Intercedensides A−C, Three New Cytotoxic Triterpene Glycosides from the Sea Cucumber *Mensamaria intercedens* Lampert

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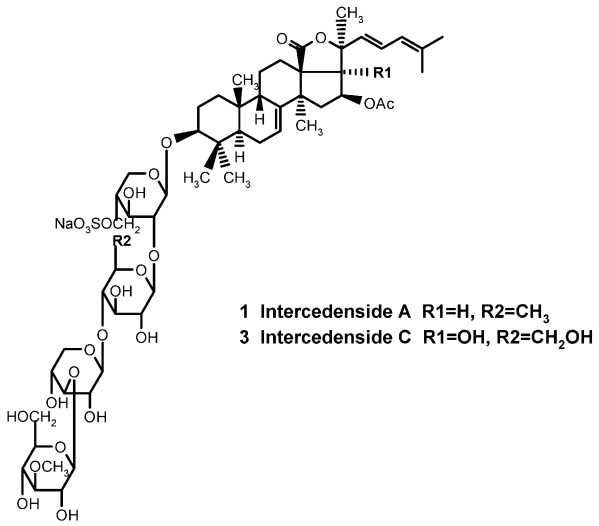
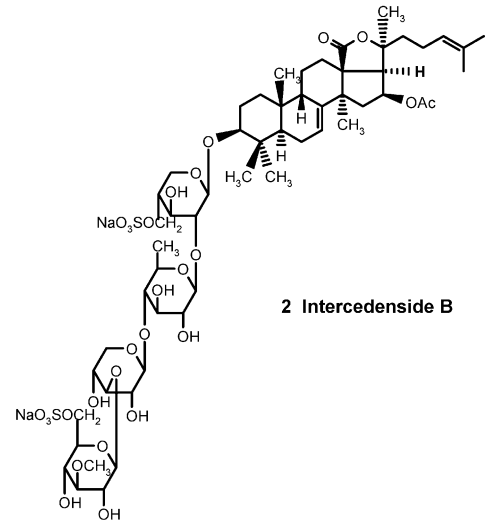
Received February 14, 2003

Three new triterpene glycosides, intercedensides A (**1**), B (**2**), and C (**3**), were isolated from the sea cucumber *Mensamria intercedens* Lampert, which is found in the South China Sea, and their structures have been elucidated by spectroscopic analysis (NMR and ESIMS) and chemical transformations. Intercedensides A (**1**) and C (**3**) have a conjugated double bond (22*E*,24-diene) in the side chain of the aglycon. Intercedenside B (**2**) has two β-D-xylose and two sulfate groups in the carbohydrate chain. All three glycosides showed significant cytotoxicity against 10 human tumor cell lines with ED_{50} in the range 0.6−4.0 μg/mL. Intercedenside A **(1)** exhibited significant in vivo antineoplastic activity against mouse Lewis lung cancer and mouse S180 sarcoma. On the basis of these initially promising results, intercedensides A−C merit further study as potential anticancer agents.

Triterpene glycosides are the predominant secondary metabolites of holothurians. More than 100 of these glycosides have been described, and the majority contain a sugar chain of up to six monosaccharide units linked to the C-3 of the aglycon, which is usually a triterpene of the lanosterol type with a 18(20) lactone.[1] These compounds exhibit various biological activities, including antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory effects.[2]

Mensamaria intercedens Lampert, a Cucumariidae-type sea cucumber, is widely distributed in the South China Sea, especially in Zhaoan Gulf and Dongshan Gulf, Fujian Province, People's Republic of China.[3] While screening extracts from marine invertebrate animals, a crude ethanolic extract from *M. intercedens* showed a significant deforming effect [minimum morphological deformation concentration (MMDC) ≤ 64 μg/mL, 5-FU as positive control with MMDC ≤ 5μg/mL] against *Pyricularia oryzae* P-2b.[4] In this paper, we report the extraction, isolation, purification, and structural elucidation of three new sulfated triterpene glycosides, intercedensides A (**1**), B (**2**), and C (**3**), and their semisynthetic desulfated derivative, as well as results from antineoplastic in vitro and in vivo screening of these compounds.





Results and Discussion

An ethanolic extract of *M. intercedens* Lampert was sequentially submitted to column chromatography on DA-101 resin (Nankai University, Tianjin, People's Republic of China), silica gel, and reversed-phase silica (Lichroprep RP-18, 40−63 μm). Final purification of individual compounds was achieved by reversed-phase HPLC on Zobax SB C-18 to give intercedenside A (**1**), intercedenside B (**2**), and intercedenside C (3**)**.

Intercedenside A (**1**), the major component of *M. intercedens* Lampert, was obtained as a colorless amorphous powder. Its molecular formula was determined as $C_{55}H_{83}O_{25}SNa$ from pseudomolecular ion peaks at *m*/*z* 1221.4702 [M

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10.1021/np030064y CCC: $25.00
Published on Web 07/25/2003

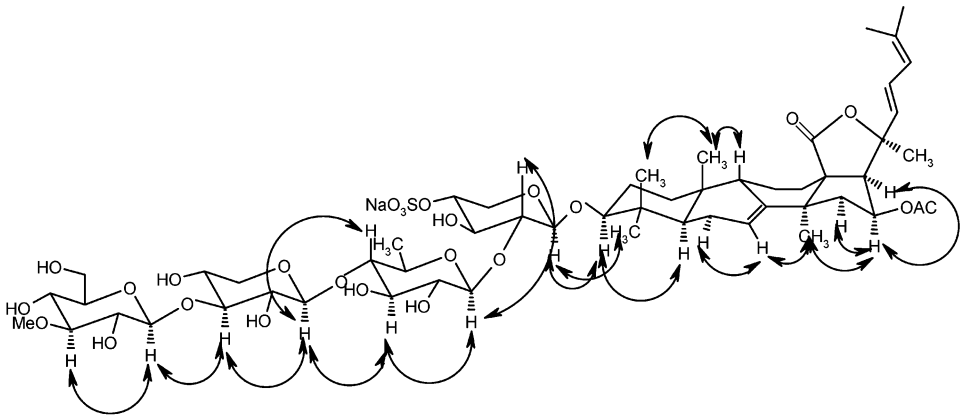

Figure 1. NOESY correlations of compound **1**.

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

position	δ_C	δ_H (m, *J* in Hz)	HMBC
1	36.2	1.43(2H, br)	
2	27.1	1.90(1H, m, β), 2.08(1H, m, α)	
3	89.2	3.24(1H, d, 9.6)	C: 1Xyl$_1$
4	39.6		
5	47.8	1.0(1H, m)	C: 4, 30, 31
6	23.3	2.0(2H, m)	
7	120.6	5.64(1H, bs)	
8	145.9		
9	47.4	3.44(1H, d, 13.8)	
10	35.6		
11	22.6	1.47(1H, m), 1.76(1H, m)	
12	30.8	2.02(1H, m), 2.15(1H, m)	
13	58.5		
14	48.2		
15	43.8	1.75(1H, m, β, 7.2, 11.4) 2.46(1H, dd, α, 7.8, 11.4)	
16	72.8	5.99(1H, ddd, 7.2, 7.8, 7.8)	C: 17, CH_3COO
17	57.6	3.11(1H, d, 7.8)	C: 13, 21
18	179.3		
19	24.0	1.21(3H, s)	C: 9, 10
20	84.0		
21	29.2	1.70(3H, s)	C: 17, 20, 22
22	132.2	5.78(1H, d, 12)	C: 20,24
23	120.5	6.11(1H, t, 12)	C: 20, 25
24	121.3	6.47(1H, d, 12)	C: 26, 27,
25	137.0		
26	26.2	1.72(3H, s)	C: 23, 24, 25, 27
27	17.7	1.68(3H, s)	C: 23, 24, 25, 26
30	17.8	1.10(3H, s)	C: 3, 4, 5, 31
31	28.7	1.26(3H, s)	C: 3, 4, 5, 30
32	32.7	1.09(3H, s)	C: 13, 14
CH_3COO	169.8		
CH_3COO	21.4	1.96(3H, s)	

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

position	δ_C	δ_H (m, *J* in Hz)	HMBC
Xyl (1→C-3)			
1	105.1	4.71(1H, d, 7.2)	C: C-3
2	83.5	3.99(1H, m)	C: 1Qui
3	75.4	4.27(1H, m)	C: 2Xyl$_1$
4	75.8	5.10(1H, m)	
5	64.2	3.71(1H, m) 4.71(1H, d, 10.2)	
Qui (1→2Xyl$_1$)			
1	105.4	5.04(1H, d, 6.6)	C: 2Xyl$_1$
2	76.2	3.95(1H, m)	
3	75.1	4.22(1H, m)	
4	86.0	3.57(1H, dd, 8.4, 7.8)	C: 1Xyl$_2$
5	71.8	4.10(1H, m)	
6	17.9	1.69(3H, d, 6.2)	C: 4,5Qui
Xyl (1→4Qui)			
1	105.1	4.82(1H, d, 7.8)	C: 4Qui
2	73.8	4.00(1H, m)	
3	87.0	4.19(1H, m)	C: 1 MeGlu
4	68.9	4.13(1H, m)	
5	66.5	3.68(1H, m) 4.28(1H, m)	
MeGlu (1→3Xyl$_2$)			
1	105.5	5.35(1H, d, 7.2)	C: 3Xyl$_2$
2	74.9	3.99(1H, m)	C: 3 MeGlu
3	87.9	3.66(1H, m)	C: 2,4 MeGlu, OMe
4	70.5	4.08(1H, m)	C: OMe
5	78.0	3.97(1H, m)	C: 4 MeGlu
6	61.9	4.24(1H, m) 4.45(1H, d, 10.2)	
OMe	60.8	3.84(3H, s)	C: 3 MeGlu

+ Na]$^+$ in positive-ion mode HRESIMS and at *m*/*z* 1175 [M − Na]$^-$ in the negative-ion mode ESIMS. A fragment ion peak at *m*/*z* 1119 [M − SO_3Na + Na + H]$^+$ in the positive-ion mode ESIMS indicated the presence of a sulfate groups in the glycoside. The IR spectrum showed the presence of hydroxyl (3438 cm^{-1}), carbonyl (1743 cm^{-1}), olefinic (1649 cm^{-1}), and sulfate (1239, 1070 cm^{-1}) groups.

The ^{1}H 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 145.9 (C-8) and 120.6 (C-7); δ_H 5.64 (1H, bs, H-7)] and for an acetoxy group [δ_C 169.8 and 21.4; δ_H 1.96 (3H,S)] were present. The acetoxy group was located at C-16 based on a cross-peak at δ 5.99/169.8 (H-16/CH_3CO) in the HMBC spectrum, and a 16β configuration was confirmed by cross-peaks at δ 2.46/5.99 (H-15α/H-16α) and 3.11/5.99 (H-17α/H-16α) in the NOESY spectrum (Figure 1) and from the coupling constants for H-16 with H-17α (7.8 Hz), H-15α (7.8 Hz), and H-15β (7.2 Hz).[5] The TOCSY spectrum of **1** indicated that three olefinic protons [δ 5.78 (1H, d, *J* = 12 Hz, H-22), 6.11 (1H, t, *J* = 12 Hz, H-23), 6.47 (1H, d, *J* = 12 Hz, H-24)] comprised a three-spin system; correspondingly, a conjugate double bond (22*E*,24-diene) should be present in the aglycon side chain. The *E* stereochemistry of the Δ^{22} double bond was deduced from the large coupling constant for H-22 with H-23 (12 Hz). This conclusion was also confirmed by the cross-peaks at δ 5.78/84.0 (H-22/C-20), 6.11/84.0 (H-23/C-20), 5.78/121.3 (H-22/C-24), 6.11/137.0 (H-23/C-25), 6.47/17.7 (H-24/C-27), and 6.47/26.2 (H-24/C-26) in the HMBC spectrum.

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,

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC Correlations for the Aglycon Moiety of Intercedenside B (**2**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

position	δ_C	δ_H (m, *J* in Hz)	HMBC
1	35.4	1.27(2H, br)	
2	26.3	1.80(1H, m, β), 1.90(1H, m, α)	
3	88.9	3.15(1H, dd, 3.6,12)	C: $1Xyl_1$
4	38.9		
5	47.7	0.87(1H, dd, 9.6, 12.8)	C: 4, 30, 31
6	23.0	1.86(2H, m)	
7	119.9	5.57(1H, bs)	
8	145.0		
9	46.7	3.24(1H, d, 13.8)	
10	34.9		
11	22.0	1.44(1H, m), 1.68(1H, m)	
12	30.8	2.09(2H, m)	
13	58.7		
14	47.0		
15	43.1	1.57(1H, m, β, 7.2, 12) 2.55(1H, dd, α, 8.4, 12)	
16	75.2	5.80(1H, ddd, 7.2, 8.4, 8.4)	C: 17, CH_3COO
17	53.9	2.69(1H, d, 8.4)	C: 13, 21
18	180.1		
19	23.4	1.06(3H, s)	C: 9, 10
20	85.4		
21	27.7	1.56(3H, s)	C: 17, 20, 22
22	38.1	1.78(1H, m), 2.36(1H, m)	C: 20, 21
23	22.7	1.81(1H, m), 1.98(1H, m)	
24	123.3	4.95(1H, d, 7.8)	C: 26, 27,
25	132.0		
26	25.0	1.57(3H, s)	C: 24, 25, 27
27	17.4	1.59(3H, s)	C: 24, 25, 26
30	16.8	0.96(3H, s)	C: 3, 4, 5, 31
31	28.2	1.13(3H, s)	C: 3, 4, 5, 30
32	31.8	1.14(3H, s)	C: 13, 14
CH_3COO	170.8		
CH_3COO	20.7	1.95(3H, s)	

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 anomeic proton resonances with coupling constants (*J* values) 6.6−7.8 Hz (Table 2). The presence of xylose, quinovose, and 3-*O*-methyl glucose 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 4.71/89.2 (H-1′/C-3), 5.04/83.5 (H-1″/C-2′), 4.82/86.0 (H-1‴/C-4″), and 5.35/87.0 (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 fragment ion peaks at *m*/*z* 921, 789, 643, and 511 in the positive-ion mode ESIMS, corresponding to the sequential losses of 3-*O*-methylglucosyl, xylosyl, quinovosyl, and xylosyl 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[6] and desulfated intercenside A (**1a**). Downfield esterification shifts were observed for the C-4′ signal (xyl_1, from δ 68.2 to 75.8 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-quinovopyranosyl(1→2)-4′-*O*-sulfate-β-D-xylopyranosyl}-holosta-7,22*E*,24-triene-3β-ol sodium salt.

Intercedenside B (**2**) was obtained as a colorless amorphous powder. The molecular formula of intercedenside B (**2**) was determined as $C_{55}H_{84}O_{28}S_2Na_2$ from pseudomolecular ion peaks at *m*/*z* 1325.2384 $[M + Na]^+$ in positive-ion mode HRESIMS and at *m*/*z* 1279 $[M - Na]^-$ in the negative-ion mode ESIMS. Fragment ion peaks at *m*/*z* 1177 $[M - Na - SO_3Na + H]^-$ and 1051 $[M - 2Na - 2SO_3Na + H]^-$ in the negative-ion mode ESIMS indicated the presence of two sulfate groups in the glycoside. The IR spectrum showed the presence of hydroxyl (3502 cm^{-1}), carbonyl (1746 cm^{-1}), olefinic (1650 cm^{-1}), and sulfate groups (1239, 1067 cm^{-1}).

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

position	δ_C	δ_H (m, *J* in Hz)	HMBC
Xyl (1→C-3)			
1	103.8	4.67(1H, d, 7.2)	C: C-3
2	81.7	3.95(1H, t, 9.8)	C: 1Qui
3	74.1	4.21(1H, t, 9)	C: $2Xyl_1$
4	75.8	4.89(1H, m)	
5	63.2	3.71(1H, m) 4.71(1H, m)	
Qui (1→2Xyl)			
1	104.1	4.93(1H, d, 7.8)	C: $2Xyl_1$
2	74.2	3.90(1H, m)	
3	74.9	3.80(1H, m)	
4	85.0	3.47(1H, t, 9.0)	C: 6Qui, $2Xyl_2$
5	71.1	3.62(1H, m)	
6	17.2	1.57(3H, d, 6.2)	C: 4,5Qui
Xyl (1→4Qui)			
1	103.9	4.74(1H, d, 7.8)	C: 4Qui
2	72.8	3.83(1H, m)	
3	86.4	4.02(1H, m)	C: 1 MeGlu
4	68.3	3.88(1H, m)	
5	65.1	3.58(1H, m) 4.14(1H, m)	
MeGlu (1→3Xyl)			
1	104.3	5.09(1H, d, 7.8)	C: $3Xyl_2$
2	73.7	3.75(1H, m)	C: 3 MeGlu
3	85.7	3.64(1H, m)	C: 2,4 MeGlu, OMe
4	69.4	3.91(1H, m)	C: OMe
5	74.7	4.01(1H, m)	C: 4 MeGlu
6	66.8	4.62(1H, dd, 6.0, 11.4) 4.88(1H, m)	
OMe	60.8	3.84(3H, s)	C: 3 MeGlu

The NMR data of **2** (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 **2** was identified as 16β-acetoxyholosta-7,24-diene-3β-ol. Like in compound **1**, the oligosaccharide chain of **2** differed from those previously identified in sea cucumber glycosides.[5] The four β-monosaccharide units were again identified as xylose, quinovose, and 3-*O*-methyl glucose in a 2:1:1 ratio based on the ^{1}H and ^{13}C spectra, which showed four anomeric carbon and four anomeic 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)−qui(1→2)−xyl(1→3)−aglycon] in compound **2** was determined by careful analysis of the HMBC cross-peaks, δ 4.67/88.9 (H-1′/C-3), 4.93/81.7 (H-1″/C-2′), 4.74/85.0 (H-1‴/C-4″), and 5.09/86.4 (H-1⁗/C-3‴).

Comparison of the ^{13}C NMR data of compound **2** with those of known related glycosides[6] and with desulfated intercenside B (**2a**) showed that the carbon signals at C-4′ (xyl_1) and C-6⁗ (3-*O*-methyl-glc) had shifted downfield from δ 68.1 to 75.8 and from δ 61.9 to 66.8 ppm, respectively, consistent with esterification by the sulfate groups.

Therefore, the structure of compound **2** was deduced as 16β-acetoxy-3-*O*-{6⁗-*O*-sulfate-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-3β-ol disodium salt.

Table 5. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC Correlations for the Aglycon Moiety of Intercedenside C (**3**) (in pyridine-d_5/D_2O, 4:1, 600/150 MHz)

position	δ_C	δ_H (m, *J* in Hz)	HMBC
1	35.4	1.30(1H, m, α), 1.39(1H, m, *β*)	
2	26.8	1.82(1H, m, *β*), 2.00(1H, m, α)	
3	88.8	3.17(1H, dd, 4.2, 12)	C: $1Xyl_1$
4	39.3		
5	48.7	0.88(1H, m)	C: 4, 30, 31
6	22.9	1.89(2H, m)	
7	119.7	5.62(1H, bs)	
8	147.4		
9	47.7	3.40(1H, d, 14.4)	
10	35.8		
11	22.3	1.49(1H, m), 1.77(1H, m)	
12	25.4	1.97(1H, m), 2.64(1H, m)	
13	59.3		
14	47.6		
15	43.1	1.79(1H, m, *β*) 2.52(1H, dd, α, 7.8, 9.0)	
16	83.5	6.07(1H, dd, 7.2, 9.0)	C: 17, CH_3COO
17	86.7		
18	178.3		
19	24.0	1.11(3H, s)	C: 9, 10
20	85.9		
21	27.9	1.70(3H, s)	C: 17, 20, 22
22	132.4	6.15(1H, d, 16.2)	C: 20, 24
23	122.5	6.55(1H, dd, 10.8, 16.2)	C: 20, 25
24	125.3	5.74(1H, d, 10.8)	C: 26, 27
25	134.5		
26	25.5	1.58(3H, s)	C: 23,24,25,27
27	17.8	1.45(3H, s)	C: 23, 24, 25, 26
30	17.1	1.00(3H, s)	C: 3, 4, 5, 31
31	28.5	1.12(3H, s)	C: 3, 4, 5, 30
32	30.7	1.46(3H, s)	C: 13, 14
CH_3COO	170.5		
CH_3COO	21.1	1.94(3H, s)	

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

position	δ_C	δ_H (m, *J* in Hz)	HMBC
Xyl (1→C-3)			
1	104.7	4.67(1H, d, 6.6)	C: C-3
2	81.6	4.13(1H, m)	C: 1Glc
3	75.0	4.27(1H, m)	C: $2Xyl_1$
4	75.9	5.06(1H, td, 4.8, 9.6)	
5	64.0	3.72(1H, m) 4.77(1H, m)	
Glc (1→2Xyl)			
1	104.7	5.14(1H, d, 7.8)	C: $2Xyl_1$
2	75.2	3.96(1H, m)	
3	68.9	4.08(1H, m)	
4	80.2	4.15(1H, m)	C: $2Xyl_2$
5	76.2	3.70(1H, m)	
6	60.8	4.30(1H, m) 4.39(1H, m)	
Xyl (1→4Glc)			
1	104.3	4.96(1H, d, 7.8)	C: 3Glc
2	73.4	3.90(1H, m)	
3	86.7	4.09(1H, m)	C: 1 MeGlu
4	68.7	3.94(1H, m)	
5	66.1	3.53(1H, m) 4.07(1H, m)	
MeGlu (1→3Xyl)			
1	104.9	5.18(1H, d, 7.8)	C: $3Xyl_2$
2	74.6	3.89(1H, m)	C: 3 MeGlu
3	87.3	3.65(1H, m)	C: 2,4 MeGlu, OMe
4	70.4	3.91(1H, m)	C: OMe
5	77.7	3.88(1H, m)	C: 4 MeGlu
6	61.8	4.06(1H, m) 4.41(1H, m)	
OMe	60.6	3.79(3H, s)	C: 3 MeGlu

Intercedenside C (**3**) was obtained as a colorless amorphous powder. The molecular formula was determined as $C_{55}H_{83}O_{27}SNa$ from the pseudomolecular ion peaks at *m/z* 1253.4638 $[M + Na]^+$ in positive-ion mode HRESIMS and at *m/z* 1207 $[M - Na]^-$ in the negative-ion mode ESIMS. The IR spectrum showed the presence of hydroxyl (3438 cm^{-1}), carbonyl (1742 cm^{-1}), olefinic (1650 cm^{-1}), and sulfate (1237, 1070 cm^{-1}) groups.

The NMR data of compound **3** (Table 5) suggested that the aglycon moiety of **3** was closely related to that of compound **1**. However, in the ^{13}C NMR data of compound **3**, the quaternary carbon signal at *δ* 86.7 (C-17) is shifted downfield by 29.1 ppm from that in compound **1** (*δ* 57.6), indicating the presence of a hydroxy group at this position in compound **3**, and furthermore, the H-17 signal at *δ* 3.11 in **1** was absent in the ^{1}H NMR spectrum of **3**. This conclusion was confirmed from the TOCSY, DQFCOSY, and HMBC spectrum. In the TOCSY spectrum, three protons [*δ*1.79 (1H, m, H-15*β*); 2.52 (1H, dd, *J* = 7.8, 9.0 Hz, H-15α); 6.07 (1H, dd, *J* = 7.2, 9.0 Hz, H-16)] comprised a three-spin system, and the HMBC spectrum showed a cross-peak at *δ* 6.07/86.7 (H-16/C-17).

The presence of four *β*-monosaccharide units in compound **3** was deduced from the ^{1}H and ^{13}C spectra, which showed four anomeric carbon and four anomeic proton resonances with coupling constants (*J* values) 6.6−7.8 Hz (Table 6). However, the oligosaccharide chain was different from those of compounds **1** and **2**, and the presence of xylose, glucose, and 3-*O*-methyl glucose 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. Protons in the monosaccharides were assigned and correlated with their corresponding carbons using HMQC, HMBC, TOCSY, and DQF-COSY experiments. The sequence of the sugar residues in compound **3** was determined by careful analysis of HMBC correlations; cross-peaks at *δ* 4.67/88.8 (H-1′/C-3), 5.14/81.6 (H-1″/C-2′), 4.96/80.2 (H-1‴/C-4″), 5.18/86.7 (H-1⁗/C-3‴) indicated that the sequence should be 3-*O*-methyl−glc(1→3)−xyl(1→4)−glc(1→2)−xyl(1→3)−aglycon.

An esterification shift in the C-4′ signal (xyl_1, from *δ* 68 to 75.9) determined the position of the sulfate group based on comparison of the ^{13}C NMR data of compound **3** with those reported for other glycosides.[7] Therefore, the structure of compound **3** was deduced as 16*β*-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*E*,24-triene-3*β*,17α-diol sodium salt.

This work represents the first study on the glycosidic contents of this South China sea cucumber. Intercedensides A (**1**) and C (**3**) are new triterpene glycosides, which contain conjugated double bonds in the side chain of the glycoside. Intercedenside B (**2**) has the same aglycon as liouvilloside A, which was isolated from the Antarctic sea cucumber *Staurocucumis liouvillei* (Dendrochirotida, Cucumariidae), but these two compounds contain different oligosaccharide chains.[5]

Glycosides **1**, **2**, and **3** were tested for in vitro cytotoxicity against 10 human tumor cell lines (A549, MCF-7, IA9, CAKI-1, U-87-MG, PC-3, KB, KB-VIN, SK-MEL-2, HCT-8) using the SRB method.[8] The ED_{50} values are listed in Table 9. Significant activity was found against all tumor cell lines. Compounds **1** and **3** showed similar potencies, while compound **2** was generally more potent in all cell lines. Iatercedenside A (**1**) also exhibited significant in vivo antineoplastic activity against mouse Lewis lung cancer and mouse S180 sarcoma, with 48.39% and 57.48% reduc-

Table 7. ^{13}C and ^{1}H NMR Chemical Shifts for the Aglycon Moiety of DS-Intercedenside A (**1a**) and DS-Intercedenside B (**2a**) (in pyridine-d_5, 400/100 Mz)

	1a		**2a**	
position	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)
1	36.3	1.39(2H, br)	35.3	1.25(2H,br)
2	27.3	1.80(1H, m, β) 2.06(1H, m, α)	26.4	1.81(1H, m, β), 1.92(1H, m, α)
3	89.3	3.24(1H, dd, 3.2, 11.6)	89.0	3.15(1H, dd, 3.6, 12)
4	39.7		38.7	
5	47.9	0.95(1H, m)	47.5	0.88(1H, m)
6	23.4	1.98(2H, m)	23.2	1.76(2H, m)
7	120.5	5.55(1H, bs)	120.0	5.60(1H, bs)
8	145.9		145.2	
9	47.5	3.36(1H, d, 13.8)	46.6	3.24(1H, d, 13.8)
10	35.6		35.0	
11	22.7	1.45(1H, m) 1.72(1H, m)	22.1	1.44(1H, m) 1.70(1H, m)
12	30.9	1.94(1H, m) 2.03(1H, m)	31.0	2.10(2H, m)
13	58.5		58.6	
14	48.2		47.1	
15	43.8	1.60(1H, β) 2.34(1H, dd, α, 8.4, 12.6)	43.2	1.60(1H, m) 2.63(1H, m)
16	72.8	5.89(1H, dd, 8.4, 12.6)	75.2	5.76(1H, dd, 7.2, 8.4)
17	57.6	2.99(1H, d, 8.8)	54.0	2.73(1H, d, 9.0)
18	179.3		179.4	
19	24.0	1.12(3H, s)	23.2	1.07(3H, s)
20	84.0		85.3	
21	29.2	1.59(3H, s)	27.6	1.58(3H, s)
22	132.2	5.67(1H, d, 12)	38.0	1.79(1H, m) 2.40(1H,m)
23	120.7	6.00(1H, t, 12)	22.5	1.83(1H, m) 2.00(1H, m)
24	121.3	6.37(1H, d, 12)	123.3	5.10(1H, d, 8.0)
25	137.0		132.2	
26	26.2	1.62(3H, s)	25.2	1.55(3H, s)
27	17.7	1.57(3H, s)	17.4	1.60(3H, s)
30	17.3	1.08(3H, s)	16.9	1.01(3H, s)
31	28.8	1.25(3H, s)	28.3	1.12(3H, s)
32	32.7	1.00(3H, s)	31.9	1.15(3H, s)
CH_3COO	169.8		169.7	
CH_3COO	21.4	1.90(3H, s)	21.0	1.07(3H, s)

tion in tumor weight, respectively, at a dose of 120 mg/kg (ip, $p < 0.01$). Based on these initially promising results, intercedensides A−C merit further study as potential anticancer agents.

Experimental Section

General Experimental Procedures. Melting points were determined on a XT5-XMT apparatus. Optical rotations were measured on a Perkin-Elmer MC-241 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/H_2O (4:1) on Inova-600 and Inova-400 spectrometers. The ESIMS (positive and negative ion modes) was obtained on a Micromass Quattro 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 B-686 chromatography pump 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 use. The sea cucumber was identified by Prof. J. R. Fang and Dr. P. R. Wu (Fujian Institute of Oceanic Research, 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, China.

Table 8. ^{13}C and ^{1}H NMR Chemical Shifts for the Sugar Moiety of DS-Intercedenside A (**1a**) and DS-Intercedenside B (**2a**) (in pyridine-d_5, 400/100 MHz)

	1a		**2a**	
position	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)
Xyl (1→C-3)				
1	105.4	4.73(1H, d, 7.2)	105.1	4.73(1H, d, 7.6)
2	84.3	3.98(1H, m)	84.0	3.96(1H, m)
3	78.2	4.13(1H, m)	78.5	4.12(1H, m)
4	68.2	4.00(1H, m)	68.1	4.04(1H, m)
5	66.5	3.57(1H, m) 4.20(1H, m)	66.1	3.56(1H, m) 4.18(1H, m)
Qui (1→2Xyl)				
1	105.2	4.71(1H, d, 6.6)	104.9	4.71(1H, d, 6.8)
2	75.1	3.90(1H, m)	75.2	3.87(1H, m)
3	75.6	3.92(1H, m)	76.2	3.92(1H, m)
4	86.0	3.59(1H, m)	87.1	3.60(1H, m)
5	71.8	3.67(1H, m)	71.4	3.68(1H, m)
6	18.0	1.64(3H, d, 6.2)	19.1	1.68(3H, d, 6.2)
Xyl (1→4Qui)				
1	105.8	5.17(1H, d, 7.8)	105.4	5.16(1H, d, 7.2)
2	73.5	3.87(1H, m)	73.1	3.77(1H, m)
3	87.5	4.09(1H, m)	87.7	4.09(1H, m)
4	70.7	4.02(1H, m)	70.3	4.01(1H, m)
5	66.8	3.56(1H, m) 4.26(1H, m)	66.4	3.57(1H, m), 4.23(1H, m)
MeGlu (1→3Xyl)				
1	105.6	5.05(1H, d, 7.2)	105.2	5.05(1H, d, 6.8)
2	74.3	3.87(1H, m)	74.7	3.83(1H, m)
3	88.0	3.61(1H, m)	87.8	3.63(1H, m)
4	70.8	4.07(1H, m)	70.2	4.04(1H, m)
5	76.6	3.85(1H, m)	76.5	3.88(1H, m)
6	62.2	4.23(1H, m) 4.35(1H, m)	61.9	4.24(1H, m) 4.32(1H, m)
OMe	60.8	3.74(3H, s)	60.3	3.74(3H, s)

Extraction and Isolation. The sea cucumber *Mensamria intercedens* Lampert specimens (100 kg, wet weight) were defrosted, cleaned, cut into small pieces, and extracted at room temperature three times with 85% ethanol (80 L, 7 days for each extraction). The first extract was concentrated, and the residue was dissolved in water. The solution was passed through a DA101 resin column (Nankai University, Tianjin, China), and the inorganic salts and polar impurities were eluted with water. Then the glycoside fraction (25.2 g) was eluted with 50% ethanol. The combined extracts of the second and the third times were concentrated. The dry extract was partitioned between water and dichloromethane and the water layer further partitioned between water and *n*-butanol. The *n*-butanol extract (38.2 g) combined with the glycoside fraction of the first extract (crude glycoside-containing mixture, 63.4 g) was further 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 fractions A (7.46 g), B (4.01 g), C (3.01 g), D (2.55 g), and E (1.08 g).

Fractions A and B were further purified by MPLC with a column of reversed-phase silica (Lichroprep RP-18, 40−63 μm). Final purification of the glycosides in fractions A and B was achieved by HPLC. Fraction A afforded 50 mg of pure intercedenside A (**1**) (t_R = 24.6 min) and 58.6 mg of intercedenside C (**3**) (t_R = 25.8 min) using MeOH/H_2O (49:51) as the mobile phase and a flow rate of 1.5 mL/min. Fraction B gave 42 mg of pure intercedenside B (**2**) (t_R = 26.6 min) using MeOH/H_2O (43:57) as the mobile phase and a flow rate of 1.5 mL/min.

Intercedenside A (**1**)**:** colorless amorphous powder, mp 184−186 °C, $[\alpha]_D^{20}$ −27.3 (*c* 0.6, pyridine); ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m*/*z* 1221 $[M + Na]^+$, 1119 $[M - SO_3Na + Na + H]^+$, 921 [1119 − 3-OMe-Glc

Table 9. ED_{50} Values of Compounds **1**, **2**, and **3** Against Human Tumor Cells in Vitro (μg/mL)

compd	A549	MCF-7	IA9	CAKI-1	U-87-MG	PC-3	KB	KB-VIN	SK-MEL-2	HCT-8
1	1.7	3.5	0.96	1.2	4.0	3.2	3.5	3.5	3.6	2.4
2	0.71	1.4	0.61	0.77	1.9	1.5	1.9	2.0	1.9	1.2
3	1.6	3.7	1.5	1.5	3.6	3.0	3.6	3.8	3.4	2.1

− Na + H]$^+$, 789 [921 − Xyl]$^+$, 643 [789 − Qui]$^+$, 511 [643 − Xyl]$^+$; ESIMS (negative ion mode) *m/z* 1175 [M − Na]$^-$, 999 [M − Na − 3-OMe-Glc]$^-$, 587 [M − Na − aglycon + H]$^-$.

Intercedenside B (2): colorless amorphous powder, mp 218−220 °C, $[\alpha]_D^{20}$ −14.2 (*c* 0.5, pyridine); ^{1}H and ^{13}C NMR, see Tables 3 and 4; ESIMS (positive ion mode) *m/z* 1325 [M + Na]$^+$; ESIMS (negative ion mode) *m/z* 1279 [M − Na]$^-$, 1177 [M − Na − SO3Na + H]$^-$, 1051[M − 2Na − 2-SO_3Na + H]$^-$, 1001 [M − Na − SO_3Na − 3-OMe-Glc + H]$^-$.

Intercedenside C (3): colorless amorphous powder, mp 192−193 °C, $[\alpha]_D^{20}$ −32.3 (*c* 0.54, pyridine); ^{1}H and ^{13}C NMR data, see Tables 5 and 6; ESIMS (positive ion mode) *m/z* 1253 [M + Na]$^+$, 1133 [M − OSO_3Na − H + Na]$^+$, 1061 [M − O-3-OMe-Glc + Na]$^+$, 957 [1133 − 3-OMe-Glc]$^+$; ESIMS (negative ion mode) *m/z* 1207 [M − Na]$^-$, 603 [M − Na − aglycon + H]$^-$.

Acid Hydrolysis of Intercedenside A (1), Intercedenside B (2), and Intercedenside C (3). 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 extract 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 the reaction mixtures were cooled, 1.5 mL of Ac_2O 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. Xylose, quinovose, and 3-*O*-Me-glucose were identified for intercedenside A (**1**) and intercedenside B (**2**) in a 2:1:1 ratio, and xylose, glucose, and 3-*O*-Me-glucose was identified for intercedenside C (**3**) in a 2:1:1 ratio.

Desulfation of Intercedenside A (1). The glycoside **1** (30 mg) was dissolved in a mixture of pyridine/dioxane (1:1) and heated under reflux for 4 h. The reaction mixture was partitioned between water and *n*-butanol. The *n*-butanol extract was evaporated under reduced pressure, and the residue was purified by reversed-phase HPLC (Zobax 300 SB-C_{18}, MeOH/H_2O, 4:1), to give the pure desulfated derivative **1a** (16 mg).

Desulfated intercedenside A (1a): colorless amorphous powder, mp 206−207 °C, $[\alpha]_D^{20}$ −58.2 (*c* 0.6, pyridine); ^{1}H and ^{13}C NMR, see Tables 7 and 8; ESIMS (positive ion mode) *m/z* 1119 [M + Na]$^+$; ESIMS (negative ion mode) *m/z* 1095 [M − H]$^-$, 919 [M − H − 3-O-Me-glc]$^-$, 787 [919 − Xyl]$^-$.

Desulfation of Intercedenside B (2). The glycoside **2** (32 mg) was desulfated as described above and purified by reversed-phase HPLC (Zobax 300 SB-C_{18}, MeOH/H_2O, 4:1), to give the pure desulfated derivative **2a** (11.9 mg).

Desulfated intercedenside B (2a): colorless amorphous powder, mp 231−232 °C, $[\alpha]_D^{20}$ −32.7 (*c* 0.5, pyridine); ^{1}H and ^{13}C NMR, see Tables 7 and 8; ESIMS (positive ion mode) *m/z* 1121[M + Na]$^+$; ESIMS (negative ion mode) *m/z* 1097 [M − H]$^-$.

Acknowledgment. This investigation was supported by a grant from Shanghai Foundation for New Drug Research awarded to Y.H.Y. (02DZ19101) and in part by a grant from the National Cancer Institute, awarded to K.H.L. (NIH, CA 17625). The authors wish to thank K. F. Bastow and Y. Nakanishi of UNC, School of Pharmacy, for performing in vitro cytotoxicity screening. We are also grateful to J. R. Fang and P. R. Wu of the Fujian Institute of Oceanic Research for the taxonomic identification of the sea cucumber.

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NP030064Y