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Megastigmane and flavone glycosides from *Acanthus ilicifolius*

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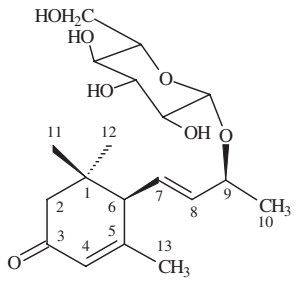
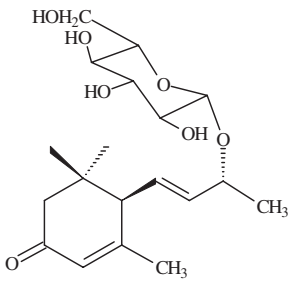
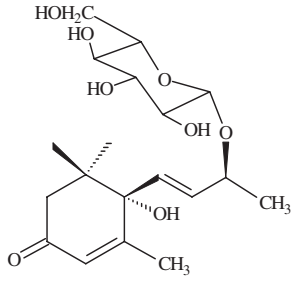
Acanthus ilicifolius L. (Acanthaceae) is a spiny herb of mangrove widely distributed in southeastern Asia. In Chinese traditional medicine, it is used as anti-inflammatory and anti-hepatitis agent. In preliminary investigations of this plant, a triterpenoid saponin [1], 2-benzoxazolinone [2], acanthicifoline [3], two phenylethanoid glycosides and seven lignin glucosides [4], five benzoxazinoid glucosides [5] have been isolated.



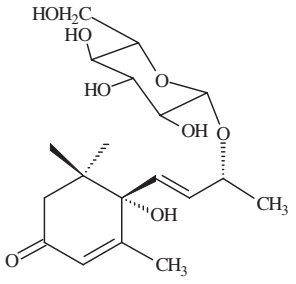
1 (6*R*, 9*S*)



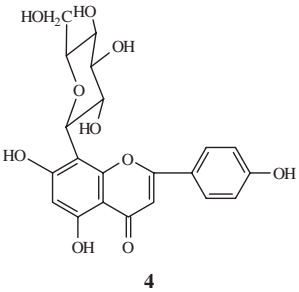
2 (6*R*, 9*R*)



3 (6*S*, 9*S*)



(6*S*, 9*R*)-roseoside



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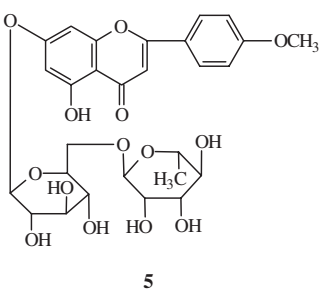

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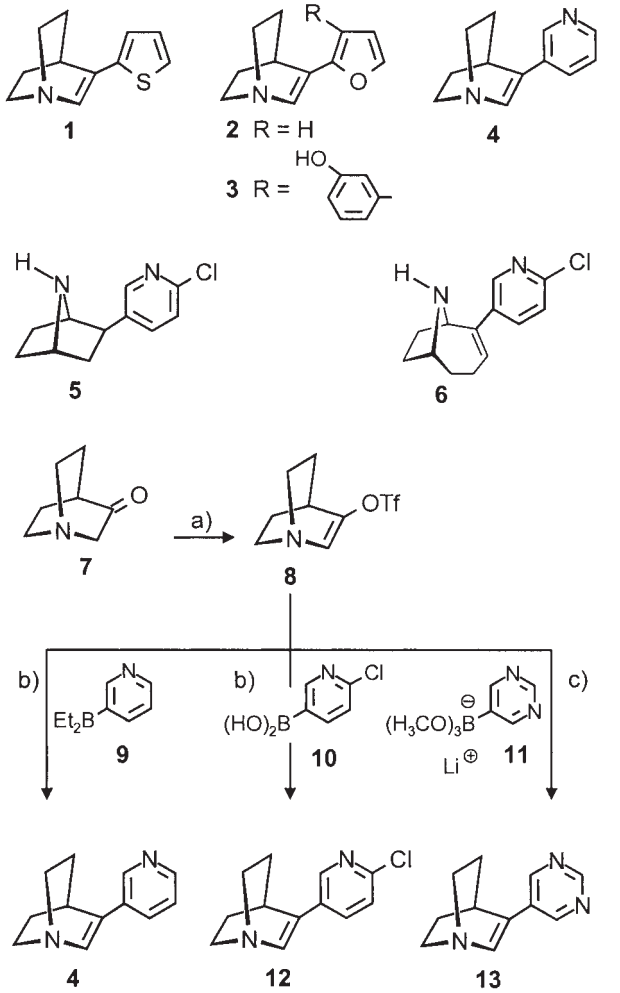
During our ongoing research on this plant, three megastigmane glucosides, (6*R*,7*E*,9*S*)-9-hydroxy-megastigman-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (**1**), (6*R*,7*E*,9*R*)-9-hydroxy-megastigman-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (**2**), (6*S*,7*E*,9*S*)-6,9-dihydroxy-megastigman-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (**3**), have been isolated from the aerial parts of this plant, along with two flavone glycosides, vitexin (**4**) and acacetin 7-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-glucopyranoside (**5**). Among them two megastigmane glucosides (**1**, **2**) and two flavone glycosides (**4**, **5**) were identified by comparison of their MS and NMR spectra data with the literature values [6–9].

Compound **3** was obtained as an amorphous powder. Positive-mode HR-ESI mass of **3** established the molecular formula $C_{19}H_{30}O_8$. The ^1H and ^{13}C NMR (Table) spectra of **3** showed the presence of one β-D-glucopyranosyl unit from the signals at δ_C 101.2 and δ_H 4.27 (1 H, d, J = 7.8 Hz). The ^{13}C NMR together with DEPT mode measurement revealed a megastigmane (α-ionol) aglycone of 13 carbon atoms. Comparison of the ^{13}C NMR spectral data of **3** with those of (6*S*,9*R*)-roseoside [7] revealed the same relative arrangement of the ring carbons and protons. The difference of the chemical shifts at C-9 (δ 74.6) and C-10 (δ 22.2) with C-9 (δ 77.3) and C-10 (δ 21.2) of

Scheme



Reagents and conditions: (a) 1. LDA, THF, −80 °C; 2. 2-n(Tf_2)-5-chloropyridine, 12 h, −80 °C → RT. 94%. (b) $Pd(PPh_3)_2Cl_2$, THF, 2M aqueous Na_2CO_3, 18 h, 80 °C, 53% and 67%. (c) analogous (b) in THF : ethanol = 3 : 1, 68%

Table: ^{1}H and ^{13}C NMR data of compounds 1, 2, 3 as well as ^{13}C NMR data of (6*S*, 9*R*)-roseoside in methanol-d_4

No.	**1**		**2**		**3**		roseoside* ^{13}C
	^{1}H δ (mult., J (Hz))	^{13}C δ_C	^{1}H δ (mult., J (Hz))	^{13}C δ_C	^{1}H δ (mult., J (Hz))	^{13}C δ_C	δ_C
1		37.2		37.1		42.4	42.4
2	2.56 (1 H, d, J = 16.4)	48.4	2.42 (1 H, d, J = 16.4)	48.3	2.62 (1 H, d, =17.2)	50.7	50.7
	2.14 (1 H, d, J = 16.4)		2.03 (1 H, d, J = 16.4)		2.18 (1 H, d, =17.2)		
3		201.9		202.0		201.2	201.1
4	5.97 (1 H, s)	126.1	5.87 (1 H, s)	126.2	5.86 (1 H, s)	127.1	127.2
5		165.4		165.5		167.1	167.2
6	2.78 (1 H, d, J = 9.0)	56.8	2.66 (1 H, d, J = 9.0)	56.8		80.0	80.0
7	5.84 (1 H, dd, J = 15.4, 9.4)	131.1	5.63 (1 H, dd, J = 15.4, 9.0)	128.8	5.98 (1 H, d, J = 15.6)	133.6	131.5
8	5.67 (1 H, dd, J = 15.4, 6.4)	137.0	5.78 (1 H, dd, J = 15.4, 6.4)	138.2	5.75 (1 H, dd, J = 15.6, 7.2)	133.7	135.3
9	4.56 (1 H, m)	74.7	4.39 (1 H, m)	77.0	4.53 (1 H, m)	74.6	77.3
10	1.37 (3 H, d, J = 6.4)	22.2	1.28 (3 H, d, J = 6.4)	21.0	1.29 (3 H, d, J = 6.8)	22.2	21.2
11	1.11 (3 H, s)	27.5	0.99 (3 H, s)	27.6	1.03 (3 H, s)	23.4	23.4
12	1.07 (3 H, s)	28.0	1.02 (3 H, s)	28.0	1.01 (3 H, s)	24.7	24.7
13	2.06 (3 H, d, J = 1.1)	23.8	1.92 (3 H, d, J = 1.1)	23.8	1.94 (3 H, d, J = 1.1)	19.6	19.5
1′	4.37 (1 H, d, J = 7.8)	101.2	4.34 (1 H, d, J = 7.8)	102.5	4.27 (1 H, d, J = 7.8)	101.2	102.7
2′		74.9		75.3		74.9	75.2
3′		78.3		78.0		78.3	78.0
4′		71.7		71.5		71.7	71.6
5′		78.1		78.1		78.1	78.1
6′	3.92 (1 H, dd, J = 11.8, 2.4)	62.8	3.81 (1 H, dd, J = 11.8, 2.4)	62.7	3.86 (1 H, dd, J = 11.8, 2.4)	62.8	62.8
	3.70 (1 H, dd, J = 11.8, 6.4)		3.64 (1 H, dd, J = 11.8, 6.4)		3.65 (1 H, dd, J = 11.8, 6.8)		

* representing (6*S*,9*R*)-roseoside

(6*S*,9*R*)-roseoside (Table) led to conclude the absolute configuration at C-9 to be *S*. Moreover, the optical rotation value of **3** with $[\alpha]_D^{25}$ −18° (MeOH; c 0.6) different from that of (6*S*,9*R*)-roseoside ($[\alpha]_D^{25}$ +111.2° [7]) supported the above deduction. Therefore, the structure of **3** was assigned as (6*S*,7*E*,9*S*)-6,9-dihydroxy-megastigman-4,7-dien-3-one-9-*O*-β-D-glucopyranoside, namely (6*S*,9*S*)-roseoside. ^{1}H-^{1}H COSY, HSQC and HMBC experiments also confirmed the above structure.

Simulating three-dimensional structures of three megastigmane glucosides **1**–**3** and (6*S*, 9*R*)-roseoside using Cambridgesoft Chem3D software showed that the upfield shift of C-9 and downfield shift of C-10 in 9*S*-magstigmane glucosides compared with that of corresponding 9*R*-magstigmane ones could be mainly explained as the nuclear spatial blocking effects.

Experimental

1. Apparatus

NMR spectra were recorded on a Bruker ARX-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). ESI-MS spectra were carried out on a Bruker Esquire-LC ion trap mass spectrometer operated in positive ion mode. HR-ESI mass were measured on a Bruker APEX II spectrometer in the same ion mode. Optical rotations were measured with an AA-10R digital polarimeter. Preparative HPLC was carried out on columns of ODS (250 × 10mm i.d., YMC) with a Waters 996 photodiode array detector.

2. Plant material

Acanthus ilicifolius L. was collected in July 2001 from Qinglan port of Hainan Province, southern China. The material was identified by Prof. Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample was kept in the Herbarium of South China Sea Institute of Oceanology.

3. Extration 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 H_2O and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively. The *n*-butanol extract (120 g) was subjected to column chromatography of a highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, 30% EtOH, 60% EtOH and 95% EtOH, successively. The fractions eluted with different concentration of EtOH were combined (70 g) and subjected to column chromatography using silica gel, Unicorn-ODS and Pharmacia-Sephadex LH-20 gel, then followed by preparative HPLC-ODS chromatography to afford compound **1** (240 mg), **2** (40 mg), **3** (289 mg), **4** (20 mg), and **5** (120 mg).

4. Enzymatic hydrolysis

To a solution of **1** (50 mg) in 6 ml acetate buffer (pH 5.0) 60 mg β-glucosidase was added, and the mixture was incubated with stirring at 37 °C for 48 h. Then the mixture was extracted with Et_2O (3×12 ml). The combined ether layers were evaporated to afford an aglycone as oily liquid (21 mg) with $[\alpha]_D^{25}$ +72.5° ($CHCl_3$, c 0.5). In the aqueous phase D-glucose was identified by comparing the optical rotation and TLC R_f value with that of authentic sample. Through the same procedure, 10 mg of **2** was hydrolyzed to give D-glucose and an aglycone (5 mg) with $[\alpha]_D^{25}$ +188.5° ($CHCl_3$, c 0.3).

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