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Constituents of the Aerial Parts of *Lonicera etrusca* Growing in Saudi Arabia

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Abstract – Phytochemical investigation of the aerial parts of *Lonicera etrusca* resulted in the isolation of three iridoids including two aglycones, loganin aglycone (log-1) (**1**) and lonicerin (log-2) (**2**), and the known common glycoside loganin (**4**). The study also afforded a coumarin derivative, 7-hydroxycoumarin (**3**), and a flavonoid glycoside, luteolin-7-*O*-β-D-glucoside (**5**). The structures were determined utilizing physical, chemical and spectral methods.

Keywords – *Lonicera etrusca*, Caprifoliaceae, Iridoids, Loganin aglycone, Lonicerin

Introduction

Family Caprifoliaceae is a relatively small family belonging to the order Dipsacales. Caprifoliaceae comprises about 6 genera and 800 species. The plants belonging to the family are mainly hardy ornamental shrubs or vines, many popular garden shrubs, especially *Abelia*, *Lonicera*, and *Weigela*. A few have become invasive weeds outside of their native ranges such as *Lonicera japonica* (Heywood, 2001). The berries of these plants have colours rang from orange to black. Fruit characters are found to be particularly important in the classification of individual genera (Manchester and Donoghue, 1995). *Lonicera* species is presented in Saudi Arabia only by *Lonicera etrusca* Santi (Chaudhary, 2000; Migahid, 1996). Previous phytochemical investigation of *Lonicera* species resulted in the isolation of iridoids (Katano, *et al*., 2001; Zuleta, *et al*., 2003; Kumar, *et al*., 2000; Prasad, *et al*., 2000), saponins (Xiang, *et al*., 2000; Li-Mei, *et al*., 2008), flavonoids (Kumar, *et al*., 2005; Monica, *et al*., 2007), coumarins (Matsuda and Kikuchi, *et al*., 1995) and phenolic acids (Zhilong, *et al*., 2008; Peng, *et al*., 2000). Previous phytochemical study of *Lonicera etrusca* resulted in the isolation of the secoiridoid glucosides loniceroside, morroniside, sweroside, secologanic acid, secoxyloganin, and secologanoside inaddition to loganin and loganic acid as iridoid glucosides (Basaran, *et al*., 1988). No biological or ethnopharmacological data were available in the literature about the plant.

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The present study aims to isolate and identify the secondary metabolites of *Lonicera etrusca* growing in Saudi Arabia.

Experimental

General – Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a Unicum Heyios á UV-Visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 (Central Lab at the College of Pharmacy, King Saud University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard Bruker program. ESIMS were obtained using Liquid Chromatography/Mass Spectrometer (Quattro micro API) equipped with a Z-spray electrospray ion source (Micromass®, Quattro micro™, WATERS). HRFABMS of **1** and **2** were measured using a JEOL JMS-HX-110 instrument. Silica gel 60/230 - 400 mesh (EM Science) and Sephadex® LH-20 (Pharmacia Fine Chemicals) were used for column chromatography, while silica gel 60 F254 (Merck) was used for TLC. Centrifugal preparative TLC (CPTLC; using Chromatotron (Harrison Research Inc. model 7924): 1 - 4 mm silica gel P254 disc. PTLC was performed on pre-coated RP-18 F_{254} plates (MERCK).

Plant material – The plants of *Lonicera etrusca* Santi (Caprifoliaceae) were collected in March 2003 from Taif, southern region of Saudi Arabia. The plant was identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#11221) was deposited at the herbarium of the Research Center for Medicinal, Aromatic and Poisonous Plants of the same College.

Extraction and Isolation – The air dried samples (1.15 kg) were exhaustedly extracted with 90% ethanol (12 L) at room temperature. The ethanol extract was evaporated under vacuum to yield 70 g of dark green residue. Part of the residue (50 g) was dissolved in MeOH/H_2O (6 : 4) and extracted with hexane (3 × 400 mL), $CHCl_3$ (3 × 500 mL) followed by *n*-butanol (2 × 300 mL). The $CHCl_3$ soluble fraction (15 g) was chromatographed over a silica gel column (500 g, 5 cm id) eluting with $CHCl_3$, followed by $CHCl_3$/MeOH mixtures in a gradient system. Thirty two fractions 200 mL each were collected. Fractions 9 - 18 (700 mg) eluted with 5% MeOH were subjected to CPTLC (2 mm silica gel disc) using 30% EtOAc in $CHCl_3$ followed by CPTLC using 30% EtOAc in hexane to give 90 mg of **1** and 8 mg of **2**. Fractions 24 - 27 (200 mg) eluted with 15% MeOH in $CHCl_3$ were purified over Sephadex® LH-20 column (50 g, 2 cm id) eluted with 10% H_2O in MeOH to yield 20 mg of **3** after crystallization from MeOH. The *n*-butanol soluble fraction (5 g) was chromatographed over Sephadex® LH-20 column (200 g, 5 cm id) eluted with 10% H_2O in MeOH. Fourty fractions 100 mL each were collected, screened by TLC and similar fractions were pooled. Fractions 9 - 14 (450 mg) were further purified over silica gel column (50 g, 2 cm id) eluted with 20% MeOH in CH_2Cl_2 to yield 145 mg of **4** after crystallization from MeOH. Fractions 28 - 35 (950 mg) were further purified over silica gel column (100 g, 2 cm id) eluted with 20% MeOH in CH_2Cl_2 to yield 248 mg of **5** after crystallization from MeOH.

Acetylation of 1 – Ten mg of **1** were dissolved in 1.0 mL pyridine and few drops of acetic anhydride were added. The reaction mixture was kept overnight at room temperature. The solvent was evaporated under stream of nitrogen to provide 10 mg of a homogenous mixture of **1a**.

Hydrolysis of 4 – Ten mg of **4** were dissolved in 0.5 mL of MeOH and then 0.5 mL 0.1 N HCl was added. The mixture was heated at reflux for 4 hr. The reaction mixture was then diluted with water and extracted with $CHCl_3$. The residue left after evaporation of $CHCl_3$ (4 mg) were identical with **1**.

Loganin aglycone (log-1) (1) – $C_{11}H_{16}O_5$, Colorless semisolid. $[\alpha]_D$ −45° (*c* = 0.71, $CHCl_3$). ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1. ESIMS (rel. abund. %): 251 (M^+ + Na, 100), 229 (M^+ + H, 28). HRESIMS (rel. int. %): 251.0896 [M^+ + Na] ($C_{11}H_{16}O_5$ + Na, calcd. 251.0895).

Lonicerin (log-2) (2) – $C_{12}H_{20}O_6$, Colorless semisolid. ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1. ESIMS (rel. abund. %): 283 (M^+ + Na, 100). HRESIMS (rel. int. %): 283.1155 [M^+ + Na] ($C_{12}H_{20}O_6$ + Na, calcd. 381.1158).

7-Hydroxycoumarin (umbelliferone) (3) – $C_9H_6O_3$, pale

Table 1. ^{1}H- and ^{13}C-NMR data (δ) of **1**, **1a**, **2** and **4**

Pos.	1[a]		1a[a]		2[b]		4[b]	
	^{1}H[c]	^{13}C	^{1}H[c]	^{13}C	^{1}H[c]	^{13}C	^{1}H[c]	^{13}C
1	4.99 (d, *J* = 4.0)	95.6	6.10 (d, *J* = 3.5)	90.8	4.74 (bs)	102.3	5.29 (d, *J* = 4.0)	97.8
3	7.43 (s)	151.6	7.36 (s)	150.1	5.10 (d, *J* = 9.0)	91.0	7.41 (s)	152.1
4	–	111.7	–	112.6	2.21 (dd, *J* = 9.0,12.0)	53.7	–	114.1
5	3.19 (q, *J* = 8.0)	31.4	3.14 (q, *J* = 7.0)	30.2	2.59 (m)	37.8	3.12 (q, *J* = 8.0)	32.2
6α	1.57 (m)	42.2	1.67 (m)	39.2	1.72 (dq, *J* = 8.0, 3.0)	40.4	1.66 (m)	42.7
6β	2.32 (dd, *J* = 7.5,13.5)		2.35 (dd, *J* = 7.0,12.0)		1.93 (m)		2.28 (dd, *J* = 7.5, 13.5)	
7	4.15 (m)	74.3	5.21 (t, *J* = 5.0)	76.9	4.18 (dt, *J* = 6.0, 3.0)	74.4	4.06 (m)	74.8
8	1.91 (m)	41.4	2.00 (m)	39.2	1.94 (m)	40.3	1.88 (m)	42.2
9	1.97 (m)	46.3	1.99 (m)	45.0	1.79 (bq, *J* = 7.0)	47.6	2.05 (m)	46.6
10	1.17 (d, *J* = 7.0)	13.2	1.08 (d, *J* = 7.0)	12.6	1.04 (d, *J* = 7.0)	12.6	1.11 (d, *J* = 6.5)	13.4
11	–	168.1	–	167.1		175.1	–	169.6
COO<u>C</u>H$_3$	3.72 (s)	51.3	3.74 (s)	51.3	3.71 (s)	52.3	3.71 (s)	51.6
OCH$_3$	–	–	–	–	3.45 (s)	55.4	–	–

[a] Data were collected in $CDCl_3$.
[b] Data were collected in CD_3OD.
[c] *J* values in parenthesis in Hz.

yellow crystals, m.p. 224 - 225 °C (MeOH). $UV_{\lambda max}^{MeOH}$: 324, 295, 242, 217; NaOMe: 350, 303, 257. EIMS (rel. int. %): 162 (M^+, 35).

Loganin (4) – $C_9H_6O_3$, white solid, m.p. 221 - 222 °C (MeOH). $[\alpha]_D$ −81° (*c* = 0.83, MeOH). ^{1}H- and ^{13}C-NMR: Table 1 and sugar ^{1}H-NMR (500 MHz, CD_3OD): δ 4.67 (d, *J* = 7.5 Hz, H-1'), 3.21 (t, *J* = 8.5 Hz, H-2'), 3.31 (m, H-3'- H-5'), 3.67 (m, H-6'), 3.91 (bd, *J* = 12 Hz, H-6'). Sugar ^{13}C-NMR (125 MHz, CD_3OD): δ 100.1 (C-1'), 75.0 (C-2'), 78.4 (C-3'), 71.7 (C-4'), 78.1 (C-5'), 62.8 (C-6'). ESIMS (rel. int. %): 251 (M^+ + Na, 100), 221 (M^+ + H, 28).

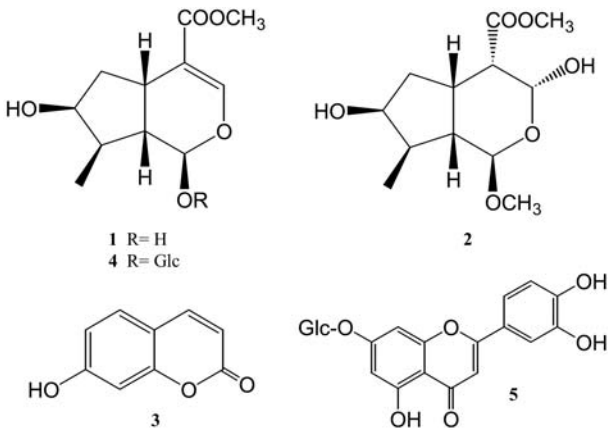
Luteolin 7-*O*-glucoside (5) – $C_{21}H_{20}O_{11}$, yellow crystals, m.p. 244 - 246 °C (MeOH). $UV_{\lambda max}^{MeOH}$: 254, 268 (sh), 348; NaOMe: 262, 403; $AlCl_3$: 273, 298 (sh), 330, 427; $AlCl_3$/HCl: 268, 295, 361, 388; NaOAc: 274, 325, 388. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.19 (t, *J* = 8.5 Hz, H-2"), 3.30 (m, H-3"- H-5"), 3.47 m (H-4", H-6"), 3.91 (d, *J* = 10.5 Hz, H-6'), 5.08 (d, *J* = 7 Hz, H-1"), 6.45 (1 H, d, *J* = 1.5 Hz, H-8), 6.75 (1 H, s, H-3), 6.79 (1 H, d, *J* = 1.5 Hz, H-6), 6.91 (1 H, d, *J* = 8.5 Hz, H- 6'), 7.42 (1 H, d, *J* = 1.5 Hz, H- 2'), 7.45 (1 H, dd, *J* = 1.5, 8.5 Hz, H-5'). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 60.6 (C-6"), 69.6 (C-4"), 73.1 (C-2"), 76.4 (C-3"), 77.1 (C-5"), 94.7 (C-8), 99.5 (C-1"), 99.9 (C-6), 103.1 (C-3), 105.3 (C-10), 113.5 (C-2'), 116.0 (C-5'), 119.1 (C-6'), 121.1 (C-1'), 145.8 (C-3'), 150.0 (C-4'), 156.9 (C-5), 161.1 (C-9), 162.9 (C-2), 164.5 (C-7), 181.8 (C-4). ESIMS (rel. int. %): 471 (M^+ + Na, 100), 449 (M^+ + H, 10).

Results and Discussion

The dried aerial parts of *Lonicera etrusca* were extracted with ethanol. The dried extract was partitioned using liquid-liquid fractionation techniques. The $CHCl_3$ and *n*-butanol soluble fractions were subjected to various chromatographic purification steps to provide five compounds.

Compounds **3 - 5** were identified as 7-hydroxycoumarin (umbelliferone) (Pouchert and Behnke, 1992), loganin (Prasad, *et al*., 2000) and luteolin 7-*O*-glucoside (Mizuno, *et al*., 1987; Markham, *et al*., 1978), respectively, by comparison with literature data and authentic material in case of **3**.

Compound **1** was isolated as a colorless semisolid. HRESIMS showed an (M^+ + Na) at 251.0896 *m/z* for the molecular formula $C_{11}H_{16}O_5$. The eleven carbons were clear in the ^{13}C-NMR (Table 1) and were sorted by DEPT experiments into 2 × CH_3 including one methoxyl group, 1 × CH_2, 6 × CH including one dioxygenated, one



oxygenated and one olefinic methins. In the ^{1}H-NMR of **1** (Table 1) the two protons at δ_H 4.99 (d, *J* = 4.0) and 4.15 (m) were assigned for the dioxygenated and oxygenated methins respectively. The chemical shifts of the two left quaternary carbons at δ_C 151.6 and 168.1 were diagnostic for olefinic and carbonyl carbons, respectively (Silverstein, *et al.*, 2005). The data of **1** were consistent with iridoid skeleton with carboxylic group at C-11 and hydroxyl group at C-7 (Boros and Stermitz, 1990). In an HMBC experiment the methyl group at δ_H 3.72 showed correlation with the carboxylic carbon at δ_C 168.1 indicating that the C-11 carboxylic group in methylated. The data of **1** were similar to those reported for loganin except the absence of sugar part signals (Prasad, *et al*., 2000). Acetylation of **1** resulted in a diacetyl derivative (**1a**) confirming the presence of two free hydroxyl groups. In the ^{1}H-NMR of **1a** both H-1 and H-7 were downfield shifted to δ_H 6.10 and 5.21, respectively indicating that C-1 and C-7 hydroxyls were free in **1**. Acid hydrolysis of loganin (**4**) produced aglycone similar to **1** as indicated by TLC and spectral data.

Compound **2** was isolated in a minute amount. The HRESIMS of **2** showed an M^+ + Na at 283.1155 *m/z* for the molecular formula $C_{12}H_{20}O_6$. The major difference between the ^{1}H- and ^{13}C-NMR of **2** comparing with **1** (Table 1) was the replacement of the olefinic signals with two methins including a second dioxygenated methin. That means hydration of the double bond with introduction of the hydroxyl group to C-3. HSQC experiment correlates the proton at δ_H 4.74 (bs) to the carbon at δ_C 102.3 and the proton at δ_H 5.10 (d, *J* = 9.0) to the carbon at δ_C 91.0. The appearance of the proton at δ_H 4.74 as a broad singlet and its HMBC correlation with the methoxyl at δ_C 55.4 enable its assignments to C-1 α-position and indicated that C-1 hydroxyl is methoxylated. HMBC correlation of the proton at δ_H 5.10 with the

carbonyl at δ_C 175.1 confirmed its location at C-3. The $J_{3,4}$ = 9 Hz and $J_{4,5}$ = 12 Hz were diagnostic for β-orientation of the three protons (Silverstein, *et al.*, 2005). The location of the second methyl group (δ_H 3.71 and δ_C 52.3) was deduced to the carboxylic group based on its HMBC correlations with the carbonyl at δ_C 175.1.

This is the first isolation of **1** and **2** from the plant species. However, both **1** and **2** were recently obtained from the microbial metabolism of loganin by intestinal bacteria. The spectral data of our isolates were in good agreement with the reported data (Li, *et al*., 2008). However, the names given for the two compounds: log-1 and log-2 are somewhat confusing. The names loganin aglycone correlating to the original glycoside loganin for **1** and lonicerin referring to the genus for **2** will be more appropriate.

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