New Aliphatic Alcohol and (*Z*)-4-Coumaric Acid Glycosides from *Acanthus ilicifolius*

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Received June 3, 2003; accepted July 28, 2003

From the aerial parts of *Acanthus ilicifolius*, a new aliphatic alcohol glycoside (ilicifolioside C) and two new (*Z*)-4-coumaric acid glycosides, (*Z*)-4-coumaric acid 4-*O*-*β*-D-glucopyranoside and (*Z*)-4-coumaric acid 4-*O*-*β*-D-apiofuranosyl-(1″→2′)-*O*-*β*-D-glucopyranoside were isolated. The structural elucidations were based on the analyses of spectroscopic data. *Z*-Form 4-coumaric acid glycosides were first isolated from plant.

Key words *Acanthus ilicifolius*; Acanthaceae; aliphatic alcohol glycoside; (*Z*)-4-coumaric acid glycoside; ilicifoliosides C

Acanthus ilicifolius L. (Acanthaceae) is a spiny herb of mangrove widely distributed in southeastern Asia. In traditional Chinese medicine, it is used as anti-inflammatory and anti-hepatitis agents. Previous pharmaceutical studies on this plant revealed that the crude alcoholic extract of its leaves showed antioxidant, hepatoprotective, antitumour and anticarcinogenic effects.[1,2] The constituents of this plant had been previously investigated and shown to contain a triterpenoidal saponin,[3] 2-benzoxazolinone,[4] acanthicifoline,[5] five benzoxazinoid glucosides,[6] two phenylethanoid glycosides and seven lignan glucosides.[7] Recently we reported the isolation and structural elucidation of a new aliphatic alcohol glycoside, a new and five known phenylethanoid glycosides from the aerial parts of this plant.[8] As part of our continuing search for bioactive natural products from tropical medicinal plants, we now describe the isolation and structural elucidation of a new aliphatic alcohol glycoside (**1**) and two new (*Z*)-4-coumaric acid glycosides (**2**, **3**) from the same plant.

The ethanolic extract of the aerial parts of *A. ilicifolius* was subjected to extraction and solvent portioning as described in the Experimental. The resulting aqueous layer was applied to column chromatography using D_{101} macroporous adsorbing resin, silica gel, octadecylsilyl silica gel and Sephadex LH-20 gel, followed by prep. HPLC-ODS to yield compounds **1**, **2** and **3** (Fig. 1).

Compound **1**, an amorphous powder, showed its pseudomolecular peaks at *m*/*z* 637 $[M+Na]^+$ and *m*/*z* 653 $[M+K]^+$ in its electrospray ionization (ESI)-MS spectra (positive ion mode). Its molecular formula was established as $C_{25}H_{42}O_{17}$ by high resolution (HR)-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **1** showed the presence of three sugar moieties [δ_H 4.38 (d, J=7.5 Hz), 4.51 (d, J=8.0 Hz), 4.73 (d, J=8.0 Hz) and δ_C 101.5, 104.2, 105.0] (Table 1), which were determined to be a *β*-D-xylopyranose and two *β*-D-glucopyranose units, in addition to eight-carbon signals for the aliphatic aglycone. Acid hydrolysis of **1** afforded D-glucose and D-xylose, which were identified by TLC and comparison of the optical rotation with authentic samples.

Distortionless enhancement by polarization transfer (DEPT) experiments indicated the presence of five methylenes (δ 25.7, 27.2, 35.3, 38.7, 117.4), two methines (δ 83.8, 140.1) and one quaternary carbon (δ 178.0) in the aglycone moiety of **1**, among which δ_C 140.1 (CH), 117.4 (CH_2) suggested a terminal double bond. The IR spectrum showed absorption bands due to a carboxyl group (3100, 1720 cm^{-1}), which was supported by the ^{13}C-NMR signal at δ_C 178.0. The chemical shifts of the aglycone carbons were quite similar to those of ilicifolioside B[8] isolated from the same plant. However, the absence of the methylene carbon at δ 62.9 together with the presence of one more carboxyl carbon at δ 178.0 were observed in **1**, indicating that the terminal hydroxyl was substituted by a carboxyl group. Consequently, the aglycone was established as 6-hydroxyl-7-octenoic acid, which was confirmed by the ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) experiments (Fig. 2). The absolute configuration at C-6 was suggested to be in the *R*-form by comparing the optical rotation value of this aglycone ($[\alpha]_D^{25}$ −28.5°) obtained from acid hydrolysis with that of ilicifolioside B ($[\alpha]_D^{25}$ −22.5°). Additionally, the position of glycosylation was assigned to C-6 due to the downfield shift (δ_C 83.8) of this carbon signal.

The assignments of protons and carbons pertaining to above three monosaccharide units were fixed with the aid of ^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple quantum

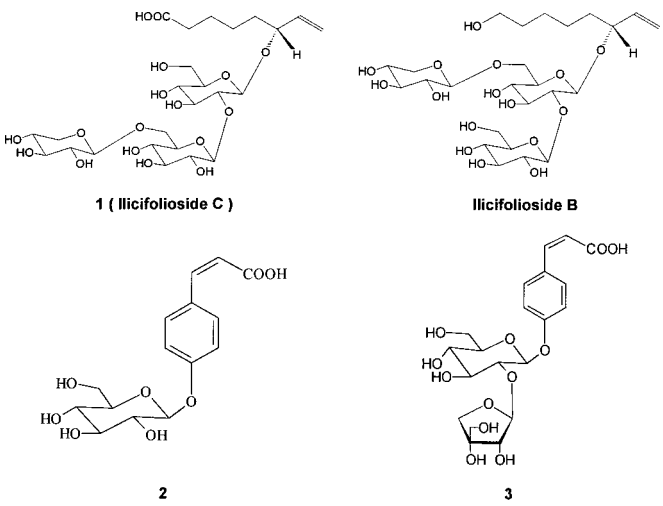

Fig. 1. Structures of Compounds **1**—**3** and Ilicifolioside B

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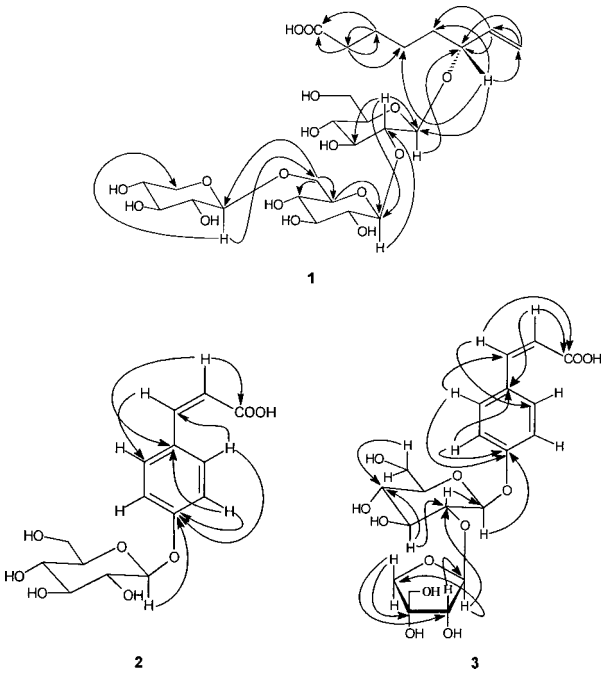

Fig. 2. The HMBC Correlations of Compounds **1**—**3**

coherence (HMQC) and HMBC experiment as shown in Table 1 [Glc-1 (δ_H 4.51, δ_C 101.5), Glc-2 (δ_H 4.73, δ_C 104.2), Xyl (δ_H 4.38, δ_C 105.0)]. Comparison of the ^{13}C-NMR spectra data of **1** with those of ilicifolioside B[8] (Table 1) revealed that D-xylose unit was not attached to the C-6 of Glc-1, but to the C-6 of Glc-2. Moreover, the sequence of the trisaccharide chain connected to the C-6 of the aglycone was finally established by the following HMBC correlations (Fig. 2): H-1 (δ 4.51) of Glc-1 with C-6 (δ 83.8) of the aglycone, H-1 (δ 4.73) of Glc-2 with C-2 (δ 81.5) of Glc-1, H-1 (δ 4.38) of Xyl with C-6 (δ 69.4) of Glc-2. Thus, the structure of compound **1** was assigned as (6*R*)-6-hydroxyl-7-octenoic acid 6-*O*-β-D-xylopyranosyl-(1‴→6″)-*O*-β-D-glucopyranosyl-(1″→2′)-*O*-β-D-glucopyranoside, named ilicifolioside C.

Compound **2**, an amorphous powder, showed its pseudomolecular peaks at *m/z* 349 $[M+Na]^+$ and *m/z* 365 $[M+K]^+$ in its positive ESI-MS spectra. Its molecular formula was established as $C_{15}H_{18}O_8$ by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectral data of **2** exhibited a nine-carbon aromatic aglycone and a monosaccharide unit [δ_H 5.20 (d, J=7.5 Hz) and δ_C 100.4] (Table 2). ^{1}H-NMR spectral data of **2** revealed an AA′BB′ system at δ 7.14 (2H, d, J=8.5 Hz) and 7.50 (2H, d, J=8.5 Hz), indicating the presence of a 1,4-disubsituted symmetrical aromatic ring in the aglycone moiety. Two coupling olefinic protons at δ 6.08 (1H, d, J=12.5 Hz) and 6.50 (1H, d, J=12.5 Hz) suggested a *Z*-form double bond. Moreover, the quaternary carbon at δ 177.8 suggested the presence of a carboxyl group, which was confirmed by the unsaturation index and elemental composition of **2**. From these results, the structure of the aglycone was identified as (*Z*)-4-coumaric acid. Acid hydrolysis of **2** gave D-glucose, identical by TLC and comparison of the optical rotation with authentic sample. Furthermore, the HMBC experiment displayed a long-range correlation from the anomeric proton [δ_H 5.20 (d, J=7.5 Hz)] of β-D-glucose unit to C-4 (δ_C 156.5) of the aglycone (Fig. 2). Therefore, the structure of compound **2** was assigned as (*Z*)-4-coumaric acid 4-*O*-β-D-glucopyranoside.

Compound **3**, an amorphous powder, showed its pseudomolecular peaks at *m/z* 481 $[M+Na]^+$ and *m/z* 497 $[M+K]^+$ in its positive ESI-MS spectra. Its molecular formula was established as $C_{20}H_{26}O_{12}$ by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectral data of **3** exhibited a nine-carbon aromatic aglycone and two monosaccharide units [δ_H 5.28 (d, J=7.5 Hz), δ_H 5.46 (d, J=2.5 Hz) and δ_C 99.2, 109.8] (Table 2). The ^{13}C-NMR spectral data of **3** were almost the same as those of **2**, except for the additional signals of a pentose unit. DEPT experiments indicated the presence of one quaternary carbon (δ 79.9), two methylenes (δ 64.2, 74.2) and two methines (δ 109.8, 77.4) in this pentose unit, identical with that of β-D-apiofuranose.

Acid hydrolysis of **3** afforded D-glucose and D-apiose, which were identified by TLC and comparison of the optical rotation with authentic samples. Furthermore, the HMBC experiment displayed the following correlations (Fig. 2): H-1 (δ 5.28) of β-D-glucopyranose with C-4 (δ 156.5) of the aglycone, H-1 (δ 5.46) of β-D-apiofuranose with C-2 (δ 79.0) of β-D-glucopyranose. Therefore, the structure of compound **3** was assigned as (*Z*)-4-coumaric acid 4-*O*-β-D-apiofuranosyl-(1″→2′)-*O*-β-D-glucopyranoside.

4-Coumaric acid is an important biosynthetic precursor of many natural products, which include coumarin, lignan, flavonoid and phenylethanoid. Usually its glycosides are reported to exist in the *E*-form. And its *Z*-form ones are not reckoned stable in nature. Up to the present its *Z*-form glycosides have not been isolated from the living organism. The isolation and structural elucidation of two *Z*-form 4-coumaric acid glycosides from the plant *A. ilicifolius* first reveals that both the *E*-form and *Z*-form ones exist naturally.

Experimental

General Procedures IR spectra were obtained in KBr on a Perkin-Elmer 599 B spectrophotometer. NMR spectra were recorded in methanol-d_4, mixture of 1,4-dioxane and deuterated water (1 : 1 v/v) using a Varian INOVA-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) or a Bruker ARX-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with tetramethylsilane as internal standard. ESI-MS spectra were measured on a Bruker APEX II spectrometer in positive ion mode. Optical rotations were measured with an AA-10R digital polarimeter. Preparative HPLC was carried out on ODS columns (250×10 mm i.d., YMC) with a Waters 996 photodiode array detector. For CC, silica gel (200—300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.), octadecylsilyl silica gel (80—100 μm) (Unicorn), Sephadex LH-20 gel (Pharmacia) and D_{101} macroporous adsorbing resin (Tianjing Chem. Ind. Co. Ltd.) were used. The solvent systems were: (I) EtOAc–MeOH–H_2O (4 : 1 : 0.1) (II) $CHCl_3$–MeOH–H_2O (6 : 4 : 0) (III) $CHCl_3$–MeOH–H_2O (6 : 4 : 0.25) (IV) $CHCl_3$–MeOH–H_2O (6 : 4 : 0.5), (V) $CHCl_3$–MeOH–H_2O (6 : 4 : 1), (VI) 20% MeOH, (VII) 10% MeOH, (VIII) 8% MeOH. The spray reagent used for TLC was 5% H_2SO_4 and 5% phosphomolybdic acid in 95% ethanol.

Plant Material *Acanthus ilicifolius* L. was collected in July 2001 from Sanya of Hainan Province, southern China. The identification of the plant was performed by Prof. Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample (NO. GKLMMM-001) is kept in the Herbarium of South China Sea Institute of Oceanology.

Extraction and Isolation The dried aerial part (10.0 kg) of *A. ilicifolius* was extracted with hot 95% and 50% EtOH three times, respectively. After removal of the solvent by evaporation, the residue (1.3 kg) was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively. The resulting aqueous layer (780 g) was subjected to CC of D_{101} macroporous adsorbing resin and eluted with H_2O, 30% EtOH, 60% EtOH successively. The fractions eluted with different concentration of ethanol were combined (16 g) and subjected

Table 1. ^{1}H-, ^{13}C-NMR Spectral Data of Ilicifolioside C (**1**) and ^{13}C-NMR Spectral Data of Ilicifolioside B (400 MHz for ^{1}H and 100 MHz for ^{13}C, methanol-d_4)

Carbon No.	Ilicifolioside C (**1**)		Ilicifolioside B
	^{1}H δ [mult., J (Hz)]	^{13}C δ	^{13}C δ
Aglycone			
1		178.0	62.9
2	2.20 (2H, t, 7.5)	38.7	26.9
3	1.64 (2H, m)	27.2	33.6
4	1.42 (2H, m)	25.7	25.7
5	1.73 (1H, m), 1.60 (1H, m)	35.3	35.8
6	4.18 (1H, dd, 13.5, 6.5)	83.8	83.5
7	5.87 (1H, ddd, 17.0, 11.0, 7.5)	140.1	140.6
8	5.28 (1H, d, 17.0)	117.4	116.8
	5.18 (1H, d, 11.0)		
Glc-1			
1′	4.51 (1H, d, 8.0)	101.5	101.8
2′	3.55 (1H, t, 9.6)	81.5	82.5
3′	3.62 (1H)[b]	77.8[a]	77.6[a]
4′	3.58 (1H)[b]	70.8	71.2
5′	3.36 (1H)[b]	77.8[a]	77.5[a]
6′	3.72 (1H, dd, 12.5, 5.5)	62.6	69.4
	3.88 (1H, dd, 12.5, 2.5)		
Glc-2			
1″	4.73 (1H, d, 8.0)	104.2	105.0
2″	3.32 (1H)[b]	75.7	76.1
3″	3.60 (1H)[b]	77.4[a]	77.9[a]
4″	3.42 (1H)[b]	70.8	71.4
5″	3.44 (1H)[b]	76.5	78.2[a]
6″	3.77 (1H, dd, 11.5, 5.5)	69.4	62.9
	4.05 (1H, dd, 12, 2.0)		
Xyl			
1‴	4.38 (1H, d, 7.5)	105.0	105.3
2‴	3.26 (1H)[b]	74.6	74.8
3‴	3.40 (1H)[b]	77.3[a]	77.7[a]
4‴	3.34 (1H)[b]	71.4	71.6
5‴	3.24 (1H)[b]	66.6	66.8
	3.91 (1H, dd, 16.0, 10.5)		

a) Assignments may be interchanged in each column. *b*) Overlapped signals are reported without designated multiplicity.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **2** and **3** (500 MHz for ^{1}H and 125 MHz for ^{13}C)[a]

No.	**2**		**3**	
	^{1}H δ [mult., J (Hz)]	^{13}C δ	^{1}H δ [mult., J (Hz)]	^{13}C δ
Aglycone				
1		131.6		131.4
2	7.50 (1H, 8.5)	130.2	7.50 (1H, 8.5)	130.2
3	7.14 (1H, 8.5)	116.7	7.13 (1H, 8.5)	116.5
4		156.5		156.5
5	7.14 (1H, 8.5)	116.7	7.13 (1H, 8.5)	116.5
6	7.50 (1H, 8.5)	130.2	7.50 (1H, 8.5)	130.2
7	6.50 (1H, 12.5)	130.3	6.53 (1H, 12.5)	130.3
8	6.08 (1H, 12.5)	126.3	6.08 (1H, 12.5)	126.3
9		177.8		177.8
Glc				
1′	5.20 (1H, d, 7.5)	100.4	5.28 (1H, d, 7.5)	99.2
2′	3.60 (1H, t, 9.6)	73.4	3.72 (1H, t, 9.6)	79.0
3′	3.70 (1H, t, 9.6)	76.5	3.70 (1H)[b]	76.5
4′	3.55 (1H, t, 9.6)	69.9	3.57 (1H, t, 9.6)	69.7
5′	3.68 (1H, m)	76.0	3.72 (1H)[b]	76.2
6′	3.81 (1H, dd, 12.5, 5.5)	61.0	3.80 (1H, d, 12.5)	61.0
	3.96 (1H, dd, 12.5, 2.5)		3.98 (1H, dd, 12.5, 2.5)	
Apif				
1″			5.46 (1H, d, 2.5)	109.8
2″			4.08 (1H, d, 2.5)	77.4
3″				79.9
4″			3.94, 4.05 (1H, each, 10.5)	74.2
5″			3.65 (2H, s)	64.2

a) Recorded in a mixture of 1,4-dioxane and deuterated water (1 : 1 v/v). *b*) Overlapped signals are reported without designated multiplicity.

to CC of silica gel (system II—V) to afford thirty fractions. Fractions 15 to 20 were combined and further separated on Pharmacia-Sephadex LH-20 (system VII) and Unicorn-ODS (system VI) CC, then followed by prep. HPLC-ODS chromatography (system VIII) to afford compounds **1** (60 mg), **2** (90 mg), **3** (55 mg).

Ilicifolioside C (**1**): Amorphous powder, $[\alpha]_D^{25}$ −48° (c=0.6, MeOH). IR (KBr) cm^{-1}: 3400 (br), 3100 (OH), 1720 (C=O). HR-ESI-MS m/z: 653.2054 $[M+K]^+$ (Calcd for $C_{25}H_{42}O_{17}K$: 653.2059). ^{1}H- and ^{13}C-NMR (methanol-d_4): Table 1.

(*Z*)-4-Coumaric Acid 4-*O*-β-D-Glucopyranoside (**2**): Amorphous powder, $[\alpha]_D^{25}$ −52° (c=0.7, H_2O). IR (KBr) cm^{-1}: 3400 (br), 2930, 1635, 1604, 1558, 1508, 1447, 1414, 1352, 1240, 1075, 1044 861 and 603. HR-ESI-MS m/z: 365.0631 $[M+K]^+$ (Calcd for $C_{15}H_{18}O_8K$: 365.0638). ^{1}H- and ^{13}C-NMR (mix. of 1,4-dioxane and D_2O): Table 2.

(*Z*)-4-Coumaric Acid 4-*O*-β-D-Apiofuranosyl-(1″→2′)-*O*-β-D-Glucopyranoside (**3**): Amorphous powder, $[\alpha]_D^{25}$ −68° (c=0.8, H_2O). IR (KBr) cm^{-1}: 3398 (br), 2933, 1635, 1606, 1558, 1508, 1437, 1412, 1349, 1241, 1076, 839 and 604. HR-ESI-MS m/z: 497.1056 $[M+K]^+$ (Calcd for $C_{20}H_{26}O_{12}K$: 497.1061). ^{1}H- and ^{13}C-NMR (mix. of 1,4-dioxane and D_2O): Table 2.

Acid Hydrolysis of Ilicifolioside C (1), 2 and 3 Compound **1** (20 mg) was treated with a mixture of 1 : 1 2 M HCl and 1,4-dioxane (5 ml) at 100 °C for 3 h. The reaction mixture was neutralized by addition of Ag_2CO_3 and filtered. The filtrate was concentrated and the residue suspended in water (10 ml) was extracted with diethyl ether (20 ml, twice). Then the extract concentrated to dryness afforded the aglycone of **1**, whose optical rotation value ($[\alpha]_D^{25}$ −28.5°) was identical with that of ilicifolioside B[8] ($[\alpha]_D^{25}$ −22.5°). The aqueous layer containing monosaccharides was concentrated and applied to a silica gel column (system I) to afford D-glucose (4 mg, *Rf* 0.16, $[\alpha]_D^{25}$ +50°) and D-xylose (3 mg, *Rf* 0.29, $[\alpha]_D^{25}$ +20°), comparing with authentic samples.

By the same method, Compound **2** (30 mg) provided D-glucose (8 mg, *Rf* 0.16, $[\alpha]_D^{25}$ +50°) and **3** (25 mg) provided D-apiose (5 mg, *Rf* 0.45, $[\alpha]_D^{25}$ +7.6°) besides D-glucose (5 mg, *Rf* 0.16, $[\alpha]_D^{25}$ +50°).

Acknowledgements This research was financially supported by a grant (code: 2001CCA04700) from the National Key Program for Base Research (973 Program), a grant (code: 2001AA620403) from the National High Technology Research and Development Program of China (863 Program) and the other one (code: KZCX3-SW-216) from Important Project of Chinese Academy of Sciences. Mass spectra were provided by Institute of Chemistry, Chinese Academy of Sciences. The NMR spectra were provided by the Center of Analysis and Measurement, Sun Yat-Sen University.

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