Cucurbitacin B 2-Sulfate and Cucurbitacin Glucosides from the Root Bark of *Helicteres angustifolia*

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A new sulfated cucurbitacin, cucurbitacin B 2-sulfate (1) and a new cucurbitacin glucoside, cucurbitacin G 2-*O*-β-D-glucopyranoside (2) together with two known cucurbitacin glucosides, arvenin I and arvenin III were isolated from the root bark of *Helicteres angustifolia*. The structures of these compounds were established on the basis of spectroscopic and chemical evidence. These four compounds taste of strong bitterness. Compound 1 is a first sulfated cucurbitacin found in plants.

Key words *Helicteres angustifolia*; Sterculiaceae; cucurbitacin; sulfate; bitter

Helicteres angustifolia L., known as one of the tumor inhibitory plants,[1] is a common folk medicine possessing analgesic, anti-inflammatory and anti-bacterial effects[2] in Taiwan. The methanol extract[3] and cucurbitacin derivatives[4] of the roots of this plant were found to have potent cytotoxic activities. To date, phytochemical studies of this plant have described the isolation of triterpenoids,[5] pregnane, coumarin and lupane derivatives.[4]

Our previous studies on the constituents of this plant have led to the isolation of sesquiterpenoid quinones,[6] flavonoid glycosides.[7] The present paper describes the isolation and characterization of a new sulfated cucurbitacin, cucurbitacin B 2-sulfate (**1**) and a new cucurbitacin glucoside, cucurbitacin G 2-*O*-β-D-glucopyranoside (**2**) together with two known compounds, arvenin I and III[8] from the *n*-BuOH extract of the root bark of this plant. These four compounds taste of strong bitterness. Compound **1** is a first sulfated cucurbitacin found in plants.

Results and Discussion

As in the previously reported procedure,[7] the fractions 5—6 (3.8 g) was chromatographed on silica gel column and preparative TLC to afford **1**, **2**, arvenin I and III.

Cucurbitacin B 2-sulfate (**1**), colorless amorphous powder, showed IR absorption of hydroxyl (3450 cm^{-1}), carbonyl (1720, 1700 cm^{-1}) and a strong broad band due to S=O group (1250 cm^{-1}). The UV spectrum showed absorption at λ_{max} (MeOH) 230 nm. The molecular formula was assigned as $C_{32}H_{46}O_{11}S$, indicated by a quasimolecular ion peak at *m/z* 637.2643 ($[M-H]^-$, $C_{32}H_{45}O_{11}S$) in high-resolution (HR)-FAB-MS (negative ion) of **1**. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) showed characteristic signals of cucurbitacin due to eight methyl groups, an olefinic proton (H-6), *trans*-coupled olefinic protons (H-23, H-24), three carbonyl carbons (C-3, C-11, C-22), four oxygenated carbons (C-2, C-16, C-20, C-25) and an acetyl group. The above data indicated **1** is a sulfated derivative of cucurbitacin. Solvolysis[9] of **1** with 1,4-dioxane in pyridine afforded a desulfated product **1a** which showed a quasimolecular ion peak at *m/z* 557 $[M-H]^-$ in FAB-MS. Compound **1a** was identified as cucurbitacin B by comparison the spectroscopic data of **1a** with the reported data.[10,11] These facts confirmed that **1** was a sulfated derivative of cucurbitacin B. The sulfate group of **1** was linked to 2-OH on the basis of following sulfation shifts. The signals at δ 5.98 due to H-2 of **1** showed downfield shift by 1.11 ppm in comparison with the signals at δ 4.87 due to H-2 of **1a**. The ^{13}C-NMR spectral data of **1** showed that the signal at δ 77.5 due to C-2 carbon atom showed downfield shift by 5.1 ppm, while the signals at δ 34.9 and δ 210.5 due to C-1 and C-3 carbon atoms showed upfield shift by 2.0 and 2.9 ppm, respectively, in comparison with those of **1a**. Therefore, the structure of compound **1** was determined as cucurbitacin B 2-sulfate.

Cucurbitacin G 2-*O*-β-D-glucopyranoside (**2**), colorless amorphous powder, showed IR absorption of hydroxyl (3400 cm^{-1}), carbonyl (1720, 1700 cm^{-1}). High-resolution (HR)-FAB-MS (negative ion) of **2** showed a quasimolecular ion peak at *m/z* 695.3677 ($[M-H]^-$, $C_{36}H_{55}O_{13}$) which confirmed the molecular formula as $C_{36}H_{56}O_{13}$. The ^{1}H- and ^{13}C-NMR spectra of **2** showed a glucose moiety. The signals at δ 5.17 (d, J=7.6 Hz) due to anomeric proton indicated the β-configuration of the glucosyl moiety. On acid hydrolysis of **2**, D-glucose was obtained as the sugar moiety. The ^{1}H- and ^{13}C-NMR spectra of **2**, bearing same glycosylation at position 2-OH, showed almost the same spectral data (Table 1) as those of arvenin I and III, except the signals due to side chains. These facts revealed that **2** is a cucurbitacin 2-*O*-β-D-glucopyranoside. A substructure, $-CH_2CHOH-$, was confirmed in side chain through a ^{1}H–^{1}H COSY experiment, by the correlation of cross peaks due to signals δ 3.62 (dd, J=15.5, 9.6

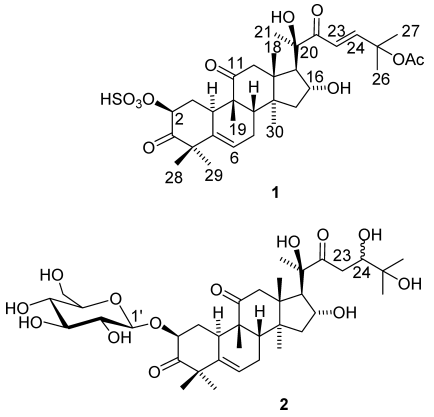

Fig. 1. Structures of Compounds **1** and **2**

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Table 1. ^{13}C- and ^{1}H-NMR Data for Compounds **1** and **2** in C_5D_5N

Position	**1**		**2**	
	δ_C	δ_H (mult; *J*, Hz)	δ_C	δ_H (mult; *J*, Hz)
1	34.9	2.86 m, 1.63[c)]	35.2	2.58 m, 1.65[c)]
2	77.5	5.98 dd (13.0, 5.6)	77.9	5.49 dd (13.0, 5.6)
3	210.5		211.6	
4	51.1		51.1	
5	140.7		140.8	
6	120.5	5.63 br d (5.3)	120.4	5.64 br d (5.3)
7	24.2	2.26 m, 1.94[c)]	24.2	2.25 m, 1.88[c)]
8	42.9	1.90[c)]	42.8	1.90[c)]
9[a)]	49.0		48.83	
10	34.5	3.11 br d (12.4)	34.2	3.06 br s
11	212.8		212.8	
12	49.2	3.23, 2.79, d (14.6)	49.1	2.90, 2.62, d (14.6)
13	51.8		51.7	
14[a)]	48.9		48.79	
15	46.3	1.90[c)], 1.57[c)]	46.1	1.90[c)], 1.57[c)]
16	70.6	5.03 br t (7.6)	70.7	5.02 br t (7.6)
17	59.4	2.99 d (7.0)	57.4	2.98 d (7.0)
18	20.5	1.16 s	20.2	1.16 s
19	19.8	0.99 s	19.9	1.05 s
20	79.7		80.4	
21	25.4	1.56 s	25.1	1.58 s
22	204.3		215.9	
23	122.5	7.36 d (15.7)	41.3	3.62 dd (15.5, 9.6)
				3.36 br d (15.5)
24	150.0	7.41 d (15.7)	76.0	4.57 dd (9.6, 1.8)
25	79.9		72.0	
26[b)]	26.2	1.60 s	24.6	1.47 s
27[b)]	26.7	1.69 s	27.6	1.51 s
28	21.8	1.33 s	21.8	1.35 s
29	28.8	1.52 s	28.9	1.53 s
30	18.8	1.35 s	18.8	1.37 s
OAc	170.0			
	21.9	1.93 s		
1′			104.2	5.17 d (7.6)
2′			76.0	4.10 br t (8.0)
3′			78.5	4.35 m
4′			71.3	3.84 m
5′			78.8	4.43 dd (12.0, 2.0)
6′			62.6	4.28 m

a, *b*) Assignments in each column may be interchangeable. *c*) Signal pattern unclear due to overlapping.

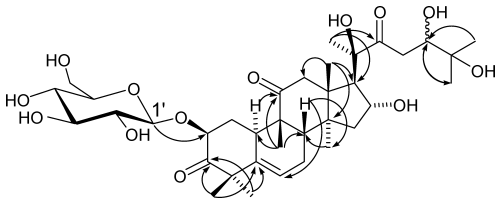

Fig. 2. Significant ^{3}J Correlations in HMBC Spectrum of **2**

Hz) and δ 3.36 (br d, J=15.5 Hz), signals δ 4.57 (dd, J=9.6, 1.8 Hz) and δ 3.62, δ 3.36. The ^{13}C-NMR spectrum of **2** showed signals at δ 41.3, 76.0 due to –CH_2CHOH– moiety. These facts revealed the presence of a hydroxyl group at C-23 or C-24 in the side chain of **2**. Detailed analysis of ^{1}H–^{1}H COSY and DEPT experiments and comparing the ^{1}H- and ^{13}C-NMR data due to the side chain of **2** with those of cucurbitacin G, 3-*epi*-isocucurbitacin G[12)] and cucurbitacin J 2-*O*-β-glucopyranoside[13)] confirmed the hydroxyl group to be linked to C-24. The location of the hydroxyl group on C-24 was further confirmed by ^{3}J correlation HMBC experiment (Fig. 2). The ^{13}C-NMR signal at δ 76.0 (C-24) showed cross peaks with the ^{1}H-NMR signals at δ 1.47 (H-26) and 1.51 (H-27), and ^{13}C-NMR signal at δ 24.6 (C-26) showed cross-peak with the ^{1}H-NMR signal at δ 4.57 (H-24). The stereochemistry of hydroxylation at C-24 could not be determined. Consequently, the structure of **2** was determined as cucurbitacin G 2-*O*-β-D-glucopyranoside.

Experimental

General Experimental Procedures Optical Rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were measured on a Hitachi 200 spectrophotometer. IR spectra were taken on a Perkin Elmer 781 infrared spectrophotometer. NMR spectra were recorded on a Bruker AM-400 and AV-500 spectrometers. FAB-MS and HR-FAB-MS were recorded on a JMS-HX-110 spectrometer. Gas liquid chromatography was done on a Hewlett-Packard 5890 gas chromatography. Column chromatography was performed on silica gel 60 (Merck, 70—230 mesh), TLC and preparative TLC were performed on precoated silica gel plates (Merck, kieselgel 60 F_{254}, 0.25, 1.00 mm respectively).

Plant Material The entire fresh plants of *Helicteres angustifolia* were collected in Puli, Nantou County, Taiwan and authentified by Prof. Chang-Sheng Kuoh, Department of Biology, National Cheng Kung University. A voucher specimen (CNACNP0512) was deposited in the natural product laboratory of Department of Applied Chemistry, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan.

Extraction and Isolation Dried powdered root bark of *H. angustifolia* (3.8 kg) was extracted with $CHCl_3$ (3×5 l) under reflux. The residue was extracted with MeOH (5×5 l) under reflux. The concentrated MeOH extract (201 g) was suspended in water. The suspension was extracted with EtOAc and *n*-butanol, successively. The *n*-butanol soluble fraction (35 g) was chromatographed on a silica gel column with $CHCl_3$/MeOH/H_2O (65 : 35 : 10, lower layer) as eluent and 250 ml were collected for each fraction. Fractions 5—6 (3.8 g) were collected and chromatographed on a silica gel column with EtOAc/MeOH mixture of increasing polarity as eluent. Elution was monitored by TLC with $CHCl_3$/MeOH/H_2O (10 : 2 : 0.1). The fractions containing cucurbitacins were collected and further purified by silica gel column chromatography, eluting with EtOAc/MeOH (20 : 1) and preparative TLC, developing with $CHCl_3$/MeOH/H_2O (8 : 2 : 0.2) to give **1** (38 mg), **2** (6 mg), arvenin I (58 mg) and III (213 mg).

Cucurbitacin B 2-Sulfate (**1**): Colorless amorphous powder; $[\alpha]_D^{25}$+30.9° (c=1.37 MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.25) nm; IR (KBr) ν_{max} 3450, 2990, 2960, 1720, 1700, 1660, 1630, 1580, 1460, 1410, 1370, 1250, 1060, 1020, 980 cm^{-1}. ^{1}H-NMR (C_5D_5N, 400 MHz): see Table 1; ^{13}C-NMR (C_5D_5N, 100 MHz): see Table 1; HR-FAB-MS (negative ion) *m/z* 637.2643 (Calcd 637.2638 for $C_{32}H_{45}O_{11}S$ [M−H]$^-$).

Solvolysis of 1 Compound **1** (9.2 mg) was dissolved in pyridine (1.0 ml)/1,4-dioxane (1.0 ml), heated at 90 °C for 8 h and then evaporated to dryness. The dried mixture was dissolved in EtOAc/H_2O (1 : 1). The EtOAc soluble fraction was concentrated and then chromatographed on preparative TLC with $CHCl_3$/MeOH (12 : 1) as eluent to afford desulfated product **1a** (4.5 mg). Compound **1a** was identified as cucurbacin B by comparison the spectroscopic data of **1a** with the reported data.[10,11)]

Cucurbitacin G 2-*O*-β-D-Glucopyranoside (**2**): Colorless amorphous powder; $[\alpha]_D^{25}$+12.3° (c=0.2 MeOH); IR (KBr) ν_{max} 3400, 2980, 2930, 1720, 1700, 1650, 1580, 1460, 1430, 1390, 1370, 1340, 1220, 1080, 1050 cm^{-1}. ^{1}H-NMR (C_5D_5N, 400 MHz): see Table 1; ^{13}C-NMR (C_5D_5N, 100 MHz): see Table 1; HR-FAB-MS (negative ion) *m/z* 695.3711 (Calcd 695.3718 for $C_{36}H_{55}O_{13}$ [M−H]$^-$).

Acid Hydrolysis of 2 A solution of **2** (1.5 mg) in 3% HCl (3 ml) was heated on a boiling water bath for 4 h. The mixture was evaporated *in vacuo*. The residue was dissolved in dry pyridine (1.0 ml) and the trimethylsilyl ethers were prepared by addition of hexamethyldisilazane (0.6 ml) and trimethylchlorosilane (0.3 ml) successively. The mixture was evaporated *in vacuo*; 0.5 ml of *n*-heptane was added. The insoluble material was filtered off. The filtrate was shown to contain TMS-ethers of D-glucose by GLC[14)] [packed glass column, 3% OV-101 on Chromosorb W-HP 80—100 mesh, 2 mm×2 m; column temperature, 150—250 °C at 10 °C/min; carrier gas, N_2; t_R 6.19, 6.76 min].

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