SHORT PAPER

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Iridoid glucosides from Chinese herb *Lonicera chrysatha* and their antitumor activity†

Yuli Wang, Qingyi Wei, Li Yang* and Zhong-Li Liu

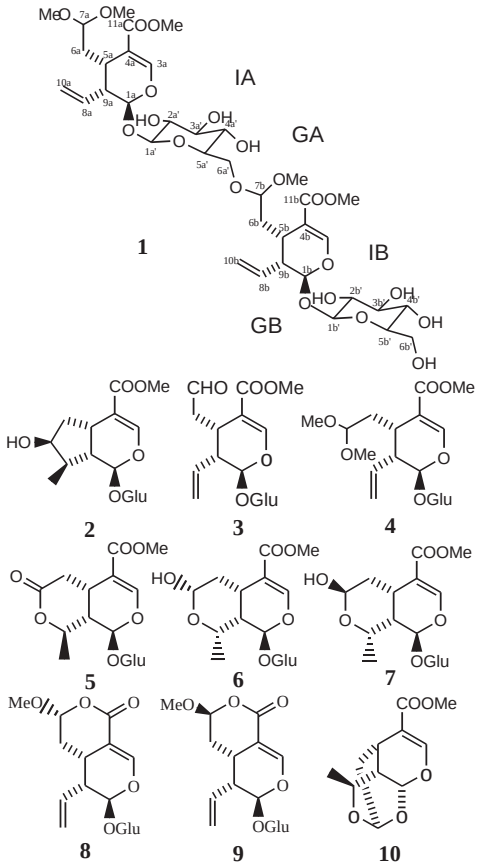
National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu 730000, China

A new bis-iridoid glucosides, secologanin methyl hemiacetal-yl-6´- secologanin dimethyl acetal (chrysathain, **1**), was isolated from the methanol extract of the leaves of Chinese medicinal plant *Lonicera chrysatha* along with nine known iridoid glucosides, *i.e.*, loganin (**2**), secologanin (**3**), secologanin dimethylacetal (**4**), 8-epi-kingiside (**5**) 7α-morroniside (**6**), 7β-morroniside (**7**), vogeloside (**8**) 7-epi-vogeloside (**9**) and sarracenin (**10**). Their structures were elucidated by spectroscopic methods. Compounds **1**–**5** showed moderate *in vitro* antitumour activity against human promyelocytic leukemia (HL-60) cells.

Many species of the genus *Lonicera* have been used in Chinese traditional medicines for the treatment of fever and skin ulcers.[1] It has been reported that aqueous extracts of some species of *Lonicera* exhibit antibacterial, antiviral and immunological enhancement effects.[1-4] In our effort to find biologically active components from Chinese medicinal plants,[5-7] we found that the methanol extract of the leaves of *Lonicera chrysatha* afforded a new bis-iridoid glucoside, secologanin methyl hemiacetal-yl-6'-secologanin dimethyl acetal (chrysathain, **1**), as well as nine known iridoid glucosides, *i.e.*, loganin (**2**), secologanin (**3**), secologanin dimethylacetal (**4**), 8-epi-kingiside (**5**) 7α-morroniside (**6**), 7β-morroniside (**7**), vogeloside (**8**) 7-epi-vogeloside (**9**) and sarracenin (**10**). Amongst these **6**, **7** and **10** were found in the genus *Lonicera* for the first time (Scheme 1). We report herein the isolation and structural elucidation of the new compound and the antitumor activities of compounds **1**–**5**.

The crushed dry leaves of *Lonicera chrysatha*, were collected from Yuzhong county, Gansu province, China in August 2001 and identified by Professor Yong-Hong Zhang at the Department of Chemistry, Lanzhou University. They were extracted with methanol followed by silica gel column chromatographic separation to give compounds **1**–**10**.

Compound **1** was obtained as an amorphous powder, $[\alpha]^{2}_{D}{}^{7}$ –199.0°(C. 1.2, CH_3OH). The HR-ESI-MS spectrum exhibits an M+Na ion peak at *m/z* 859.3271 corresponding to a molecular formula of $C_{37}H_{56}O_{21}$ (calcd. for M+Na: 859.3206). Its IR spectrum shows absorption bands for hydroxyl groups (3395 cm^{-1}), double bonds (1631 cm^{-1}) and conjugated ester carbonyl groups (1705, 1288 and 1077 cm^{-1}). The ^{13}C NMR and DEPT spectra of **1** exhibit 37 carbon signals (5×CH_3, 6×CH_2, 22×CH, 4×C). In conjunction with its ^{1}H NMR spectrum it is clear that compound **1** possesses five methoxyl groups (δ_C ~ 52 and δ_H ~ 3.5), two terminal double bonds (δ_C ~ 120 and δ_H ~ 5.3), two cyclic double bonds connected to oxygen (δ_C ~ 111 and 153, δ_H ~ 7.5), two ester carbonyl carbons (δ_C ~ 169) and two glucose moieties (δ_C ~ 62–100, δ_H ~ 3.2–4.8). Comparison with the NMR data of secologanin and secologanin dimethyl acetal [8,9] suggests that **1** is a new bis-iridoid glucosides, secologanin methyl hemiacetal-yl-6'-secologanin dimethyl acetal and named as chrysathain. Some ^{1}H and ^{13}C NMR signals are overlapped but can be distinguished from their 2D NMR spectra. The assignments are confirmed by its H,H-COSY, HMQC and HMBC spectra as discussed below.



Scheme 1

The most deshielded ^{1}H NMR signal of **1** at δ 7.45 (2H) was assigned as H-3a and H-3b since it is typical for the olefinic proton of an α,β-unsaturated ester. The corresponding C-3a,b (δ 153.3) is assigned from the HMQC spectrum. In the HMBC spectrum the H-3 protons are correlated with C-1, C-4 and C-5, and the C-1a,b (δ 97.8) are correlated with H-3a,b, H-5a,b, H-8a,b and H-9a,b, respectively. Therefore, the structure of the dihydropyran ring was confirmed. The HMBC correlations of the terminal double bond protons H-10a (δ 5.26) and H-10b (δ 5.28) with C-8a,b, C-9a,b, C-1a,b and C-5a,b respectively, and the H,H-COSY correlations of H-8a,b with H-8a,b and H-10a,b, confirm that the two terminal double bonds are connected to C-9a and C-9b respectively. Similarly, the connections of the two methoxycarbonyl groups at C-4a and C-4b, the dimethyl acetal group at C-6a and the methyl hemiacetal group at C-6b were confirmed by their

* To receive any correspondence. E-mail: yangl@lzu.edu.cn

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H,H-COSY and HMBC correlations. The HMQC correlations of H-1a with C-1a' and H-1a' with C-1a indicate that the iridoid A (IA) is connected to the C-1a' of glucose A (GA). The HMBC correlations of H-1b with C-1b' and H-1b' with C-1b indicate that the iridoid B (IB) is connected to the C-1b' of glucose B (GB). The chemical shifts of 6a' (δ_C 66.7, δ_H 3.61 and 3.90) and 6b' (δ_C 62.7, δ_H 3.70 and 3.93) in the two glucoses are significantly different because C-6a' is connected to the iridoid B through an oxygen at C-7b, while C-6b' is connected to a hydroxyl group. These are also confirmed by HMBC correlations of H-6a' with C-7b and H-7b with C-6a'. The clear NOESY correlations of H-5a with H-9a, H-5b with H-9b, and no such correlations with H-1a,b, demonstrate that H-5a,b and H-9a,b are β-oriented, while H-1a,b are α-oriented as in the case of other known secologanins.[8,9]

Structures of compounds **2** (loganin[8,9]), **3** (secologanin[8,9]), **4** (secologanin dimethylacetal[8,9]), **5** (8-epi-kingiside[10]), **6** (7α-morroniside[11]), **7** (7β-morroniside[11]), **8** (vogeloside[8]), **9** (7-epi-vogeloside[8]) and **10** (sarracenin[12]) were identified by comparison of their ^{1}H and ^{13}C NMR, MS and IR spectroscopic data and optical rotations with those reported in the literature. It should be noted that some chemical shift assignments for sarracenin[12] (**10**) require correction based on our H,H-COSY, HMQC and HMBC data. The signal at δ 22.1 should be assigned to C-5 instead of C-6, and that at δ 35.1 should be assigned to C-6 instead of C-5, based on the HMQC correlation of C-5 with the methine proton H-5 (δ 2.98), and that of C-6 with the methylene protons H-6a and H-6e (δ 1.69 and 2.34 respectively). In addition, the signal at δ 5.78 should be assigned to H-1 instead of H-7, and that of δ 4.99 should be assigned to H-7 instead of H-1, based on the HMQC correlation of H-1 with C-1 (δ 91.7) and that of H-7 with C-7 (δ 88.1), and the HMBC correlations of H-1 with C-3 and C-5, C-1 with H-3, H-7 and H-8, H-7 with C-8 and C-1, and C-7 with H-6e.

The *in vitro* antitumour activity of compounds **1**–**5** against human promyelocytic leukemia (HL-60) cells was tested by the methylthiazoylyl tetrazolium (MTT) assay[13] (Fig. 1). It is seen that HL-60 cell proliferation was inhibited by these compounds in a concentration-dependent manner and the IC_{50} values of compounds **1**, **2**, **4** and **5** are around 70 μg/ml.

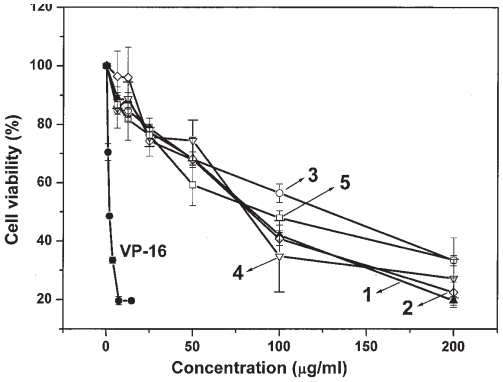

Fig. 1 Effects of compounds **1**–**5** on viability of HL-60 cells. The percent viability was determined by MTT assay as described in the experimental section. Values are the mean ± SE of triplicate samples. ▲ : **1**; ◇ : **2**; ○ : **3**; ▽ : **4**; □ : **5**; ● : VP-16 (positive control).

Experimental

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken on a Nicolet 170 SX IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer with TMS as internal standard. HR-ESI-MS and EI-MS spectra were obtained on a Bruker APEX II FT-MS and HP 5988 MS spectrometers respectively.

Extraction and isolation procedures: The air-dried leaves of *Lonicera chrysatha* (2.5 kg) were crushed into small pieces and extracted with methanol at room temperature to give a black-brown residue (94 g) after removing the solvent. This residue was separated by silica gel (200–300 mesh) column chromatography with gradient elution of chloroform–methanol (from 30:1 to 3:1), giving **1** (41 mg), **2** (33 mg), **3** (74 mg), **4** (600 mg), **5** (34 mg), **6** (15 mg),**7** (8 mg). **8** (2 mg), **9** (71 mg) and **10** (3 mg).

Chrysathain (**1**): amorphous powder, $[\alpha]^2{}_D{}^7$ –199.0°(*ca* 1.2, CH_3OH). HR-ESI-MS: M+Na$^+$ Found: 859.3271, Calcd for $C_{37}H_{56}O_{21}$ + Na: 859.3206; ν_{max}/cm^{-1}: 3395, 1705, 1631, 1288, 1077; δ_H (400 MHz, CD_3OD, TMS): 7.45 s (2H, H-3a,b), 5.73 (ddd, *J*=5.2, 8.0, 13.6 Hz) (H-8a), 5.70 m (H-8b), 5.53 (d, *J*=4.0 Hz) (H-1a), 5.47 (d, *J*=4.0 Hz) (H-1b), 5.32 (d, *J*=16.4 Hz) (2H, H-10a,b *trans*-), 5.28 (d, *J*=10.0 Hz) (H-10b *cis*-), 5.26 (d, *J*=10.0 Hz) (H-10a *cis*-), 4.70 (*J*=5.6 Hz) (H-1a'), 4.68 (d, *J*=5.6 Hz) (H-1b'), 4.64 (t *J*=7.8 Hz) (H-7b), 4.48 (t *J*=7.8 Hz) (H-7a), 3.93 (m), 3.70 (m) (2H, H-6b'), 3.90 (m), 3.61 (dd, *J*=8.0, 12.0 Hz) (2H, H-6a'), 3.71 (s) (6H, 11a, b-MeO), 3.43 (m) (H-5b'), 3.38 (m) (H-4b'), 3.36 (m) (3H, H-3a',b', H-5a'), 3.32 (s) (3H, 7a-MeO), 3.30 (s) (3H, 7a-MeO), 3.28 (s) (3H, 7b-MeO), 3.26 (m) (H-2a'), 3.25 (m) (H-2b'), 3.24 (m) (H-4a'), 2.94 (dt, *J*=6.4, 8.4 Hz) (H-5a), 2.93 (dt *J*=6.4, 8.4 Hz) (H-5b), 2.70 (ddd, *J*=4.0, 5.2, 8.4 Hz) (H-9a), 2.66 (m) (H-9b), 2.04 (dd, *J*=6.4, 7.8 Hz), 1.75 (dd, *J*=6.5, 11.9) (2H, H-6b), 2.00 (dd, *J*=6.4, 7.8 Hz), 1.64 (dd, *J*=6.6, 12.4 Hz) (2H, H-6a); δ_C (100 MHz, CD_3OD, TMS): 169.2 (C-11a,b), 153.3 (C-3a,b), 135.7 (C-8a), 135.6 (C-8b), 120.0 (C-10b), 119.8 (C-10a), 111.7 (C-4a,b), 104.4 (C-7a), 104.0 (C-7b), 100.1 (C-1'a,b), 97.8 (C-1a,b), 78.2 (C-3a'), 77.9 (C-3b'), 77.7 (C-5a'), 77.0 (C-5b'), 74.5 (C-2a',b'), 71.4 (C-5a',b'), 66.7 (C-6a'), 62.7 (C-6b'), 53.9 (7b-MeO), 52.8 (7a-MeO), 51.8 (11a,b-MeO), 45.2 (C-9a,b), 33.7 (C-6a), 33.5 (C-6b), 29.7 (C-5b), 29.3 (C-5a).

The structures of compounds **2**–**10** were characterised by their m.p., IR, MS, ^{1}H and ^{13}C NMR data and compared with those reported in literature.[8-12] In case of sarracenin[12] (**10**), however, the correct NMR assignments shall be: δ_H (400 MHz, $CDCl_3$, TMS): 7.46 (s (H-3), 5.78 (d, *J*=1.6 Hz) (H-1), 4.99 (d, *J*=3.3 Hz) (H-7), 4.21 (q *J*=6.4 Hz) (H-8), 3.75 (s (11-OCH_3), 2.98 (ddd, *J*=1.6, 5.2, 9.2 Hz) (H-5), 2.34 (dd, *J*=9.6, 13.8 Hz) (H-6a), 1.69 m (H-6e), 1.66 m (H-9), 1.34 (d, *J*=6.5 Hz) (3H, H-10); δ_C (100 MHz, $CDCl_3$, TMS): 166.8 (C-11), 150.1 (C-3), 112.3 (C-4), 91.7 (C-1), 88.1 (C-7), 69.0 (C-8), 51.4(11-OCH_3), 35.1(C-6), 32.3 (C-9), 22.1 (C-5), 18.7 (C-10).

Cytotoxicity assay: Human promyelocytic leukemia (HL-60) cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were cultured at 37°C under a humidified atmosphere of 5 % CO_2 in RPMI 1640 medium supplemented with 10 % fetal serum and dispersed in a 96-well flat-microtiter plate with 1×10^4 cells/well. Then the cells were incubated with various concentrations of compounds **1**-**5** or etoposide (VP-16) which was used as a positive control. After 48 h exposure to the toxins cell viability was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay[13] by measuring the absorbance at 570 nm with a Bio-Rad 550 ELISA microplate Reader. Each test was performed in triplicate.

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