Psoralenoside and Isopsoralenoside, Two New Benzofuran Glycosides from *Psoralea corylifolia*

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Two new benzofuran glycosides, called psoralenoside and isopsoralenoside, were isolated from the fruits of *Psoralea corylifolia*, together with nine known compounds. Their structures were elucidated by detailed spectral analyses including extensive two dimensional (2D) NMR spectra.

Key words *Psoralea corylifolia*; psoralenoside; isopsoralenoside; benzofuran glycoside

The fruits of *Psoralea corylifolia* L. (Fabaceae), well-known as a traditional Chinese medicine "Buguzhi", are widely applied for the cure of gynaecological bleeding, vitiligo and psoriasis. In recent years, many studies focused on analyses of its quality,[1—3] phytochemistry,[4,5] and bioactivities.[6—18] Our liquid chromatography mass spectroscopy (LC-MS) analyses on the chemical profile of the fruits led to the discoveries of two benzofuran glycosides. They were isolated and identified to be the glucosides of psoralen and isopsoralen by detailed spectral analyses, respectively, along with nine known compounds.

In the MeOH extract of the fruits of *Psoralea corylifolia*, two interesting compounds (**1**, **2**) were found by high performance liquid chromatography (HPLC) analyses. They were shown as major peaks in the HPLC chromatogram, but had never been reported. Moreover, they were absent in the boiled water extract of the fruits, but their polarity was close to sugar. The high performance liquid chromatography electrospray ionization mass spectroscopy (HPLC-ESI-MS) analyses showed that they presented the same MS spectra, in which three major ion peaks were displayed clearly. As shown in Fig. 1, one was at *m/z* 384, corresponding to $[M+NH_4]^+$. Another was at *m/z* 205, due to $[M-glc+H]^+$. The base peak was at *m/z* 187, assignable to $[M-glc-H_2O+H]^+$, or $[psoralen/isopsoralen+H]^+$. In the high resolution electrospray ionization mass spectroscopy (HR-ESI-MS), compound **1** showed the $[M+K]^+$ peak at *m/z* 405.0601, in accord with the molecular formula $C_{17}H_{18}O_9$ (Calcd 405.0582 for $C_{17}H_{18}O_9K$), and compound **2** displayed the $[M+Na]^+$ peak at *m/z* 389.0869, suggesting the same molecular formula $C_{17}H_{18}O_9$ (Calcd 389.0843 for $C_{17}H_{18}O_9Na$). These observations suggested that compounds **1** and **2** might be glycosides related to psoralen and isopsoralen, respectively. This deduction was confirmed by the de-

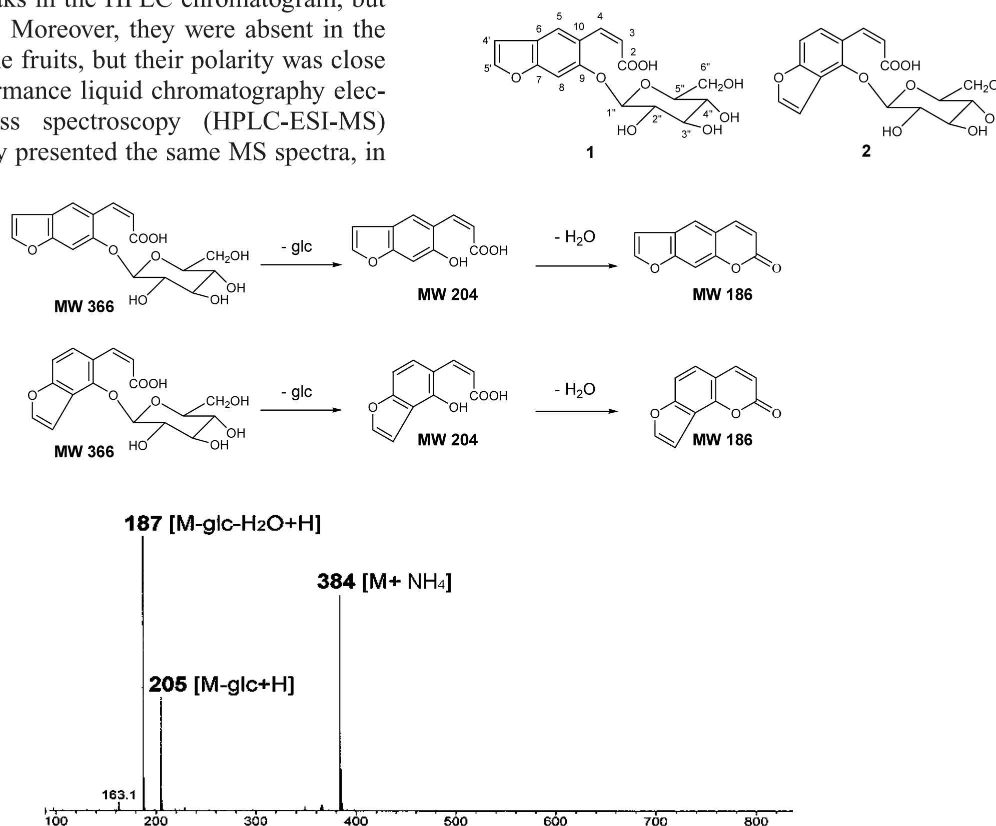

Fig. 1. The HPLC-ESI-MS Spectra of Compounds **1** and **2**

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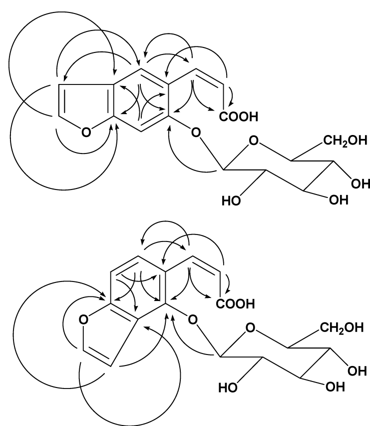

Fig. 2. HMBC Correlations of **1** and **2**

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** in $CDCl_3$ (δ in ppm, J in Hz)

C	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
2		177.1		178.0
3	6.07, d, 12.4	127.8	6.07, d, 12.4	129.0
4	6.95, d, 12.4	129.8	7.03, d, 12.4	128.7
5	7.88, s	123.1	7.67, d, 9.2	128.0
6		123.8	7.20, d, 9.2	108.9
7		157.2		158.6
8	7.36, s	100.6		122.5
9		155.1		150.6
10		124.9		125.8
4′	6.75, d, 2.0	108.1	7.18, d, 2.0	107.5
5′	7.65, d, 2.0	146.9	7.65, d, 2.0	146.7
1″	4.98, d, 7.6	103.7	4.95, d, 7.6	106.6
2″	3.53—3.55, m	75.5	3.54—3.56, m	76.5
3″	3.51—3.53, m	78.8	3.45—3.49, m	79.2
4″	3.47—3.49, m	72.0	3.45—3.49, m	72.4
5″	3.49—3.51, m	78.7	3.45—3.49, m	79.1
6″	3.92, dd, 12.0, 3.0; 3.73, dd, 12.0, 5.6	63.1	3.83, dd, 12.0, 3.0; 3.72, dd, 12.0, 5.6	63.6

tailed analyses of their NMR spectra.

The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited two groups of signals consistent with the structural moieties of psoralen and glucose.[19] The glucose was located at C-9 by the key heteronuclear multiple bond connectivity (HMBC) correlation (Fig. 2) between the anomeric proton H″-1 (δ 4.98, d, J=7.6 Hz) with C-9 (δ 155.1). Furthermore, the glucose was β-orientated according to the coupling constant J=7.6 Hz. A similar benzofuran glucoside, namely psoralic acid O-β-D-glucopyranoside, had been reported from the fruits of *P. plicata*,[19] with the only difference from **1** being at the *trans*-cinnamic acid residue. The coupling constant (J) of the *trans*-coupled olefinic protons was 16.0 Hz, while that of **1** was 12.4 Hz, which suggested that the cinnamic acid moiety was *cis*-coupled. This deduction was confirmed by the clear NOE effect arising from these two *cis*-coupled protons (δ 6.07 and 6.95, d, J=12.4 Hz) in the ROESY spectrum of **1**. Therefore, compound **1** was determined to be the *cis*-isomer of psoralic acid O-β-D-glucopyranoside, called psoralenoside. In the same way, compound **2** was elucidated to be *cis*-isomer of isopsoralic acid O-β-D-glucopyranoside, and called isopsoralenoside. The elucidation was supported by the acid hydrolysis of **1** and **2** which gave a mixture of psoralen, isopsoralen, and D-glucose. Their NMR data were unambiguously assigned on the basis of extensive 2D-NMR analysis (Table 1).

Nine known compounds were also isolated. By comparing them with the reported data,[3,5] they were identified as psoralen, isopsoralen, psoralidin, corylifolin, corylin, corylifolinin, isobavachalcone, corylifol A, and bakuchiol.

It was very difficult to isolate these benzofuran glycosides. In the previous phytochemical studies on the fruits of *Psoralea plicata*,[19] several similar benzofuran glycosides were reported. These glycosides, however, were obtained as acetates. Acetylization was carried out to improve the separation by changing the chemical and chromatographic properties of these glycosides. In other words, the glycosides with free hydroxyls had not been isolated. In our experiments, the target compounds were first isolated by repeated column chromatography (CC) on ODS. But the isolates were proved impure by NMR analysis, though their related HPLC analyses showed only one peak. The impurity was finally revealed by an evaporative light scattering detector (ELSD), and was removed by CC on Sephadex LH-20 eluting with MeOH.

Experimental

General Procedures ESI-MS was recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on a Brucker AM-400 and a DRX-500 instrument with TMS as internal standard, respectively. The HPLC-ESI-MS analyses were carried out using an Agilent 1100 series which consisted of a binary pump and photodiode array detector (DAD), coupled to an Agilent MSD-Trap SL through a Brucker atmospheric pressure chemical ionization (APCI) interface. The APCI-MS spectra were acquired over a range of m/z 100—900 in positive ion mode. The temperature of dry gas and APCI were set at 350 °C and 400 °C, respectively. The nebulizer gas pressure was 50 psi and the dry gas flow rate was 5 l/min. The HPLC system was directly connected to the MS without stream splitting. The injection volume of sample was 10 μl. The Agilent 1100 series HPLC system equipped with a Zorbax® XDB-C_8 analytical column (4.6×150 mm, 5 μm, Agilent Technologies, U.S.A.), a C_{18} guard column (4.6×12.5 mm, 5 μm, Agilent Technologies), a DAD, and an Alltech ELSD 2000 detector (U.S.A.) was set up for the analysis. The analysis was performed at 20 °C during the whole process. The mobile phase was a mixture of methanol and 0.1% acetic acid at a flow rate of 1.0 ml/min. Linear gradient elution from 10% to 88% methanol (v/v) in 40 min was applied.

Plant Material The dried fruits of *P. corylifolia* were purchased in Shenzhen, China, and authenticated by Dr. C. F. Qiao, Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China. A specimen (No. CMED-0178-26) was deposited at the Chinese Medicine Laboratory, HKJCICM.

Extraction and Isolation The dried powder of the fruits of *P. corylifolia* (1 kg) was refluxed twice with 5 l EtOAc. The residue was then refluxed twice with 5 l of MeOH. The MeOH extract was filtered and evaporated to dryness by rotary evaporation below 60 °C under reduced pressure. The residue was suspended in water. The water solution was loaded on a Diaion HP-20 macroporous resin column, eluting with water. The glycoside fraction (200 mg) was collected on the basis of HPLC detection. Repeated separation of the target fraction on an ODS column eluting with 5% MeOH, and further purification by Sephadex LH-20 column chromatography eluting with MeOH yielded compounds **1** (21 mg) and **2** (30 mg). All the fractions were detected using HPLC.

Psoralenoside (**1**): A white amorphous powder. $[\alpha]_D^{28}$ −64° (c=0.023, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 2922, 1637, 1554, 1464, 1423, 1345, 1294, 1192, 1162, 1075, 1041, 898, 840, 765, 730, 623. ESI-MS m/z: 405 $[M+K]^+$; HR-ESI-MS m/z: 405.0601 $[M+H]^+$, (Calcd 405.0582 for $C_{17}H_{18}O_9K$).

Isopsoralenoside (**2**): A white amorphous powder. $[\alpha]_D^{20}$ +3° (c=0.024, MeOH). IR (KBr) ν_{max} cm^{-1}: 3399, 2922, 1633, 1555, 1466, 1425, 1345, 1279, 1208, 1162, 1072, 894, 854, 753, 612. ESI-MS m/z: 389 $[M+Na]^+$; HR-ESI-MS m/z: 389.0869 $[M+Na]^+$, (Calcd 389.0843 for $C_{17}H_{18}O_9Na$).

Acid Hydrolysis of 1 and 2[20] A solution of **1** and **2** (2.0 mg of each) in 1 M HCl (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction

mixture was extracted with EtOAc. With psoralen, isopsoralen, and D-glucose (Sigma) being reference compounds, the EtOAc extract and acid-water layer were examined under several TLC conditions; psoralen and isopsoralen were found in the EtOAc extract and D-glucose was detected in the water layer.

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