Philinopsides A and B, Two New Sulfated Triterpene Glycosides from the Sea Cucumber *Pentacta quadrangularis*

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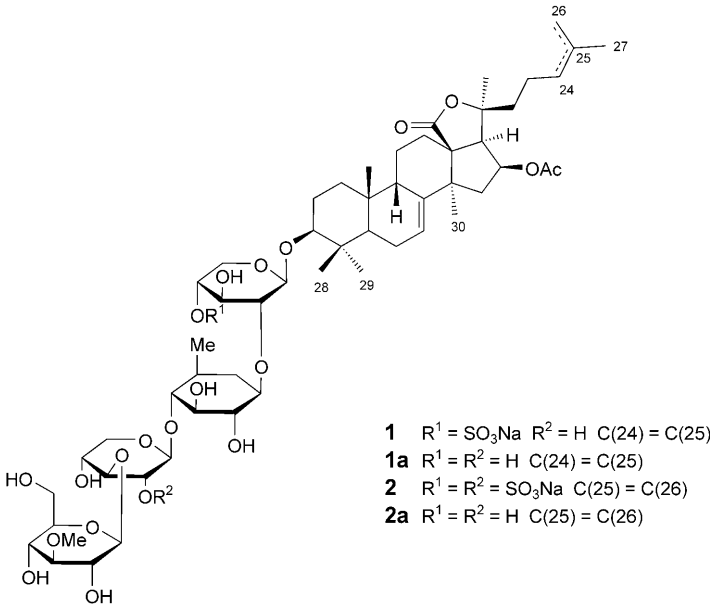
Two new sulfated triterpene glycosides, philinopsides A (**1**) and B (**2**), were isolated from the sea cucumber *Pentacta quadrangularis*. Their structures were established by extensive spectral analysis (2D-NMR and MS) and chemical evidence. Philinopside A (**1**) and B (**2**) showed significant cytotoxicity (ED_{50} 0.75–3.50 μg/ml) against ten tumor cell lines. Compound **1** also significantly inhibited the proliferation, migration, and tube formation of human microvascular endothelial cells.

Introduction. – As a part of our ongoing investigation on biologically active triterpene glycosides from sea cucumbers, we decided to focus our attention on the saponins of the South China sea cucumber *Pentacta quadrangularis*, collected near Guangdong province, China. In this paper, we describe the isolation of philinopside A (**1**) and philinopside B (**2**), the main components of the polar extracts, whose structures were determined by ^{1}H- and ^{13}C-NMR and 2D (DQCOSY, HMQC, HMBC, and NOESY) NMR spectra, and ESI-MS studies, as well as by comparison with NMR data of related saponins.

Results and Discussion. – The EtOH extracts of *P. quadrangularis* (5 kg, dry weight) were sequentially submitted to column chromatography (*DA-101* resin and silica gel) giving the fraction containing philinopsides A (**1**) and B (**2**). Compounds **1** and **2** were further isolated and purified by reversed-phase HPLC (*Zobax-SB-C_{18}*).

Philinopside A (**1**) was obtained as a colorless amorphous powder. Its molecular formula was determined as $C_{55}H_{85}NaO_{25}S$ from the pseudomolecular-ion peak at *m*/*z* 1223.4896 $(M+Na)^+$ in the HR-ESI-MS (positive-ion mode). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*) suggested the presence of a triterpene aglycon with two olefinic bonds and one ester and one lactone carbonyl group bonded to an oligosaccharide chain composed of four sugar units. A comparison of the spectral data of **1** with those of published saponins showed that the aglycon part of **1** was identical to that



of liouvilloside A from *Staurocucumis liouvillei* [1], featuring the characteristic C(24)=C(25) bond. Compound **1** was treated with 2N HCl to give D-xylose, D-quivonose (=6-deoxy-D-glucose) and 3-*O*-methyl-D-glucose in the ratio 2:1:1. The monosaccharides were identified by GC in the form of the corresponding aldononitrile peracetates. The site of linkage of the sulfo group in the sugar unit of **1** was demonstrated by its solvolysis with dioxane/pyridine to desulfophilinopside (**1a**) (NMR data in *Tables 3* and *4*). The sugar moiety of **1a** was identical to that of desulfated intercedenside A from *Mensamria intercedens* [3]. When the ^{13}C-NMR signals of the sugar moiety of **1** were compared with those of desulfophilinopside A (**1a**) (*Table 4*), an esterification shift (from 68.3 to 75.3) was observed at the signals of C(4) (Xyl). The structure of **1** was finally elucidated on the basis of extensive spectroscopic analysis and chemical evidence as (3β,9β,16β)-16-(acetyloxy)-3-{[3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-quinovopyranosyl-(1 → 2)-4-*O*-sulfo-β-D-xylopyranosyl]oxy}-18-oxo-18,20-epoxylanosta-7,24-diene monosodium salt (**1**).

The ^{1}H- and ^{13}C-NMR spectra of **1** showed resonances for a C(7)=C(8) bond (δ(C) 145.6 (C(8)) and 120.5 (C(7)); δ(H) 5.64 (br. *s*, H–C(7))) and a 16β-(acetyloxy) group (δ(C) 169.6 and 21.5; δ(H) 1.96 (*s*, 3 H)) closely related to those of frondoside D isolated from *Cucumaria frondosa* [2]. The location of the AcO group at C(16) was deduced from the chemical shift of the signal at δ 5.91 (*dt*, *J* = 9.6, 9.0, H–C(16)), which showed coupling to signals at δ 2.62 (*d*, *J* = 9.0, H–C(17)), 2.57 (*dd*, *J* = 9.6, 12.1, H_α–C(15)), and 1.75 (*m*, H_β–C(15)) in the ^{1}H,^{1}H-COSY plot. The 16β configuration of the AcO group was confirmed by NOESY experiments. Two C-atoms at δ(C) 132.1 (C(25)) and 124.4 (C(24)), along with two olefinic Me signals at δ(C) 25.7 and 17.9 (Me(26) and Me(27)), supported the presence of an additional C(24)=C(25) bond. Analysis of ^{1}H,^{1}H-COSY, HMQC, and NOESY data allowed the assignment of all ^{1}H- and ^{13}C-NMR resonances and established the relative configuration of all chiral centers of the aglycon.

Table 1. *^{1}H- and ^{13}C-NMR Data for the Aglycon Moiety of Philinopside A* (**1**). δ in ppm, J in Hz.

	δ(C)[a])	δ(H)[b])	^{1}H,^{1}H-COSY	^{1}H,^{1}H-NOESY	^{1}H,^{13}C-HMBC
CH_2(1)	35.8 (*t*)	1.47–1.54 (*m*)	CH_2(2)		
CH_2(2)	26.8 (*t*)	1.89–2.02 (*m*)	CH_2(1), H_α–C(3)	Me(19)	
H–C(3)	88.1 (*d*)	3.24 (*dd*, J = 4.2, 11.9)	H_α–C(2), H_β–C(2)	H–C(5), Me(29), H_α–C(1)	C(1) (Xyl[1])
C(4)	39.8 (*s*)				
H–C(5)	47.7 (*d*)	1.01 (*t*, J = 7.6)	CH_2(6)	H_α–C(3), Me(29), H–C(7)	C(4), C(28), C(29)
CH_2(6)	22.4 (*t*)	1.89–2.02 (*m*)	H–C(5), H–C(7)		
H–C(7)	120.5 (*d*)	5.64 (br. *s*)	CH_2(6)	H–C(17), Me(29), Me(30), H–C(5)	
C(8)	145.6 (*s*)				
H–C(9)	47.3 (*d*)	3.46 (*d*, J = 14.2)	H_α–C(11), H_β–C(11)		
C(10)	35.7 (*s*)				
CH_2(11)	22.4 (*t*)	1.49–1.60 (*m*, H_α) 1.75–1.93 (*m*, H_β)	H–C(9), CH_2(12) H–C(9), CH_2(12)		
CH_2(12)	30.2 (*t*)	2.08–2.21 (*m*)	H_α–C(11), H_β–C(11)		
C(13)	59.5 (*s*)				
C(14)	47.6 (*s*)				
CH_2(15)	43.5 (*t*)	1.71–1.79 (*m*, H_α) 2.57 (*dd*, J = 9.6, 12.1, H_β)	H_α–C(15), H_α–C(16) H_β–C(15), H_α–C(16)		
H–C(16)	73.9 (*d*)	5.91 (*dt*, J = 9.6, 9.0)	H_α–C(15), H_β–C(15), H_α–C(17)	H_α–C(17), Me(30)	C(17), Me*C*O
H–C(17)	53.8 (*d*)	2.62 (*d*, J = 9.0)	H_α–C(16)	H_α–C(16), H–C(7), Me(30), Me(21)	C(13), C(21)
C(18)	179.6 (*s*)				
Me(19)	24.2 (*q*)	1.31 (*s*)		H_β–C(2), Me(28)	
C(20)	85.2 (*s*)				
Me(21)	28.9 (*q*)	1.47 (*s*)		H–C(17)	
CH_2(22)	38.8 (*t*)	2.47–2.59 (*m*)	CH_2(23)		
CH_2(23)	24.2 (*t*)	1.94–2.03 (*m*)	CH_2(22), H–C(24)		
H–C(24)	124.4 (*d*)	5.11 (*s*)			C(26), C(27)
C(25)	132.1 (*s*)				
Me(26)	25.7 (*q*)	1.58 (*s*)	H–C(24)		
Me(27)	17.9 (*q*)	1.65 (*s*)			
Me(28)	17.5 (*q*)	1.26 (*s*)		Me(19)	C(3), C(5), C(29)
Me(29)	28.9 (*q*)	1.10 (*s*)		H–C(3), H–C(5), H–C(7)	C(3), C(5), C(28)
Me(30)	32.4 (*q*)	1.21 (*s*)		H–C(16), H–C(17), H–C(7)	C(13), C(14)
Me*C*O	169.9 (*s*)				
*Me*CO	21.5 (*q*)	1.96 (*s*)			

[a]) Recorded at 150 MHz in (D_5)pyridine/D_2O 4:1. Multiplicity by DEPT. [b]) Recorded at 600 MHz in (D_5)pyridine/D_2O 4:1.

Table 2. *^{1}H- and ^{13}C-NMR Data for the Sugar Moieties of Philinopside A* (**1**) *and B* (**2**). δ in ppm, J in Hz.

	1		**2**	
	δ(C)[a])	δ(H)[b])	δ(C)[a])	δ(H)[b])
Xyl4SO$_3$Na^1 ($1 \rightarrow$ C(3)):				
H–C(1)	105.3 (*d*)	4.80 (*d*, *J* = 7.0)	104.7 (*d*)	4.82 (*d*, *J* = 7.0)
H–C(2)	83.8 (*d*)	4.07–4.15 (*m*)	83.1 (*d*)	4.06–4.14 (*m*)
H–C(3)	76.4 (*d*)	4.03–4.15 (*m*)	75.8 (*d*)	4.04–4.14 (*m*)
H–C(4)	75.3 (*d*)	5.15–5.23 (*m*)	75.3 (*d*)	5.16–5.23 (*m*)
CH$_2$(5)	64.5 (*t*)	4.71–4.79 (*m*, H$_\alpha$), 3.81 (*d*, *J* = 6.6, H$_\beta$)	64.1 (*t*)	4.69–4.77 (*m*, H$_\alpha$), 3.80 (*d*, *J* = 6.8, H$_\beta$)
Qui^2 ($1 \rightarrow 2$):				
H–C(1)	105.6 (*d*)	5.14 (*d*, *J* = 7.6)	102.5 (*d*)	4.99 (*d*, *J* = 7.6)
H–C(2)	75.6 (*d*)	3.98–4.07 (*m*)	81.1 (*d*)	4.05–4.14 (*m*)
H–C(3)	75.8 (*d*)	4.31–4.40 (*m*)	74.5 (*d*)	4.29–4.38 (*m*)
H–C(4)	86.1 (*d*)	3.69 (*t*, *J* = 9.0)	87.7 (*d*)	3.67 (*t*, *J* = 9.0)
H–C(5)	71.9 (*d*)	4.03–4.15 (*m*)	71.6 (*d*)	4.00–4.09 (*m*)
Me(6)	18.0 (*q*)	1.82 (*d*, *J* = 6.2)	18.4 (*q*)	1.85 (*d*, *J* = 6.2)
Xyl^3 ($1 \rightarrow 4$)[d]):				
H–C(1)	105.3 (*d*)	4.93 (*d*, *J* = 7.6)	104.9 (*d*)	5.05 (*d*, *J* = 7.6)
H–C(2)	73.9 (*d*)	3.76–3.84 (*m*)	73.9 (*d*)	3.86–3.95 (*m*)
H–C(3)	87.2 (*d*)	4.22–4.32 (*m*)	87.7 (*d*)	4.17–4.26 (*m*)
H–C(4)	69.0 (*d*)	4.18–4.27 (*m*)	68.9 (*d*)	4.15–4.24 (*m*)
CH$_2$(5)	66.7 (*t*)	4.33–4.42 (*m*, H$_\alpha$), 3.70–3.79 (*m*, H$_\beta$)	66.8 (*t*)	4.26–4.35 (*m*, H$_\alpha$), 3.68–3.76 (*m*, H$_\beta$)
Glu3Me^4 ($1 \rightarrow 3$):				
H–C(1)	105.7 (*d*)	5.46 (*d*, *J* = 7.9)	105.5 (*d*)	5.40 (*d*, *J* = 7.9)
H–C(2)	75.2 (*d*)	4.27–4.38 (*m*)	75.1 (*d*)	4.28–4.38 (*m*)
H–C(3)	88.1 (*d*)	3.74–3.83 (*m*)	87.9 (*d*)	3.24–3.33 (*m*)
H–C(4)	70.7 (*d*)	4.06–4.16 (*m*)	70.9 (*d*)	4.14–4.23 (*m*)
H–C(5)	78.3 (*d*)	4.04–4.13 (*m*)	77.9 (*d*)	4.08–1.17 (*m*)
CH$_2$(6)	62.1 (*t*)	4.31–4.40 (*m*, H$_\alpha$), 4.45 (*dd*, *J* = 18.0, 11.4, H$_\beta$)	62.0 (*t*)	4.19–4.28 (*m*, H$_\alpha$), 4.50 (*dd*, *J* = 17.4, 12.1, H$_\beta$)
MeO	60.61 (*q*)	3.84 (*s*)	59.6 (*q*)	3.79 (*s*)

[a]) Recorded at 150 MHz in (D$_5$)pyridine/D$_2$O 4:1. Multiplicity by DEPT. [b]) Recorded at 600 MHz in (D$_5$)pyridine/D$_2$O 4:1. [d]) In case of **2**, Xyl^3 is 4-sulfo substituted.

The sugar portion of **1** displayed ^{1}H- and ^{13}C-NMR resonances suggesting the presence of four monosacharide units (four anomeric C-atoms at δ(C) 105.3, 105.6, 105.3, and 105.7 and four anomeric protons at δ(H) 4.80 (*d*, *J* = 7.0 Hz), 5.14 (*d*, *J* = 7.6 Hz), 4.93 (*d*, *J* = 7.6 Hz), 5.46 (*d*, *J* = 7.9 Hz)). The β-D-configurations at the anomeric C-atoms were deduced from the coupling constant values (*J* = 7.1–7.8 Hz).

The ESI-MS (positive-ion mode) of **1** showed a pseudomolecular ion at *m*/*z* 1223 ($[M+Na]^+$). Fragments corresponding to the loss of the sugar moieties and SO_3Na^+ from the $[M+Na]^+$ peak were also observed at 1120 ($[M+Na-SO_3Na]^+$), 945 ($[M+Na-SO_3Na-Glc3Me+H]^+$), 814 ($[M+Na-SO_3Na-Glc3Me-Xyl+H]^+$), and 667 ($[M+Na-SO_3Na-Glc3Me-Xyl-Qui+H]^+$), showing that the 3-*O*-methyl-D-glucose must be terminal (*Fig. 1*). To check the sequence of sugars indicated by ESI-MS and determine the points of interglycosidic attachment, we used a combination of NOESY and HMBC experiments. From HMBC cross-peaks at 4.80/88.1 (H–C(1′)/C(3)), 5.14/83.8 (H–C(1″)/C(2′)), 4.93/86.1 (H–C(1‴)/C(4″)), and 5.46/87.2 (H–C(1⁗)/C(3‴)), the sequence of the sugar residues of **1** should be Glc3Me-($1 \rightarrow 3$)-Xyl-($1 \rightarrow 4$)-Qui-($1 \rightarrow 2$)-Xyl-($1 \rightarrow 3$)-aglycon.

Table 3. *^{13}C- and ^{1}H-NMR Data for the Aglycon Moieties of Desulfophilinopside A* (**1a**) *and Didesulfophilinopside B* (**2a**). δ in ppm, J in Hz.

	1a		**2a**	
	δ(C)[a])	δ(H)[b])	δ(C)[a])	δ(H)[b])
CH_2(1)	36.0 (*t*)	1.42 (br.)	36.3 (*t*)	1.45 (br.)
CH_2(2)	27.1 (*t*)	1.88–1.97 (*m*, H_α)	27.3 (*t*)	1.75–1.86 (*m*, H_α)
		2.03–2.11 (*m*, H_β)		2.07–2.15 (*m*, H_β)
H–C(3)	89.3 (*d*)	3.26 (*dd*, J = 4.2, 11.9)	89.0 (*d*)	3.29 (*d*, *J* = 8.8)
C(4)	39.3 (*s*)		39.3 (*s*)	
H–C(5)	48.5 (*d*)	0.98–1.07 (*m*)	48.1 (*d*)	0.94–2.03 (*m*)
CH_2(6)	23.0 (*t*)	1.97–2.08 (*m*)	23.3 (*t*)	1.65–1.76 (*m*)
H–C(7)	121.6 (*d*)	5.65 (*d*, *J* = 0.8)	120.3 (*d*)	5.62 (br.)
C(8)	145.2 (*s*)		144.7 (*s*)	
H–C(9)	46.6 (*d*)	3.73 (*d*, *J* = 14.0)	47.2 (*d*)	3.46 (*d*, *J* = 13.6)
C(10)	35.6 (*s*)		36.2 (*s*)	
CH_2(11)	22.5 (*t*)	1.53–1.63 (*m*, H_α)	22.6 (*t*)	1.45–1.54 (*m*, H_α)
		1.66–1.75 (*m*, H_β)		1.86–1.95 (*m*, H_β)
CH_2(12)	29.8 (*t*)	1.99–2.08 (*m*, H_α)	31.5 (*t*)	1.98–2.09 (*m*, H_α)
		2.19–2.28 (*m*, H_β)		2.11–2.19 (*m*, H_β)
C(13)	56.7 (*s*)		59.3 (*s*)	
C(14)	45.7 (*s*)		47.5 (*s*)	
CH_2(15)	52.0 (*t*)	2.62 (*d*, *J* = 9.6, H_α)	43.7 (*t*)	2.59 (*d*, *J* = 9.0, H_α)
		1.66–1.75 (*m*, H_β)		1.68–1.77 (*m*, H_β)
H–C(16)	75.6 (*d*)	5.89 (*dd*, *J* = 8.8, 9.2)	75.0 (*d*)	5.94 (*dd*, *J* = 8.4, 8.4)
H–C(17)	53.2 (*d*)	2.69 (*d*, *J* = 9.2)	52.9 (*d*)	2.63 (*d*, *J* = 8.8)
C(18)	178.5 (*s*)		179.1 (*s*)	
Me(19)	24.1 (*q*)	1.24 (*s*)	23.9 (*q*)	1.18 (*s*)
C(20)	83.3 (*s*)		84.9 (*s*)	
Me(21)	26.3 (*q*)	1.43 (*s*)	28.3 (*q*)	1.52 (*s*)
CH_2(22)	38.5 (*t*)	1.59–1.69 (*m*, H_α)	38.8 (*t*)	1.98–2.07 (*m*, H_α)
		1.76–1.85 (*m*, H_β)		2.51–2.63 (*m*, H_β)
CH_2(23)	22.5 (*t*)	1.79–1.90 (*m*, H_α)	23.8 (*t*)	1.92–2.01 (*m*, H_α)
		1.79–1.90 (*m*, H_β)		1.98–2.07 (*m*, H_β)
H–C(24)	124.2 (*d*)	5.05–5.14 (*m*)	38.0 (*t*)	1.95-2.03 (*m*)
C(25)	131.9 (*s*)		145.6 (*s*)	
Me(26)	25.5 (*q*)	1.66 (*s*)	110.5 (*t*)	4.78 (*s*)
Me(27)	17.6 (*q*)	1.10 (*s*)	22.2 (*q*)	1.69 (*s*)
Me(28)	17.3 (*q*)	1.11 (*s*)	17.3 (*q*)	1.07 (*s*)
Me(29)	28.8 (*q*)	1.28 (*s*)	28.8 (*q*)	1.19 (*s*)
Me(30)	31.9 (*q*)	1.17 (*s*)	32.2 (*q*)	1.14 (*s*)
Me*C*OC	169.8 (*s*)		169.7 (*s*)	
*Me*CO	21.4 (*q*)	1.07 (*s*)	21.0 (*q*)	2.00 (*s*)

[a]) Recorded at 100 MHz in (D_5)pyridine. Multiplicity by DEPT. [b]) Recorded at 400 MHz in (D_5)pyridine.

Philinopside B (**2**) was obtained as a colorless powder. On the basis of HR-ESI-MS, the molecular formula $C_{55}H_{84}Na_2O_{28}S_2$ was assigned to **2**. The NMR-spectral features of **2** (*Tables 5* and *2*) and of didesulfophilinopside B (**2a**) (*Tables 3* and *4*) are similar to those of **1** and **1a**, respectively, except for signals due to the side chain and sulfo group. The structure of **2** was determined by its MS and NMR experiments as

Table 4. *^{13}C- and ^{1}H-NMR Data for the Sugar Moieties of Desulfophilinopside A* (**1a**) *and Didesulfophilinopside B* (**2a**). δ in ppm, J in Hz.

	1a		**2a**	
	δ(C)[a])	δ(H)[b])	δ(C)[a])	δ(H)[b])
Xyl[1] (1 → C(3)):				
H–C(1)	105.3 (*d*)	4.73 (*d*, *J* = 7.2)	104.7 (*d*)	4.73 (*d*, *J* = 7.2)
H–C(2)	83.8 (*d*)	4.07–4.16 (*m*)	84.0 (*d*)	4.07–4.15 (*m*)
H–C(3)	78.4 (*d*)	4.05–4.13 (*m*)	78.0 (*d*)	4.05–4.13 (*m*)
H–C(4)	68.3 (*d*)	5.15–5.24 (*m*)	68.2 (*d*)	5.14–5.24 (*m*)
CH_2(5)	66.3 (*t*)	3.53–3.62 (*m*, H_α), 4.13–4.23 (*m*, H_β)	66.1 (*t*)	3.54–3.62 (*m*, H_α), 4.13–4.22 (*m*, H_β)
Qui[2] (1 → 2):				
H–C(1)	105.3 (*d*)	4.72 (*d*, *J* = 7.6)	104.9 (*d*)	4.79 (*d*, *J* = 7.2)
H–C(2)	75.2 (*d*)	3.78–3.87 (*m*)	81.1 (*d*)	3.84–3.93 (*m*)
H–C(3)	75.8 (*d*)	3.89–3.98 (*m*)	74.5 (*d*)	3.87–3.96 (*m*)
H–C(4)	87.1 (*d*)	3.60 (*t*, *J* = 9.0)	87.4 (*d*)	3.63 (*t*, *J* = 9.2)
H–C(5)	71.4 (*d*)	3.62–3.73 (*m*)	71.4 (*d*)	3.64–3.73 (*m*)
Me(6)	18.4 (*q*)	1.82 (*d*, *J* = 6.2)	19.1 (*q*)	1.68 (*d*, *J* = 6.4)
Xyl[3] (1 → 4):				
H–C(1)	105.8 (*d*)	5.17 (*d*, *J* = 7.6)	105.9 (*d*)	5.15 (*d*, *J* = 7.6)
H–C(2)	73.5 (*d*)	3.76–3.85 (*m*)	73.2 (*d*)	3.69–3.78 (*m*)
H–C(3)	87.2 (*d*)	4.02–4.11 (*m*)	87.7 (*d*)	4.08–4.16 (*m*)
H–C(4)	70.7 (*d*)	4.07–4.16 (*m*)	69.8 (*d*)	4.04–4.13 (*m*)
CH_2(5)	66.8 (*t*)	3.52–3.61 (*m*, H_α), 4.21–4.31 (*m*, H_β)	66.8 (*t*)	3.53–3.62 (*m*, H_α), 4.19–4.28 (*m*, H_β)
Glu3Me[4] (1 → 3):				
H–C(1)	105.7 (*d*)	5.06 (*d*, *J* = 7.4)	105.5 (*d*)	5.04 (*d*, *J* = 7.9)
H–C(2)	74.6 (*d*)	3.77–3.86 (*m*)	75.1 (*d*)	3.83–3.92 (*m*)
H–C(3)	87.8 (*d*)	3.63–3.72 (*m*)	87.9 (*d*)	3.62–3.72 (*m*)
H–C(4)	70.7 (*d*)	4.02–4.11 (*m*)	70.9 (*d*)	4.05–4.13 (*m*)
H–C(5)	76.3 (*d*)	3.81–3.90 (*m*)	77.9 (*d*)	3.81–3.90 (*m*)
CH_2(6)	62.0 (*t*)	4.18–4.27 (*m*, H_α), 4.31–4.39 (*m*, H_β)	62.0 (*d*)	4.19–4.28 (*m*, H_α), 4.34–4.42 (*m*, H_β)
MeO	60.3 (*q*)	3.74 (*s*)	60.8 (*q*)	3.75 (*s*)

[a]) Recorded at 100 MHz in (D_5)pyridine. Multiplicity by DEPT. [b]) Recorded at 400 MHz in (D_5)pyridine.

(3β,9β,16β)-16-(acetyloxy)-3-{[3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-2-*O*-sulfo-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-quinovopyranosyl-(1 → 2)-4-*O*-sulfo-β-D-xylopyranosyl]oxy}-18-oxo-18,20-epoxylanosta-7,25(26)-diene disodium salt (**2**).

The ^{13}C- and ^{1}H-NMR spectra of **2** showed four monosaccharide units (four anomeric C-atoms at δ(C) 104.7, 102.5, 104.9, and 105.5 and four anomeric protons at δ(H) 4.82 (*d*, *J* = 7.0 Hz), 4.99 (*d*, *J* = 7.6 Hz), 5.05 (*d*, *J* = 7.6 Hz), and 5.40 (*d*, *J* = 7.9 Hz)). The β-D-configurations at the anomeric C-atoms were deduced from the coupling constant values (*J* = 7.0–7.9 Hz). The ^{13}C-NMR spectrum of **2** showed a disubstituted terminal C=C bond at δ(C) 145.7 (C(25)) and 110.0 (C(26)). The ^{1}H-NMR spectrum of **2** also showed an olefinic methyl signal at δ(H) 1.69 (*s*, Me(27)), which was identical to that of the side chain of the aglycons of several triterpene glycosides isolated from the sea cucumbers, *Stichopus japonicus* [4], *Cucumaria japonica* [5], and *Hemoiedema spectabilis* [6]. The NOESY spectrum of **2** allowed to establish the relative configuration of all chiral centers of the aglycon. In accordance with

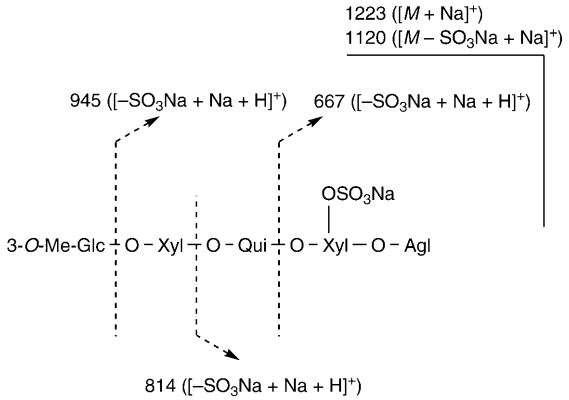


Fig. 1. *ESI-MS* (positive-ion mode) *fragmentation of philinopside A* (**1**)

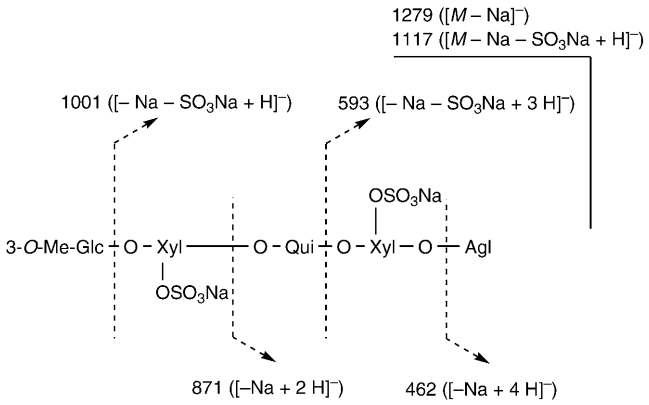


Fig. 2. *ESI-MS* (negative-ion mode) *fragmentation of philinopside B* (**2**)

the structure proposed, the ESI-MS (positive-ion mode) of **2** showed the pseudomolecular ion at *m*/*z* 1325 ($[M+Na]^+$). The negative-ion mode ESI-MS of **2** also exhibited several significant ions at *m*/*z* 1279 ($[M-Na]^-$), 1177 ($[M-Na-SO_3Na+H]^-$), 1001 ($[M-Na-SO_3Na-Glc3Me+H]^-$), 871 ($[M-Na-SO_3Na-Glc3Me-Xyl+2\,H]^-$), 593 ($[M-Na-2SO_3Na-Glc3Me-Xyl-Qui+3\,H]^-$), 462 ($[M-Na-2SO_3Na-Glc3Me-Xyl-Qui-Xyl+4\,H]^-$) (*Fig. 2*). The positions of the interglycosidic linkages in the linear oligosaccharide moieties of **2** were established by NOESY and HMBC. The positions of the two sulfato groups in **2** were ascertained in the same manner as for **1**.

Philinopside A (**1**) and B (**2**) were tested for in *vitro* cytotoxicity against ten human tumor cell lines (CAKI, HOS, KB-VIN, KB, SM-MEL-2, U87-MG, HCT-8, IA9, A549, and PC3) by using SRB methods [7] and vincristine as a positive control. The ED_{50} values are listed in *Table 6*. Compound **1** and **2** showed significant activity against all tumor cell lines. In addition, philinopside A (**1**) significantly inhibited the proliferation, migration, and tube formation of human microvascular endothelial cells (HMECs) in a dose-dependent manner, with average IC_{50} values of 1.4 ± 0.17, 0.89 ± 0.23, and 0.98 ± 0.19

Table 5. *^{1}H- and ^{13}C-NMR Data for the Aglycon Moiety of Philinopside B* (**2**). δ in ppm, J in Hz.

	δ(C)[a])	δ(H)[b])	^{1}H,^{1}H-COSY	^{1}H,^{1}H-NOESY	^{1}H,^{13}C-HMBC
CH_2(1)	35.6 (*t*)	1.44–1.57 (*m*)	CH_2(2)		
CH_2(2)	27.0 (*t*)	1.89–2.01 (*m*)	CH_2(1), H_α–C(3)	Me(19)	
H–C(3)	89.5 (*d*)	3.25 (*dd*, *J* = 4.2, 11.9)	H_α–C(2), H_β–C(2)	H–C(5), Me(29), H–C(1)	C(1)(Xyl[1])
C(4)	39.6 (*s*)				
H–C(5)	48.0 (*d*)	0.99 (*t*, *J* = 7.6)	CH_2(6)	H_α–C(3), Me(2), H–C(7)	C(4), C(28), C(29)
CH_2(6)	22.2 (*t*)	1.86–1.97 (*m*)	H–C(5), H–C(7)		
H–C(7)	120.4 (*d*)	5.60 (br. *s*)	CH_2(6)	H–C(5), H–C(17), Me(29), Me(30)	
C(8)	145.6 (*s*)				
H–C(9)	47.1 (*d*)	3.44 (*d*, *J* = 14.0)	H_α–C(11), H_β–C(11)		
C(10)	35.6 (*s*)				
CH_2(11)	22.7 (*t*)	1.51–1.59 (*m*, H_α), 1.76–1.83 (*m*, H_β)	H–C(9), CH_2(12) H–C(9), CH_2(12)		
CH_2(12)	30.4 (*t*)	2.05–2.18 (*m*)	H_α–C(11), H_β–C(11)		
C(13)	59.3 (*s*)				
C(14)	47.5 (*s*)				
CH_2(15)	43.7 (*t*)	1.68–1.75 (*m*, H_α), 2.55–2.62 (*m*, H_β)	H_α–C(15), H_α–C(16) H_β–C(15), H_α–C(16)		
H–C(16)	73.8 (*d*)	5.91 (*dd*, *J* = 9.6, 9.0)	H_α–C(15), H_β–C(15), H_α–C(17)	H–C(17), Me(30)	C(17), Me*C*O
H–C(17)	53.7 (*d*)	2.64 (*d*, *J* = 9.0)	H_α–C(16)	H–C(16), H–C(7), Me(21), Me(30)	C(13)
C(18)	179.5 (*s*)				
Me(19)	24.0 (*q*)	1.32 (*s*)		H_β–C(2), Me(28)	
C(20)	85.0 (*s*)				
Me(21)	28.2 (*q*)	1.47 (*s*)		H–C(17)	C(17), C(20), CH_2(22)
CH_2(22)	38.8 (*t*)	2.33–2.40 (*m*, H_α), 1.84–1.93 (*m*, H_β)	CH_2(23)		
CH_2(23)	22.7 (*t*)	1.51–1.63 (*m*)	CH_2(22), CH_2(24)		
CH_2(24)	38.4 (*t*)	1.98 (*t*, *J* = 7.8)	CH_2(23)		
C(25)	145.7 (*s*)				
CH_2(26)	110.0 (*t*)	4.75–4.83 (*m*)	Me(27)	Me(27), CH_2(24)	C(24), C(23)
Me(27)	22.2 (*q*)	1.69 (*s*)			
Me(28)	17.3 (*q*)	1.02 (*s*)		Me(19)	C(3), C(5), C(29)
Me(29)	28.9 (*q*)	1.21 (*s*)		H–C(3), H–C(5), H–C(7)	C(3), C(5), C(28)
Me(30)	32.4 (*q*)	0.93 (*s*)		H–C(16), H–C(17), H–C(7)	C(13), C(14)
Me*C*O	169.8 (*s*)				
*Me*CO	21.2 (*q*)	1.94 (*s*)			

[a]) Recorded at 150 MHz in (D_5)pyridine/D_2O 4:1. Multiplicity by DEPT. [b]) Recorded at 600 MHz in (D_5)pyridine/D_2O 4:1.

Table 6. ED_{50} *Values* [μg/ml] *of Philinopside A* (**1**) *and B* (**2**) *against Human Tumor Cells* in vitro

Cell line	CAK1	HOS	KB-VIN	KB	SK-MEL-2	U87-MG	HCT-8	IA9	A549	PC3
1	3.00	1.80	3.30	3.50	3.20	3.20	1.70	1.79	1.70	1.70
2	2.00	1.10	3.00	3.00	2.60	2.40	0.93	0.90	0.75	1.70

μM, respectively [8]. Based on these promising preliminary results, philinopside A (**1**) and B (**2**) merit further study as potential anticancer agents.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh) from *Qing Dao Hai Yang Chemical Group Co.* Anal. TLC: precoated silica gel *G60 F-254* plates from *Yan Tai Zi Fu Chemical Group Co*. MPLC: *Büchi* chromatography pump *B-686* equipped with a *Lobar* column (*Lichroprep RP-18*, 40–63 μm). HPLC: *Agilent-1100* system equipped with a refractive index detector; *Zobax-300-SB-C_{18}* column (250×9.4 mm i.d.). M.p.: *XT5-XMT* apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-341* polarimeter. IR Spectra: *Perkin-Elmer-683* IR spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR spectra: *Inova-600* spectrometers; at 600 (^1H) and 150 MHz (^{13}C); chemical shifts δ in ppm, coupling constants J in Hz; assignments supported by $^1H,^1H$-COSY, HMQC, HMBC, and NOESY experiments. ESI-MS and HR-EI-MS: *Micromass-Quatro* mass spectrometer. GC/MS: *Finnigan-Voyager* GC/MS spectrometer with a *HP-5* column (30 m×0.25 mm i.d.).

Animal Material. Specimens of *P. quadrangularis* were collected at different locations around the South China sea near Guangdong provice, China, in May 2000. The organism was identified by Prof. *J. R. Fang* of the Fujian Institute of Oceanic Research, China. A voucher specimen (reg. No. SA200042) is preserved at the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, China.

Extraction and Purification. The sea cucumbers (5 kg, dry weight) were defrosted and extracted twice with 70% EtOH (20 l). The combined EtOH extract was evaporated and the aq. residue dissolved in H_2O (3 l). The H_2O-soluble fraction was passed through a *DA101*-resin column (60×30 cm; Nankai University, Tianjin, P.R. China) and eluted with dist. H_2O until a negative Cl^- ion reaction was obtained, followed by elution with 95% EtOH (3 l). The combined EtOH eluate was evaporated to give a glassy material (16 g) that was subjected to CC (*Sepahedex LH-20* (3×50 cm), MeOH/ H_2O 2 : 1). The fraction containing saponins was resubjected to CC (dry column (2×50 cm) of silica gel, lower phase of $CHCl_3$/ MeOH/H_2O 7 : 3 : 1). Each subfraction containing saponins was purified by reversed-phase HPLC (*Zobax SBC-18*, 60% MeOH): pure **1** (123 mg) and **2** (63 mg).

Philinopside A (=*(3β,9β,16β)-16-(Acetyloxy)-3-{[3-*O*-methyl-β-*D*-glucopyranosyl-(1 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)*-O*-β-*D*-quinovopyranosyl-(1 → 2)-4-*O*-sulfo-β-*D*-xylopyranosyl]oxy}-18-oxo-18, 20-epoxylanosta-7,24-diene Monosodium Salt*; **1**): White amorphous powder. M.p. 222–225°. $[\alpha]_D^{20} = -16.7$ ($c = 0.5$, pyridine. IR (KBr): 3419 (OH), 2932 (CH_2, CH_3), 1747 (C=O), 1233 (SO_3Na), 1039 (–O–). ^1H- and ^{13}C-NMR. *Tables 1* and *2*. HR-ESI-MS: 1223.4896. ESI-MS (pos.): 1223 ($[M+Na]^+$), 1120 ($[M+Na-SO_3\ \ Na]^+$), 945 ($[M+Na-SO_3\ \ Na]^+$), 945 ($[M+Na+H-SO_3$-Na – Glc3Me]$^+$), 667 ($[M+Na+H-SO_3Na$ – Glc3Me-Xyl ([Qui]$^+$).

Philinopside B (=*(3β,9β,16β)-16-(Acetyloxy)-3-{[3-*O*-methyl-β-*D*-glucopyranosyl-(1 → 3)*-O*-2-*O*-sulfo-β-*D*-xylopyranosyl-(1 → 4)*-O*-β-*D*-quinovopyranosyl-(1 → 2)-4-*O*-sulfo-β-*D*-xylopyranosyl]oxy}-18-oxo-18,20-epoxylanosta-7,25(26)-diene Disodium Salt*; **2**): White amorphous powder. M.p. 218–220°.

$[\alpha]_D^{20} = -13.4$ ($c = 0.5$, pyridine). ^{1}H- and ^{13}C-NMR. *Tables 2* and *5*. HR-ESI-MS: 1325.2384. ESI-MS (pos.): 1325 ($[M+Na]^+$), 1149 ($[M+Na-Glc3Me]^+$). ESI-MS (neg.): 1279 ($[M-Na]^-$), 1177 ($[M-Na-SO_3Na+H]^-$), 1001 ($[M-Na-SO_3Na-Glc3Me]^-$), 871 ($[M-Na-SO_3Na-Glc3Me-Xyl+2H]^-$), 593 ($[M-Na-2SO_3\ Na-Glc3Me-Xyl-Qui+3H]$), 462 ($[M-Na-2SO_3\ Na-Glc3Me-Xyl-Qui-Xyl+4H]$).

Acid Hydrolyzation of Philinopside A (**1**) *and Philinopside B* (**2**). Glycoside **1** or **2** (5 mg) was heated in a screwcap vial with 2N HCl (5 ml) at 120° for 1 h. The aglycon was extracted with CH_2Cl_2, and the aq. residue was evaporated. Each sugar mixture was treated with pyridine (1 ml) and $NH_2OH \cdot HCl$ (2 mg) at 100° for 1 h. The mixture was cooled and peracetylated with Ac_2O (1 ml) at 100° for 1 h. The resulting aldononitrile peracetate was evaporated and the residue submitted to GC/MS analysis, with aldononitrile peracetates of standard 3-*O*-methyl-D-glucose, D-xylose, and D-quinovose as reference samples. Both **1** and **2** gave peaks of aldononitrile peracetates of the above three standard sugars in a ratio 1:2:1.

Desulfation of Philinopside A (**1**) *and Philinopside B* (**2**). Glycoside **1** or **2** (20 mg) was dissolved in pyridine/dioxane 1:1 (5.0 ml) and heated under reflux for 4 h. The mixture was partitioned between H_2O and BuOH. The BuOH extract was evaporated and the residue purified by reversed-phase HPLC (*Zobax-300-SB-C_{18}*, 80% MeOH/H_2O): pure **1a** (8 mg) or **2a** (7.5 mg).

Desulfophilinopside A (**1a**): White amorphous powder. M.p. 231–232°. $[\alpha]_D^{20} = -31.8$ ($c = 0.5$, pyridine). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS (pos.): 1121 (100, $[M+Na]^+$). ESI-MS (pos.): 1097 ($[M-H]^-$.

Didesulfophilinopside B (**2a**): White amorphous powder. M.p. 222–225°. $[\alpha]_D^{20} = -26.7$ ($c = 0.5$, pyridine). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS (pos.): 1121 (100, $[M+Na]^+$).

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