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Some Sesquiterpenoids and 5α,8α-Epidioxysterols from *Solanum lyratum*

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From the stems of *Solanum lyratum* Thunb. (Solanaceae), two sesquiterpenoids together with two 5α,8α-epidioxy sterols have been isolated and identified as atractylenolide I, dehydrocarissone, ergosterol peroxide, 9,11-dehydroergosterol peroxide. These compounds never previously isolated from this genus.

Key words: *Solanum lyratum*, Solanaceae, Sesquiterpenoid, 5α,8α-Epidioxy sterol, Atractylenolide I, Dehydrocarissone, Ergosterol peroxide, 9,11-Dehydroergosterol peroxide

INTRODUCTION

The whole plant of *Solanum lyratum* Thunb. (Solanaceae) are called "Baimaoteng" in chinese medicine and have been used as a remedy for various cancers in the Shanghai region of China (Shougakukan, 1985). In addition, European *Solanum dulcamara* L., in the same genus as *Solanum lyratum* Thunb., has been widely used in folk medicine for treating cancers and warts (Kupchan *et al.*, 1965). The antitumor substances and components of *Solanum dulcamara* and *Solanum lyratum* have been studied extensively by several reseachers (Kupchan *et al.*, 1965; Murakami *et al.*, 1981; Murakami *et al.*, 1985; Yahara *et al.*, 1985; Yahara *et al.*, 1986). This paper deals with the isolation and the structure elucidation of sesquiterpenoids together with 5α, 8α-epidioxy sterols from the stems of this plant.

MATERIALS AND METHODS

General Experimental Procedures

Melting points were taken on a electrothermal melting point apparatus and are uncorrected. IR spectra were determined on a MIDAC 117 spectrophotometer. MS spectra were obtained with Hewlett Packard 5980 II GC/MS system. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Gemini-300 spectrometer.

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Isolation

The stems of *Solanum lyratum* were collected in Junnam province in 1988. The air-dried plant material (990 g) was finely ground and extracted with MeOH at room temp. The obtained extracts were concentrated *in vacuo* to afford a residue which was suspended in H_2O and successively partitioned with $CHCl_3$, EtOAc, and BuOH. The $CHCl_3$ fraction was first separated by silica gel column chromatography into seven fractions (CH_2Cl_2 only, CH_2Cl_2 : Me_2CO=19 : 1, 9 : 1, 4 : 1, CH_2Cl_2 : MeOH=6 : 1, 4 : 1, 2 : 1). Fr. 1 was rechromatographed over SiO_2 eluting with Hex. : EtOAc=15 : 1, followed by prep. TLC with Hex. : Et_2O=9 : 1 to give compound **1** (12 mg). Fr. 4 was fractionated by MPLC on SiO_2 (CH_2Cl_2 : EtOAc=5 : 1 to 1 : 1). The intermediate frs. were further purified by silica gel column chromatography with Hex.-Me_2CO to give compound **2** (3.5 mg). Repeated silica gel column chromatography (Hex. : EtOAc=3 : 1) of the Fr. 3 gave a mixture of compound **3** (5.5 mg) and **4** (3.5 mg), which were separated by silica gel column chromatography eluting with Tolu. : Et_2O : Me_2CO=10 : 4 : 0.5.

Compound 1: Colorless needles (from Hex.), mp. 110-111℃; IR ν_{max} (KBr) cm^{-1} 1773, 1651 (lactone carbonyl), 1441, 1111, 1017; MS m/z (rel. int.) 230 $[M]^+$ (76), 215 $[M\text{-}CH_3]^+$ (100), 201 $[M\text{-}CHO]^+$ (43), 187 (32), 159 (30), 91 (49), 41 (71); ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, H-14), 1.90 (d, J=1.6 Hz, H-13), 2.35 (m, H-3), 2.52 (m, H_b-6), 2.69 (dd, J=16.7, 4.1 Hz, H_a-6), 4.62 (br. s, H-15), 4.91 (br. s, H-15'), 5.60 (s, H-9); ^{13}C-NMR: see Table I.

Table 1. ^{13}C-NMR spectral data for Compound **1** and **2** in $CDCl_3$

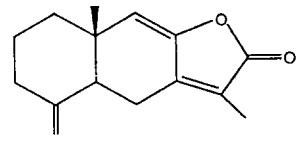
Carbon No.	Compound 1	Compound 2
C-1	39.1	156.5
C-2	23.0	126.3
C-3	36.2	–
C-4	148.0	129.3
C-5	48.4	160.1
C-6	22.7	28.7
C-7	148.3	50.5
C-8	148.0	22.0
C-9	119.1	38.0
C-10	38.1	40.3
C-11	120.4	72.4
C-12	171.4	27.0[a]
C-13	8.5	27.7[a]
C-14	18.6	23.4
C-15	107.5	10.4

[a]Assignement may be interchangable

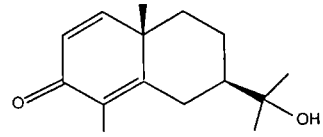
Compound 2: Colorless oil; IR ν_{max} (KBr) cm^{-1} 3412 (OH), 1657 (unsaturated C=O), 1620 (C=C), 1462, 1373, 1136; MS m/z (rel. int.) 234[M]$^+$ (1), 219 [M-CH_3]$^+$ (3), 216 [M-H_2O]$^+$ (46), 201 (50), 173 (80), 161 (37), 145 (54), 91 (57), 59 [C_3H_7O]$^+$ (100); ^{1}H-NMR ($CDCl_3$): δ 1.23 (s, H-14), 1.28 (s, H-12 or H-13), 1.29 (s, H-12 or 13), 1.93 (s, H-15), 2.04 (t, J=13.2 Hz, H-6a), 2.98 (d, J=13.2 Hz, H-6e), 6.24 (d, J=9.8 Hz, H-1), 6.76 (d, J=9.8 Hz, H-2); ^{13}C-NMR: see Table 1.

Compound 3: White amorphous powder; IR ν_{max} (KBr) cm^{-1} 3314 (OH), 1458, 1379, 1074; MS m/z (rel. int.) 428 [M]$^+$ (19), 410 [M-H_2O]$^+$ (81), 395 [M-H_2O-CH_3]$^+$ (25), 378 [M-H_2O-O_2]$^+$ (20), 303 [M-side chain]$^+$ (18), 285 (57), 271 (13), 267 (100), 253 (35), 251 (33); ^{1}H-NMR ($CDCl_3$): δ 0.82 (s, H-18), 0.82 (d, J=6.3 Hz, H-26 or H-27), 0.83 (d, J=6.5 Hz, H-26 or H-27), 0.88 (s, H-19), 0.91 (d, J=6.8 Hz, H-28), 1.00 (d, J=6.6 Hz, H-21), 3.97 (m, H-3), 5.14 (dd, J=15.3, 7.8 Hz, H-23), 5.23 (dd, J=15.3, 7.0 Hz, H-22), 6.24 (d, J=8.4 Hz, H-6), 6.50 (d, J=8.4 Hz, H-7); ^{13}C-NMR ($CDCl_3$): δ 12.9 (C-18), 17.6 (C-28), 18.2 (C-19), 19.7 (C-27), 20.0 (C-26), 20.7 (C-11), 20.9 (C-21), 23.4 (C-15), 28.7 (C-16), 30.2 (C-2), 33.1 (C-25), 34.7 (C-1), 37.0 (C-4), 39.4 (C-12), 39.8 (C-20), 42.8 (C-24), 44.6 (C-13), 51.1 (C-14), 51.7 (C-9), 56.2 (C-17), 66.5 (C-3), 79.5 (C-5), 82.2 (C-8), 130.8 (C-6), 132.3 (C-23), 135.2 (C-22), 135.5 (C-7).

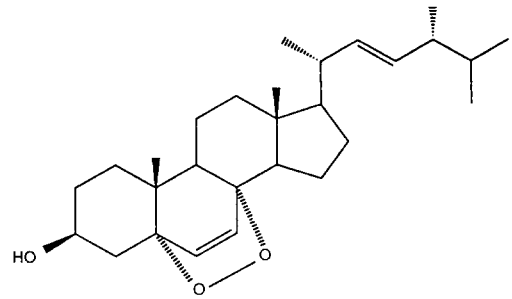
Compound 4: White amorphous powder; IR ν_{max} (KBr) cm^{-1} 3412 (OH), 1460, 1373, 1078; MS m/z (rel. int.) 426 [M]$^+$ (8), 408 [M-H_2O]$^+$ (4), 394 [M-O_2]$^+$ (24), 376 [M-O_2-H_2O]$^+$ (30), 361 [M-O_2-H_2O-CH_3]$^+$ (6), 251 (71), 69 (100); ^{1}H-NMR ($CDCl_3$): δ 0.74 (s, H-18), 0.83 (d, J=6.7 Hz, H-26 or H-27), 0.84 (d, J=6.7 Hz, H-26 or H- 27), 0.92 (d, J=6.9 Hz, H-28), 1.00 (d, J=6.6 Hz, H-21), 1.10 (s, H-19), 4.02 (m, H-3), 5.16 (dd, J=15.3, 7.7 Hz, H-23), 5.25 (dd, J=15.1, 7.0 Hz, H-22), 5.44 (dd, J=6.0, 2.0 Hz, H-11), 6.30 (d, J=8.6 Hz, H-6), 6.6 (d, J=8.6 Hz, H-7); ^{13}C-NMR ($CDCl_3$): δ 13.0 (C-18), 17.6 (C-28), 19.6 (C-27), 20.0 (C-26), 20.7 (C-21), 20.9 (C-15), 25.6 (C-19), 28.6 (C-16), 30.6 (C-2), 32.6 (C-1), 33.1 (C-25), 36.1 (C-4), 39.9 (C-20), 41.2 (C-12), 42.8 (C-24), 43.6 (C-13), 48.2 (C-14), 55.9 (C-17), 66.4 (C-3), 119.7 (C-11), 130.8 (C-6), 132.5 (C-23), 135.1 (C-22), 135.5 (C-7).



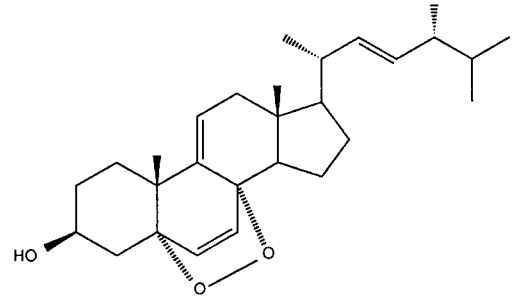
Compound 1 ; Atractylenolide I



Compound 2 ; Dehydrocarissone



Compound 3 ; Ergosterol Peroxide



Compound 4 ; 9,11 - Dehydro Ergosterol Peroxide

RESULTS AND DISCUSSION

Column chromatography of $CHCl_3$ soluble fraction of the MeOH extract afforded four compounds, which were identified by comparison of spectral data with

those reported in literature.

The IR spectrum of compound **1** showed the presence of a conjugated γ-lactone carbonyl group (1773, 1651 cm^{-1}). The GC-MS of **1** gave a molecular ion peak at m/z 230 and base peak at m/z 215 resulting from the loss of methyl fragmentation. The ^{1}H-NMR spectrum showed the signals of a tertiary methyl protons at δ 0.93 (3H, s), olefinic methyl protons at δ 1.90 (3H, d, J=1.6 Hz), exocyclic methylene protons at δ 4.62 and 4.91 (each 1H, br. s), isolated olefinic proton at δ 5.60 (1H, s). ^{1}H-^{1}H COSY showed correlation between the resonance at δ 1.90 and the signal at δ 2.52, which was further correlated to a signal at δ 2.69. The latter signals were correlated to the resonance at δ 2.35. The multiplicity of the H-5 at δ 2.35 supported that **1** was a Eudesmane derivatives that lactone closure was at C-8 and not C-6. The ^{13}C-NMR and DEPT spectra showed the signals of two methyl carbons, five methylene carbons, two methine carbons, five quaternary carbons and one carbonyl carbon. On the basis of the results, the structure of **1** was elucidated as atractylenolide I, which was isolated from *Atractylodes Rhizoma* (Peter *et al.*, 1989; Yoshihiro *et al.*, 1989) and reported to show an antiinflammatory effect (Endo *et al.*, 1979; Bohlmann *et al.*, 1980). This is the first time that **1** has been isolated from Solanum genus.

Compound **2** exhibited absorption bands due to an α,β- and α',β'- unsaturated ketone (1657 cm^{-1}) and hydroxyl groups (3412 cm^{-1}) in its IR spectrum. The EI mass spectrum of **2** showed a molecular ion peak at m/z 234 and characteristic fragment ion peaks at m/z 219 and 216 resulting from the loss of water and methyl fragmentation. The ^{1}H-NMR spectral data of **2** showed signals of tertiary methyl protons at δ 1.23 (3H, s), vinyl methyl proton at δ 1.93 (3H, s) and two olefinic protons at δ 6.24 and 6.76 (each 1H, d, J=9.8 Hz). The characteristic fragment ion at m/z 59 (base peak), the IR absorption band at 3412cm^{-1} and ^{1}H-NMR signal at δ 1.28 (3H, s) and 1.29 (3H, s) suggested that compound **2** has the hydroxyisopropyl moiety . Based on the above results, the structure of **2** was determined to be dehydrocarissone and finally verified by comparision of the ^{13}C-NMR data with those of the reported in literature (Uegaki *et al.*, 1988) . This is the first report of the isolation of dehydrocarissone (**2**) from natural plants, although it has been isolated from TMV- inoculated leaves of *Nicotiana undulata* (Uegaki *et al.*, 1988) .

Compound **3** showed broad absorption peak at 3314 cm^{-1} due to the hydroxyl group in its IR spectrum. The mass spectrum of **3** showed a molecular ion peak at m/z 428 and fragment ion peaks at m/z 410 $[M\text{-}H_2O]^+$, 378 $[M\text{-}O_2\text{-}H_2O]^+$ and 303 [M-side chain (C_9H_{17})]$^+$. An intense peak at m/z 378 is accounted for the retro-Diels-Alder type fragmentation from $[M\text{-}H_2O]$ fragment (Gunatilaka *et al.*, 1981). The ^{1}H-NMR spectrum of **3** showed signals for C-6 and C-7 protons at δ 6.24 and δ 6.50 (each 1H, d, J=8.4 Hz). The high field region displayed four methyl doublet peak at δ 0.82, 0.83, 0.91, 1.00 and two methyl singlet peak at δ 0.82, 0.88. A broad multiplet at δ 3.97 is due to the C-3 proton and doublet of doublet peak at δ 5.23 (1H, dd, J=15.3, 7.0 Hz), and δ 5.14 (1H, dd, J =15.3, 7.8 Hz) are typical for the trans Δ^{22} protons in the side chain (Stonard *et al.*, 1980; Gunatilaka *et al.*, 1981). Based on these results of above mentioned and comparison of the data which were reported (Chun-Nan *et al.*, 1991; Fisch *et al.*, 1973; Gonzalez *et al.*, 1983; Kahlos *et al.*, 1989; Yoshihisa *et al.*, 1991; Yoshihisa *et al.*, 1992), compound **3** was identified as the ergosterol peroxide.

Compound **4** showed very similar spectral data to those of **3**. It showed a molecular ion peak at m/z 426, and fragmentation ion peaks at m/z 408 $[M\text{-}H_2O]^+$, 394 $[M\text{-}O_2]^+$ in the EI-MS spectrum. This suggested that **4** is the homologue of **3** having one additional double bond. The ^{1}H-NMR spectrum of **4** showed characteristic signals of two angular methyl protons at δ 0.74 (C-18) and δ 1.10 (C-19). In comparision with the chemical shift of **4** with those of **3**, C-18 proton shift of **4** is upfield by 0.07 ppm, while the C-19 proton shift is downfield by 0.21 ppm. These chemical shift difference are very similar to the relationship between androstane and its 9 (11)-ene homologue (Bhacca *et al.*, 1964). Consequently, **4** is defined as 9, 11-dehydroergosterol peroxide (Fisch *et al.*, 1973; Tomofumi *et al.*, 1988). This is the first report of the isolation of Compound **3** and **4** from Solanum lyratum.

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