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Triterpenoid saponins with antifeedant activities from stem bark of *Catunaregam spinosa* (Rubiaceae) against *Plutella xylostella* (Plutellidae)

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ABSTRACT

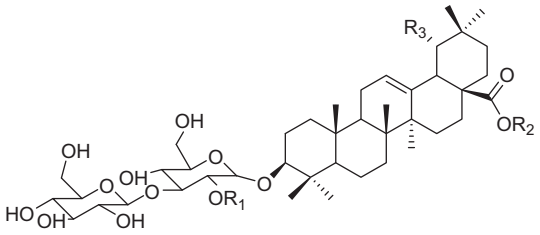
Seven triterpenoid saponins, including four new compounds, catunarosides A–D (**1–4**), and three known compounds, swartziatrioside (**5**), aralia-saponin V (**6**), araliasaponin IV (**7**) were isolated from the stem bark of *Catunaregam spinosa*, a Chinese mangrove associate. Their structures were elucidated on the basis of their spectral data and hydrolysis experiments. The antifeedant activities of compounds **1–7** against *Plutella xylostella* were also evaluated.

1. Introduction

Catunaregam spinosa (Thunb.) Tirveng (Rubiaceae), known as 'Madana and Mainphal' in Indian and 'Sacrifício de Cristo' in Brazil, is a folk medicine plant distributed in tropical and subtropical regions.[1,2] Its fruits are well known for their antispasmodic, antidysenteric, antifertility and immunomodulatory properties.[3,4] The plant is used to cure emetic, asthma, jaundice, gonorrhea.[5] Previous phytochemical investigations on this plant have led to the isolation and identification of coumarin glucosides, iridoid glucosides, triterpenoid saponins and lignans.[1,3,6–10] Triterpenoid saponins account for 9.5% of the EtOH extracts from fruits of *C. spinosa*.[2] In our phytochemical research on the stem bark of *C. spinosa*, four new oleanane-type triterpenoid saponins, catunarosides A–D, together with swartziatrioside, aralia-saponin V and araliasaponin IV were isolated (Fig. 1), and their antifeedant activities against *Plutella xylostella* were also evaluated.

2. Results and discussion

Catunaroside A (**1**) was obtained as an amorphous powder. It has ten degrees of unsaturation as deduced from the molecular formula $C_{47}H_{76}O_{18}$ determined by HRESIMS. The IR spectrum revealed absorption bands at 1741 cm^{-1} (C=O) and 1645 cm^{-1} (C=C). Therefore, the molecule has eight rings. The ^{13}C NMR spectrum showed seven methyl carbons at δ 27.7, 16.4, 15.4, 17.4, 24.8, 28.8 and 24.9, two olefinic carbons at δ 123.1 and 144.9. The seven methyl groups (δ 1.28, 1.07, 0.84, 1.03, 1.68, 1.20 and 1.12) and an olefinic proton



1	R_1=β-D-Xyl	R_2=H	R_3=OH
2	R_1=α-L-Rha	R_2=H	R_3=H
3	R_1=α-L-Rha	R_2=β-D-Glc	R_3=OH
4	R_1=β-D-Xyl	R_2=β-D-Glc	R_3=OH
5	R_1=β-D-Xyl	R_2=H	R_3=H
6	R_1=β-D-Glc	R_2=β-D-Glc	R_3=H
7	R_1=β-D-Xyl	R_2=β-D-Glc	R_3=H

Figure 1. Structures of compounds **1–7**.

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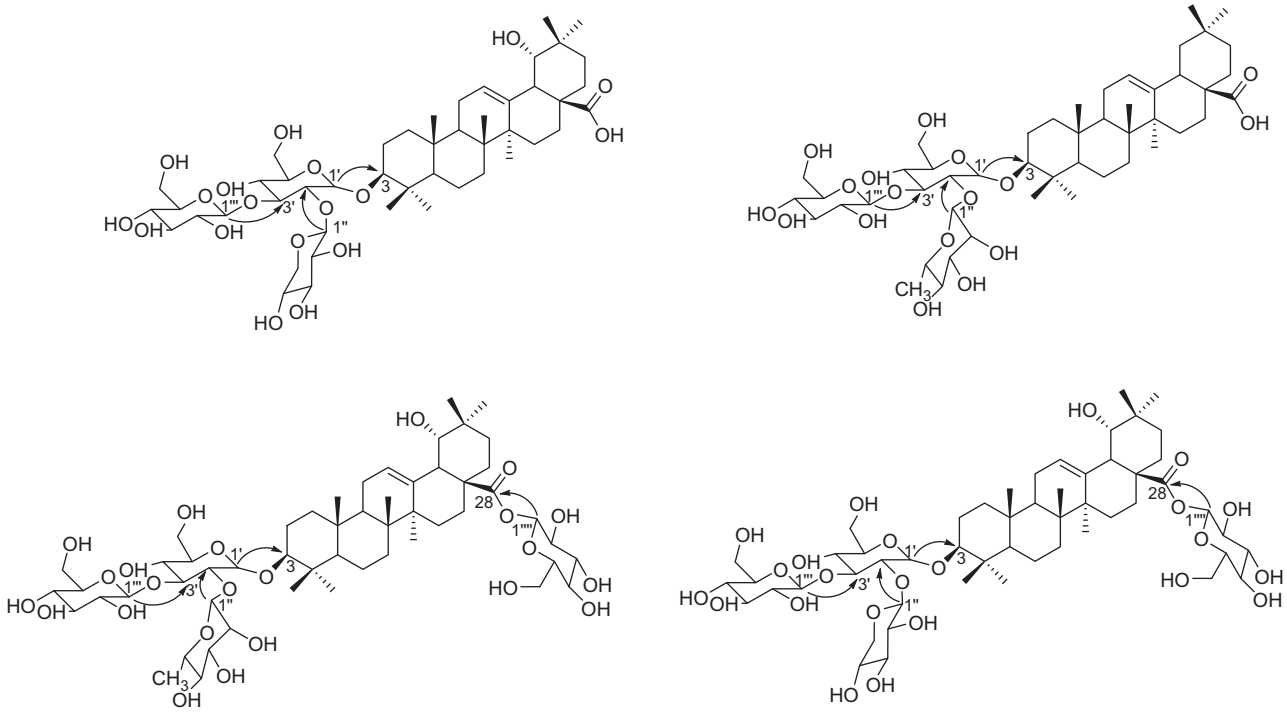

Figure 2. Key HMBC correlations for the sugar sequence of **1**–**4** (from H to C).

(δ 5.55, br s) were also observed in the ^{1}H NMR spectrum. The information from the ^{1}H NMR spectrum coupled with the ^{13}C NMR spectrum indicated that **1** possessed a siaresinolic acidic aglycon.[11] The chemical shifts of C-3 (δ 89.6) and C-28 (δ 180.9) revealed that **1** was a monodesmosidic glycoside. In the ^{13}C NMR spectrum, 27 of 47 resonances were assigned to the oligosaccharide moieties. The ^{1}H NMR and ^{13}C NMR spectra of **1** exhibited three sugar anomeric protons at δ 4.83 (d, J = 7.7 Hz), 5.37 (d, J = 7.9 Hz) and 5.60 (d, J = 7.7 Hz), and carbons at δ 105.1, 104.8 and 104.6. The monosaccharides were identified as D-glucose and D-xylose by TLC and GC-MS analysis, The glycan part signals on NMR spectrum of **1** were identical with those of swartziatrioside (**5**),[12] and the sequence of the glycan part was deduced from the following HMBC correlations: H-1″ (δ 5.37) of terminal glucose with C-3′ (δ 88.9) of inner glucose, H-1‴ (δ 5.60) of terminal xylose with C-2′ (δ 79.0) inner glucose, H-1′ (δ 4.83) of inner glucose with C-3 (δ 89.6) of the aglycon (Fig. 2). The β-anomeric configurations of the glucose and xylose units were determined by the $^3J_{H1,H2}$ coupling constants (7.7–7.9 Hz). On the basis of all the foregoing evidence, compound **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-siaresinolic acid.

Compound **2** was analyzed to have the molecular formula of $C_{48}H_{78}O_{17}$ by its HRESIMS spectrum (*m/z*: 949.5341 $[M+Na]^+$). The signals of seven methyl carbons at δ 28.0, 17.1, 15.6, 17.4, 26.2, 33.3, 23.7, two olefinic carbons δ 122.5 and 144.8, and one carbonyl carbon at δ 180.8 were present in the ^{13}C NMR spectrum. In combination with the ^{1}H NMR spectrum, the aglycon part of this compound was determined as oleanolic acid, based on comparisons of the ^{1}H, ^{13}C and DEPT NMR signals of **2** with those from 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl]-olean-12-en-3β-ol-28-oic acid.[9] From the signals in the anomeric region of the ^{13}C NMR spectrum (resonances at δ 104.9, 103.9 and 101.7), compound **2** had three sugar residues. Acid hydrolysis suggested that the monosaccharides of this compound were L-rhamnose and D-glucose, which were identified by GCMS analysis of their chiral derivatives. According to the characteristic proton signal at δ 1.70 (3H, d, J = 6.0 Hz) in the ^{1}H NMR spectrum, **2** could be deduced to contain one L-rhamnose and two D-glucose residues. The ^{13}C NMR chemical shifts of C-3 (δ 88.7) and C-28 (δ 180.8) indicated that **2** was a monodesmosidic glycoside in which the sugar moiety was connected at C-3.[13] The sequence of the oligosaccharide chain, as well as the glycoside sites, was determined by an HMBC experiment. In the HMBC spectrum, the following correlations were observed: H-1′ (δ 4.87) of the inner glucose and C-3 (δ 88.7) of aglycon part; H-1″ (δ 5.15) of the terminal glucose and C-3′ (δ 89.6) of inner glucose; H-1‴ (δ 6.51) of the terminal rhamnose and C-2′ (δ 78.7) of inner glucose (Fig. 2). The β-anomeric configurations of the glucose units and α-anomeric configuration of the rhamnose unit were separately determined by their $^3J_{H1,H2}$ coupling constants (7.5 and 7.8 Hz) and the $^1J_{C1,H1}$ coupling constant (169 Hz). On the basis of this evidence, the structure of compound **2**, named as catunaroside B, was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-oleanolic acid.

Compound **3** was shown to have the molecular formula $C_{54}H_{88}O_{23}$ based on its HRESIMS spectrum. A comparison of the ^{1}H and ^{13}C NMR spectra of **3** with those of **1** clearly revealed that the aglycon of **3** was identical to that of **1**. Acid hydrolysis of **3** gave L-rhamnose and D-glucose, which were determined by comparing the R_f values in TLC analysis and the retention times in GC–MS analysis with that of authentic samples. Four anomeric proton signals at δ 4.85 (d, J = 7.5 Hz), 5.13 (d, J = 7.9 Hz), 6.49 (br s) and 6.37 (d, J = 8.5 Hz) in the ^{1}H NMR spectrum were observed. The downfield ^{13}C NMR chemical shift at δ 88.8 and the upfield ^{13}C NMR chemical shift at δ 177.3 suggested that **3** was a bisdesmosidic glycoside with glycosidic linkages at C-3 through an ether bond and at C-28 through an ester bond.[14] By comparison of their signals in ^{13}C NMR spectrum with those of **2**, the glycan parts were the same, except one more glucose residue, which was connected to C-28. The sequence of the glycan parts and glycoside sites were also proven by the following HMBC correlations: H-1′ (δ 4.85) of the inner glucose and C-3 (δ 88.8) of aglycon part; H-1″ (δ 5.13) of the glucose and C-3′ (δ 89.5) of inner glucose; H-1‴ (δ 6.49) of the rhamnose and C-2′ (δ 78.7) of inner glucose (Fig. 2); H-1⁗ (δ 6.37) of the glucose

and C-28 (δ 177.3) of aglycon part (Fig. 2). The $^1J_{C1,H1}$ coupling constant of the rhamnose unit (170 Hz) confirmed that the anomeric proton was equatorial (α-pyranoid anomeric form). The β-anomeric configurations of the D-glucose were determined by their $^3J_{H1,H2}$ coupling constants (7.5–8.5 Hz). On the basis of the above evidence, the structure of compound **3** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-siaresinolic acid 28-*O*-β-D-glucopyranoside.

Compound **4** has the molecular formula of $C_{53}H_{86}O_{23}$ based on its HRESIMS spectrum. The ^{1}H and ^{13}C NMR signals of **4** were assigned after DEPT, HSQC and HMBC experiments. A comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **3** clearly revealed that the aglycon part of **4** was identical to that of **3**, and **4** was suggested to be a 3,28-bisdesmoside with four monosaccharide units. It has the same linkage sequence of sugar moiety and the same ester-glycoside chain as araliasaponin IV,[15] which was further confirmed by acid hydrolysis and the key HMBC correlations (Fig. 2). From the above information, the structure of **4** was determined as 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-siaresinolic acid 28-*O*-β-D-glucopyranoside.

Compounds **5**–**7**, an amorphous powder, had similar IR spectra with **1**–**4**. Their ^{1}H and ^{13}C NMR signals were also assigned after DEPT, HSQC and HMBC experiments. They were elucidated as swartziatrioside (**5**),[12] aralia-saponin V (**6**)[16] and araliasaponin IV (**7**)[15] separately by HRESIMS, IR data, 1D and 2D NMR data. Compounds **1**–**4** were new compounds, which are named as catunarosides A–D.

The antifeedant activities of compounds **1**–**7** against second-instar larvae of *P. xylostella* were tested. The results are shown in Table 4. All the isolated compounds show potent antifeedant activity against second-instar larvae of the diamondback moth. Compound **5**, with the AFC_{50} value 106.73 μg/mL, is the most active one of all compounds. Our findings support the earlier suggestion that triterpenoid saponins are repellent or deterrent to some insect herbivores.[17]

Bisdesmosidic saponins have two sugar chains, usually one at the C-3 carbon, and one at C-28. They lack many of the properties and activities of monodesmosidic saponins, and can be converted into the biologically active monodesmosidic form by removal of the sugar at the C-28 position.[18] Our results show bidesmosidic saponins **3**, **4**, **6** and **7** exhibit weaker antifeedant activity than monodesmosidic saponins **1**, **2** and **5**. These findings suggest bidesmosidic saponins in *C. spinosa* would be deglycosylated to monodesmosidic saponins at C-28 position when the plant is attacked by *P. xylostella*.

3. Experimental

3.1. General

Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Plant), macroporous resin (D101, Nankai University Chemical Plant), RP-18 silica gel (40–60 mesh, Merck) or on Sephadex LH-20 (Pharmacia). IR spectra were obtained using Bruker EQUINOX55 spectrometer. Optical rotations were taken on an ATAGO Polax-2L polarimeter. NMR spectra were recorded on a Bruker DRX-500, in pyridine-d_5, either at 500 (^{1}H) or 125 (^{13}C) MHz, using tetramethylsilane (TMS) as the internal standard. ESI mass spectra were collected on MDS SCIEX API 2000 LC/MS/MS instrument and Bruker BioTOF Q spectrometer for HR-ESI mass spectra. Precoated Si gel plates were used for analytical TLC. Preparative HPLC (Waters-600) equipped with a Waters-996 photodiode array detector was made by using an ODS column (YMC-Pack ODS-5-A, 250 × 10 mm i.d., 5 μm; YMC).

3.2. Plants and insects

The stem bark of *C. spinosa* was collected in February 2006 from Sanya, Hainan Province, PR China. The specimen was identified by professor Si Zhang, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen has been deposited in the South China Sea Institute of Oceanology, Chinese Academy of Sciences (accession number: GKLMMM020). The *P. xylostella* tested were obtained from established laboratory stock culture maintained

Table 1
^{1}H NMR spectroscopic data (δ) for the sugar moieties of compounds **1**–**7** (600 MHz in pyridine-d_5)

Position	1	2	3	4	5	6	7
3-*O*	Glu	Glu	Glu	Glu	Glu	Glu	Glu
1′	4.83 (d, 7.7)	4.87 (d, 7.5)	4.85 (d, 7.5)	4.82 (d, 7.5)	4.83 (d, 7.5)	4.83 (d, 7.5)	4.82 (d, 7.5)
2′	4.19	4.04	4.03	4.23	4.19	4.34	4.2
3′	4.25	4.22	4.21	4.22	4.24	4.24	4.23
4′	4.06	4.28	4.26	4.04	4.04	4.01	4.00
5′	3.85	4.10	4.11	3.82	3.84	3.80	3.81
6′	4.56, 4.29	4.48, 4.27	4.48, 4.28	4.54, 4.27	4.54, 4.26	4.48, 4.30	4.50, 4.22
2′-*O*	Xyl	Rha	Rha	Xyl	Xyl	Glu	Xyl
1″	5.60 (d, 7.7)	6.51 br s	6.49 br s	5.60 (d, 7.5)	5.59 (d, 7.4)	5.72 (d, 8.0)	5.56 (d, 7.5)
2″	4.08	4.62	4.60	4.05	4.05	4.06	4.03
3″	4.32	4.82	4.82	4.29	4.29	4.25	4.24
4″	4.26	4.31	4.29	4.23	4.24	4.14	4.17
5″	3.60, 4.30	4.77	4.75	3.60, 4.31	3.60, 4.31	3.87	3.55, 4.26
6″		1.70 (d, 6.0)	1.69 (d, 6.0)			4.44	
3′-*O*	Glu	Glu	Glu	Glu	Glu	Glu	Glu
1‴	5.37 (d, 7.9)	5.15 (d, 7.8)	5.13 (d, 7.5)	5.37 (d, 7.5)	5.36 (d, 7.8)	5.37 (d, 7.5)	5.34 (d, 8.0)
2‴	4.07	3.90	3.88	4.04	4.05	4.02	4.03
3‴	4.24	4.04	4.01	4.24	4.23	4.19	4.17
4‴	4.18	4.19	4.18	4.16	4.15	4.12	4.14
5‴	4.05	4.12	4.10	4.03	4.04	4.17	4.02
6‴	4.47, 4.25	4.56, 4.27	4.55, 4.26	4.45, 4.25	4.45, 4.24	4.23	4.42, 4.20
28-*O*			Glu	Glu		Glu	Glu
1⁗			6.37 (d, 8.5)	6.38 (d, 8.0)		6.33 (d, 8.0)	6.29 (d, 7.5)
2⁗			4.20	4.22		4.18	4.17
3⁗			4.36	4.37		4.22	4.18
4⁗			4.28	4.23		4.31	4.32
5⁗			4.36	4.37		4.31	4.31
6⁗			4.41	4.43		4.37	4.37

Table 2
^{13}C NMR spectroscopic data (δ) for the aglycon moieties of compounds **1–4** (125 MHz in pyridine-d_5)

Position	1	2	3	4	Position	1	2	3	4
1	38.5	39.0	39.0	38.6	16	28.4	23.7	28.1	28.0
2	26.6	26.7	26.7	26.5	17	46.1	46.7	46.5	46.5
3	89.6	88.7	88.8	89.7	18	44.8	42.0	44.6	44.6
4	39.7	39.5	39.5	39.6	19	81.3	46.5	81.2	81.1
5	56.0	56.1	56.3	56.0	20	35.7	31.0	35.6	35.5
6	18.6	18.5	18.8	18.7	21	29.2	34.1	29.1	29.0
7	33.3	33.2	33.2	33.0	22	33.7	33.2	33.1	33.2
8	40.0	39.7	40.2	40.2	23	27.7	28.0	28.1	27.8
9	48.2	48.0	48.3	48.2	24	16.4	17.1	17.0	16.4
10	37.1	37.0	37.2	37.1	25	15.4	15.6	15.6	15.4
11	24.1	23.7	24.1	24.1	26	17.4	17.4	17.6	17.5
12	123.1	122.5	123.1	123.7	27	24.8	26.2	25.0	24.9
13	144.9	144.8	144.3	144.3	28	180.9	180.8	177.3	177.0
14	42.1	42.2	42.2	42.1	29	28.8	33.3	28.7	28.7
15	29.2	28.3	29.0	29.0	30	24.9	23.7	24.7	24.7

for >15 generations at 25 ± 1 °C temperature, 75 ± 10% RH and a 14:10 h (L: D) photoperiod. Cabbage plants (*Brassica oleraceae*) were grown in greenhouse at Institute of Plant Protection and Microbiology, Zhejiang Academy of Agriculture Science, PR China, without any application of insecticides.

3.3. Extraction and isolation

The *n*-BuOH extract of air-dried and powered plant material (9.0 kg) was extracted by the same method as previously reported.[7] The *n*-BuOH soluble fraction (170 g) was passed through a macroporous resin (D101) column eluted with H_2O and EtOH–H_2O (3:7, 6:4, 95:5), whereas the EtOH–H_2O (6:4, 35 g) eluted portion was subjected to silica gel CC eluting with $CHCl_3$–MeOH (90:10→50:50) to give fractions A–F. Fraction D was separated by ODS silica gel CC eluting with MeOH–H_2O (50:50) and purified by preparative HPLC using MeOH–H_2O–H_3PO_4 (65:35:0.1) to give compounds **6** (15 mg) and **7** (132 mg); Fraction F was separated by silica gel CC eluting with $CHCl_3$–MeOH–H_2O (80:20:5), then purified by preparative HPLC using MeOH–H_2O–H_3PO_4 (55:45:0.1) to give compounds **3** (176 mg) and **4** (13 mg). The EtOH–H_2O (95:5, 10 g) eluting portion was subjected to silica gel CC eluting with $CHCl_3$–MeOH (98:2→50:50) to give fractions a–g. Fraction d was separated by ODS silica gel CC eluted with MeOH–H_2O (50:50→80:20), then fractions 29–30 and fractions 41–45 were purified by preparative HPLC, respectively, using MeOH–H_2O–H_3PO_4 (65:35:0.1) and MeOH–H_2O–H_3PO_4 (62:38:0.1) to give compounds **1** (96 mg), **5** (13 mg), and **2** (6 mg).

3.3.1. Catunaroside A (1)

Colorless amorphous powder, $[\alpha]_D^{20}$ +10.4 (*c* 0.8, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400, 2920, 1741, 1645, 1038; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.25(1H, dd, *J* = 4.5, 11.6 Hz, H-3), 5.55 (1H, br s, H-12), 3.63 (1H, br s, H-18), 3.60 (1H, br s, H-19), 1.28 (1H, s, H-23), 1.07 (1H, s, H-24), 0.84 (1H, s, H-25), 1.03 (1H, s, H-26), 1.68 (1H, s, H-27), 1.20 (1H, s, H-29), 1.12 (1H, s, H-30); ^{1}H NMR data of the sugar part, see Table 1; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2 and Table 3; ESIMS (positive mode) *m/z*: 951 $[M+Na]^+$, 967 $[M+K]^+$; HRESIMS (positive mode) *m/z*: 951.4938 ($[M+Na]^+$, $C_{47}H_{76}O_{18}Na^+$; calcd 951.4930).

3.3.2. Catunaroside B (2)

Colorless amorphous powder, $[\alpha]_D^{20}$ +32.4 (*c* 0.8, MeOH); IR (KBr) ν_{max} cm^{-1}: 3410, 2925, 1739, 1640, 1030; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.40(1H, dd, *J* = 3.9, 12.0 Hz, H-3), 5.47 (1H, br s, H-12), 3.39 (1H, dd, *J* = 10.1, 3.8 Hz, H-18), 1.30 (1H, s, H-23), 1.17 (1H, s, H-24), 0.82 (1H, s, H-25), 0.98 (1H, s, H-26), 1.26 (1H, s, H-27), 0.95 (1H, s, H-29), 1.01 (1H, s, H-30); ^{1}H NMR data of the sugar part, see Table 1; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2 and Table 3; ESIMS (positive mode) *m/z*: 949 $[M+Na]^+$, 965 $[M+K]^+$; HRESIMS (positive mode) *m/z*: 949.5349 ($[M+Na]^+$, $C_{48}H_{78}O_{17}Na^+$; calcd 949.5341).

Table 3
^{13}C NMR spectroscopic data (δ) for the sugar moieties of compounds **1–7** (125 MHz in pyridine-d_5)

Position	1	2	3	4	5	6	7
3-*O*	Glu	Glu	Glu	Glu	Glu	Glu	Glu
1′	105.1	104.9	104.9	105.0	105.0	104.9	105.1
2′	79.0	78.7	78.7	79.0	79.0	79.3	79.0
3′	88.9	89.6	89.5	89.0	88.9	88.7	88.9
4′	70.2	76.9	77.0	70.2	70.1	70.0	70.2
5′	77.8	69.8	69.9	77.8	77.8	77.6	77.8
6′	62.3	62.7	62.7	62.2	62.3	63.4	62.3
2′-*O*	Xyl	Rha	Rha	Xyl	Xyl	Glu	Xyl
1″	104.6	101.7	101.7	104.6	104.6	103.8	104.7
2″	76.2	72.5	72.5	76.2	76.2	75.4	76.3
3″	79.3	72.5	72.5	79.3	79.3	78.6	79.4
4″	71.4	73.9	73.9	71.4	71.4	72.6	71.5
5″	67.2	69.8	69.9	67.2	67.2	77.8	67.2
6″		18.6	18.6			62.2	
3′-*O*	Glu	Glu	Glu	Glu	Glu	Glu	Glu
1‴	104.8	103.9	103.9	104.7	104.7	104.7	104.8
2‴	75.4	77.8	77.8	75.4	75.4	76.4	75.4
3‴	78.6	75.2	75.2	78.6	78.6	78.6	78.6
4‴	71.6	78.4	78.4	71.6	71.6	71.6	71.6
5‴	78.6	71.5	71.5	78.6	78.6	78.6	78.6
6‴	62.6	62.3	62.4	62.6	62.6	62.6	62.7
28-*O*			Glu	Glu		Glu	Glu
1⁗			95.9	95.9		95.7	95.8
2⁗			74.2	74.2		74.2	74.2
3⁗			78.9	78.9		78.9	78.9
4⁗			71.1	71.1		71.1	71.2
5⁗			79.3	79.3		79.3	79.3
6⁗			62.2	62.3		62.3	62.4

Table 4
Antifeedant activities of compounds **1–7** against *P. xylostella* larva

Compound no.	Regression equation	*r*	AFC_{50} (μg/ml)
1	$Y = 4.673+0.137x$	0.986	243.23
2	$Y = 4.250+0.297x$	0.999	369.63
3	$Y = 4.300+0.235x$	0.992	946.13
4	$Y = 4.673+0.137x$	0.952	608.42
5	$Y = 4.299+0.346x$	0.981	106.73
6	$Y = 4.286+0.191x$	0.992	5467.82
7	$Y = 4.250+0.297x$	0.996	335.54

3.3.3. Catunaroside C (3)

Colorless amorphous powder, $[\alpha]_D^{20}$ +37.5 (*c* 0.4, MeOH); IR (KBr) ν_{max} cm^{-1}: 3408, 2926, 1743, 1644, 1035; ^{1}H NMR (500 MHz,

pyridine-d_5): δ 3.38 (1H, dd, J = 3.8, 11.5 Hz, H-3), 5.50 (1H, br s, H-12), 3.52 (1H, br s, H-18), 3.57 (1H, br s, H-19), 1.25 (1H, s, H-23), 1.18 (1H, s, H-24), 0.88 (1H, s, H-25), 1.13 (1H, s, H-26), 1.62 (1H, s, H-27), 1.14 (1H, s, H-29), 0.98 (1H, s, H-30); ^{1}H NMR data of the sugar part, see Table 1; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2 and Table 3; ESIMS (positive mode) m/z: 1127 [M+Na]$^+$; HRESIMS (positive mode) m/z: 1127.5562 ([M+Na]$^+$, $C_{54}H_{88}O_{23}Na^+$; calcd 1127.5565).

3.3.4. Catunaroside D (4)

Colorless amorphous powder, $[\alpha]_D^{20}$ +68.5 (c 0.15, MeOH); IR (KBr) ν_{max} cm^{-1}: 3408, 2929, 1741, 1645, 1038; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.25 (1H, dd, J = 3.5, 11.5 Hz, H-3), 5.50 (1H, br s, H-12), 3.52 (1H, br s, H-18), 3.58 (1H, br s, H-19), 1.28 (1H, s, H-23), 1.09 (1H, s, H-24), 0.87 (1H, s, H-25), 1.13 (1H, s, H-26), 1.65 (1H, s, H-27), 1.14 (1H, s, H-29), 0.98 (1H, s, H-30); ^{1}H NMR data of the sugar part, see Table 1. ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 2 and Table 3. ESIMS (positive mode) m/z: 1114 [M+Na]$^+$, 1127 [M+K]$^+$; HRESIMS (positive mode) m/z: 1113.5471 ([M+Na]$^+$, $C_{53}H_{86}O_{23}Na^+$; calcd 1113.5463).

3.3.5. Swartziatrioside (5)

Colorless amorphous powder, $[\alpha]_D^{20}$ +35.0 (c 0.15, MeOH); IR (KBr) ν_{max} cm^{-1}: 3403, 2929, 1740, 1645, 1038; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.28 (1H, dd, J = 4.0, 11.0 Hz, H-3), 5.47 (1H, br s, H-12), 3.29 (1H, dd, J = 13.0, 4.0 Hz, H-18), 1.28 (1H, s, H-23), 1.07 (1H, s, H-24), 0.81 (1H, s, H-25), 0.98 (1H, s, H-26), 1.30 (1H, s, H-27), 0.96 (1H, s, H-29), 1.01 (1H, s, H-30); ^{1}H and ^{13}C NMR data of the sugar part, see Table **1** and Table **3**; ESIMS (positive mode) m/z: 935 [M+Na]$^+$, 951 [M+K]$^+$.

3.3.6. Aralia-saponin V (6)

Colorless amorphous powder, $[\alpha]_D^{20}$ −15.0 (c, 0.2, MeOH); IR (KBr) ν_{max} cm^{-1}: 3409, 2924, 1738, 1641, 1038; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.27 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.42 (1H, br s, H-12), 3.19 (1H, dd, J = 12.5, 3.8 Hz, H-18), 1.25 (1H, s, H-23), 1.08 (1H, s, H-24), 0.81 (1H, s, H-25), 1.08 (1H, s, H-26), 1.25 (1H, s, H-27), 0.91 (1H, s, H-29), 0.88 (1H, s, H-30), 4.83 (1H, d, J = 7.5 Hz, H-1′); ^{1}H and ^{13}C NMR data of the sugar part, see Table **1** and Table **3**; ESIMS (positive mode) m/z: 1127 [M+Na]$^+$.

3.3.7. Araliasaponin IV (7)

Colorless amorphous powder, $[\alpha]_D^{20}$ +80.0 (c = 0.5, MeOH); IR (KBr) ν_{max} cm^{-1}: 3411, 2928, 1743, 1640, 1032; ^{1}H NMR (500 MHz, pyridine-d_5): δ 3.26(1H, dd, J = 4.0, 11.0 Hz, H-3), 5.42 (1H, br s, H-12), 3.19 (1H, dd, J = 13.0, 4.0 Hz, H-18), 1.27 (1H, s, H-23), 1.07 (1H, s, H-24), 0.84 (1H, s, H-25), 1.08 (1H, s, H-26), 1.27 (1H, s, H-27), 0.92 (1H, s, H-29), 0.88 (1H, s, H-30); ^{1}H and ^{13}C NMR data of the sugar part, see Table **1** and Table **3**; ESIMS (positive mode) m/z: 1098 [M+Na]$^+$.

3.4. Acid Hydrolysis of 1–7 and determination of absolute configuration of monosaccharides

Compounds **1**–**7** (5 mg each) were heated at reflux in 5 mL of 4 N HCl for 8 h (kept sealed) in a water bath (100 °C). After cooling, the reaction mixtures were extracted with EtOAc (5 mL) saturated with H_2O. The aqueous layers were adjusted to pH 6 with $NaHCO_3$. After being concentrated under reduced pressure, each H_2O layer (monosaccharide portion) was identified by comparison of the R_f value with that of authentic samples eluting with $CHCl_3$–MeOH–H_2O (8:7:1) and EtOAc–MeOH–AcOH–H_2O (13:3:4:3) solvent system, visualized with ethanol–10% H_2SO_4 spraying and then heating. The chiral derivatives were prepared by the reported method.[19] The GC–MS analysis was carried on Shimadzu QP-2010 GC–MS. Column: Rtx-5MS (30 m, 0.25 mm i.d., 0.25 μm). Column temperature: 100–180 °C, with a rate of 10 °C/min, then 180–240 °C, with a rate of 3 °C /min, keeping at 240 °C for 5 min, and the carrier gas was He (1.2 mL/min), split ratio 1/50, injection temperature: 250 °C. Injection volume: 1 μL. The absolute configurations of the monosaccharides were confirmed to be L-rhamnose, D-xylose, and D-glucose, by comparison of the retention times of monosaccharide derivatives with those of standard samples: L-rhamnose (9.69 min), D-xylose (12.45 min) and D-glucose (13.61 min), respectively.

3.5. Antifeedant assay

A conventional non-choice leaf disc method experiment was conducted to evaluate the antifeedant activities.[20] Each sample was dissolved in water containing 0.05% Tween 80 (Polysorbate 80) to obtain serial concentrations of 0.1, 1, 10, 100 and 1000 ppm. Water containing 0.05% Tween 80 was used as a control separately. Leaf disks (1.5 cm diameter) cut from cabbage leaves with a cork borer, were dipped for 10 s in the sample and control solutions and dried in the air for 3 h at room temperature. Four leaf disks were transferred to Petri dishes (9 cm diameter and 2 cm depth) with a moist filter paper on the bottom. Five second instar larvae of *P. xylostella* were starved for 2 h and then placed alongside the leaf disks at the center of the Petri dish. Each treatment was replicated six times and conducted at 25 ± 1 °C temperature, 75 ± 10% RH and a photoperiod of 14:10 h (L:D). The amount of leaf consumed within 48 h was recorded. The area of each disk consumed during this period was determined by placing leaf remains on the graph paper and then counting the squares within the consumed area. Then the antifeedant index (%) was calculated as $(C - T)/C \times 100$ where C is the consumed leaf area of negative control disks and T is the consumed leaf area of treated and positive control disks. Leaf area consumed in treatment was corrected from the control. The antifeedant concentration of 50% (AFC_{50}) values generated by linear regression was determined by probit analysis (SPSS V13.0).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.07.022.

References

1. Hamerski, L.; Furlan, M.; Silva, D. H. S.; Cavakgeriro, A. J.; Eberlin, M. N.; Tomazela, D. M.; Bolzani, V. da S. *Phytochemistry* **2003**, *63*, 397–400.
2. Atal, C. K.; Lamba, S. S. *Indian J. Pharm.* **1960**, *22*, 120–122.
3. Dubois, M. A.; Benze, S.; Wagner, H. *Planta Med.* **1990**, *56*, 451–454.
4. Satpute, K. L.; Jadhav, M. M.; Karodi, R. S.; Katare, Y. S.; Patil, M. J.; Rub, R.; Bafna, A. B. *J. Pharmacognosy Phytother.* **2009**, *1*, 36–40.
5. Kirtikar, K. R.; Basu, B. D., 2nd ed. In *Indian Medicinal Plants*; International Book Distributors Dehradun: India, 1999; Vol. 2, pp 1760–1764.
6. Sati, S. P.; Chaukiyal, D. C.; Sati, O. P. *J. Nat. Prod.* **1989**, *52*, 376–379.
7. Gao, G. C.; Luo, X. M.; Wei, X. Y.; Qi, S. H.; Yin, H.; Xiao, Z. H.; Zhang, S. *Helv. Chim. Acta* **2010**, *93*, 339–344.
8. Gao, G. C.; Qi, S. H.; Zhang, S.; Yin, H.; Xiao, Z. H.; Li, M. Y.; Li, Q. X. *Pharmazie* **2008**, *63*, 542–544.
9. Sati, O. P.; Rana, U.; Chaukiyal, D. C.; Madhusudanan, K. P.; Bhakuni, D. S. *Planta Med.* **1987**, *53*, 530–531.
10. Sotheeswaran, S.; Bokel, M.; Kraus, W. *Phytochemistry* **1989**, *28*, 1544–1546.
11. Yaguchi, E.; Miyase, T.; Ueno, A. *Phytochemistry* **1995**, *39*, 185–189.
12. Abdel-kader, M. S.; Bahler, B. D.; Malone, S.; Werkhoven, M. C.; Wisses, J. H.; Neddermann, K.; Bursuker, M. I.; Kingston, D. G. *J. Nat. Prod.* **2000**, *63*, 1461–1464.

13. Lemmich, E.; Cornett, C.; Furu, P.; Jorstian, C. L.; Knudsen, A. D.; Olsen, C. E.; Salih, A.; Thiilborg, S. T. *Phytochemistry* **1995**, *39*, 63–68.
14. Ouyang, M. A.; Liu, Y. Q.; Wang, H. Q.; Yang, C. R. *Phytochemistry* **1998**, *49*, 2483–2486.
15. Miyase, T.; Shiokawa, K. I.; Zhang, D. M.; Ueno, A. *Phytochemistry* **1996**, *41*, 1411–1418.
16. Song, S. J.; Nakamura, N.; Ma, C. M.; Hattori, M.; Xu, S. X. *Phytochemistry* **2001**, *56*, 491–497.
17. Geyter, E. D.; Lambert, E.; Geelen, D.; Smagghe, G. *Pest Tech.* **2007**, *1*, 96–105.
18. Osbourn, A. *Trends Plant Sci.* **1996**, *1*, 4–9.
19. Nie, W.; Luo, J. G.; Kong, L. Y. *Carbohydr. Res.* **2010**, *345*, 68–73.
20. Sengonca, C.; Liu, Y.; Zhu, Y. J. *J. Pest Sci.* **2006**, *79*, 3–8.