Studies on a Medicinal Parasitic Plant: Lignans from the Stems of *Cynomorium songaricum*

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Eight phenolic compounds including two new lignan glucopyranosides together with a known alkaloid were isolated from the stems of *Cynomorium songaricum* RUPR. (Cynomoriaceae). Their chemical structures were elucidated on the basis of spectral and chemical evidence. The chemotaxonomic significance of these metabolites is discussed.

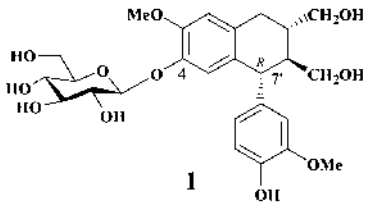
Key words *Cynomorium songaricum*; Cynomoriaceae; parasitic plant; lignan; chemotaxonomy

Parasitic plants are defined as vascular plants that have developed specialized organs for the penetration of the tissues of other vascular plants (hosts) and the establishment of connections to the vascular strands of the host for the absorption of nutrients by the parasite. Parasitic plants are commonly divided into holoparasites that lack chlorophyll, do not carry out photosynthesis, and absorb organic matter from the hosts, and hemiparasites that are green, capable of photosynthesis, and absorb mainly inorganic nutrients from hosts. From ancient times, a number of parasitic plants have been widely used as traditional medicines in China. For the purpose of understanding the unusual secondary metabolism in parasitic plants and elucidating the pharmacologically active constituents of traditional Chinese medicines derived from parasitic plants, we initiated studies of the chemical constituents of *Cynomorium songaricum* RUPR. (Cynomoriaceae). *C. songaricum* belongs is an achlorophyllous holoparasite that is distributed in the northwestern part of China. Its stems are used as tonics and for the treatment of kidney disorders.[1] Among its chemical constituents, steroids, triterpenes,[2] fructosides,[3] flavanoids, and condensed tannins[4] have been reported previously. This paper deals with the structural elucidation of two new phenolic compounds (**1** and **2**) isolated from the stems of *C. songaricum* and their chemotaxonomic significance.

Results and Discussion

MeOH extracts and 70% aqueous acetone extracts of the stems of *C. songaricum* were combined and subjected to partition between the EtOAc and H_2O layers. The H_2O layer and EtOAc layer were individually fractionated and purified by a combination of column chromatographies as described in the Experimental Methods section. Compounds **1** and **2**, together with nicoloside,[5] gallic acid, phloridzin,[4] and rutin were isolated from the H_2O layer. Methyl protocatechuicate, *p*-hydroxy benzoic acid, and (−) catechin were obtained from the EtOAc layer. The known compounds were identified by comparison with authentic samples or reported spectral and physical data.

Compound **1**, a pale yellow amorphous powder, showed an $[M+Na]^+$ and $[M+H]^+$ ion peak at *m/z* 545 and 523, respectively, in the positive FAB-MS spectrum, which corresponds to the molecular formula $C_{26}H_{34}O_{11}$, together with carbon signals in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of **1** showed signals due to two methoxyl groups [δ_H 3.76, 3.53 (each 3H, s)], an anomeric proton of sugar [δ_H 5.51 (1H, d, J=7 Hz)], 1,3,4-trisubstituted [δ_H 7.02 (1H, d, J=2 Hz), 7.01 (1H, d, J=8), 6.86 (1H, dd, J=2, 8 Hz)], and 1,3,4,6-tetrasubstituted [δ_H 7.09 (1H, s), 6.90 (1H, s)] benzene rings. In the ^{13}C-NMR spectrum (Table 1), signals arising from a glucopyranose moiety, two aromatic nuclei, and six aliphatic carbons along with two methoxyl groups were observed, suggesting that the aglycone of **1** is a lignan. Enzymatic hydrolysis of **1** by cellulasefurnished (−)-isolariciresinol (**1a**) ($[\alpha]_D^{15}$ −34.6°, acetone)[6] and D-glucose [$[\alpha]_D^{20}$ +27.6° (H_2O)], confirmed that **1** is a glucopyranoside of **1a**. When downfield shifts of C-1 and C-4 of **1** were compared with those of **1a** (Table 1), they indicated a glycosidic linkage at the C-4 hydroxyl group. The anomeric configuration of glucopyranose was determined to be β from the $^3J_{H1,H2}$ value (7 Hz). The absolute configuration of C-7′ of **1** was further established to be *R*, since a positive Cotton effect at 291 nm[7] was observed in the CD spectrum. Thus compound **1** was determined to be (−)-isolariciresinol 4-*O*-β-D-glucopyranoside.



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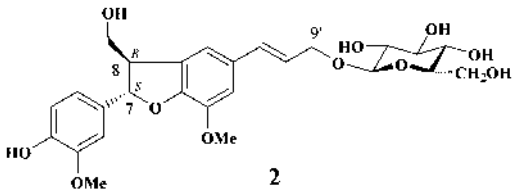

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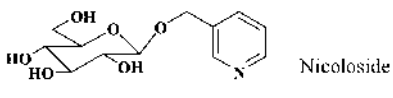

Nicoloside

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Table 1. ^{13}C-NMR (75 MHz) Sectral Data of **1**, **1a**, and **2**

No.	**1**[a)]	**1a**[a)]	**2**[b)]
1	137.2	128.1	134.5
2	116.3	116.4	110.5
3	148.7	148.7	149.1
4	148.1	147.0	147.5
5	117.9	117.9	116.1
6	137.9	138.0	119.7
7	33.6	33.7	89.3
8	40.1	40.4	56.7
9	65.5	65.5	64.8
1′	134.0	134.3	132.3
2′	112.9	112.6	112.1
3′	146.7	146.5	145.5
4′	145.7	146.2	149.4
5′	113.7	113.7	130.3
6′	122.9	123.0	116.7
7′	47.6	47.9	134.2
8′	47.9	48.1	124.2
9′	61.2	61.8	70.9
OMe	56.2	56.1	55.1
OMe	55.8	55.8	56.4
Glc-1	101.8	—	103.1
-2	74.7	—	75.1
-3	78.2	—	77.9
-4	70.3	—	71.7
-5	78.5	—	78.1
-6	61.8	—	62.8

a) In C_5D_5N, *b*) in CD_3OD.

Compound **2** was isolated as a pale yellow amorphous powder. Its positive FAB-MS spectrum exhibited an $[M+Na]^+$ ion peak at *m/z* 543, indicating that the degree of unsaturation of **2** is one greater than that of **1**. In the ^{1}H-NMR spectrum of **2**, in addition to the signals arising from two methoxyl groups, a β-glucopyranose moiety, 1,3,4-trisubstituted and 1,3,4,5-tetrasubstituted benzene rings which are similar to those of **1**, two olefinic proton signals [δ_H 6.61 (1H, d, J=16 Hz), 6.22 (1H, d*t*, J=16, 6 Hz)] of a *trans* double-bond were observed. The presence of glucose was also confirmed by acid hydrolysis of **2**, followed by cochromatography with an authentic sample of glucose on high performance liquid chromatography (HPLC). ^{13}C-NMR data (Table 1) indicated that **2** is also a lignan glucopyranoside. By carefully analyzing the ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{13}C-NMR spectrum, the aglycone of **2** was determined to be dehydrodiconiferyl alcohol.[8)] The coupling constant (6 Hz) of H-7 and H-8 supported a *trans* configuration between C-7 and C-8.[9)] Since H-9′ signals shifted downfield by 1.1 ppm compared with that of dehydrodiconiferyl alcohol,[8)] the location of the glucose was established to be at the C-9′ hydroxyl position. The absolute configuration of the dihydrofuran ring was determined to be 7*S*, 8*R* from the positive Cotton effect at 278 nm in the CD spectrum.[9)] On the basis of the above evidence, compound **2** was determined to be (7*S*, 8*R*) dehydrodiconiferyl alcohol 9′-β-glucopyranoside.

We have isolated eight henolic compounds, including two new lignan glucopyranosides (**1**, **2**), together with a known alkaloid from the stems of *C. songaricum*, a parasitic medicinal plant of the family Cynomoriaceae. In our ongoing studies on the chemical constituents of parasitic medicinal plants, we have recently isolated compound **1** and a neolignan glucopyranoside with the same dihydrobenzofuran skeleton as compound **2** from *Balanophora japonica* (Balanophoraceae) (Jiang *et al.*, unpublished data). Moreover, phloridzin, a dihydrochalcone glucoside, has been also reported from a Balanophora plant.[10)] This chemical evidence suggests that Cynomoriaceae is taxonomically related to Balanophoraceae. The polyphenolic constituents of *Balanophora* will be reported in the near future.

Experimental Methods

^{1}H- (500 and 300 MHz) and ^{13}C- (125 MHz and 75 MHz) NMR: Varian UNITY plus 500 and Varian GEMINI 300 spectrometers with tetramethylsilane (TMS) as the internal standard. EI-MS and FAB-MS: JEOL JMX DX-303 mass spectrometer. CD: JASCO J-725 apparatus. Optical rotations: JASCO DIP-370 digital polarimeter. CC: Kieselgel 60 (70—230 mesh, Merck), MCI-gel CHP 20P (75—150 μm, Mitsubishi Kasei), Chromatorex ODS (100—200 mesh, Fuji Silysia Chemical), Sephadex LH-20 (25—100 mm, Pharmacia), and TSK gel Toyopearl HW-40 (Tosoh Corp.). TLC: Precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck). HPLC was performed with a JASCO 880-PU pump equipped with an 830-RI RI detector, using a column of TOSOH TSKgel NH_2 60 (4.5×250 mm) with a solvent system of H_2O–CH_3CN (4 : 6) (1 ml/min).

Plant material: Stems of *C. songaricum* were purchased from a drugstore in Tianjin, China, in June, 1997, and identified by one of the authors (TJ).

Extraction and separation: The stems (2.0 kg) were chopped into small pieces and extracted with MeOH, then extracted with 70% aqueous acetone at room temperature. The combined extracts were suspended in H_2O, then extracted with EtOAc. The resulting EtOAc extract (60.5 g) was subjected to silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 10 : 0 : 0—9 : 1 : 0.1—6 : 4 : 1) to give fr. 1 (34.3 g), fr. 2 (8.5 g), fr. 3 (4.2 g) and fr. 4 (10.2 g). Protocatechuiate (46.9 mg) was isolated from fr. 2 by a combination of chromatographies over MCI-gel CHP 20P (50—100% MeOH), Chromatorex ODS (40—100% MeOH) and Sephadex LH-20 (0—8% MeOH in $CHCl_3$). By similar chromatographic procedures, *p*-hydroxy benzoic acid (24.2 mg) was obtained from fr. 3 and (−)catechin (48.8 mg) from fr. 4.

The water layer was chromatographed on a Sephadex LH-20 column with 0—100% MeOH (10% stepwise increase in MeOH concentration) to give frs. 1 (55.0 g), 2 (2.6 g), 3 (14.6 g), and 4 (9.2 g). Fraction 2 was chromatographed on MCI-gel CHP 20P (0—100% MeOH), Sephadex LH-20 (0—100% MeOH), and Toyopearl HW-40 (0—50%) column to give compounds **1** (22.3 mg) and **2** (15.0 mg), nicoloside (26.6 mg), and gallic acid (6.9 mg). Using a similar isolation method, phloridzin (13.0 mg) and rutin (19.8 mg) were isolated from fr. 3.

Compound **1**: A pale yellow amorphous powder, $[\alpha]_D^{15}$ −33.0° (MeOH; *c* 0.2). Positive FAB-MS: *m/z* 545 $[M+Na]^+$, 523 $[M+H]^+$. CD ($c=8\times10^{-3}$, MeOH) $[\theta]^{18}$ (nm): −7656 (241), −6567 (273), +12540 (291). ^{1}H-NMR (300 MHz, C_5D_5N): δ_H 7.09 (1H, s, H-2), 7.02 (1H, d, J=2 Hz, H-2′), 7.01 (1H, d, J=8, H-5′), 6.90 (1H, s, H-5), 6.86 (1H, dd, J=2, 8 Hz, H-6′), 5.51 (1H, d, J=7 Hz, glc-1), 3.76, 3.53 (each 3H, s, –OMe), 3.17 (2H, m, H_2-7), 2.47 (2H, m, H_2-8). ^{13}C-NMR data, see Table 1.

Enzymatic Hydrolysis of **1**: An aqueous solution (5 ml) containing **1** (10 mg) and cellulase (30 mg) was incubated at 37 °C for 12 d. The reaction mixture was evaporated to dryness *in vacuo*, and then subjected to silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 10 : 0 : 0—7 : 3 : 0.5) to give D-glucose (1.5 mg): $[\alpha]_D^{20}$ +27.6° (H_2O; *c* 0.1, measured 24 h after dissolving in H_2O) and (−)-isolariciresinol (**1a**, 4.8 mg): a pale yellow amorphous powder, $[\alpha]_D^{15}$ −34.6° (acetone; *c* 0.4). Positive FAB-MS: *m/z* 383 $[M+Na]^+$. ^{1}H-NMR (300 MHz, C_5D_5N): δ 7.18 (1H, dd, J=2, 8 Hz, H-6′), 7.06 (1H, s, H-2), 6.96 (1H, d, J=2 Hz, H-2′), 6.95 (1H, d, J=8, H-5′), 6.88 (1H, s, H-5), 3.80, 3.55 (each 3H, s, –OMe), 3.16 (2H, m, H_2-7), 2.58 (2H, m, H_2-8). ^{13}C-NMR data, see Table 1.

Compound **2**: A pale yellow amorphous powder, $[\alpha]_D^{15}$ −19.5° (MeOH; *c* 0.4). Positive FAB-MS: *m/z* 543 $[M+Na]^+$. CD ($c=10.8\times10^{-3}$, MeOH) $[\theta]^{18}$ (nm): +21586 (278). ^{1}H-NMR (500 MHz, CD_3OD): δ 6.98 (1H, br s, H-6′), 6.95 (1H, br s, H-2′), 6.94 (1H, d, J=2 Hz, H-2), 6.82 (1H, dd, J=2, 8 Hz, H-6), 6.76 (1H, d, J=8, H-5), 6.61 (1H, d, J=16 Hz, H-7′), 6.22 (1H, dt, J=16, 6 Hz, H-8′), 5.52 (1H, d, J=6 Hz, H-7), 4.49, 4.30 (each 1H, J=6, 13 Hz, H_2-9′), 4.36 (1H, d, J=8 Hz, glc-1), 3.88 (1H, dd, J=2, 12 Hz, glc-6a), 3.88, 3.82 (each 3H, s, –OMe), 3.84 (1H, dd, J=3, 11 Hz, H-9a), 3.78 (1H, dd, J=4, 11 Hz, H-9b), 3.68 (1H, dd, J=5, 12 Hz, glc-6b), 3.49 (1H, m, H-8), 3.35 (1H, t, J=8 Hz, glc-3), 3.31 (1H, t, J=8 Hz, glc-4), 3.27 (1H, m, glc-5), 3.23 (1H, t, J=8 Hz, glc-2). ^{13}C-NMR data, see Table 1.

Acid Hydrolysis of **2**: Compound **2** (2.1 mg) in 1 N HCl 1 ml and EtOH 1 ml was refluxed for 3 h. The mixture was passed through an Amberlite IR 400 (OH^-) column and then washed with H_2O. The eluate was evaporated to drynes and then dissolved in a small amount of H_2O–CH_3CN (4 : 6) solvent to perform HPLC. The peak (retention time 5.75 min) of glucose was detected and confirmed by cochromatography with an authentic sample of glucose.

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