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

Nobutoshi TANAKA,* Ken-ichi HOSOI, Daizo TANAKA, and Mimei TAKAHASHI

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Funakawara-machi, Ichigaya, Shinjuku-ku, Tokyo 162, Japan. Received October 5, 1995; accepted December 5, 1995

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 6α,9α-epidioxy-14α-hydroperoxyergosta-4,7,22-trien-3-one (2), which is transformed successively to 6α,7α;8α,9α-diepoxy-14α-hydroperoxyergosta-4,22-dien-3-one (3) and 14α-hydroperoxy-9α-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; 6α,9α-epidioxy-14α-hydroperoxy-ergosta-4,7,22-trien-3-one; 6α,7α;8α,9α-diepoxy-14α-hydroperoxyergosta-4,22-dien-3-one; 14α-hydroperoxy-9α-hydroxy-ergosta-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 We found that all the fruit bodies of more than 100 fungal species showed the presence of **1** on TLC (unpublished data).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)-triunsaturated 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 $CHCl_3$, 1% H_2SO_4 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 $CHCl_3$ (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 °C, $[\alpha]_D$ +126° (c=0.5, $CHCl_3$), was formulated as $C_{28}H_{40}O_5$ 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 ^{13}C-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 ^{13}C–^1H, ^1H–^1H and long-range ^{13}C–^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 α-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 C_5D_5N and compared with that in $CDCl_3$. 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

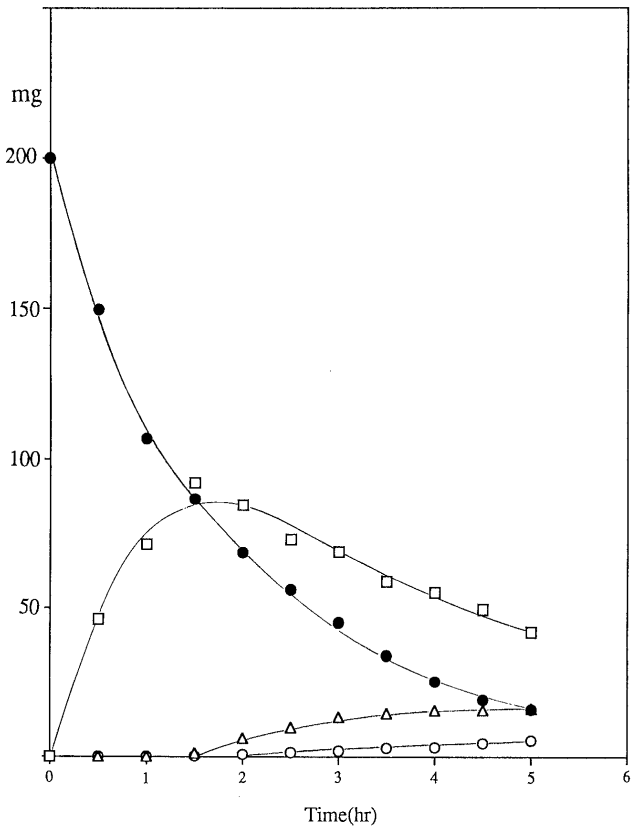

Fig. 1. Time Course of Each Compound

●, ergosta-4,6,8(14),22-tetraen-3-one (**1**); □, compound **2**; △, compound **3**; ○, compound **4**.

* To whom correspondence should be addressed.

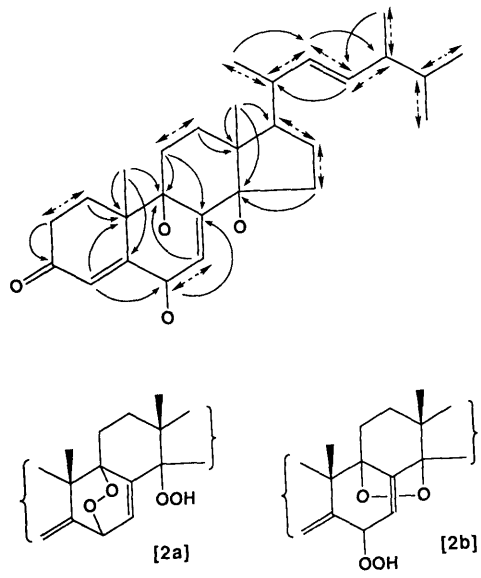

Fig. 2. ^{1}H-^{1}H COSY (←——→) and Long-Range ^{13}C ^{1}H COSY (^{1}H⌒^{13}C) Connections for **2**, and Possible Structures **2a** and **2b**

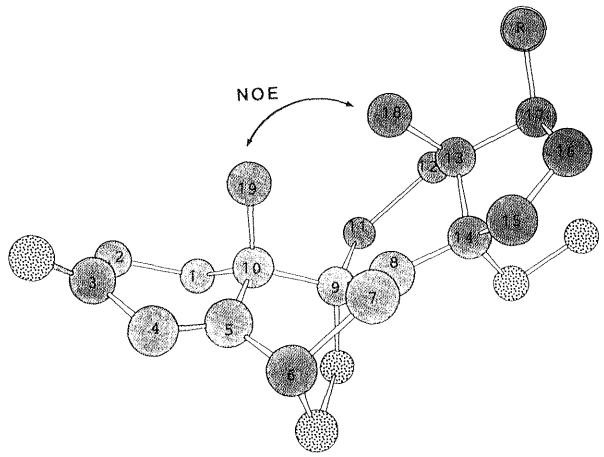

Fig. 3. Chem 3D Model of Compound **2**

of H-12α, H-15α and H-17α showed downfield shifts of 0.19, 0.42 and 0.23 ppm, respectively, indicating an α-orientation of the hydroperoxy group, while H-12β and H-15β, of which the configuration was confirmed by NOE correlation with the signals of H_3-19 and H_3-18, respectively, showed shifts of 0.02 and 0.07 ppm. Thus, the structure of **2** was determined as 6α,9α-epidioxy-14α-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 $C_{28}H_{40}O_5$ by elemental analysis and a signal count in the ^{13}C-NMR spectrum ($6CH_3+6CH_2+9CH+7C$). The UV spectrum [λ_{max} (MeOH): 230 nm (log ε 4.12)] showed the absorption of an αβ-unsaturated ketone. The ^{13}C-NMR spectrum showed the presence of the same side chain as in **2** and an αβ-unsaturated ketone [196.7 (C), 133.5 (CH), 156.7 (C)]. Among the remaining signals, those at δ 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 δ 49.1 and 46.5 appear at rather higher field than those of usual oxygenated carbons, the chemical shifts of the correlated

Table 1. ^{13}C-NMR Data in $CDCl_3$

Carbon No.	Compound			
	1	**2**	**3**	**4**
1	34.2	27.1	30.8	27.5
2	34.2	34.1	33.5	34.1
3	199.5	198.7	196.7	199.4
4	123.0	124.1	133.5	134.7
5	164.3	161.5	156.7	154.7
6	124.5	76.7	46.5	186.9
7	134.0	130.7	49.1	133.0
8	124.4	141.9	59.6	157.1
9	44.3	84.2	67.9	73.6
10	36.8	42.7	41.1	44.3
11	19.0	22.5	20.2	26.5
12	35.6	27.1	27.0	26.9
13	44.0	45.1	45.3	47.2
14	156.1	95.6	93.0	97.3
15	25.4	23.9	23.8	25.1
16	27.7	27.4	27.7	27.6
17	55.7	50.0	48.6	50.5
18	19.0	16.6	16.7	16.9
19	16.7	24.2	20.8	22.7
20	39.3	39.9	40.1	39.8
21	21.2	21.1	21.3	21.2
22	135.0	135.1	134.9	134.7
23	132.6	132.6	132.8	133.0
24	42.9	42.7	42.7	42.8
25	33.1	33.0	33.0	33.0
26	19.7	19.7	19.7	19.7
27	20.0	19.9	19.9	19.9
28	17.7	17.5	17.5	17.5

proton signals [δ 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, ^{1}H–^{1}H and long-range ^{13}C–^{1}H COSY, the structure was considered to be a 6,7,8,9,14-tetraoxygenated 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 (δ 3.50) and H-7 (δ 3.59) were correlated with the methyl signal of C-19 (δ 1.37) and C-18 (δ 0.87), respectively, in the NOESY spectrum, the α-configuration of the epoxy ring at C-6 and C-7 was determined. The correlation of the H-12β signal (δ 1.95) with the H_3-19 signal (δ 1.37) in the NOESY spectrum indicated the α-configuration of the other epoxy ring. As shown in Fig. 1, **3** was formed from **2**,[5] and so the hydroperoxy group was expected to exist in α-configuration. Thus, the structure of **3** was determined as 6α,7α;8α,9α-diepoxy-14α-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 $C_{28}H_{40}O_5$ from the HR-EI-MS. The ^{13}C-NMR spectrum showed the presence of the same side chain as in **2**, two αβ-unsaturated ketones [δ 199.4 (C), 134.7 (CH), 154.7 (C), 186.9 (C), 133.0 (CH), 157.1 (C)] and two oxygenated carbons [δ 97.3 (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 diene endoperoxides has been reported to produce a bisepoxide and a ketoepoxide, of which the

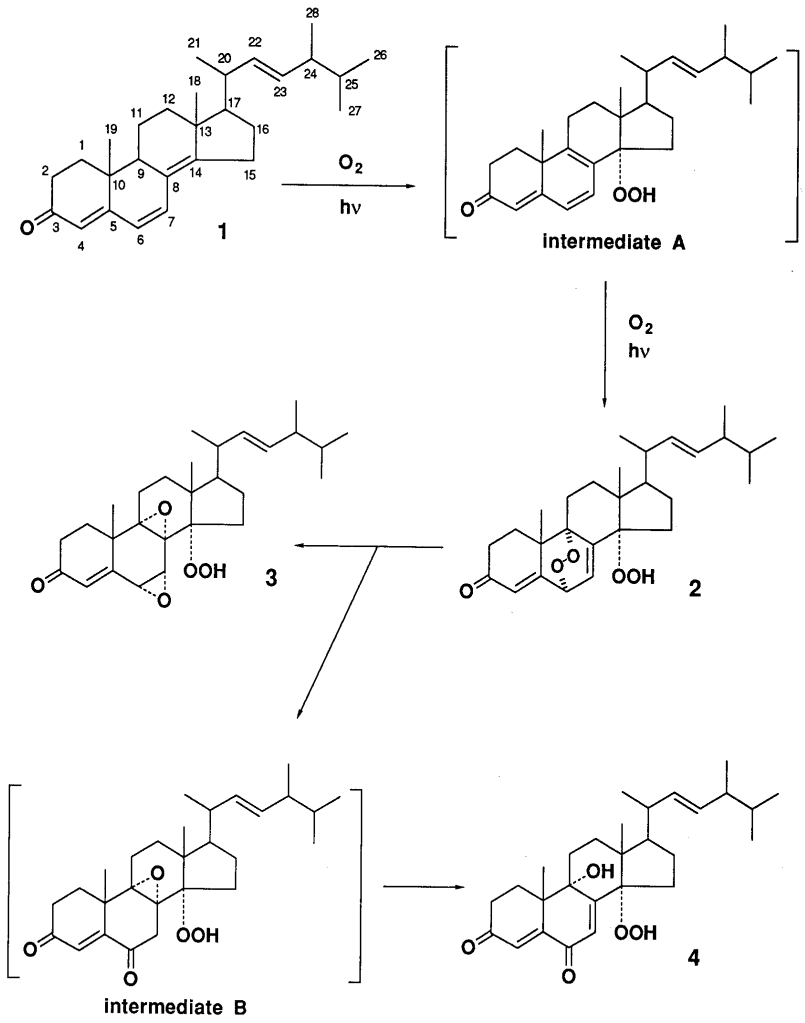

Chart 1

latter is unstable and changes to a keto alcohol.[6,7] As the structure of **3** is corresponds to the diepoxide from **2**, **4** should be assigned as the corresponding keto alcohol, 14α-hydroperoxy-9α-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,[8] **2**, **3** and **4** as well as the singlet oxygen should be harmful to organisms. This phototoxicity 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-6AV).

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 1h. After cooling, the mixture was filtered, and the filtrate was washed with 5% $NaSO_3$, 5% NaOH and water, then dried over $NaSO_4$ 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) ν_{max} cm^{-1}: 2055, 2850, 1665, 1585, 1460, 970. UV (EtOH) λ_{max}: 348 (log ε 4.47). ^{1}H-NMR ($CDCl_3$) δ: 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_3-26). 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 × 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 $CHCl_3$ to obtain **2** (46 mg), **3** (16 mg) and **4** (14 mg).

6α,9α-Epidioxy-14α-hydroperoxyergosta-4,7,22-trien-3-one (2) Colorless needles from *n*-hexane, mp 179—183 °C, $[\alpha]_D$ +126° (c=0.5, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3440, 2955, 1677, 1457, 1368, 1226, 1139, 972, 861. UV (EtOH) λ_{max}: 245 nm (log ε 4.12). ^{1}H-NMR ($CDCl_3$) 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, H_3-19), 1.00 (3H, d, J=6.7 Hz, H_3-21), 0.94 (3H, s, H_3-18), 0.92 (3H, d, J=6.7 Hz, H_3-28), 0.84 (3H, d, J=6.7 Hz, H_3-27), 0.83 (3H, d, J=7.0 Hz, H_3-26). EI-MS m/z: 456 (M^+), 440, 438, 422. HR-EI-MS m/z: 456.288 (M^+), Calcd for $C_{28}H_{40}O_6$: 456.287.

6α,7α;8α,9α-Diepoxy-14α-hydroperoxyergosta-4,22-dien-3-one (3) Colorless needles from benzene, mp 196—199 °C, $[\alpha]_D$ +66° (c=0.5, THF). IR (KBr) ν_{max} cm^{-1}: 3415, 2955, 1667, 1448, 1370, 1246, 932. UV (EtOH) λ_{max}: 230 nm (log ε 4.12). ^{1}H-NMR ($CDCl_3$) δ: 6.33 (1H, d, J=0.6 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, H_3-19), 0.98 (3H, d, J=6.7 Hz, H_3-21), 0.92 (3H, d, J=6.7 Hz, H_3-28), 0.87 (3H, s, H_3-18), 0.84 (3H, d, J=7.0 Hz, H_3-27), 0.83 (3H, d, J=7.0 Hz, H_3-26). EI-MS m/z: 438 (M^+-H_2O), 423, 395, 353. *Anal.* C, 73.57; H, 8.89; Calcd for $C_{28}H_{40}O_6$: C, 73.65; H, 8.83.

14α-Hydroperoxy-9α-hydroxyergosta-4,7,22-triene-3,6-dione (4) Colorless needles from benzene, mp 182—185 °C, $[\alpha]_D$ −112° (c=0.25, EtOH). IR (KBr) ν_{max} cm^{-1}: 3425, 2965, 1664, 1457, 1370, 1267, 964. UV (EtOH) λ_{max}: 268 nm (log ε 4.21). ^{1}H-NMR ($CDCl_3$) δ: 6.62 (1H, d, J=0.6 Hz, H-4), 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, H_3-19), 1.02 (3H, d, J=6.7 Hz, H_3-21), 0.93 (3H, d, J=6.7 Hz, H_3-28), 0.86 (3H, s, H_3-18), 0.85 (3H, d, J=6.7 Hz, H_3-27), 0.83 (1H, d, J=6.7 Hz, H_3-26). EI-MS m/z: 456 (M^+), 440, 438, 422. HR-EI-MS m/z: 456.287 (M^+), Calcd for $C_{28}H_{40}O_5$: 456.287.

Stability of 1 The stability of **1** (10 mg) under the following conditions was examined with monitoring by TLC. 1) Refluxing for 3 h in MeOH (2 ml) containing 1% MeONa. 2) Refluxing for 4 h in benzene (2 ml) containing 1% benzylmercaptan. 3) Refluxing for 2 h in $CHCl_3$ (2 ml) containing 1% *p*-toluenesulfonic acid. 4) Refluxing for 4 h in MeOH (2 ml) containing 1% H_2SO_4. No product was identified under any condition.

References and Notes

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