.4 (C), 134.7 (CH), 154.7 (C), 186.9 (C), 133.0 (CH), 157.1 (C)] and two oxygenated carbons [\(\delta\) 897.3 (C), 73.6 (C)]. Considering the chemical shifts and the molecular formula, the oxygen functions were assigned as a hydroperoxy group and a hydroxy group. The photochemical rearrangement of dience endoperoxides has been reported to produce a bisepoxide and a ketoepoxide, of which the
latter is unstable and changes to a keto alcohol. As the structure of 3 is corresponds to the diepoxide from 2, 4 should be assigned as the corresponding keto alcohol, 14z-hydroperoxy-9z-hydroxyergosta-4,7,22-triene-3,6-dione. This structure was also supported by the long-range $^{13}$C-$^1$H COSY.

Based on the structures of the main products, the reaction was considered to proceed as follows. On irradiation with UV light, 1 acts as a sensitizer and also as a starting material. The excited singlet oxygen causes ene-reaction on the C-ring of 1 to produce an intermediate endo-diene hydroperoxide (intermediate A in Chart 1), which reacts immediately with one more oxygen to provide 2. Then, the rearrangement of 2 provides 3 and 4. The participation of the excited singlet oxygen in the first reaction was proved by the fact that the reaction was quenched by addition of 1,4-diazabicyclo[2.2.2]octane, a typical quencher of singlet excited oxygen atoms.

These photochemical properties of 1 may be linked to the biological role of 1, which has not yet been established. As described for other hydroperoxides, 3, 4, 2 as well as the singlet oxygen should be harmful to organisms. This photositoxicity may be important in the biological role of 1 in nature.

**Experimental**

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic polarimeter. The $^{13}$C-NMR spectra were measured with a JEOL GSX-500 spectrometer. The $^1$H-NMR spectra were measured with a JEOL GSX-500 (multiplicity, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet). UV spectra were recorded on a Hitachi 323 spectrometer and IR spectra on a Shimadzu IR-460 spectrometer. Mass spectra were measured with a JEOL SX-102 spectrometer. HPLC was run on a Shimadzu LC-9A apparatus with a UV detector (Shimadzu SPD-6A).

**Ergosta-4,6,8(14),22-tetraen-3-one (1)** A mixture of ergosterol (5 g) and 2,3-dichloro-5,6-dicyano-p-benzoquinone (5 g) in benzene (100 ml) was heated under reflux for 1 h. After cooling, the mixture was filtered, and the filtrate was washed with 5% NaSO$_4$, 5% NaOH, and water, then dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was chromatographed on alumina using benzene-CHCl$_3$ to obtain 1 (1.5 g). Pale yellow plates from n-hexane, mp 119-122°C. IR (KBr) $\nu_{max}$ cm$^{-1}$: 2055, 2850, 1665, 1585, 1460, 970. UV (EtOH) $\lambda_{max}$: 348 (log e 4.47). $^1$H-NMR (CDCl$_3$) $\delta$: 6.61 (1H, d, $J$ = 9.5 Hz, H-7), 6.03 (1H, d, $J$ = 9.5 Hz, H-6), 5.74 (1H, s, H-4), 5.46 (1H, dd, $J$ = 15.3, 7.3 Hz, H-23), 5.20 (1H, dd, $J$ = 15.3, 7.9 Hz, H-22), 1.06 (3H, d, $J$ = 6.7 Hz, H$_3$-21), 1.00 (3H, s, H$_3$-19), 0.96 (3H, s, H$_3$-18), 0.93 (3H, d, $J$ = 7.0 Hz, H$_3$-28), 0.85 (3H, d, $J$ = 7.0 Hz, H$_3$-27), 0.83 (3H, d, $J$ = 6.7 Hz, H$_3$-20). EI-MS m/z: 392 (M$^+$), 268, 253.

**Photoreaction of 1** A solution of 1 (200 mg) in CHCl$_3$ (50 ml) was stirred under irradiation with UV light (100 W high-pressure mercury arc) through Pyrex at room temperature. The reaction mixture was sampled every 30 min for HPLC examination. The conditions of the HPLC were as follows: column, Tosoh SILICA-60 (150 x 4.6 mm I.P.); mobile phase, 10% n-hexane in CHCl$_3$; flow rate, 1 ml/min; detection, 254 nm; injection volume, 5 µl. The amounts of the products were determined from calibration curves prepared with authentic samples. After 5 h, the reaction was stopped and the CHCl$_3$ was evaporated off.
under reduced pressure. The residue was chromatographed on silica gel using n-hexane and CHCl₃ to obtain 2 (46 mg), 3 (16 mg) and 4 (14 mg).

6a,9a-Epideoxy-14a-hydroperoxyergosta-4,7,22-trien-3-one (2)
Colorless needles from n-hexane, mp 179–183°C, [α]D 0 +126° (c = 0.5, CHCl₃). IR (KBr) νmax cm⁻¹: 3440, 2955, 1677, 1457, 1368, 1226, 1139, 972, 861. UV (EtOH) λmax nm (log ε): 245 nm (4.12). 1H-NMR (CDCl₃) δ: 6.83 (1H, d, J = 6.1 Hz, H-7), 5.97 (1H, s, H-4), 5.27 (1H, dd, J = 15.3, 7.6 Hz, H-23), 5.18 (1H, dd, J = 15.3, 8.2 Hz, H-22), 4.98 (1H, d, J = 6.1 Hz, H-6), 1.30 (3H, s, H₂-19), 1.00 (3H, d, J = 6.7 Hz, H₂-21), 0.94 (3H, s, H₂-18), 0.92 (3H, d, J = 6.7 Hz, H₂-28), 0.84 (3H, d, J = 6.7 Hz, H₂-27), 0.83 (3H, d, J = 7.0 Hz, H₂-26). EI-MS m/z: 456 (M⁺), 440, 438, 422. HR-El-MS m/z: 456.288 (M⁺). Caled for C₂₉H₄₄O₂: C, 74.8; H, 9.7.

6a,7a,8a,9a-Diepoxy-14a-hydroperoxyergosta-4,22-dien-3-one (3)
Colorless needles from benzene, mp 196–199°C, [α]D 0 +66° (c = 0.5, THF). IR (KBr) νmax cm⁻¹: 3415, 2955, 1667, 1448, 1370, 1246, 932. UV (EtOH) λmax nm (log ε): 230 nm (4.12). 1H-NMR (CDCl₃) δ: 6.33 (1H, d, J = 6.6 Hz, H-4), 5.27 (1H, dd, J = 15.3, 7.6 Hz, H-23), 5.16 (1H, dd, J = 15.3, 8.2 Hz, H-22), 3.59 (1H, d, J = 3.7 Hz, H-7), 3.50 (1H, dd, J = 3.7, 0.6 Hz, H-6), 1.37 (3H, s, H₂-19), 0.98 (3H, d, J = 6.7 Hz, H₂-21), 0.92 (3H, d, J = 6.7 Hz, H₂-28), 0.87 (3H, s, H₂-18), 0.84 (3H, d, J = 7.0 Hz, H₂-27), 0.83 (3H, d, J = 7.0 Hz, H₂-26). EI-MS m/z: 438 (M⁺ - H₂O), 423, 395, 353. Anal. C, 73.57; H, 8.89; Caled for C₂₉H₄₂O₂: C, 73.65; H, 8.83.

14a-Hydroperoxy-9a-hydroxyergosta-4,7,22-trien-3,6-dione (4)
Colorless needles from benzene, mp 182–185°C, [α]D 0 -112° (c = 0.25, EtOH). IR (KBr) νmax cm⁻¹: 3425, 2965, 1664, 1457, 1370, 1267, 964. UV (EtOH) λmax nm (log ε): 268 nm (4.21). 1H-NMR (CDCl₃) δ: 6.22 (1H, s, H-7), 5.29 (1H, dd, J = 15.3, 7.6 Hz, H-23), 5.17 (1H, dd, J = 15.3, 8.2 Hz, H-22), 1.41 (3H, s, H₂-19), 1.02 (3H, d, J = 6.7 Hz, H₂-21), 0.93 (3H, d, J = 6.7 Hz, H₂-28), 0.86 (3H, s, H₂-18), 0.85 (3H, d, J = 6.7 Hz, H₂-27), 0.83 (1H, d, J = 6.7 Hz, H₂-26). EI-MS m/z: 456 (M⁺), 440, 438, 422. HR-El-MS m/z: 456.287 (M⁺). Caled for C₂₉H₄₄O₂: 456.287.