Furocoumarin Glycosides from the Leaves of *Ficus ruficaulis* Merr. var. *antaoensis*

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From the methanolic extract of the leaves of *Ficus ruficaulis* Merr. var. *antaoensis*, 5-*O*-*β*-D-glucopyranosyl-6-hydroxyangelicin (**1**), 6-*O*-*β*-D-glucopyranosyl-5-hydroxyangelicin (**2**), 5,6-*O*-*β*-D-diglucopyranosylangelicin (**3**), 8-*O*-*β*-D-glucopyranosyl-5-hydroxypsoralen (**4**), 5-*O*-*β*-D-glucopyranosyl-8-hydroxypsoralen (**5**), 3,4,5-trihydroxydehydro-α-ionol-9-*O*-*β*-D-glucopyranoside (**6**), rutin (**7**), and isoquercitrin (**8**) were isolated. The structures of compounds **1**−**4** were elucidated by the analysis of their spectroscopic data. Their *in vitro* antiproliferation activities were also evaluated.

Ficus ruficaulis Merr. var. *antaoensis*, a variable species indigenous to Taiwan, is distributed only in the areas of Nanjenshan, Hengchun, and Lanyu Island. It is a medium-sized deciduous tree belonging to the family Moraceae.[1] Since it is seldom seen in the field, the tree is rarely put into a practical use, even in folk medicines. However, it has been shown from pharmacological experiments that the water extract of the leaves of this plant exhibits antiproliferation activity on human mononuclear cells in our previous studies.[2] The leaves may contain bioactive agents with immunomodulatory activities worthwhile to be investigated phytochemically. Therefore, a series of phytochemical examinations on a methanolic extract of the leaves of this plant were undertaken and have led to the isolation and characterization of four new furocoumarin glucosides (**1**−**4**) along with four known compounds (**5**−**8**). This paper describes the isolation and structural elucidation of the new compounds.

Results and Discussion

From the methanolic extract of fresh leaves of *F. ruficaulis*, eight major compounds, including four new furocoumarin glucosides, were identified. The compounds were isolated by sequential separation on Sephadex LH-20 and reversed-phase HPLC. Compound **5** was obtained as a light yellow powder whose UV, IR, MS, and ^{1}H and ^{13}C NMR data were consistent with those of 5-*O*-*β*-D-glucopyranosyl-8-hydroxypsoralen, which has been previously isolated from *Heracleum rapula* Franch.[3] Spectroscopic data of 3,4,5-trihydroxydehydro-α-ionol-9-*O*-*β*-D-glucopyranoside (**6**) were matched with those reported.[4] Rutin (**7**) was identical with the compound previously reported.[5] Compound **8** was identified as isoquercitrin, and its spectroscopic data were in good agreement with published data.[6]

Compound **1**, a light yellow amorphous powder, showed a molecular ion peak at *m/z* 381 in the positive FABMS spectrum. Its strong yellow-green fluorescence under UV 365 nm excitation and the aromatic region in its ^{1}H and ^{13}C NMR spectra were typical of furocoumarins.[7] The IR spectrum indicated the presence of a hydroxyl group (3377 cm^{-1}) and a carbonyl group (1695 cm^{-1}). Its UV absorption maxima at 218, 252, and 308 nm indicated a 5,6-dioxygenated angelicin skeleton,[8] which was supported by the peak at *m/z* 217 for $C_{11}H_5O_5$ in the positive FABMS spectrum. Analysis of the ^{1}H NMR spectrum for **1** showed signals characteristic of a disubstituted furan ring at δ_H 7.87 (H-2′, d, J = 2.3 Hz) and 7.10 (H-3′, d, J = 2.3 Hz) and an AX coupling system at δ_H 6.37 (H-3, d, J = 9.8 Hz) and 8.57 (H-4, d, J = 9.8 Hz), in addition to an anomeric doublet at δ_H 4.71 (H-1″, d, J = 7.9 Hz) and complex signals appearing at δ_H 3.30−3.84 which corresponded to protons of the glucopyranosyl moiety. By considering the coupling constant of the anomeric proton, the sugar moiety should be the *β*-anomer. No aromatic proton was observed, suggesting that both C-5 and C-6 are substituted. This is in agreement with the placement of oxygenated functional groups at each of these carbons. The ^{13}C NMR spectrum of **1** showed 12 carbon signals at δ_C 100−165 due to the 11 carbons of the angular furocoumarin nucleus and the anomeric carbon of the glucose, and five carbon signals at δ_C 60−80 due to the sugar moiety. In the HMBC spectrum, the anomeric proton at δ_H 4.71 showed a ^3J correlation with the carbon signal at δ_C 138.3 (C-5), which also connected with δ_H 8.57 (H-4). These data unambiguously established that the glucose was attached at C-5 of the furocoumarin nucleus. Accordingly, the structure of **1** was deduced as 5-*O*-*β*-D-glucopyranosyl-6-hydroxyangelicin.

Compound **2** was also assigned a molecular formula of $C_{17}H_{16}O_{10}$, by HRFABMS. The UV spectrum of **2** exhibited absorption maxima at 221, 252, and 313 nm, similar to that of **1**, also suggesting that **2** possessed an angular furocoumarin skeleton with two oxygenated substituents at C-5 and C-6. By comparison of the ^{13}C NMR data of **2** and **1**, differences involved the chemical shifts of C-5 and C-6. C-5 at δ_C 146.8 was deduced from the HMBC spectrum due to its correlation with H-4. According to the chemical shift changes induced by the glycosylation effect,[9] the hydroxyl and glucopyranosyl groups located at C-5 (δ_C 146.8) and C-6 (δ_C 128.5), respectively, in **2** are at reverse positions

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compared to their positions in **1**. Therefore, the structure of **2** was elucidated as 6-*O*-*β*-D-glucopyranosyl-5-hydroxyangelicin.

Compound **3** also showed UV absorption bands characteristic of a 5,6-dioxygenated angelicin derivative similar to **1** and **2**. The ^{1}H NMR spectrum of **3** indicated the presence of an angelicin moiety, which was revealed by the following signals: δ_H 6.40 (H-3, d, J = 9.9 Hz), 8.30 (H-4, d, J = 9.9 Hz), 7.23 (H-3′, d, J = 2.0 Hz), and 8.12 (H-2′, d, J = 2.0 Hz). Besides these signals, two doublets at δ_H 5.10 (H-1″, d, J = 7.9 Hz) and 5.39 (H-1‴, d, J = 7.7 Hz) were attributed to two anomeric protons. The coupling patterns of the aliphatic proton signals at δ_H 3.07−5.55, which were assigned by the aid of the ^{1}H−^{1}H COSY spectrum, were characteristic of a glucopyranoside. These data, coupled with the FABMS data, suggested that **3** is a diglucoside of angelicin. Two glucosyl groups in **3** were assigned at C-5 and C-6 of the angelicin moiety on the basis of the remarkable upfield shifts of the C-5 (δ 140.7) and C-6 (δ 131.3) signals, and they were also confirmed by HMBC measurements. Thus, compound **3** was characterized as 5,6-*O*-*β*-D-diglucopyranosylangelicin. Although various furocoumarin glycosides have been found in plants, diglycosides such as **3** are rare.

Compound **4**, with the same molecular formula as **1**, also exhibited an IR absorption band at 1695 cm^{-1}, indicating the presence of a carbonyl. However, its UV spectrum, showing absorption maxima at 220, 246.5, 272, 287, and 317 nm, quite similar to those of **5**, indicated a 5,8-dioxygenated psoralen skeleton.[3,10] The downfield region of the ^{1}H NMR spectrum showed two pairs of doublets at δ_H 6.25 and 8.23 (J = 9.8 Hz) attributed to H-3 and H-4, respectively, while the second pair at δ_H 7.18 and 7.93 (each 1H, d, J = 2.0 Hz) confirmed the presence of the benzofuran moiety. The upfield region contained one sugar unit, as evident from the presence of an anomeric proton signal at δ_H 5.30 (H-1″, d, J = 7.5 Hz). From comparison of the ^{13}C NMR spectroscopic data of compounds **4** and **5**, the glucopyranosyl moiety of **4** was proposed to be located at the C-8 position. In the HMBC spectrum, the anomeric proton signal at δ_H 5.30 (H-1″) showed correlation with the carbon signal at δ_C 121.9 (C-8). Therefore, the structure of **4** was elucidated as 8-*O*-*β*-D-glucopyranosyl-5-hydroxypsoralen.

The inflammatory response is a nonspecific immune response triggered by pathogenic microorganism infection or tissue injury and provides early protection in restricting the tissue damage to the site of infection or tissue injury.[11] Several immune cells including lymphocytes, neutrophils, monocytes, eosinophils, and basophils are involved in the inflammatory response, which in response to invasive organisms, if sufficiently intense or inappropriately prolonged, could paradoxically aggravate the injury or even cause death. The use of anti-inflammatory medications must therefore be discreet. Blockage of the lymphocyte activation and proliferation is an anti-inflammatory mechanism.[12] In this investigation the isolated pure compounds **1**−**5** from *F. ruficaulis* were tested for their antiproliferation activity on human mononuclear cells involving T lymphocytes, B lymphocytes, and macrophages isolated from peripheral blood.[13] Heparinized human peripheral blood (50 mL) was obtained from healthy donors. Mononuclear cells were isolated from human peripheral blood by the Ficoll-Hypaque gradient density method.[14] The lymphoproliferation test was performed according to the published method.[15] Compound **1**, having an IC_{50} value of 33.4 μM, was moderately active in contrast to cyclosporine A, with an IC_{50} value of 12 nM. However, compounds **2**−**5** did not show any activity up to 100 μM concentration.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer Model 341 polarimeter. UV spectra were measured in H_2O on a Hitachi U-2000 spectrophotometer. The IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. The NMR spectra were recorded in MeOH-d_4 or DMSO-d_6 at room temperature on a Bruker DRX-500 SB spectrometer, and the solvent resonances were used as internal shift references. FABMS and HRFABMS were recorded on a JEOL SX-102A instrument using *m*-nitrobenzyl alcohol (NBA) as the matrix. Sephadex LH-20 (Pharmacia Biotech) was used for open column chromatography. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). HPLC was performed using an ODS column (Hyperprep HS C18, 10 mm i.d. × 250 mm, Thermo Electron Corp., Bellefonte, PA; detector, RI).

Plant Material. Leaves of *F. ruficaulis* were sampled from the National Museum of Natural Science in Taichung in October 2003 and were identified by Dr. Chen Chang, an assistant researcher in the Department of Botany, National Museum of Natural Science. Voucher specimens (No. 20031014) have been deposited at the Graduate Institute of Pharmacognosy Science, Taipei Medical University, Taipei, Taiwan.

Extraction and Isolation. Fresh leaves (1 kg) were extracted three times with 2.5 L of MeOH for 1 week. The MeOH extract was adjusted to 85% in aqueous solution for an *n*-hexane partition, which generated two fractions soluble in aqueous MeOH and *n*-hexane. Subsequently, the aqueous MeOH-soluble fraction was evaporated to dryness under vacuum (58.5 g) and further partitioned between EtOAc and H_2O. The EtOAc layer was evaporated to dryness (17.7 g) and redissolved in MeOH for chromatographic separation. The first separation step was carried out using gel filtration chromatography on a Sephadex LH-20 column (3 × 55 cm) and eluted by MeOH with a flow rate of 13 mL/min.

Fractions collected were checked by TLC using EtOAc−HOAc−H_2O (85:10:10) for development. Vanillin-sulfuric acid (light blue spots) and a UV 365 nm lamp were used in the detection of furocoumarin glycosides. Subsequently, the fractions from the above separation were combined to give three subfractions, I, II, and III. Subfraction I was rechromatographed on a reversed-phase HPLC column with MeCN−H_2O (16:84) as eluent to yield **3** (2.3 mg) and **6** (8.0 mg). HPLC of subfraction II on the same column with MeCN−H_2O (20:80) as eluent afforded **1** (45.4 mg), **2** (12.0 mg), **4** (7.5 mg), **5** (6.5 mg), and **7** (105.0 mg). Subfraction III was purified by using the same column and eluent as used on subfraction II to yield **8** (2.5 mg).

5-*O*-*β*-D-Glucopyranosyl-6-hydroxyangelicin (1): amorphous light yellow powder; $[\alpha]^{20}_D$ +4.1° (*c* 0.15, pyridine); IR (KBr) ν_{max} 3377, 1695, 1581, 1454, 1064, 1034; UV λ_{max} (H_2O) (log ϵ) 218 (4.4), 252 (4.2), 308 (3.9) nm; ^{1}H and ^{13}C NMR data, see Table 1; FABMS $[M + H]^+$ *m/z* 381; HRFABMS *m/z* $[M + H]^+$ 381.0821 (calcd for $C_{17}H_{17}O_{10}$ 381.0823).

6-*O*-*β*-D-Glucopyranosyl-5-hydroxyangelicin (2): amorphous light yellow powder; $[\alpha]^{20}_D$ −40.0° (*c* 0.04, pyridine); IR (KBr) ν_{max} 3381, 1714, 1626, 1338, 1124, 1072; UV λ_{max} (H_2O) (log ϵ) 221 (4.0), 252 (4.0), 313 (3.6) nm; ^{1}H and ^{13}C NMR data, see Table 1; FABMS $[M + H]^+$ *m/z* 381; HRFABMS *m/z* $[M + H]^+$ 381.0821 (calcd for $C_{17}H_{17}O_{10}$ 381.0823).

5,6-*O*-*β*-D-Diglucopyranosylangelicin (3): amorphous white powder; $[\alpha]^{20}_D$ −30.0° (*c* 0.01, pyridine); IR (KBr) ν_{max} 3360, 1716, 1558, 1541, 1055; UV λ_{max} (H_2O) (log ϵ) 217 (4.4), 250 (4.1), 301 (3.9) nm; ^{1}H and ^{13}C NMR data, see Table 1; FABMS $[M + H]^+$ *m/z* 543; HRFABMS *m/z* $[M + H]^+$ 543.1368 (calcd for $C_{23}H_{27}O_{15}$ 543.1351).

8-*O*-*β*-D-Glucopyranosyl-5-hydroxypsoralen (4): amorphous light yellow powder; $[\alpha]^{20}_D$ −28.8° (*c* 0.07, pyridine); IR (KBr) ν_{max} 3327, 1695, 1491, 1446, 1340, 1066, 1055; UV λ_{max} (H_2O) (log ϵ) 220 (4.1), 246.5 (3.8), 272 (4.0), 287 (3.7), 317 (3.8)

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data (CD_3OD, 500 MHz) for Compounds **1**−**3** [δ in ppm, mult. (J in Hz)]

	1			**2**			**3**[a]		
position	^{13}C[b]	^{1}H[c]	HMBC (H→C)	^{13}C[b]	^{1}H[c]	HMBC (H→C)	^{13}C[b]	^{1}H[c]	HMBC (H→C)
2	163.1 s			163.2 s			159.7 s		
3	114.2 d	6.37 d (9.8)	2, 10	112.4 d	6.30 d (9.7)	2, 10	113.3 d	6.40 d (9.9)	2, 10
4	143.1 d	8.57 d (9.8)	2, 5, 9	142.4 d	8.28 d (9.7)	2, 5, 9	141.6 d	8.30 d (9.9)	2, 5, 9
5	138.3 s			146.8 s			140.7 s		
6	135.2 s			128.5 s			131.3 s		
7	148.2 s			151.6 s			148.8 s		
8	116.2 s			110.7 s			114.2 s		
9	141.6 s			145.7 s			142.6 s		
10	111.4 s			107.7 s			110.9 s		
2′	148.0 d	7.87 d (2.3)	3′, 7, 8	146.1 d	7.74 d (2.0)	3′, 7, 8	147.4 d	8.12 d (2.0)	3′, 7, 8
3′	104.7 d	7.10 d (2.3)	2′, 7, 8	104.6 d	7.03 d (2.0)	2′, 7, 8	103.7 d	7.23 d (2.0)	2′, 7, 8
1″	108.3 d	4.71 d (7.9)	5	106.9 d	4.96 d (7.9)	6	104.0 d	5.10 d (7.9)	5
2″	75.4 d	3.59 t (7.9)		75.3 d	3.57 t (8.0)	3″	73.9 or 74.1 d	3.38	
3″	77.9 d	3.45	4″	77.8 d	3.44	4″	76.3 d	3.25	
4″	70.9 d	3.47	3″	70.9 d	3.48	3″	69.7 or 69.9 d	3.15	
5″	78.6 d	3.30		78.5 d	3.30		77.2 or 77.5 d	3.07 or 3.13	
6″a	62.2 t	3.78 dd (5.0, 12.0)		62.1 t	3.73 dd (4.5, 12.0)		60.7 t	3.38	
6″b	62.2 t	3.84 dd (2.3, 12.0)		62.1 t	3.80 dd (2.3, 12.0)		60.7 t	3.55	
1‴							102.5 d	5.39 d (7.7)	6
2‴							73.9 or 74.1 d	3.38	
3‴							76.3 d	3.25	
4‴							69.7 or 69.9 d	3.15	
5‴							77.2 or 77.5 d	3.07 or 3.13	
6‴a							60.7 t	3.38	
6‴b							60.7 t	3.55	

[a] Measured in DMSO-d_6. [b] Multiplicities were obtained from DEPT experiments. [c] Signals without multiplicity were picked up from ^{1}H−^{1}H COSY or HMQC spectra.

nm; ^{1}H NMR data (DMSO-d_6) δ 8.23 (d, J = 9.8 Hz, H-4), 7.93 (d, J = 2.0 Hz, H-2′), 7.18 (d, J = 2 Hz, H-3′), 6.25 (d, J = 9.8 Hz, H-3), 5.30 (d, J = 7.5 Hz, H-1″), 3.52 (H-6″b), 3.37 (H-6″a), 3.31 (H-2″), 3.25 (H-3″), 3.17 (H-4″), 3.10 (H-5″); ^{13}C NMR data (DMSO-d_6) δ 160 (s, C-2), 148.4 (s, C-7), 145.3 (d, C-2′), 143.1 (s, C-9), 142.7 (s, C-5), 140.1 (d, C-4), 121.9 (s, C-8), 113.6 (s, C-6), 110.9 (d, C-3), 104.9 (d, C-3′), 104.2 (s, C-10), 102.3 (d, C-1″), 77.4 (d, C-5″), 76.6 (d, C-3″), 73.9 (d, C-2″), 69.7 (d, C-4″), 60.7 (t, C-6″); FABMS [M + H]$^+$ m/z 381; HRFABMS m/z [M + H]$^+$ 381.0822 (calcd for $C_{17}H_{17}O_{10}$ 381.0823).

Monosaccharide Composition Analysis of 1−5. Compounds **1**−**5** (100 μg) were treated with 0.5 M methanolic HCl at 80 °C for 16 h to give a methyl glycoside derivative. Free hydroxyl groups were trimethylsilylated using the Sylon HTP trimethylsilylation reagent (Supelco, Bellefonte, PA) for 20 min at room temperature. After removal of the excess reagent and organic solvent by condensation, the resulting volatile derivatives were dissolved in *n*-hexane, and this solution was used for GC/MS analysis. GC/MS analysis of the trimethylsilylated derivatives was performed on a fused silica capillary column using a temperature gradient of 60−140 °C at 25 °C/min, increased to 250 °C at 5 °C/min, and then increased to 300 °C at 10 °C/min. Several authentic monosaccharides including arabinose, rhamnose, fructose, xylose, mannose, galactose, and glucose were used as standards, and arabitol was the internal standard. When compared with the trimethylsilylated derivatives of the standards, the monosaccharide composition of **1**−**5** was confirmed to be glucose. The retention time of the trimethylsilyl methyl glucose was found to be 16.22 and 16.55 min.

Acid Hydrolysis. Compound **1** (5 mg) was hydrolyzed by 1 M H_2SO_4 (2 mL) at room temperature overnight. The reaction mixture was then partitioned with EtOAc (2 × 2 mL). The lower layer was neutralized using 1 M $Ba(OH)_2$ (2 mL) and filtered with glass wool, and the filtrate was evaporated to give D-glucose: $[\alpha]^{25}_D$ +42.7° (*c* 0.1, H_2O).

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