Intercedensides D−I, Cytotoxic Triterpene Glycosides from the Sea Cucumber *Mensamaria intercedens* Lampert

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Six new triterpene glycosides, intercedensides D−I (**1**−**6**), were isolated from the whole bodies of the sea cucumber *Mensamria intercedens* Lampert, which is found in the South China Sea. Their structures were elucidated by extensive spectroscopic analysis (NMR and ESIMS) and chemical methods. lntercedensides D (**1**), E (**2**), G (**4**), and H (**5**) have a conjugated double bond system (22*Z*,24-diene) in the aglycon side chain, while intercedensides F (**3**) and I (**6**) have only a single double bond (24, 25) in this same chain. lntercedensides D−H (**1**−**5**) showed significant cytotoxicity (ED_{50} 0.96−5.0 μg/mL) against 10 human tumor cell lines.

Holothurians (sea cucumbers) are a rich source of triterpene glycosides, the main secondary metabolites. The triterpene is usually of the lanosterol-type with a 18(20)-lactone. A sugar chain of up to six monosaccharide units is generally linked to the C-3 of the aglycon. Such compounds have been associated with antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory properties.[2]

Mensamaria intercedens Lampert is a Cucumariidae-type sea cucumber, which is found extensively in the South China Sea, particularly in Taiwan Strait, Zhaoan Gulf, and Dongshan Gulf, Fujian Province, People's Republic of China.[3] After a report of the deforming effect with *M. intercedens* against *Pyricularia oryzae* P-2b,[4] we previously investigated an ethanolic extract from this species and reported the isolation of three new cytotoxic sulfated triterpene glycosides, intercedensides A−C.[5] In this paper, we report our continued study of *M. intercedens* and the isolation, purification, and structural elucidation of six new sulfated triterpene glycosides, intercedensides D−I (**1**−**6**), and their in vitro cytotoxicity activities against 10 human tumor cell lines.

Results and Discussion

The 85% ethanolic extract of the whole bodies of *M. intercedens* Lampert was successively chromatographed on DA-101 resin (Nankai University, Tianjin, P. R. China), silica gel, and reversed-phase silica (Lichroprep RP-18, 40−63 μm). Finally, reversed-phase HPLC on Zobax SB C-18 afforded intercedenside D (**1**), intercedenside E (**2**), intercedenside F (**3**), intercedenside G (**4**), intercedenside H (**5**), and intercedenside I (**6**).

lntercedenside D (**1**) was obtained as a colorless amorphous powder. Its molecular formula was determined as $C_{55}H_{83}O_{27}SNa$ from pseudomolecular ion peaks at *m/z* 1253.4613 $[M + Na]^+$ in positive-ion mode HRESIMS and at *m/z* 1207 $[M - Na]^-$ in negative-ion mode ESIMS. A positive-ion fragment peak at *m/z* 1133 $[M - OSO_3Na - H + Na]^+$ indicated the presence of a sulfate group in the glycoside. The IR spectrum showed the presence of hydroxyl (3436 cm^{-1}), carbonyl (1745 cm^{-1}), olefinic (1653 cm^{-1}), and sulfate (1235, 1068 cm^{-1}) groups.

The ^1H and ^{13}C NMR spectral data of **1** (Table 1) suggested the presence of a triterpenoid aglycon with three olefinic bonds, one ester, and one lactone carbonyl group bonded to an oligosaccharide chain composed of four sugar units. Resonances for a 7(8)-double bond [δ_C 147.5 (C-8) and 119.6 (C-7); δ_H 5.59 (1H, bs, H-7)] and for an acetoxy group [δ_C 170.6 and 21.0; δ_H 1.93 (3H, s)] were present. The acetoxy group was located at C-16 on the basis of a cross-peak at δ 6.07/170.6 (H-16/CH_3CO) in the HMBC spectrum. The TOCSY spectrum of **1** indicated that three olefinic protons [δ_H 5.80 (1H, d, $J = 12$ Hz, H-22), 6.08 (1H, t, $J = 12$ Hz, H-23), 6.73 (1H, d, $J = 12$ Hz, H-24)] comprised a three-spin system; correspondingly, a conjugated double bond system (22*Z*,24-diene) should be present in the aglycon side chain. The *Z* stereochemistry of the Δ^{22} double bond was deduced from the coupling constant between H-22 and H-23 ($J = 12$ Hz),[6] compared with the analogous coupling constant of the related intercedenside C ($J = 16$ Hz),[5] which was assigned as an *E* double bond. [Intercedenside A was also assigned with a 22*E* double bond in a prior paper.[5] However, in light of its smaller coupling constant ($J = 12$ Hz), the stereochemistry likely should be revised to 22*Z*.] This conclusion was also confirmed by the cross-peaks at δ_H 5.80/86.0 (H-22/C-20), 6.08/86.0 (H-23/C-20), 5.80/122.0 (H-22/C-24), 6.08/135.4 (H-23/C-25), 6.73/17.2 (H-24/C-27), and 6.73/26.0 (H-24/C-26) in the HMBC spectrum and the cross-peak at δ_H 6.08/5.80 (H-23/H-22) in the NOESY spectrum.

The identities of the sugar moieties and position of the sulfate group were elucidated from extensive analysis of the NMR data (^{13}C, ^1H, DQF-COSY, TOCSY, HMBC, and

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Table 1. ^{13}C and ^{1}H NMR Chemical Shifts for the Aglycon Moieties of Intercedenside D (**1**) and Intercedenside E (**2**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

	1		**2**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	35.2	1.25 (1H, m, α) 1.33 (1H, m, β)	35.4	1.28 (1H, m, α) 1.37 (1H, m, β)
2	26.7	1.82 (1H, m, β) 1.97 (1H,m, α)	26.6	1.83 (1H, m, β) 1.97 (1H, m, α)
3	88.9	3.15 (1H, dd, 3.6, 12)	88.8	3.18 (1H, dd, 4.2, 12)
4	39.1		39.2	
5	47.8	0.84 (1H, m)	47.9	0.90 (1H, m)
6	22.8	1.86 (2H, m)	22.9	1.89 (2H, m)
7	119.6	5.59 (1H, bs)	119.7	5.61 (1H, bs)
8	147.5		147.6	
9	47.5	3.34 (1H, d, 9.6)	47.7	3.38 (1H, d, 14.4)
10	35.7		35.9	
11	22.3	1.49 (1H, m) 1.77 (1H, m)	22.4	1.45 (1H, m) 1.79 (1H, m)
12	25.4	1.98 (1H, m) 2.64 (1H, m)	25.5	1.99 (1H, m) 2.65 (1H, m)
13	58.0		58.2	
14	48.4		48.6	
15	43.3	1.69 (1H, m, β) 2.53 (1H, dd, α, 4.8, 8.4)	43.4	1.70 (1H, m, β) 2.55 (1H, dd, α, 4.8, 8.4)
16	82.8	6.07 (1H, m)	82.8	6.09 (1H, m)
17	87.4		87.5	
18	178.4		178.5	
19	23.8	1.07 (3H, s)	24.0	1.10 (3H, s)
20	86.0		86.2	
21	26.5	1.74 (3H, s)	26.8	1.75 (3H, s)
22	128.3	5.80 (1H, d, 12)	128.5	5.83 (1H, d, 12)
23	121.0	6.08 (1H, t, 12)	121.1	6.10 (1H, t, 12)
24	122.0	6.73 (1H, d, 12)	122.1	6.77 (1H, d, 12)
25	135.4		136.1	
26	26.0	1.58 (3H, s)	26.1	1.59 (3H, s)
27	17.2	1.57 (3H, s)	17.3	1.53 (3H, s)
30	17.0	0.98 (3H, s)	16.8	1.0 (3H, s)
31	28.4	1.10 (3H, s)	28.3	1.15 (3H, s)
32	30.9	1.44 (3H, s)	31.0	1.48 (3H, s)
CH_3COO	170.6		170.5	
CH_3COO	21.0	1.94 (3H, s)	21.1	1.93 (3H, s)

Table 2. ^{13}C and ^{1}H NMR Chemical Shifts for the Sugar Moieties of Intercedenside D (**1**) and Intercedenside E (**2**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

	1		**2**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
	Xyl (1 → C-3)		Xyl(1 → C-3)	
	104.7	4.66 (1H, d, 7.2)	104.8	4.60 (1H, d, 7.2)
	81.1	4.12 (1H, m)	82.3	4.00 (1H, m)
	74.9	4.25 (1H, m)	75.4	4.26 (1H, m)
	75.9	5.01 (1H, m)	75.9	5.06 (1H, m)
	63.8	3.72 (1H, m) 4.75 (1H, m)	64.1	3.71 (1H, m) 4.79 (1H, m)
	Glc (1 → 2Xyl)		Xyl_2 (1 → $2Xyl_1$)	
	104.3	5.13 (1H, d, 7.2)	105.7	5.02 (1H, d, 7.2)
	75.6	3.88 (1H, m)	74.7	3.88 (1H, m)
	71.8	4.04 (1H, m)	74.9	4.03 (1H, m)
	80.0	4.08 (1H, m)	77.0	4.11 (1H, m)
	76.0	3.69 (1H, m)	64.2	3.50 (1H, m) 4.34 (1H, m)
	60.7	4.26 (1H, m) 4.31 (1H, m)		
	Xyl_2 (1 → 4Glc)		Xyl_3 (1 → $4Xyl_2$)	
	104.1	4.91 (1H, d, 7.8)	102.8	4.76 (1H, d, 7.2)
	73.2	3.86 (1H, m)	72.6	3.90 (1H, m)
	86.4	4.06 (1H, m)	86.4	4.09 (1H, m)
	68.6	3.93 (1H, m)	68.8	3.97 (1H, m)
	65.8	3.53 (1H, m) 4.05 (1H, m)	68.0	3.53 (1H, m), 4.14 (1H, m)
	MeGlu (1 → 3 Xyl_2)		MeGlu (1 → 3 Xyl_3)	
	1 04.4	5.15 (1H, d, 7.2)	104.5	5.22 (1H, d, 7.8)
	74.4	3.84 (1H, m)	75.6	3.95 (1H, m)
	86.9	3.64 (1H, m)	87.2	3.66 (1H, m)
	70.2	3.87 (1H, m)	70.4	3.94 (1H, m)
	77.4	3.91 (1H, m)	77.7	3.87 (1H, m)
	61.6	4.02 (1H, m) 4.36 (1H, m)	61.8	4.06 (1H, m) 4.40 (1H, m)
OCH_3	60.4	3.78 (3H, s)	60.6	3.79 (3H, s)

NOESY′ HMQC) of the carbohydrate chain. The presence of β-monosaccharide units was deduced from the ^{1}H and ^{13}C spectra, which showed four anomeric carbon and four anomeric proton resonances with coupling constants (J values) of 7.2−7.8 Hz (Table 2). The presence of xylose, glucose, and 3-*O*-methylglucose in a 2:1:1 ratio was confirmed by acidic hydrolysis with aqueous 15% HCl followed by GC-MS analysis of the corresponding aldononitrile peracetates. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of DQF-COSY, TOCSY, HMQC, and HMBC experiments. The monosaccharide sequence was determined by careful analysis of HMBC correlations. Cross-peaks at δ_H 4.66/88.9 (H-1′/C-3), 5.13/81.1 (H-1″/C-2′), 4.91/80.0 (H-1‴/C-4″), and 5.15/86.4 (H-1⁗/C-3‴) indicated the following sequence of sugar residues: 3-*O*-methyl-glc(l → 3)-xyl(l → 4)-glc(1 → 2)-xyl(1 → 3)-aglycon. This conclusion was confirmed by fragment ion peaks at 1061 [M − *O* − 3-OMe − Glc + Na]$^+$, 945 [M − 3-OMe − Glc − Xyl + Na]$^+$, and 783 [M − 3-OMe − Glc − Xyl − Glc + Na]$^+$ in the positive-ion mode ESIMS, corresponding to the sequential loss of 3-*O*-methylglucosyl, xylosyl, and glucosyl units, respectively.

The site of the sulfate linkage was determined by comparing the ^{13}C NMR data of compound **1** with those of known glycosides.[7] A downfield esterification shift was observed for the C-4′ signal (xyl$_1$, from δ 68.2 to 75.9 ppm). Therefore, the structure of compound **1** was deduced as 16β-acetoxy-3-*O*-{3′-*O*-methyl-β-D-glucopyranosyl(1→ 3)-β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}holosta-7,22*Z*,24-triene-3β,7α-diol sodium salt.

lntercedenside E (**2**) was obtained as a colorless amorphous powder. Its molecular formula was determined as $C_{54}H_{81}O_{26}SNa$ from pseudomolecular ion peaks at *m/z* 1223 [M + Na]$^+$ in positive-ion mode ESIMS and at *m/z* 1177 [M − Na]$^-$ in negative-ion mode ESIMS. A fragment ion peak at *m/z* 1103 [M − OSO$_3$Na − H + Na]$^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate groups in the glycoside. The IR spectrum showed the presence of hydroxyl (3442 cm^{-1}), carbonyl (1734 cm^{-1}), olefinic (1659 cm^{-1}), and sulfate (1237, 1071 cm^{-1}) groups.

On the basis of its HMQC, DFF-COSY, and TOCSY NMR spectra, all signals of compound **2** were assigned as shown in Tables 1 and 2. From a NMR data comparison, compounds **2** and **1** have similar aglycon moieties.

The identities of the sugar moieties and position of the sulfate group were elucidated from extensive analysis of the NMR data (^{13}C, ^{1}H, DQF-COSY, TOCSY, HMBC, HMQC, and NOESY) of the carbohydrate chain. In particular, the presence of four β-monosaccharide units was deduced from the ^{1}H and ^{13}C spectra, which showed four anomeric carbon and four anomeric proton resonances with coupling constants (J values) of 7.2−7.8 Hz (Table 2). The presence of xylose and 3-*O*-methylglucose in a 3:1 ratio was confirmed by acidic hydrolysis with aqueous 15% HCl followed by GC-MS analysis of the corresponding aldononitrile peracetates. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the DQF-COSY, TOCSY, HMQC, and HMBC experiments. The monosaccharide sequence was determined by careful analysis of HMBC correlations. Cross-peaks at δ 4.60/88.8 (H-1′/C-3), 5.02/82.3 (H-1″/C-2′), 4.76/77.0 (H-1‴/C-4″), and 5.22/86.4 (H-1⁗/C-3‴) indicated the following sequence of sugar residues: 3-*O*-methyl-glc(1→3)-xyl(1→4)-xyl(1→2)-xyl(1→3)-aglycon. This conclusion was confirmed by fragment ion peaks 1031 (M − *O* − 3-OMe − Glc + Na]$^+$, 915 [M − 3-OMe − Glc − Xyl + Na]$^+$, and 783 [M − 3-OMe − Glc − Xyl − Xyl + Na]$^+$ in the positive-ion mode ESIMS, corresponding to the sequential loss of 3-*O*-methylglucosyl, xylosyl, and xylosyl units, respectively.

The site of the sulfate linkage was determined by comparing ^{13}C NMR data of compound **2** with those of known glycosides.[7] A downfield esterification shift was observed for the C-4′ signal (xyl$_1$, from δ 68.2 to 75.9 ppm). Therefore, the structure of compound **2** was deduced as 16β-acetoxy-3-*O*-{3′-*O*-methyl-β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}holosta-7,22*Z*,24-triene-3β,17α-diol sodium salt.

lntercedenside F (**3**) was obtained as a colorless amorphous powder. The molecular of intercedenside F (**3**) formula was determined as $C_{55}H_{85}O_{27}SNa$ from pseudomolecular ion peaks at *m/z* 1255.4790 [M + Na]$^+$ in positive-ion mode HRESIMS and at *m/z* 1209 [M − Na]$^-$ in negative-ion mode ESIMS. Fragment ion peaks at *m/z* 1135 [M − OSO$_3$Na − H + Na]$^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate group in the glycoside. The IR spectrum showed the presence of hydroxyl (3437 cm^{-1}), carbonyl (1731 cm^{-1}), olefinic (1660 cm^{-1}), and sulfate groups (1241, 1069 cm^{-1}).

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts for the Aglycon Moieties of Intercedenside F (**3**) and Intercedenside I (**6**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

	3		**6**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	35.7	1.25 (1H, m, α) 1.35 (1H, m, β)	35.6	1.23 (1H, m, α) 1.38 (1H, m, β)
2	26.8	1.80 (1H, m, β) 1.94 (1H, m, α)	26.6	1.83 (1H, m, β) 1.97 (1H, m, α)
3	88.8	3.16 (1H, m)	88.8	3.14 (1H, m)
4	39.2		39.0	
5	47.3	0.86 (1H, m)	47.5	0.85 (1H, m)
6	22.9	1.85 (2H, m)	23.0	1.82 (2H, m)
7	119.4	5.64 (1H, bs)	119.6	5.63 (1H, bs)
8	147.8		145.2	
9	47.1	3.37 (1H, d, 14.4)	47.3	3.28 (1H, d, 13.8)
10	35.3		35.5	
11	22.3	1.45 (1H, m) 1.75 (1H, m)	22.4	1.43 (1H, m) 1.76 (1H, m)
12	25.8	1.92 (1H, m) 2.60 (1H, m)	25.9	1.90 (1H, m) 2.48 (1H, m)
13	59.6		58.8	
14	48.7		48.6	
15	43.4	1.71 (1H, m, β) 2.63 (1H,dd, α, 4.8, 8.4)	43.2	1.72 (1H,m, β) 2.64 (1H,dd, m, α,)
16	85.2	6.16 (1H, m)	85.2	6.16 (1H, m)
17	87.2		87.0	
18	178.4		179.4	
19	23.9	1.04 (3H, s)	23.6	1.05 (3H, s)
20	87.0		86.8	
21	25.4	1.68 (3H, s)	26.3	1.69 (3H, s)
22	37.4	2.03 (1H, m) 2.50 (1H, m)	37.6	2.01 (1H, m) 2.49 (1H, m)
23	23.6	2.20 (1H, m) 2.28 (1H, m)	23.4	2.18 (1H, m) 2.30 (1H, m)
24	124.5	5.04 (1H, m)	123.9	5.08 (1H, m)
25	131.6		132.0	
26	25.3	1.55 (3H, s)	25.2	1.54 (3H, s)
27	17.4	1.43 (3H, s)	17.3	1.46 (3H, s)
30	17.1	0.98 (3H, s)	17.0	1.0 (3H, s)
31	28.5	1.07 (3H, s)	28.4	1.12 (3H, s)
32	30.4	1.45 (3H, s)	30.8	1.43 (3H, s)
CH_3*C*OO	169.9		169.7	
*CH_3*COO	20.8	1.95 (3H, s)	21.1	1.94 (3H, s)

Table 4. ^{13}C and ^{1}H NMR Chemical Shifts for the Sugar Moieties of Intercedenside F (**3**) and Intercedenside I (**6**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

	3		**6**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
	Xyl (1 → C-3)		Xyl (1 → C-3)	
	104.8	4.65 (1H, d, 7.2)	105.1	4.73 (1H, d, 7.6)
	81.4	4.13 (1H, m)	83.9	3.98 (1H, m)
	75.1	4.26 (1H, m)	75.1	4.26 (1H, m)
	75.9	5.05 (1H, m)	75.9	5.13 (1H, m)
	63.9	3.72 (1H, m)	64.1	3.69 (1H, m)
		4.76 (1H, m)		4.78 (1H, m)
	Glc (1 → 2Xyl)		Qui (1 → $2Xyl_1$)	
	104.5	5.14 (1H, d, 7.2)	104.9	4.71 (1H, d, 7.2)
	75.8	3.95 (1H, m)	76.2	3.87 (1H, m)
	71.4	4.04 (1H, m)	75.2	4.22 (1H, m)
	80.1	4.12 (1H, m)	86.1	3.60 (1H, m)
	76.1	3.69 (1H, m)	71.4	4.10 (1H, m)
	60.8	4.28 (1H, m)	19.1	1.68 (1H, m)
		4.37 (1H, m)		
	Xyl_2 (1 → 4Glc)		Xyl_2 (1 → 4Qui)	
	104.2	4.95 (1H, d, 7.8)	105.4	5.08 (1H, d, 7.2)
	73.3	3.90 (1H, m)	73.1	4.02 (1H, m)
	86.4	4.07 (1H, m)	87.3	4.16 (1H, m)
	68.6	3.93 (1H, m)	70.1	4.11 (1H, m)
	66.0	3.53 (1H, m)	66.8	3.57 (1H, m),
		4.08 (1H, m)		4.23 (1H, m)
	MeGlu (1 → 3 Xyl_2)		MeGlu (1 → 3 Xyl_2)	
	104.6	5.18 (1H, d, 7.2)	105.5	5.30 (1H, d, 7.8)
	74.5	3.86 (1H, m)	74.8	3.93 (1H, m)
	86.6	3.65 (1H, m)	87.8	3.67 (1H, m)
	70.3	3.92 (1H, m)	70.8	4.04 (1H, m)
	77.6	3.88 (1H, m)	78.3	3.93 (1H, m)
	61.7	4.05 (1H, m)	61.9	4.24 (1H, m)
		4.40 (1H, m)		4.46 (1H, m)
O*CH*$_3$	60.5	3.78 (3H, s)	60.3	3.79 (3H, s)

The NMR data of **3** (Table 3) were quite comparable to those of **1**, except for the presence of only two rather than three olefinic bonds; thus, the aglycon moiety of compound **3** was identified as 16β-acetoxyholosta-7,24-diene-3β,17α-diol.

The four β-monosaccharide units of compound **3** were identified as xylose, glucose, and 3-*O*-methylglucose in a 2:1:1 ratio based on the ^{1}H and ^{13}C spectra, which showed four anomeric carbon and four anomeric proton resonances with coupling constants (*J* values) of 7.2−7.8 Hz (Table 4) and by acidic hydrolysis with aqueous 15% HCl followed by GC-MS analysis of the corresponding aldononitrile peracetates. The sequence of the sugar residues [3-*O*-methyl-glc(1→3)-xyl(1→4)-glc(1→2)-xyl(1→3)-aglycon] in compound **3** was determined by careful analysis of the HMBC cross-peaks, δ 4.65/88.8 (H-l′/C-3), 5.14/81.4 (H-l″/C-2′), 4.95/80.1 (H-1‴/C-4″), and 5.18/86.4 (H-1⁗/C-3‴). This conclusion was confirmed by fragment ion peaks at 1064 $[M - O - 3\text{-OMe} - Glc + H + Na]^+$, 959 $[M - OSO_3Na - H - 3\text{-OMe} - Glc + Na]^+$, 947 $[M - 3\text{-OMe} - Glc - Xyl + Na]^+$, and 785 $[M - 3\text{-OMe} - Glc - Xyl - Glc + Na]^+$ in the positive-ion mode ESIMS, corresponding to the sequential losses of 3-*O*-methylglucosyl, xylosyl, and glucosyl units, respectively.

Comparison of the ^{13}C NMR data of compound **3** with those of known related glycosides[6] showed that the carbon signal at C-4′ (xyl_1) had shifted downfield from δ 68.1 to 75.9, consistent with esterification by the sulfate groups. Therefore, the structure of compound **3** was deduced as 16β-acetoxy-3-*O*-{3′-*O*-methyl-β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}holosta-7,24-diene-3β,17α-diol sodium salt.

lntercedenside G (**4**) was obtained as a colorless amorphous powder. Its molecular formula was determined as $C_{54}H_{81}O_{25}SNa$ from pseudomolecular ion peaks at *m/z* 1207.4526 $[M + Na]^+$ in positive-ion mode HRESIMS and at *m/z* 1161 $[M - Na]^-$ in negative-ion mode ESIMS. A fragment ion peak at *m/z* 1087 $[M - OSO_3Na - H + Na]^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate groups in the glycoside. The IR spectrum showed the presence of hydroxyl (3443 cm^{-1}), carbonyl (1729 cm^{-1}), olefinic (1665 cm^{-1}), and sulfate (1242, 1071 cm^{-1}) groups.

On the basis of HMQC, TOCSY, DQFCOSY, and HMBC spectra, all ^{1}H and ^{13}C signals of compound **4** were assigned as shown in Tables 5 and 6. A NMR spectral comparison of **4** with **2** showed that the two compounds differed structurally at the C-17 position. In the ^{13}C NMR spectrum of **4**, the C-16 and C-17 signals were shifted upfield by 10 and 30 ppm, respectively, compared to those of **2**, indicating that the absence of a hydroxy group at C-17. Thus, the aglycon moiety of compound **4** was identified as 16β-acetoxyholosta-7,22Z,24-trien-3β-ol.

However, NMR data comparison showed the **4** and **2** have the same sugar moieties. The 3:1 ratio of xylose and 3-*O*-methylglucose was confirmed by acidic hydrolysis with aqueous 15% HCl followed by GC-MS analysis of the corresponding aldononitrile peracetates. The monosaccharide sequence was determined by careful analysis of HMBC correlations. Cross-peaks at δ 4.66/88.8 (H-1′/C-3), 5.01/82.4 (H-1″/C-2′), 4.76/77.0 (H-1‴/C-4″), and 5.22/86.2 (H-1⁗/C-3‴) indicated the following sequence of sugar residues: 3-*O*-methyl-glc(1→3)-xyl(1→4)-xyl(1→2)-xyl(1→3)-aglycon. Therefore, the structure of compound **4** was deduced as 16β-acetoxy-3-*O*-{3′-*O*-methyl-β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}holosta-7,22*Z*,24-trien-3β-ol sodium salt.

lntercedenside H (**5**) was obtained as a colorless amorphous powder. Its molecular formula was determined as

Table 5. ^{13}C and ^{1}H NMR Chemical Shifts for the Aglycon Moieties of Intercedenside G (**4**) and Intercedenside H (**5**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

	4		**5**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	35.9	1.33 (2H, m)	35.3	1.26 (1H, m, α) 1.34 (1H, m, β)
2	26.7	1.80 (1H, m, β) 1.99 (1H, m, α)	26.5	1.80 (1H,m, β) 1.98 (1H, m, α)
3	88.8	3.19 (1H, m)	88.3	3.20 (1H, dd, 4.2, 12)
4	39.2		39.5	
5	47.6	0.91 (1H, dd, 4.2, 10.2)	47.9	0.93 (1H, m)
6	23.0	1.95 (2H, m)	23.0	1.91 (2H, m)
7	120.2	5.57 (1H, bs)	120.1	5.62 (1H, bs)
8	145.6		147.9	
9	47.2	3.33 (1H, d, 13.8)	47.5	3.37 (1H, d, 13.8)
10	35.3		35.6	
11	22.3	1.44 (1H, m, α) 1.76 (1H, m, β)	22.5	1.44 (1H, m, α) 1.72 (1H, m, β)
12	25.7	1.91 (1H, m) 2.67 (1H, m)	25.7	1.98 (1H, m) 2.65 (1H, m)
13	58.3		58.5	
14	47.9		48.3	
15	43.8	1.59 (1H, dd, β, 7.2, 12) 2.42 (1H, dd, α, 7.8, 12)	43.6	1.72 (1H, m, β) 2.53 (1H, dd, α, 4.8, 8.4)
16	72.7	5.91 (1H, m)	82.8	6.10 (1H, m)
17	57.2	3.12 (1H, d, 8.4)	87.6	
18	179.5		179.0	
19	23.7	0.90 (3H, s)	24.1	1.2 (3H, s)
20	84.0		85.9	
21	28.9	1.60 (3H, s)	27.0	1.79 (3H, s)
22	131.7	5.69 (1H, m)	128.6	5.77 (1H, d, 12)
23	120.3	6.03 (1H, m)	121.3	6.20 (1H, t, 12)
24	121.0	6.42 (1H, m)	122.5	6.69 (1H, d, 12)
25	137.0		136.4	
26	26.0	1.66 (3H, s)	26.2	1.60 (3H, s)
27	17.5	1.61 (3H, s)	17.7	1.57 (3H, s)
30	16.8	1.00 (3H, s)	17.0	1.08 (3H, s)
31	28.2	1.16 (3H, s)	28.3	1.15 (3H, s)
32	32.4	1.08 (3H, s)	31.5	1.49 (3H, s)
CH_3*C*OO	170.2		170.1	
*CH_3*COO	21.2	1.92 (3H, s)	21.1	1.94 (3H, s)

$C_{55}H_{83}O_{26}SNa$ from pseudomolecular ion peaks at *m/z* 1237 $[M + Na]^+$ in positive-ion mode ESIMS and at *m/z* 1191 $[M - Na]^-$ in negative-ion mode ESIMS. A fragment ion peak at *m/z* 1117 $[M - OSO_3Na - H + Na]^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate group in the glycoside. The IR spectrum showed the presence of hydroxyl (3437 cm^{-1}), carbonyl (1732 cm^{-1}), olefinic (1671 cm^{-1}), and sulfate (1245, 1069 cm^{-1}) groups.

On the basis of HMQC, TOCSY, DQF-COSY, and HMBC spectra, all ^1H and ^{13}C signals were assigned as shown in Table 5 and Table 6. A NMR spectral comparison of **5** with **1** showed that these compounds have similar aglycons. The presence of four β-monosaccharide units in compound **5** was deduced from the ^1H and ^{13}C spectra, which showed four anomeric carbon and four anomeric proton resonances with coupling constants (*J* values) of 6.6−7.8 Hz (Table 6). The presence of xylose, quinovose, and 3-*O*-methylglucose in a 2:1:1 ratio was confirmed by acidic hydrolysis with aqueous 15% HCl followed by GC-MS analysis of the corresponding aldononitrile peracetates. The monosaccharide sequence was determined by careful analysis of HMBC correlations. Cross-peaks at δ 4.70/88.3 (H−l′/C-3), 4.71/83.2 (H-1″/C-2′), 4.84/86.0 (H-1‴/C-4″), and 5.32/87.2 (H-1⁗/C-3‴) indicated the following sequence of sugar residues: 3-*O*-methyl-glc(1→3)-xyl(1→4)-qui(1→2)-xyl(1→3)-aglycon. This conclusion was confirmed by the following fragment MS ion peaks: 1045 $[M - O - 3\text{-OMe} - Glc + Na]^+$, 942 $[M - OSO_3Na\ 3\text{-OMe}\ Glc + Na]^+$, and 809 $[M - OSO_3Na - 3\text{-OMe} - Glc - Xyl - H + Na]^+$ in the positive-ion mode ESIMS.

The site of the sulfate linkage was determined by comparing the ^{13}C NMR data of compound **5** with those of known glycosides.[7] A downfield esterification shift was observed for the C-4′ signal (xyl_1, from δ 68.2 to 75.8 ppm). Therefore, the structure of compound **5** was deduced as 16β-acetoxy-3-*O*-{3′-*O*-methyl-β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-β-D-quinovopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}holosta-7,22*Z*,24-triene-3β,17α-diol sodium salt.

Intercedenside I (**6**) was obtained as a colorless amorphous powder. The molecular formula of intercedenside I (**6**) was determined as $C_{55}H_{85}O_{26}SNa$ from pseudomolecular ion peaks at *m/z* 1239 $[M + Na]^+$ in positive-ion mode ESIMS and at *m/z* 1193 $[M - Na]^-$ in negative-ion mode ESIMS. Fragment ion peaks at *m/z* 1119 $[M - OSO_3Na - H + Na]^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate group in the glycoside. The IR spectrum showed the presence of hydroxyl (3436 cm^{-1}), carbonyl (1739 cm^{-1}), olefinic (1678 cm^{-1}), and sulfate groups (1240, 1072 cm^{-1}).

NMR spectral comparisons of **6** with **3** and **5** (Table 3) showed that **6** and **3** have similar aglycons, and **6** and **5** (Table 4 and Table 6) have the same sugar moieties. These conclusions were confirmed by fragment ion peaks at 1047 $[M - O\text{-}3\text{-OMe} - Glc + Na]^+$, 943 $[M - OSO_3Na - 3\text{-OMe} - Glc - H + Na]^+$, 785 $[M - 3\text{-OMe} - Glc - Xyl - Qui + Na]^+$, 665 $[M - OSO_3Na - 3\text{-OMe} - Glc - Xyl - Qui - H + Na]^+$, 533 $[M - OSO_3Na - 3\text{-OMe} - Glc - Xyl - Qui - Xyl - H + Na]^+$ in the positive-ion mode ESIMS. Therefore, the structure of compound **6** was deduced as 16β-acetoxy-

Table 6. ^{13}C and ^{1}H NMR Chemical Shifts for the Sugar Moieties of Intercedenside G (**4**) and Intercedenside H (**5**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

position	4		5	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
	Xyl(1 → C-3)		Xyl(1 → C-3)	
	104.7	4.66 (1H, d, 7.2)	105.3	4.70 (1H, d, 7.2)
	82.4	4.01 (1H, m)	83.2	3.97 (1H, m)
	75.3	4.24 (1H, m)	75.2	4.26 (1H, m)
	75.8	5.07 (1H, m)	75.8	5.10 (1H, m)
	64.1	3.73 (1H, m)	64.0	3.60 (1H, m)
		4.79 (1H, m)		4.74 (1H,m)
	Glc (1 → 2Xyl)		Qui (1 → $2Xyl_1$)	
	105.8	5.01 (1H, d, 7.2)	105.2	4.71 (1H, d, 6.6)
	74.7	3.89 (1H, m)	76.1	3.91 (1H, m)
	74.9	4.05 (1H, m)	86.0	4.12 (1H, m)
	77.0	4.13 (1H, m)	71.8	3.59 (1H, m)
	64.2	3.49 (1H, m)	18.0	1.64 (3H, d, 6.2)
		4.31 (1H, m)		
	Xyl_2 (1 → 4 Xyl_2)		Xyl_2 (1 → 4Qui)	
	104.2	4.76 (1H, d, 7.8)	105.0	4.84 (1H, d, 7.8)
	72.6	3.90 (1H, m)	73.6	4.01 (1H, m)
	86.2	4.09 (1H, m)	87.2	4.18 (1H, m)
	68.8	3.97 (1H, m)	78.8	4.12 (1H, m)
	66.0	3.54 (1H, m)	66.6	3.56 (1H, m),
		4.12 (1H, m)		4.27 (1H, m)
	MeGlu (1 → 3 Xyl_3)		MeGlu (1 → 3 Xyl_2)	
	104.6	5.22 (1H, d, 7.8)	105.6	5.32 (1H, d, 7.2)
	75.6	3.95 (1H, m)	74.7	3.98 (1H, m)
	87.2	3.68 (1H, m)	88.0	3.63 (1H, m)
	70.4	3.94 (1H, m)	70.5	4.07 (1H, m)
	77.7	3.86 (1H, m)	78.1	3.94 (1H, m)
	61.8	4.06 (1H, m)	62.0	4.23 (1H, m)
		4.40 (1H, m)		4.41 (1H, m)
OCH_3	60.5	3.78 (3H, s)	60.8	3.84 (3H, s)

3-*O*-{3′-*O*-methyl-*β*-D-glucopyranosyl(1→3)-*β*-D-xylopyranosyl(1→4)-*β*-D-quinovopyranosyl(1→2)-4′-*O*-sulfate-*β*-D-xylopyranosyl}holosta-7,24-diene-3*β*,17α-diol sodium salt.

This work represents a continuing study on the glycosidic contents of this South China sea cucumber. In the current study, intercedensides D (**1**), E (**2**), G (**4**), and H (**5**) are new triterpene glycosides with a conjugated double bond system in the side chain of the aglycon. Intercedenside D (**1**) has the same structure as intercedenside C, except for different stereochemistry of the Δ^{22} double bond, which is *Z* in the former and *E* in the latter compound. Except for the 17-OH, the aglycons of intercedenside F (**3**) and intercedenside I (**6**) are very similar to the aglycon of liouvilloside A, which was isolated from the Antarctic sea cucumber *Staurocucumis liouvillei* (Dendrochirotida, Cucumariidae).[8]

Glycosides **1**, **2**, **3**, **4**, and **5** were tested for in vitro cytotoxicity against 10 human tumor cell lines (A549, MCF-7, 1A9, CAKI-1, U-87-MG, PC-3, KB, KB-VIN, SK-MEL-2, HCT-8) using the SRB method.[9] The ED_{50} values are listed in Table 7. Significant activity was found against all tumor cell lines. On the basis of these initially promising results, intercedensides D−H (**1**−**5**) merit further study as potential anticancer agents.

Table 7. ED_{50} Values of Compounds **1**−**5** against Human Tumor Cells in Vitro (μg/mL)

cell line	compound				
	1	**2**	**3**	**4**	**5**
A549	1.8	1.4	1.7	1.6	1.4
MCF-7	2.4	1.4	2.1	2.0	1.8
1A9	2.4	1.7	1.7	1.9	0.96
CAKI-1	>5	1.6	1.7	3.8	1.0
U-87-MG	4.1	2.1	3.3	3.3	3.2
PC-3	3.3	1.7	2.3	2.0	2.2
KB	3.7	1.9	3.2	3.3	3.0
KB-VIN	4.3	2.0	3.2	3.9	3.7
SK-MEL-2	4.2	1.6	2.1	2.4	2.2
HCT-8	2.9	1.1	1.9	1.8	1.9

Experimental Section

General Experimental Procedures. Melting points were determined on a XT5-XMT apparatus. Optical rotations were measured on a Perkin-Elmer MC-24 polarimeter. An IR spectrum was recorded on a Perkin-Elmer 683 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in C_5D_5N/D_2O (4:1) on Inova-600 and Inova-400 spectrometers. The ESIMS (positive- and negative-ion modes) was obtained on a Micromass Quatrro mass spectrometer. GC-MS was performed on a Finnigan Voyager GC-MS spectrometer with a HP-5 column (30 m × 0.25 mm i.d.). MPLC was performed using a Buchi chromatography pump B-686 equipped with a Lobar column (Lichroprep RP-18, 40−63 μm). Preparative HPLC was performed on an Agilent 1100 series equipped with a Quatpump, a degasser, a Rheodyne manual injector, and a refractive index detector using a Zobax 300 SB-C_{18} column (25 cm × 9.4 mm i.d.). TLC was carried out on precoated Si gel $HSGF_{254}$ ($CHCl_3$/EtOAc/MeOH/H_2O, 4:4:2.5:0.5) and RP-C_{18} plates (MeOH/H_2O, 1:1).

Animal Material. Specimens of *Mensamria intercedens* Lampert were collected at a depth of 3−30 m by a fishery bottom trawler in the Gulf of Dongshan in the South China Sea in February 2001 and deep frozen until used. The sea cucumber was identified by Prof. J. R. Fang and Dr. P. R. Wu (Fujian Institute of Oceanic Research, P. R. China). A voucher specimen (no. HYSC-2001-02) is preserved in the Department of Marine Drug Research, School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The extraction and preliminary chromatography were reported in a prior paper.[5] Briefly, the crude glycoside-containing mixture (63.4 g) obtained from *M. intercedens* was chromatographed on Si gel eluting with a $CHCl_3$/MeOH/H_2O (8:2:1 to 6.5:3.5:1) (lower phase) gradient to give several fractions. Fraction A (7.46 g) was further purified by reversed-phase silica MPLC [(Lichroprep RP-18, 40−63 μm; MeOH/H_2O (1:1)] to give fractions A_1 (0.8 g), A_2 (1.1 g), and A_3 (2.43 g). HPLC then resulted in the following pure glycosides. Fraction A_2 afforded intercedenside G (**4**) (55 mg, t_R = 26.54 min) and intercedenside H (**5**) (37 mg, t_R = 24.92 min) using MeOH/H_2O (48:52) as the mobile phase and a flow rate of 1.5 mL/min. Fraction A_3 afforded intercedenside I (**6**) (27 mg, t_R = 19.10 min), intercedenside D (**1**) (61.3 mg, t_R = 21.24 min), intercedenside E (**3**) (6.7 mg, t_R = 22.74 min), and intercedenside F (**6**) (51.5 mg, t_R = 26.22 min) using MeOH/H_2O (46:54) as the mobile phase and a flow rate of 1.5 mL/min.

Intercedenside D (1): colorless amorphous powder; mp 214−216 °C; $[\alpha]^{20}_D$ −36.3 (*c* 0.54, pyridine); ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1253 [M + Na]$^+$, 1133 [M − OSO_3Na − H + Na]$^+$, 1061 [M − *O*-3-OMe − Glc + Na]$^+$, 945 [M − 3-OMe − Glc − Xyl + Na]$^+$, 783 [M − 3-OMe − Glc − Xyl − Glc + Na]$^+$, 493 [625 − Xyl]$^+$; ESIMS (negative-ion mode) *m/z* 1207 [M − Na]$^-$, 619 [M − Na − Aglycon + H]$^-$.

Intercedenside E (2): colorless amorphous powder; mp 242−244 °C; $[\alpha]^{20}_D$ −39.4 (*c* 0.43, pyridine); ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1223 [M + Na]$^+$, 1103 [M − OSO_3Na − H + Na]$^+$, 1031 [M − *O*-3-OMe − Glc + Na]$^+$, 915 [M − 3-OMe − Glc-Xyl + Na]$^+$, 783 [M − 3-OMe − Glc − Xyl − Xyl + Na]$^+$, 653 [M − OSO_3Na − H-3-OMe − Glc − Xyl − Xyl + Na]$^+$, 463 [595 − Xyl]$^+$; ESIMS (negative-ion mode) *m/z* 1177 [M − Na]$^-$, 588 [M − *O*-3-OMe − Glc − Xyl − Xyl − Xyl − Na]$^-$.

Intercedenside F (3): colorless amorphous powder; mp 226−228 °C; $[\alpha]^{20}_D$ −33.2 (*c* 0.39, pyridine); ^{1}H and ^{13}C NMR data, see Tables 3 and 4; ESIMS (positive-ion mode) *m/z* 1255 [M + Na]$^+$, 135 [M − OSO_3Na − H + Na]$^+$, 1064 [M − *O*-3-OMe − Glc + H + Na]$^+$, 959 [M − OSO_3Na − 3-OMe − Glc − H + Na]$^+$, 947 (M − 3-OMe − Glc − Xyl + Na]$^+$ [M − 3-OMe − Glc − Xyl − Glc + Na]$^+$; ESIMS (negative-ion mode) *m/z* 1209 [M − Na]$^-$, 603 [M − Na − Aglycon + H]$^-$.

Intercedenside G (4): colorless amorphous powder; mp 241.5−243.2 °C; $[\alpha]^{20}_D$ −41.9 (*c* 0.46, pyridine); ^{1}H and ^{13}C NMR data, see Tables 5 and 6; ESIMS (positive-ion mode) *m/z* 1207 [M + Na]$^+$, 1087 [M − OSO_3Na + Na]$^+$, 1015 [M − *O*-3-OMe − Glc + Na]$^+$, 911 [M − OSO_3Na − 3-OMe − Glc + Na]$^+$, 463 [595 − Xyl]$^+$; ESIMS (negative-ion mode) *m/z* 1161 [M − Na]$^-$, 589 [M − Na − Aglycon + H]$^-$.

Intercedenside H (5): colorless amorphous powder; mp 188−190 °C; $[\alpha]^{20}_D$ (*c* 0.43, pyridine); ^{1}H and ^{13}C NMR data, see Tables 5 and 6; ESIMS (positive-ion mode) *m/z* 1237 [M + Na]$^+$, 1117 [M − OSO_3Na − H + Na]$^+$, 1045 [M − *O*-3-OMe − Glc + Na]$^+$, 942 [M − OSO_3Na − 3-OMe − Glc + H + Na]$^+$, 809 [M − OSO_3Na − 3-OMe − Glc − Xyl − H + Na]$^+$; ESIMS (negative-ion mode) *m/z* 1191 [M − Na]$^-$.

Intercedenside I (6): colorless amorphous powder; mp 221−223 °C; $[\alpha]^{20}_D$ −17.0 (*c* 0.47, pyridine); ^{1}H and ^{13}C NMR data, see Tables 3 and 4; ESIMS (positive-ion mode) *m/z* 1239 [M + Na]$^+$, 1119 [M − OSO_3Na − H + Na]$^+$, 1047 [M − *O*-3-OMe − Glc + Na]$^+$, 943 [M − OSO_3Na − 3-OMe − Glc − H + Na]$^+$, 785 [M − 3-OMe − Glc − Xyl − Qui − H + Na]$^+$, 665 [M − OSO_3Na − 3-OMe − Glc − Xyl − Qui + Na]$^+$, 533 [M − OSO_3Na − 3-OMe − Glc − Xyl − Qui − Xyl − H + Na]$^+$; ESIMS (negative-ion mode) *m/z* 1193 [M − Na]$^-$.

Acid Hydrolysis of Intercedensides D−I (1−6). Each glycoside (5 mg) was heated in an ampule with 5 mL of aqueous 15% HCl at 110 °C for 2 h. The aglycon was extracted with dichloromethane, and the aqueous residue was evaporated under reduced pressure. Then, 1 mL of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the dry residue, and the mixture was heated at 100 °C for 1 h. After cooling, Ac_2O (0.5 mL) was added, and the mixtures were heated at 100 °C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples.

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