Two New Cyclolignan Glycosides from *Acanthus ilicifolius*

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Z. Naturforsch. **59b,** 341 – 344 (2004); received November 28, 2003

Two new cyclolignan glycosides, (+)-lyoniresinol 3a-*O*-α-D-galactopyranosyl-(1 $\rightarrow$ 6)-β-D-glucopyranoside (1) and (+)-lyoniresinol 2a-*O*-α-D-galactopyranosyl-3a-*O*-β-D-glucopyranoside (2) were isolated from the aerial parts of *Acanthus ilicifolius*. Their structure elucidation is based on the analyses of spectroscopic data.

Key words: Acanthus ilicifolius, Acanthaceae, Cyclolignan Glycoside

Introduction

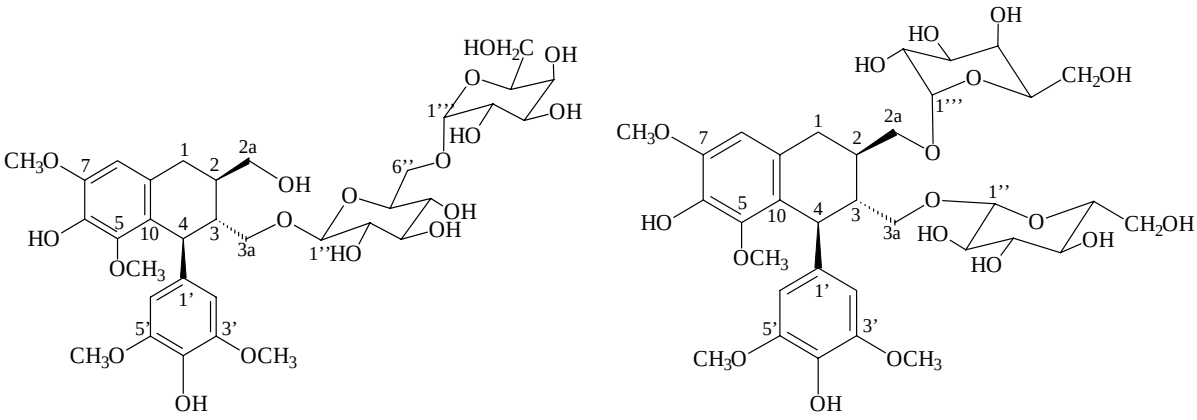
Acanthus ilicifolius L. (Acanthaceae) is a spiny herb of mangrove widely distributed in southeastern Asia. In traditional Chinese medicine, it is used as an anti-inflammatory and anti-hepatitis agent. Previous pharmaceutical studies on this plant revealed that the crude alcoholic extract of its leaves showed antioxidant, hepatoprotective, antitumour and anticarcinogenic effects [1 – 2]. The constituents of this plant had been previously investigated and shown to contain a triterpenoid 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 two new cyclolignan glycosides (**1, 2**) from the same plant.

Results and Discussion

The ethanolic extract of the aerial parts of *A. ilicifolius* was subjected to extraction and solvent portioning as described in the Experimental Section. The resulting aqueous layer was subject 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** and **2** (Fig. 1).

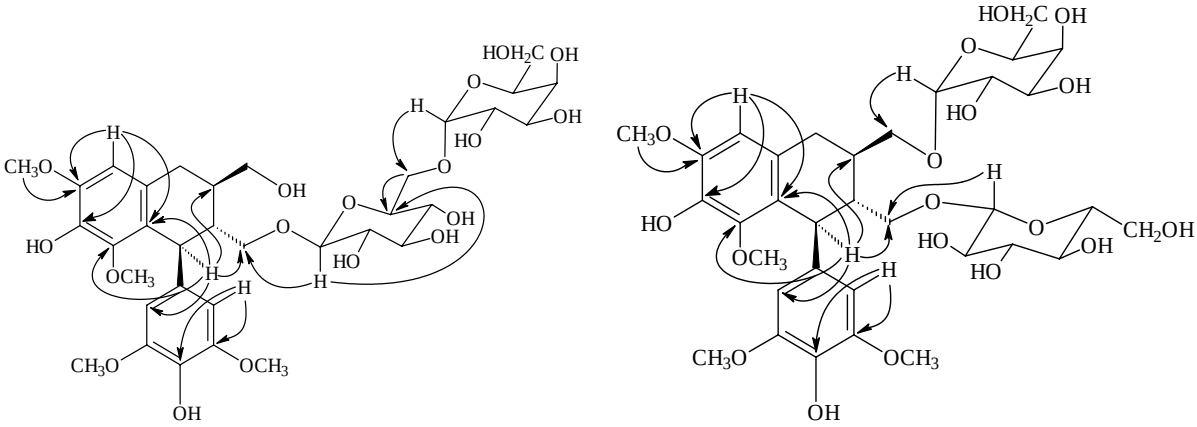
The molecular formula of compound **1** was established as $C_{34}H_{48}O_{18}$ by HRESI-MS in positive ion mode. The ^{1}H and ^{13}C NMR (Table 1) spectra of **1** showed the presence of two sugar moieties [δ 4.82 (1H, d, J = 3.8 Hz), 4.26(1H, d, J = 8.0 Hz) for ^{1}H NMR and δ 100.1, 105.0 for ^{13}C NMR] (Table 1), which were identified to be an α-D-galactopyranose and a β-D-glucopyranose unit. Acid hydrolysis of **1** afforded D-glucose and D-galactose, identical by TLC and comparison of the optical rotation with authentic samples. Its ^{13}C NMR spectrum was similar to that of (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**), previously isolated from the same plant [8], except for the additional group of signals of α-D-galactopyranosyl unit. Comparison of the ^{13}C NMR spectral data of **1** with that of (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**) (Table 1) revealed the downfield shift of C-6 (+4.7 ppm) of β-D-glucopyranose, indicating that the additional α-D-galactopyranosyl unit was substituted at C-6 of the glucose unit. Furthermore, the HMBC spectrum revealed a correlation between H-1 of the α-D-galactopyranosyl moiety and C-6 of the β-D-glucopyranosyl unit (Fig. 2). Consequently, the structure of compound **1** was elucidated to be (+)-lyoniresinol 3a-*O*-α-D-galactopyranosyl-(1 $\rightarrow$ 6)-β-D-glucopyranoside.

Compound **2** had the same molecular formula as compound **1**, established by the HRESI-MS and NMR data (Table 1). The ^{1}H and ^{13}C NMR spectra also showed the presence of two sugar moieties [δ 4.73 (1H, d, J = 3.8 Hz), 4.32(1H, d, J = 8.0 Hz) for ^{1}H NMR and δ=98.9, 103.0 for ^{13}C NMR] (Table 1),



1 **2**

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



1 **2**

Fig. 2. The significant HMBC correlations of compounds **1** and **2**.

which were identified to be an α-D-galactopyranose and a β-D-glucopyranose unit. Acid hydrolysis of **1** afforded D-glucose and D-galactose, identical by TLC and comparison of the optical rotation with authentic samples. Comparison of the ^{13}C NMR spectral data of **2** with that of (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**) (Table 1) revealed the downfield shift of C-2a ($+5.1$ ppm) of the aglycone, indicating that the additional α-D-galactopyranosyl unit was substituted at C-2a of the aglycone. Furthermore, the HMBC spectrum revealed a correlation between H-1 of the α-D-galactopyranosyl moiety and C-2a of the aglycone (Fig. 2). Therefore, the structure of compound **2** was assigned as (+)-lyoniresinol 2a-*O*-α-D-galactopyranosyl-3a-*O*-β-D-glucopy -ranoside.

Compounds **1** and **2** constitute a rare pair of positional isomers of cyclolignan glycosides containing an α-D-galactopyranosyl unit. To our knowledge **2** is the first C-2a glycosylated cyclolignan glycoside of lyoniresinol type found in nature so far.

Experimental Section

General

NMR spectra were recorded in methanol-d_4 and deuterated water using a Bruker ARX-500 spectrometer (500 MHz for ^{1}H NMR and 125 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

Carbon no.	**1** (Methanol-d_4)		**2** (D_2O)		**3** (Methanol-d_4)
Aglycone	^{1}H NMR δ H; mult.; *J*(Hz)	^{13}C NMR δC; mult	^{1}H NMR δ H; mult.; *J*(Hz)	^{13}C NMR δC; mult	^{13}C NMR δC; mult
1	2.72; dd; 15.2; 4.8 2.65; m	33.9; t	2.67; dd; 15.2; 4.8 2.61;m	31.6; t	33.8; t
2	1.70; m	40.4; d	1.80; m	36.4; d	40.6; d
2a	3.55[a] 3.62[a]	66.1; t	3.65[a] 3.82[a]	71.3; t	66.2; t
3	4.34; d; 6.4	43.1; d	4.23; d; 6.0	40.9; d	42.8; d
3a	3.42[a] 3.86[a]	71.4; t	3.58[a] 3.92[a]	71.5; t	71.4; t
4	2.03; m	46.8; d	2.24; m	44.2; d	46.7; d
5		148.6; s		147.5; s	148.6; s
6		138.8; s		136.6; s	138.9; s
7		147.6; s		146.2; s	147.6; s
8	6.54; s	107.8; d	6.65; s	108.1; d	107.8; d
9		130.1; s		130.1; s	130.2; s
10		126.4; s		124.4; s	126.4; s
1’		139.4; s		138.3; s	139.4; s
2’	6.40; s	107.0; d	6.34; s	106.0; d	106.9; d
3’		149.0; s		147.6; s	148.9; s
4’		134.6; s		134.3; s	134.5; s
5’		149.0; s		147.6; s	148.9; s
6’	6.40; s	107.0; d	6.34; s	106.0; d	106.9; d
Glc-1”	4.26; d; 8.0	105.0; d	4.32; d; 8.0	103.0; d	104.8; d
2”	3.24[a]	75.2; d	3.30[a]	73.2; d	75.2; d
3”	3.50[a]	78.2; d	3.52[a]	76.3; d	78.2; d
4”	3.70[a]	71.0; d	3.70[a]	69.4; d	71.7; d
5”	3.35[a]	76.3; d	3.36[a]	76.0; d	77.9; d
6”	3.65[a] 3.89[a]	67.5; t	3.74[a] 3.88[a]	61.2; t	62.8; d
Gal-1”’	4.82; d, 3.8	100.1; d	4.73; d, 3.8	98.9; d	
2”’	3.70[a]	70.5; d	3.75[a]	68.6; d	
3”’	3.68[a]	71.6; d	3.70[a]	69.8; d	
4”’	3.88[a]	71.4; d	3.80[a]	69.7; d	
5”’	3.82[a]	72.2; d	3.80[a]	70.8; d	
6”’	3.65[a] 3.82[a]	62.6; t	3.75[a] 3.95[a]	60.9; t	
5-OMe	3.82; s	60.0; q	3.63; s	60.2; q	60.2; q
7-OMe	3.33; s	56.6; q	3.22; s	56.4; q	56.6; q
3’, 5’-OMe	3.72; s	56.9; q	3.30; s	56.5; q	56.9; q

Table 1. ^{1}H (HMQC), ^{13}C NMR spectral data of compounds **1, 2** and ^{13}C NMR spectral data of compound **3** (500 MHz for ^{1}H and 125 MHz for ^{13}C).

[a] Overlapped signals are reported without designated multiplicity.

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 (4:1.2:0.1) (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) 15% MeOH, (VIII) 14% 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) was 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 normal 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 to CC of silica gel (system II-V) to afford

thirty fractions. Fractions 20 to 25 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** (25 mg), **2** (15 mg).

Compound **1**

Amorphous powder, $[\alpha]_D^{25} + 36.5^\circ$ (c 0.8, methanol). – ^{1}H NMR and ^{13}C NMR (methanol-d_4): See Table 1. – HR-ESI-MS, m/z : 767.2731 [M+Na]$^+$·($C_{34}H_{48}O_{18}$Na requires 767.2738).

Compound **2**

Amorphous powder, $[\alpha]_D^{25} + 40.0^\circ$ (c 0.6, methanol). – ^{1}H NMR and ^{13}C NMR (D_2O): See Table 1. – HR-ESI-MS, m/z : 767.2735 [M+Na]$^+$·($C_{34}H_{48}O_{18}$Na requires 767.2738).

Acid hydrolysis of **1** *and* **2**

Compound **1** (10 mg) was treated with a mixture of 1:1 2M 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 ($[\alpha]_D^{25} + 22^\circ$) was identical with that of (+)-lyoniresinol [9] ($[\alpha]_D^{25} + 23^\circ$). The aqueous layer containing monosaccharides was concentrated and applied to a silica gel column (system I) to afford D-glucose (4 mg, R_f 0.25, $[\alpha]_D^{25} + 50^\circ$) and D-galactose (4 mg, R_f 0.18, $[\alpha]_D^{25} + 80^\circ$), comparing with authentic samples.

By the same method, compound **2** (8 mg) provided D-glucose (3 mg, R_f 0.25, $[\alpha]_D^{25} + 50^\circ$), D-galactose (3 mg, R_f 0.18, $[\alpha]_D^{25} + 80^\circ$) and the aglycone of (+)-lyoniresinol as **1**.

Acknowledgements

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

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