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Cytotoxic Constituents from *Solanum Lyratum*

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Activity-guided fractionation of the ethanol extract of the whole plant from *Solanum lyratum* resulted in the isolation of a new pregnane derivative glycoside, 16-dehydropregnenolone 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosid uronic acid (**2**), as well as other six known compounds: 16-dehydropregnenolone (**1**), allopregenolone (**3**), protocatechuic acid (**4**), vanillic acid (**5**), caffeic acid (**6**), and scopoletin (**7**). The structures of the isolated compounds were elucidated on the basis of their spectral data and chemical evidences. Compounds **1**, **3**, **4** were isolated for the first time from this plant. Cytotoxic activities of the isolated compounds were evaluated. Compound **1** exhibited significant cytotoxic activity against A375-S2, HeLa, SGC-7901, and Bel-7402 with IC_{50} values of 13.1 ± 0.9, 21.5 ± 1.0, 40.2 ± 0.7, and 49.8 ± 1.2 μg/mL, respectively.

Key words: Solanum, *Solanum Lyratum*, Cytotoxicity, 16-Dehydropregnenolone 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid, 16-Dehydropregnenolone

INTRODUCTION

An eastern crude drug, *Solanum lyratum* Thunb. had been used to treat cancers and tumors for centuries. Previously, it was reported that the aqueous extract of *Solanum lyratum* had cytotoxic activity against HL60 cell lines (Shi *et al.*, 2002) and the EtOAc-soluble extract had apoptosis-inducing activity in Bel-7404 cell lines (Shan *et al.*, 2001). The plant is known to contain steroidal glucuronides, steroidal alkaloid glucosides, coumarin, phenolic acid and other compounds, and only a limited number of these compounds had been reported (Murakami *et al.*, 1985; Yahara *et al.*, 1985; Yung *et al.*, 1997; Kang *et al.*, 1998;Yang *et al.*, 2002). To systematically evaluate its potential anticancer activity, the bioactive ingredients were isolated from the whole plant of *Solanum lyratum* by activity-guided fractionation, and cytotoxic activity of isolated compounds were also determined in this study.

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MATERIALS AND METHODS

General experimental procedure

The NMR spectra were recorded with a Bruker-ARX 300 or 600 spectrometer operating at 300 or 600 MHz for ^{1}H- and 75 or 150 MHz for ^{13}C-NMR, respectively. Chemical shifts were reported in parts per million on the δ scale with TMS as the internal standard. ESI-MS were recorded on a Finnigan LCQ LC-MS analyzer. The IR absorption spectra were recorded in KBr discs using a Bruker IFS-55 infrared spectrophotometer. Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, Qingdao, People's Republic of China), and Sephadex LH-20 (25-100 μm, Pharmacia). TLC was performed on precoated Si gel 60 F254 plates (0.2 mm thick, Merck) and spots were detected by spraying with 10% ethanolic H_2SO_4 reagent.

Plant material

The whole plants of *Solanum lyratum* were purchased from ShangHai XuHui Chinese Herbal Medicine Factory in January, 2003. This plant was identified by professor Qi-Shi Sun of the college of Chinese Traditional Medicine, Shenyang Pharmaceutical University. A voucher specimen (No. 20030110) was deposited in the college of Pharmacy, Shenyang Pharmaceutical University.

Table I. The cytotoxic activity of extract against tumor cell lines

Extracts/fractions	IC_{50} (μg/mL)[a]					
	HeLa	A375-S2	SGC-7901	Bel-7402	L929	MCF7
Ethanol extract	320.1±1.0	210.0±1.4	176.1±1.8	287.4±0.9	460.5±1.0	>500
EtOAc fraction	20.9±1.4	9.7±1.6	65.4±0.9	106.2±1.1	76.5±0.9	129.2±0.8
n-BuOH fraction	64.4±1.6	31.7±1.2	114.8±0.7	180.2±1.0	357.7±1.4	266.3±0.5
Petrol fraction	>500	>500	-	-	>500	>500
Aqueous fraction	>500	>500	-	-	>500	>500

[a]IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

Extraction and Isolation

The air-dried aerial parts of *Solanum lyratum* (15 kg) were extracted with 95% EtOH, and the resulting extracts were combined and concentrated *in vacuo* to yield a brown residue (705 g, 4.7%). The residue was suspended in water and partitioned with petroleum ether, followed by ethyl acetate and *n*-butanol and finally the residual extract upon concentration to give petroleum ether (180 g), EtOAc (135 g), BuOH (150 g), and aqueous (210 g) fractions, respectively. Each fraction was evaluated for the cytotoxic activity on the tumor cell lines (Table I). Consequently, it was shown that the activity resided in the EtOAc fraction and the BuOH fraction. Thus, the EtOAc fraction was subjected to column chromatography on silica gel using petroleum ether-acetone (100:0~0:100) as the eluting solvent to give 15 fractions (E_1-E_{15}). The BuOH fraction was separated by silica gel using $CHCl_3$-MeOH (100:0~0:100) to give 10 fractions (N_1-N_{10}). Fraction E_1-E_{15} and N_1-N_{10} were evaluated for the cytotoxic activity against HeLa and A375-S2 (Table II).

Fraction E_8 was chromatographed on silica gel column using petroleum ether-acetone (80:1~0:1) and Sephadex LH20 using $CHCl_3$-MeOH (1:1~0:1) to offer compound **5** (10.4 mg), compound **6** (23 mg), and compound **7** (46 mg). Fraction E_9 was purified by silica gel column using $CHCl_3$-MeOH (80:0~0:1) and Sephadex LH20 using $CHCl_3$-MeOH (1:1~0:1) to give compound **4** (90 mg). Isolation of fraction E_{13} with silica gel column using $CHCl_3$-Actone (30:1~0:1) and RP-HPLC (ODS, 75% MeOH) afforded compound **2** (30 mg) and compound **3** (90 mg). Fraction N_{10} was chromatographed on silica gel column using $CHCl_3$-MeOH-H_2O (8:2:0.2) and RP-HPLC (ODS, 60% MeOH) to obtain compound **1** (14 mg).

16-Dehydropregnenolone (1)

White amorphous powder. Positive ESI-MS *m/z*: 315.4 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.93 (3H, s, H-18), 1.05 (3H, s, H-19), 2.27 (3H, s, H-21), 3.52 (1H, m, H-3), 5.37 (1H, dd, *J*=2.58, 2.64 Hz, H-6), 6.71 (1H, dd, *J*=3.12,1.86 Hz, H-16); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 37.1(C-1), 31.5 (C-2), 71.7 (C-3), 42.3 (C-4), 141.4 (C-5), 121.0 (C-6), 31.6 (C-7), 30.2 (C-8), 50.1 (C-9), 36.7 (C-10), 20.7 (C-11), 34.6 (C-12), 46.1 (C-13), 56.4 (C-14), 32.3 (C-15), 144.4 (C-16), 155.4(C-17), 15.7 (C-18), 19.3 (C-19), 196.8 (C-20), 27.1 (C-21).

16-Dehydropregnenolone 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid (2)

White amorphous powder. IR (cm^{-1}): 3408.6, 2255.6, 2128.4, 1644.0, 1410.7, 1048.0, 1025.7, 999.8, 826.3, 764.8, 617.8; Positive ESI-MS *m/z*: 681.3$[M+2Na]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.84 (3H, s, H-18), 0.98 (3H, s, H-19), 1.08 (3H, d, *J* = 6.12 Hz, H-6 of Rha), 2.21

Table II. The cytotoxic activity of E_4-E_{15} against two tumor cell lines

fractions	IC_{50} (μg/mL)[a]	
	HeLa	A375-S2
E_1- E_3	>100	>100
E_4	49.7±1.4	38.9±1.2
E_5	44.5±0.9	29.8±1.9
E_6	29.7±1.0	15.0±1.3
E_7	45.7±0.9	27.3±0.8
E_8	41.7±0.7	32.3±1.1
E_9	39.2±1.1	16.5±1.5
E_{10}	36.5±1.2	43.7±1.7
E_{11}	55.4±0.6	52.8±0.9
E_{12}	55.3±0.9	43.7±1.0
E_{13}	42.0±0.8	28.1±1.2
E_{14}	60.0±1.0	42.3±0.9
E_{15}	42.1±1.8	30.3±0.9
N_1	65.3±1.0	47.3±0.9
N_2	76.9±1.0	62.6±0.9
N_3-N_9	>100	>100
N_{10}	50.2±1.1	46.5±0.9

[a]IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

(3H, s, H-21), 4.34 (1H, d, *J* = 7.80 Hz, H-1 of Glu), 5.03 (1H, s, H-1 of Rha), 5.33 (1H, br s, H-6), 6.89 (1H, br s, H-16); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 36.9 (C-1), 29.8 (C-2), 75.9 (C-3), 40.1 (C-4), 140.9 (C-5), 121.1 (C-6), 31.1 (C-7), 29.1 (C-8), 50.1 (C-9), 36.5 (C-10), 20.3 (C-11), 34.4 (C-12), 45.6 (C-13), 56.0 (C-14), 31.9 (C-15), 145.4 (C-16), 154.3 (C-17), 15.7 (C-18), 19.0 (C-19), 196.3 (C-20), 27.1 (C-21), Glu 98.0 (C-1), 76.5 (C-2), 78.1 (C-3), 72.4(C-4), 73.3 (C-5), 172.5 (C-6), Rha 100.2 (C-1), 70.6 (C-2), 70.7 (C-3), 72.1 (C-4), 67.9 (C-5), 17.9 (C-6).

3-Hydroxy-5-pregn-16-en-20-one (3)

^{1}H-NMR (300 MHz, acetone-d_6) δ: 0.88 (3H, s, 18-H), 0.84 (3H, s, H-19), 2.25 (3H, s, H-21), 6.69 (1H, dd, *J*=1.8,3.0 Hz, H-16), 3.59 (1H, m, H-3); ^{13}C-NMR (75 MHz, acetone-d_6) δ: 36.8 (C-1), 31.9 (C-2), 71.3 (C-3), 38.1(C-4), 45.1 (C-5), 28.5 (C-6), 31.5 (C-7), 33.8 (C-8), 54.8 (C-9), 35.7 (C-10), 21.0 (C-11), 34.8 (C-12), 46.3 (C-13), 56.3(C-14), 32.2 (C-15), 144.5 (C-16), 155.5 (C-17), 15.9 (C-18), 12.2 (C-19), 196.9 (C-20), 27.1 (C-21).

Protocatechuic acid (4)

^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.92 (1H, s, -COOH), 7.34 (1H, d, *J*=1.8 Hz, H-2), 7.30 (1H, dd, *J*=8.1, 1.8 Hz, H-6), 6.79 (1H,d, *J*=8.1 Hz, H-5); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 121.7 (C-1), 116.5(C-2), 144.9(C-3), 150.0 (C-4), 115.2 (C-5), 122.0 (C-6), 167.3 (-COOH).

Vanillic acid (5)

^{1}H-NMR (300 MHz, CD_3OD) δ: 7.60 (1H, dd, *J*=1.4, 8.2 Hz, H-6), 7.56 (1H, d, *J*=1.4 Hz, H-2), 6.92 (1H, d, *J*=8.2 Hz, H-5), 3.91 (3H, s, -OCH_3); ^{13}C-NMR (75 MHz, CD_3OD) δ: 124.9 (C-1), 115.6 (C-2), 152.1 (C-3), 148.1 (C-4), 113.4 (C-5), 122.9 (C-6), 56.3 (-OCH_3), 167.6 (-COOH).

Caffeic acid (6)

^{1}H-NMR (300 MHz, acetone-d_6) δ: 7.41 (1H, d, *J*=15.8 Hz, H-7), 6.99 (1H, d, *J*=1.3 Hz, H-2), 6.95 (1H, dd, *J*=8.2, 1.3 Hz, H-6), 6.75 (1H, d, *J*=8.2 Hz, H-5), 6.16 (1H, d, *J*=15.8 Hz, H-8); ^{13}C-NMR(75 MHz, acetone-d_6) δ: 125.8 (C-1), 115.2 (C-2), 145.6 (C-3), 148.2 (C-4), 115.8 (C-5), 121.2 (C-6), 144.6 (C-7), 114.7 (C-8), 168.0 (C-9).

Scopoletin (7)

^{1}H-NMR (300 MHz, acetone-d_6) δ: 8.87 (1H, s, -OH), 7.84 (1H, d, *J*=9.6 Hz, H-4), 7.19 (1H, s, H-5), 6.80 (1H, s, H-8), 6.17 (1H, d, *J*=9.6 Hz, H-3), 3.91 (3H, s, -OCH_3); ^{13}C-NMR (75 MHz, acetone-d_6) δ: 160.0 (C-2), 111.8 (C-3), 143.2(C-4), 110.6 (C-4a), 108.5 (C-5), 150.4 (C-6), 149.0 (C-7), 102.3 (C-8), 144.0 (C-8a), 55.3 (-OCH_3).

Cell culture

HeLa (human uterine carcinoma) cell lines, A375-S2 (human malignant melanoma) cell lines, MCF7 (human breast cancer) cell lines, L929 (murine fibrosarcoma) cell lines, SGC-7901 (human gastric cancer) cell lines, and Bel-7402 (human hepatocellular carcinoma) cell lines were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, U.S.A.) including 0.03% L-glutanmine (Gibco) with 10% fetal bovine serum (FBS) (Dalian Biological Reagent Factary, Dalian, China). All cells were maintained at 37°C, 5% CO_2 in a humidified atmosphere incubator. The confluent cells were used for bioactivity assay.

Cytotoxicity assay

The inhibition of the cellular growth was estimated using 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Livermore, MD, U.S.A.) assay as described by Mosmann (Mosmann, 1983).

RESULTS AND DISCUSSION

The ethanol extract of *Solanum lyratum* exhibited moderate cytotoxic activity against A375-S2, SGC-7901, Bel-7402, HeLa, L929 cells, and no activity against MCF7 cell. As for E_1–E_{15} of EtOAC fraction, E_1–E_3, which had low polarity, showed no cytotoxic activity, whereas fraction E_4–E_{15} exhibited significant dose-dependent cytotoxic activity for HeLa and A375-S2. As for N_1-N_{10} of BuOH fraction, only N_{10} showed significant dose-dependent cytotoxic activity for HeLa and A375-S2.

By bioactivity-guided isolation using the cytotoxicity assay against several human cancer cell lines, seven compounds from *Solanum lyratum* were isolated.

Compound **1**, 16-dehydropregnenolone ($C_{21}H_{30}O_2$), was characterized by positive ESI-MS ($M+H^+$, *m/z* 315), ^{1}H-NMR, and ^{13}C-NMR. Confirmation of the compound **1** as 16-dehydropregnenolone was made by comparison of ^{13}C-NMR spectrum with that in literature (Zsuzsa *et al.*, 1995). Its ^{13}C-NMR, DEPT, and HSQC spectra revealed 21 (three CH_3-, seven CH_2-, six CH-, and five q-C) carbon signals which are indicative of a pregnenolone skeleton (Zsuzsa *et al.*, 1995). A one-proton signal at δ 3.52 in the ^{1}H-NMR and one oxygenated methine signal at δ 71.7 in the ^{13}C-NMR spectra indicated the presence of one alcohol group in the structure. Two vinylic proton signals at δ 5.37 and 6.71, and signals of two pairs of olefinic carbons at δ 121.2 (C-6), 140.4(C-5), and δ 144.4 (C-16), 155.4 (C-17) showed that the presence of two double bond at C_5 and C_{16}. The methyl protons at δ_H 2.27 (H-21) and its HMBC correlations to C-20 (δ 196.8) and C-17 (δ 155.4) pointed to a methyl α,β-unsaturated ketone. From the above evidences, the structure of **1** was concluded to be 16-dehydropregnenolone.

Compound **2** was obtained as white amorphous powder. The molecular formula $C_{33}H_{48}O_{12}$ was determined by

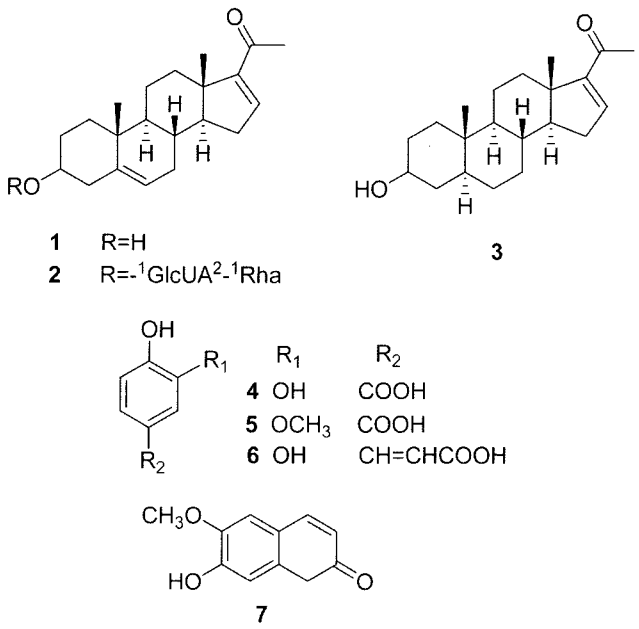

Fig. 1. The structure of seven isolated compounds

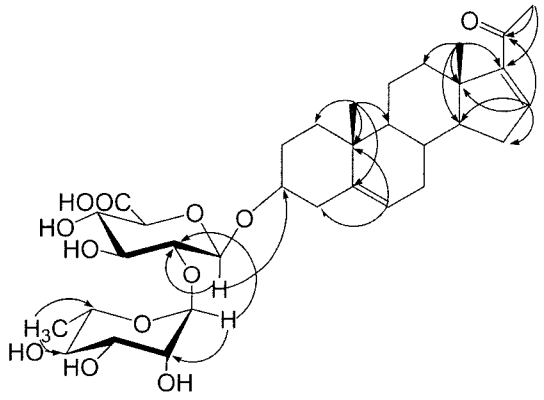

Fig. 2. The structure and selected HMBC correlation of compound **2** (from H to C)

positive ESI-MS (at *m/z* 681.3 $[M+2Na]^+$), and DEPT NMR spectral data. The ^{1}H-NMR spectrum of compound **2** exhibited signals characteristic for three methyl singlets at δ 0.84 (s, CH_3-18), 0.98 (s, CH_3-19), and 2.21 (s, CH_3-21); one methyl doublets at δ 1.08 (d, *J*=6.12, Rha C-6), two trisubstituted olefinic protons at δ 5.35 and 6.89 and two anomeric protons at δ 4.34 and 5.04. The ^{13}C-NMR spectrum of **2** showed signals for two pairs of olefinic carbons at δ 121.2 (C-6), 140.4 (C-5), and 145.4 (C-16), 154.3 (C-17), two anomeric carbons of sugars at δ 98.0 and 100.2, and a carbonyl carbon at δ 196.3 and a carboxyl carbon at δ 172.5 (glu C-6). Careful comparison of ^{1}H-NMR and ^{13}C-NMR spectral data of **2** with compound **1** allowed to establish the compound **2** as 16-dehydropregnenolone (3β-hydroxy-5, 16-pregnadien-20-one) glycoside with two sugars attached at C-3 position.

Table III. The cytotoxic activity of compounds against several tumor cell lines

Compound	IC_{50} (μg/mL)[a]			
	HeLa	A375-S2	SGC-7901	Bel-7402
1	21.5±2.3	13.1±1.9	40.2±1.7	49.8±2.2
2	>50	>50	>50	>50
3	>50	>50	>50	>50
4	>50	>50	>50	>50
5	>50	>50	>50	>50
6	>50	>50	>50	>50
7	>50	>50	>50	>50
5-Fu[b]	17.5±1.4	15.0±0.7	3.9±1.0	14.1±0.4

[a]IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.
[b]5-Fu (5-Fluorouracil) was used as a positive control.

The sugars were identified as *D*-glucuronic acid and L-rhamose by co-TLC with authentic samples after acid hydrolysis. The chemical shifts of the sugar moiety in ^{13}C-NMR also confirmed the presence of one glucuronic acid and one rhamose. The sugar linkages were determined on the basis of HMBC spectrum. The HMBC correlation was observed between a proton signal at δ 4.34 (Glu-H-1) and a carbon signal at δ 75.9 due to C-3 of the aglycone moiety, while another anomeric proton signal at δ 5.04 (rha-H-1) showed a correlation with a carbon signal at δ 76.5 due to C-2 of the inner sugar, suggesting glycosylation at C-3 of aglycone with a rha (1→2)-glu moiety. The anomeric configurations of the glucuronic acid was determined to be β for on the basis of the J_{H-H} values (7.8 Hz), and α configuration was determined by the broad singlet signal of rha-H-1 at 5.04 ppm, ^{13}C chemical shifts at δ 101.3 of C-1 of rhamnose indicated its glycosdic linkage is a α configuration. All these confirmed the fact that the aglycone of compound **2** was 16-dehydropregnenolone with a sugar moiety composed of rha (1→2) glu at C-3 position of aglycone. From the above evidences, the structure of 2 was concluded to be 16-dehydropregnenolone 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid. To the best knowledge, it has not been reported yet, and it is a new compound.

The structures of compound **3**, **4**, **5**, **6**, and **7** were identified by comparison of spectral data with those of literature values (Dawe and Wright, 1986; Park *et al.*, 1993; Park *et al.*, 1994; Yang *et al.*, 2002; Kang *et al.*, 1998; Steck and Mazurek, 1972). Compounds **1**, **3**, and **4** were isolated for the first time from this plant.

The cytotoxic activities of isolated compounds were determined against HeLa, A375-S2, SGC-7901, and Bel-7402 cells. The 50% growth inhibition (IC_{50}) values are

listed in Table III. Cytotoxic activities of compound **1** against HeLa, A375-S2, SGC-7901, and Bel-7402 cells were exhibited in a concentration-dependent manner. To the best of our knowledge, this is the first report for the cytotoxic activity of compound **1**.

ABBREVIATION

HeLa, human uterine carcinoma; A375-S2, human malignant melanoma; L929, murine fibrosarcoma; MCF7, human breast cancer; Bel-7402, human hepatocellular carcinoma; SGC-7901, human gastric cancer; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC_{50}, the 50% growth inhibition; 5-Fu, 5-Fluorouracil.

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REFERENCES

Dawe, R. D. and Wright, L. C., An improved route to pregna-5,20-dien-3β-ol(*muricin aglycone*): Carbon and proton nuclear magnetic resonance assignments for the aglycone and a number of related pregnant derivatives. *Can. J. Chem.*, 65, 666-669 (1987).

Kang, S. Y., Sung, S. H., Park, J. H., and Kim, Y. C., Hepatoprotective activity of scopoletin, a constituent of *Solanum Lyratum*. *Arch. Pharm. Res.*, 21, 718-722 (1998).

Mosmann, T., Rapid colorimetric assay for celluar growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Murakami, K., Ezima, H., and Takaishi, Y., Studies on the constituents of solanum plants. v: The constituents of *S. lyratum* Thunb. II. *Chem. Pharm. Bull.*, 33, 67-73 (1985).

Park, S. W., Yook, C. S., and Lee, H. K., Chemical components from the fruits of *Crataegus pinnatifida* var. *psllosa. Kor. J. Pharmacogn.*, 25,328-335 (1994).

Park, W. Y., Lee, S. C., Ahn, B. T., Lee, S. H., Ro, J. S., and Lee, K. S., Phenolic compounds from *Acalypha australis L. Kor. J. Pharmcogn.*, 24, 20-25 (1993).

Shan, C. M., Hu, J. J., and Du, G. H., Apoptosis of hepotoma cell line BEL-7404 induced by extracts of Solanum lyratum Thunb. *Chinese Journal Of Clinical Pharmacology and Therapeutics*, 6, 200-203 (2001).

Shi, W. R. and Yan, L., Influence of *Solanum Lyratum* Thumb on HL-60. *J. of Fujian College of TCM*, 12, 36-38 (2002).

Steck, W. and Mazurek, M., Identification of natural coumarins by nmr spectroscopy. *Lioydia*, 35, 418-439 (1972).

Yahara, S., Morooka, M., and Ikeda, M., Two new steroidal glucuronides from *Solanum lyratum* II, *Planta medica*, 52, 496-498 (1986).

Yang, J. Z., Guo, G. M., Zou, L. X., and Ding, Y., Studies on chemical constituent of *Solanum Lyratum. ZhongGuo Zhongyao Zazhi*, 27, 42-43 (2002).

Yahara, S., Murakami, N., and Yamasaki, M., A furostanol glucuronide from *Solanum lyratum. Phytochemistry*, 24, 2748 (1985).

Yung, Y. L., Feng, L. H., and Toshihiro, N., Two new soladulcidine glycosides from *Solanum lyratum. Chem. Pharm. Bull.*, 45, 1381-1382 (1997).

Zsuzsa, S., Peter, F., and Frederick, S., Complete ^{1}H and ^{13}C NMR spectra of pregnenolone. *Steroids*, 60, 442-446 (1995).