Phytochemical study of *Swertia longifolia*

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Received 26 Apr 2008; Revised 16 Sep 2008; Accepted 20 Sep 2008

ABSTRACT

Background: Swertia spp. (Gentianaceae) grow widely in eastern and southern Asian countries such as Japan, China and India and are used as traditional remedy for gastrointestinal complains because of their bitter principles. Several studies have been carried out on hypoglycemic, hepatoprotective, mono amino oxidase inhibitory and antidepressant effects of these plants and it has been shown that xanthones and iridoids are responsible for their activities.

Purpose of the study: In order to gain better knowledge of endemic plants of Flora Iranica, *Swertia longifolia* Boiss. growing in the northern parts of Iran, was subjected to phytochemical studies.

Methods: Dried and milled aerial parts of the plant were extracted with petroleum ether and ethanol of which results of petroleum ether extract has been reported previously. For purification of ethanol extract, it was acidified with acetic acid and subsequently extracted with chloroform and then with *n*-butanol. The *n*-butanol extract was analyzed using different chromatographic methods and the structures of the isolated components were established by means of spectroscopic techniques.

Results: Four components including an iridoid glycoside (loganic acid), a secoiridoid glycoside (gentiopicroside), a secoiridoid dilactone (gentiolactone) and a nucleoside (uridine) were isolated from *n*-butanol extract of the plant.

Major conclusion: Similar to other species of Swertia, iridoid and secoiridoid glycosides could be considered as major constituents of *Swertia longifolia* Boiss.

Keywords: *Swertia longifolia* Boiss., Gentianaceae, gentiolactone, gentiopicroside, loganic acid, uridine.

INTRODUCTION

The Swertia genus (Gentianaceae) comprises 170 species that grow abundantly in east and south Asia. Seventy nine species have been distributed in China of which about 20 species have been used in Chinese traditional medicine for treatment of hepatic, choleretic and inflammatory diseases. Several species are also used in India and Pakistan. The plants of Swertia genus are rich sources of xanthones, flavonoids, iridoid and secoiridoid glycosides and terpenoids (1). Many investigations have been carried out on pharmacological effects of Swertia species and some of their components in the pure form have been isolated. It has been demonstrated that they have hypoglycemic (2), hepatoprotective (3,4), anti-HIV (5,6), antitubercular (7), mono amino oxidase inhibitory (8,9) and central nervous system depressant (10) activities. In Iran, two Swertia species exist: *S. lactea* and *S. longifolia* and the second one is endemic plant of Flora Iranica which grows in wet areas of Alborz mountains (north of Iran) (11). Last studies on this plant has shown that the aerial parts of plant contain xanthones and xanthone glycosides (12,13). Another investigation has demonstrated hepatoprotective effects of methanol and petroleum ether extracts and swerchirin with xanthone structure as major component of the plant (3). In this investigation, extraction, purification and structure elucidation of iridoid and secoiridoid glycosides have been determined.

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MATERIALS AND METHODS

Plant materials

Swertia longifolia Boiss. (aerial parts) was collected in July 2003 from the North of Iran, Mazandaran province, Lavashm mountains, ca. 2900 m, Hajimehdipoor, No. 81007 (TARI) and identified by Dr. V. Mozaffarian, Botanist, from Research Institute of Forests and Rangelands (Tehran).

General experimental procedures

^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker Avance 400 NMR spectrometer with $CDCl_3$ or CD_3OD as solvents as appropriate and TMS as an internal standard. Chemical shifts are expressed in δ-values (ppm). COSY (Correlated spectroscopy), HMQC (Heteronuclear multiple quantum coherence) and HMBC (Heteronuclear multiple band correlation) spectra were obtained using the usual pulse sequences. EIMS (Electron ionization mass spectrometry) spectra were recorded on a Finnigan TSQ-Mat 70 (70 eV) spectrometer and DCIMS (Desorption chemical ionization mass spectrometry) spectra were recorded on a R210C quadripolar spectrometer. Column chromatography was performed using silica gel (Kieselgel 60, 0.063-0.2 mm, ASTM, Germany) and Sephadex LH-20 (25-100 µm, Pharmacia Fine Chemicals, Sweden). MPLC (Medium pressure liquid chromatography) was performed on a C_{18} column (50-60 µm, MN, Germany) by MPLC apparatus of D-1000 Berlin 37. VLC (Vacuum liquid chromatography) was carried out with a silica gel column (35-70 µm, SDS, Germany). Separation by SPE (Solid phase extraction) was carried out using silica gel (35-70 µm, SDS, Germany). TLC (Thin layer chromatography) employed Merck Kieselgel $60F_{254}$ and RP-18 WF_{254} S (Germany).

Extraction and isolation

Dried and milled aerial parts of plant (1 kg) were extracted sequentially in a Soxhlet apparatus with petroleum ether (60-80 ˚C, 12 hrs) and ethanol (24 hrs) and the results of the analyses of Petroleum ether extract was reported previously (12). The ethanol extract of the defatted plant, was evaporated under reduced pressure and the residue was treated with aqueous 4% acetic acid (500 ml) and the mixture was filtered. The clarified aqueous acid fraction was partitioned with chloroform and then with *n*-butanol.

Isolation of compounds from n-butanol extract

n-Butanol extract was evaporated under reduced pressure (12 g) and subjected to a Sephadex LH-20 column (2.5×55 cm) and eluted with methanol (1 l). This process yielded 5 fractions, of which two fractions were selected for further purification (fraction A, 8.95 g and B, 300.70 mg) since they contained major components as was determined by thin layer chromatography (TLC).

Purification of fraction A

Vacuum liquid chromatography (VLC) was performed on a silica gel column (7×3 cm). The solvents were CH_2Cl_2:EtOAc:MeOH 60:40:0, 60:20:20, 50:30:20, 30:50:20, 10:70:20, 0:80:20, 0:60:40, 0:50:50, 0:0:100. The volume was 350 ml for each step. The fractions obtained from CH_2Cl_2:EtOAc:MeOH 60:40:0 (fraction A_1, 42.4 mg) and 60:20:20 (fraction A_2, 1.13 g) were chosen for further purifications because they contained major components as was determined by TLC.

For separation of components of the fraction A_1, a solid phase extraction (SPE) method was used. The sample was subjected to a silica gel column (1.5×4.5 cm) and eluted with CH_2Cl_2:EtOAc:MeOH (each 24 ml) 100:0:0, 98:2:0, 96:4:0, 94:6:0, 92:8:0, 90:10:0, 88:12:0, 86:14:0, 84:16:0, 80:20:0, 76:24:0, 72:28:0, 68:32:0, 64:36:0, 60:40:0, 55:45:0, 50:50:0, 40:50:10, 30:50:20, 20:50:30, 10:50:40, 0:50:50, 0:40:60, 0:20:80, 0:0:100. The fractions obtained from CH_2Cl_2:EtOAc:MeOH 98:2:0 contained a secoiridoid dilactone 1 (1.1mg) (14,15).

Gentiolactone **1:** Amorphous, $C_{10}H_{12}O_5$, MW: 212, IR υ_{max} (Cm^{-1}): 3500, 1745, 1725, 1600; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.00 (3H, t, J = 7.4 Hz, H-10), 1.71 (1H, dq, J = 14.4, 7.2 Hz, H-8a), 1.85 (1H, dq, J = 14.4, 7.2 Hz, H-8b), 2.64 (1H, m, H-6a), 2.81 (1H, m, H-6b), 4.49 (2H, m, H-7), 5.01 (1H, ddd, J = 16.4, 3.6, 2.4 Hz, H-3a), 5.25 (1H, dt, J = 16.4, 1.8 Hz, H-3b); ^{13}C-NMR ($CDCl_3$, 100 MHz): 7.7 (C-10), 22.5 (C-6), 30.8 (C-8), 66.5 (C-7), 66.7 (C-3), 72.1 (C-9), 118.8 (C-4), 153.4 (C-5), 161.5 (C-11), 172.6 (C-1).

In order to separate components of the fraction A_2, this fraction was chromatographed over a silica gel column (3×39 cm) with CH_2Cl_2:EtOAc:MeOH 60:20:20, 50:20:30, 10:40:50, 0:0:100 as solvents. The volume was 830 ml for each step. Finally secoiridoid glycoside **2** (64.2 mg) (16) was obtained from CH_2Cl_2:EtOAc:MeOH 60:20:20 fractions and an iridoid glycoside **3** (57.2 mg) (17) was purified from CH_2Cl_2:EtOAc:MeOH 10:40:50 fractions.

Gentiopicroside **2**: Amorphous, $C_{16}H_{20}O_9$, MW: 356, EIMS: *m/z* 357 (28), 195 (100), 176 (72); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.44 (1H, s, H-3), 5.75 (1H, ddd, J = 17.2, 10.4, 6.8 Hz, H-8), 5.66 (1H, d, J = 2.8 Hz, H-1), 5.61 (1H, m, H-6), 5.23

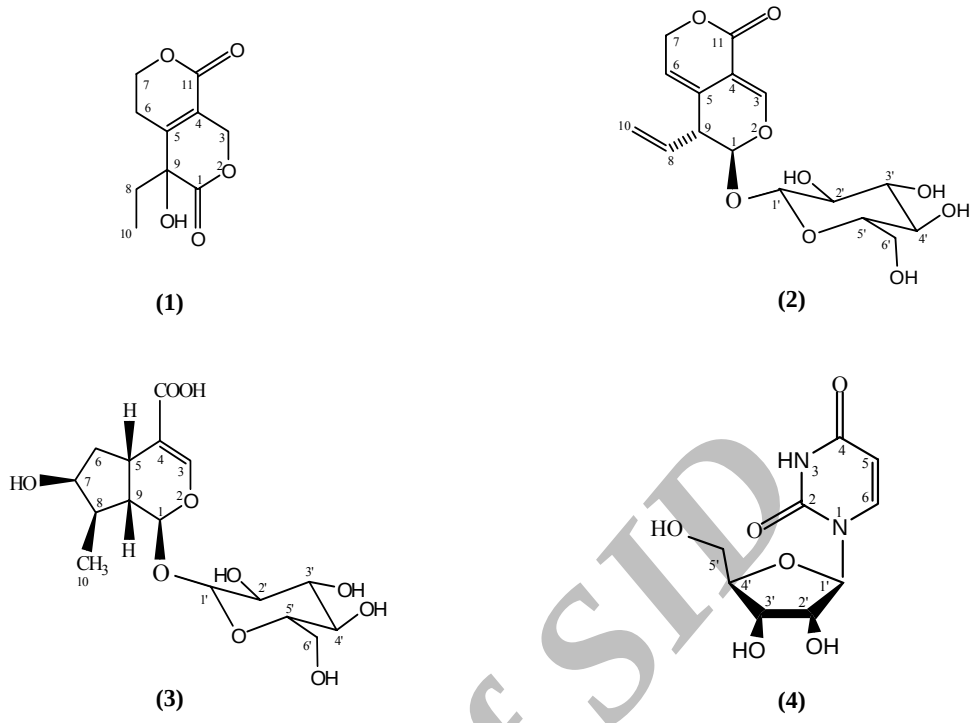

Figure 1. Structures of compounds **1-4**

(1H, dt, J = 15.6, 1.4 Hz, H-10a), 5.19 (1H, dt, J = 8.0, 1.4 Hz, H-10b), 5.07 (1H, m, H-7a), 4.98 (1H, m, H-7b), 4.64 (1H, d, J = 8.0 Hz, H-1'), 3.89 (1H, dd, J = 12.0, 2.4 Hz, H-6'a), 3.64 (1H, dd, J = 12.0, 6.4 Hz, H-6'b), 3.35 (1H, t, J = 8.8 Hz, H-3'), 3.30 (2H, m, H-5', H-9), 3.23 (1H, t, J = 8.8 Hz, H-4'), 3.14 (1H, dd, J = 9.2, 8.0 Hz, H-2'); ^{13}C-NMR (CD_3OD, 100 MHz): δ 165.0 (C-11), 149.3 (C-3), 133.6 (C-8), 125.6 (C-5), 117.2 (C-10), 115.8 (C-6), 103.5 (C-4), 98.8 (C-1'), 97.1 (C-1), 77.0 (C-3'), 76.5 (C-5'), 73.1 (C-2'), 70.1 (C-4'), 69.6 (C-7), 61.3 (C-6'), 45.2 (C-9).

Loganic acid **3**: Amorphous, $C_{16}H_{24}O_{10}$, MW: 376, DCIMS: *m/z* 394 (10), 232 (10), 215 (70), 212 (100), 198 (58), 180 (69), 153 (77), 145 (67), 127 (66); ^{1}H-NMR (CD_3OD, 400 MHz), δ 7.30 (1H, s, H-3), 5.20 (1H, d, J = 4.4 Hz, H-1), 4.60 (1H, d, J = 8.0 Hz, H-1'), 3.98 (1H, m, H-7), 3.83 (1H, dd, J = 12.0, 1.6 Hz, H-6'a), 3.61 (1H, dd, J = 12.0, 5.6 Hz, H-6'b), 3.32 (2H, m, H-5', H-3'), 3.24 (1H, m, H-4'), 3.15 (1H, dd, J = 8.8, 8.0 Hz, H-2'), 3.03 (1H, bq, J = 8.0 Hz, H-5), 2.17 (1H, ddd, J = 14.0, 8.0, 1.2 Hz, H-6β), 1.95 (1H, dt, J = 13.2, 4.0 Hz, H-9), 1.80 (1H, m, H-8), 1.60 (1H, ddd, J = 12.8, 8.0, 4.0 Hz, H-6α), 1.03 (1H, d, J = 6.8 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz), δ 170.1 (C-11), 150.3 (C-3), 113.3 (C-4), 98.6 (C-1'), 96.2 (C-1), 76.9 (C-5'), 76.6 (C-3'), 73.8 (C-7), 73.3 (C-2'), 70.2 (C-4'), 61.3 (C-6'a), 45.1 (C-9), 41.3 (C-8), 40.7 (C-6), 30.8 (C-5), 12.1 (C-10).

Isolation of components from fraction B

MPLC was performed on a C_{18} column (1×47 cm). The solvents were H_2O: MeOH 100:0 (220 ml), 95:5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50 (each 110 ml), 40:60 (160 ml), 30:70, 20:80, 10:90 (each 110 ml) and 0:100 (330 ml) using λ_{max} of 250 nm. The first fractions obtained from H_2O:MeOH 100:0 contained a pure nucleoside 4 (3.9 mg) (18,19).

Uridine **4**: Amorphous, $C_9H_{12}N_2O_6$, MW: 244, EIMS: *m/z* 244 (4), 226 (23), 214 (27), 196 (39), 179 (100), 168 (78), 133 (54), 125 (88), 113 (57), 73 (66); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.95 (1H, d, J = 8.0 Hz, H-6), 5.84 (1H, d, J = 4.4 Hz, H-1'), 5.63 (1H, d, J = 8.0 Hz, H-5), 4.11 (1H, t, J = 4.8 Hz, H-2'), 4.08 (1H, t, J = 4.8 Hz, H-3'), 3.94 (1H, m, H-4'), 3.78 (1H, dd, J = 12.0, 2.8 Hz, H-5'a), 3.67 (1H, dd, J = 12.0, 2.8 Hz, H-5'b); ^{13}C-NMR (CD_3OD, 100 MHz): δ 164.8 (C-2), 151.1 (C-4), 141.3 (C-6), 101.2 (C-5), 89.3 (C-1'), 85.0 (C-4'), 74.3 (C-2'), 70.0 (C-3'), 60.9 (C-5').

RESULTS AND DISCUSSION

The family of Gentianaceae is apparently characterized by the universal occurrence of

iridoid glycosides. The iridoids which are found consistently are secoiridoids which occasionally result from carboxylic iridoids by biosynthetic pathway (20).

Preliminary examination of the *n*-butanol extract of the aerial parts of *Swertia longifolia* by analytical TLC suggested the presence of major components mainly with iridoid structure; which were isolated by chromatographic methods. Four components were purified from the extract and their structures determined by spectroscopic means. The spectroscopic data of compounds **1-4** were in good agreement with those described in the literature for gentiolactone (14,15), gentiopicroside (16), loganic acid (17) and uridine (18,19).

Uridine is a nucleoside with pyrimidine structure which is presents in all organisms as one of the RNA bases (21). Gentiopicroside is one of the most common secoiridoid glycosides presents in Gentianaceae which has been reported from 107 of 127 species in Gentianaceae, mainly in Gentiana nad Swertia genus (20). The compound has interesting pharmacological properties such as anti-inflammatory (22), protective effects against many kinds of chemical and immunological liver injuries (23-25) and stress-induced gastric ulcers (26), bile secretion promoting property (25) and smooth muscle relaxing activity (27). It also exhibits cytoprotective effect which may cause a synergism in terms of wound healing activity of Gentiana root (28). Since gentiopicroside exists in *Swertia longifolia* as well, above-mentioned pharmacological properties could be expected from this plant. Loganic acid which is another compound of *S. longifolia*, has iridoid glycoside structure. It has been reported mainly from Gentiana species (29). However among Swertia species, loganic acid has been isolated only from *S. caroliniensis* (30, 31) and no report is available about occurrence of gentiolactone in Swertia species. Therefore, it is the first report of presence of this compound in Swertia genus. Although, several investigations have been performed on biological and pharmacological abilities of pure components isolated from plants such as iridoids and secoiridoids, no study has been carried out on effects of loganic acid and gentiolactone.

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