# Two New Cytotoxic Disulfated Holostane Glycosides from the Sea Cucumber *Pentacta quadrangularis*

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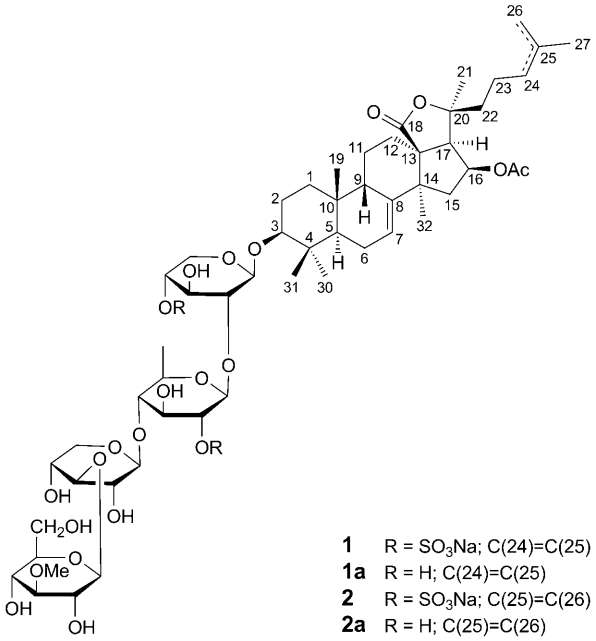
Two new disulfated triterpene glycosides, pentactasides B and C (**1** and **2**, resp.), were isolated from the sea cucumber *Pentacta quadrangularis* collected from the South China Sea. Their structures were elucidated by extensive spectral analysis (2D-NMR and MS) and chemical methods. The compounds **1** and **2** possess the same tetrasaccharide moieties with two sulfated groups, but are different in the side chains of the triterpene aglycone. Pentactasides B and C (**1** and **2**, resp.) showed significant cytotoxicities ($IC_{50}$ 0.09–2.30 μM) against different human tumor cell lines.

**Introduction.** – The predominant secondary metabolites from sea cucumbers (class Holothuroidea) are triterpene glycosides of the lanosterol type with a 18,20 lactone moiety and a sugar chain of up to six monosaccharide units linked to C(3) of the aglycone [1]. These saponins have a wide spectrum of biological effects, including antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory activities [2].

As a continuation of our search for new bioactive compounds from echinoderms [3–5], we have investigated the polar extracts of the sea cucumber *P. quadrangularis*. Recently, we have demonstrated the antitumor activity of two new disulfated triterpene glycosides from the sea cucumber *P. quadrangularis* collected in the South China Sea, near Guangdong Province, P. R. China [4][6]. We report here the isolation and structure elucidation of two new sulfated triterpene glycosides, pentactasides B and C (**1** and **2**, resp.), as well as the cytotoxic activities of these compounds and their semisynthetic desulfated derivatives *in vitro* against different tumor cell lines.

**Results and Discussion.** – Pentactaside B (**1**), a colorless amorphous powder, gave a positive result to *Liebermann–Burchard* and *Molish* tests. The molecular formula was determined as $C_{55}H_{84}Na_2O_{28}S_2$ from the $[M+Na]^+$ ion at $m/z$ 1325.6125 in the positive-ion mode HR-ESI-MS and from the $[M-Na]^-$ ion at $m/z$ 1279 in the negative-ion mode ESI-MS. Fragment-ion peaks at $m/z$ 1205 ($[M+Na-NaHSO_4]^+$) and at 1085 ($[M+Na-2\ NaHSO_4]^+$) in the ESI-MS (positive-ion mode) indicated the presence of two sulfate groups in **1**, which was confirmed by the IR spectrum with absorption bands at 1237 and 1210 $cm^{-1}$. The IR spectrum also showed an absorption band due to a $\gamma$-




lactone moiety (1742 $cm^{-1}$), and a strong broad absorption (3456 $cm^{-1}$) reminiscent of a glycosidic structure.

The $^1H$- and $^{13}C$-NMR spectra of **1** (*Tables 1* and *2*) suggested the presence of a triterpene aglycone with seven Me groups, and displayed two olefinic signals ($\delta$(H) 5.63, $\delta$(C) 145.5 and 120.4; and $\delta$(H) 5.10, $\delta$(C) 132.5 and 124.4), of an AcO group ($\delta$(H) 1.99; $\delta$(C) 169.8, 21.2), and of a lactone C=O group ($\delta$(C) 179.5). These data were similar to those of the aglycone of violaceuside III [7], from which **1** differed only at C(16). The location of the AcO group at C(16) was deduced from the chemical shift of H–C(16) ($\delta$(H) 5.91–5.98), which was coupled to H–C(17) ($\delta$(H) 2.64), $H_\alpha$–C(15) ($\delta$(H) 2.58–2.64), and $H_\beta$–C(15) ($\delta$(H) 1.72–1.81) in the TOCSY spectrum. The 16$\beta$-configuration of the AcO group was confirmed by NOESY experiments, and from the coupling constants between H–C(16) and $H_\alpha$–C(17) ($J$ = 9.2 Hz) [8]. The positions of the C=C bonds (C(7)=C(8) and C(24)=C(25)) were deduced from the NMR signals at $\delta$(C) 145.5 (C(8)) and 120.4 (C(7)) ($\delta$(H) 5.63 (br. *s*, H–C(7))), and at $\delta$(C) 124.4 (C(24)) and 132.5 (C(25)) ($\delta$(H) 5.10 (br. *s*, H–C(24))), together with the analysis of TOCSY and HMBC experiments [9].

The sugar moieties of **1** were determined to be D-xylose (Xyl), D-quinovose (Qui), and 3-*O*-methyl-D-glucose (MeGlc) in a ratio of 2 : 1 : 1, as determined by hydrolysis in aqueous 2M $CF_3COOH$, followed by preparation of the corresponding aldononitrile peracetates, which were analyzed by GC/MS. The D-configurations for the sugar units are typical among sea cucumber glycosides [10]. The $^1H$- and $^{13}C$-NMR spectra of **1** indicated four sugar units (four anomeric H-atoms at $\delta$(H) 4.79 (*d*, $J$ = 7.0), 4.99 (*d*, $J$ = 7.6), 4.87 (*d*, $J$ = 7.2), and 5.26 (*d*, $J$ = 7.6), and four anomeric C-atoms at $\delta$(C) 104.7,

Table 1. *$^1$H- and $^{13}$C-NMR Data for the Aglycone Moiety of Pentactaside B* (**1**) ($\delta$ in ppm, $J$ in Hz)

| | $\delta$(C)[a]) | $\delta$(H)[b]) | NOESY | HMBC |
|---|---|---|---|---|
| $CH_2$(1) | 36.1 (*t*) | 1.41–1.87 (*m*) | | |
| $CH_2$(2) | 27.0 (*t*) | 1.90–2.04 (*m*) | | |
| H–C(3) | 89.5 (*d*) | 3.20 (*dd*, *J* = 4.2, 12.0) | $H_\alpha$–C(1) (Xyl$^1$), $H_\alpha$–C(5), Me(30) | C(1) (Xyl$^1$) |
| C(4) | 39.6 (*s*) | | | |
| H–C(5) | 48.0 (*d*) | 0.99 (*t*, *J* = 9.6) | $H_\alpha$–C(3), Me(30) | C(4), C(30), C(31) |
| $CH_2$(6) | 23.3 (*t*) | 1.89–2.05 (*m*) | | |
| H–C(7) | 120.4 (*d*) | 5.63 (br. *s*) | | |
| C(8) | 145.5 (*s*) | | | |
| H–C(9) | 47.1 (*d*) | 3.44 (br. *d*, *J* = 12.0) | | |
| C(10) | 35.6 (*s*) | | | |
| $CH_2$(11) | 23.2 (*t*) | 1.78–2.02 (*m*) | H–C(9) | C(8), C(10), C(12) |
| $CH_2$(12) | 31.5 (*t*) | 1.94–2.11 (*m*) | $H_\alpha$–C(11), $H_\beta$–C(11) | C(11), C(13), C(14) |
| C(13) | 59.3 (*s*) | | | |
| C(14) | 47.5 (*s*) | | | |
| $CH_2$(15) | 43.7 (*t*) | 2.58–2.64 (*m*, $H_\alpha$), 1.72–1.81 (*m*, $H_\beta$) | $H_\alpha$–C(16) | |
| H–C(16) | 75.1 (*d*) | 5.91–5.98 (*m*) | $H_\alpha$–C(17), $H_\alpha$–C(15) | C(17), Me*C*O |
| H–C(17) | 54.8 (*d*) | 2.64 (*d*, *J* = 9.0) | $H_\alpha$–C(16) | |
| C(18) | 179.5 (*s*) | | | |
| Me(19) | 24.0 (*q*) | 1.19 (*s*) | $H_\beta$–C(2), Me(31), $H_\beta$–C(11) | C(5), C(9), C(10) |
| C(20) | 85.0 (*s*) | | | |
| Me(21) | 28.2 (*q*) | 1.47 (*s*) | | C(17), C(20), C(22) |
| $CH_2$(22) | 38.5 (*t*) | 1.88–2.36 (*m*) | | |
| $CH_2$(23) | 22.7 (*t*) | 1.77–1.83 (*m*) | | |
| H–C(24) | 124.4 (*d*) | 5.10 (br. *s*) | | C(26), C(27) |
| C(25) | 132.5 (*s*) | | | |
| Me(26) | 25.9 (*q*) | 1.62 (*s*) | | C(24), C(25), C(27) |
| Me(27) | 22.2 (*q*) | 1.68 (*s*) | | C(24), C(25), C(26) |
| Me(30) | 17.3 (*q*) | 1.10 (*s*) | $H_\alpha$–C(1) (Xyl$^1$), $H_\alpha$–C(3), $H_\alpha$–C(5) | C(3), C(4), C(5), C(31) |
| Me(31) | 28.7 (*q*) | 1.21 (*s*) | Me(19) | C(3), C(4), C(5), C(30) |
| Me(32) | 32.2 (*q*) | 1.12 (*s*) | $H_\alpha$–C(16) | C(8), C(13), C(14) |
| Me*C*O | 169.8 (*s*) | | | |
| *Me*CO | 21.2 (*q*) | 1.99 (*s*) | | Me*C*O |

[a]) Measured at 150 MHz in $C_5D_5N/D_2O$ 4 : 1. [b]) Measured at 600 MHz in $C_5D_5N/D_2O$ 4 : 1.

102.5, 104.9, and 105.5). The NMR signals were fully assigned by 2D-NMR experiments including DQ-COSY, TOCSY, HMQC, HMBC, and NOESY (*Table 2*). The large vicinal coupling constants ($^3J$(1,2) from 7.2 to 7.6 Hz) of the anomeric signals indicated *trans*-diaxial orientations with respect to their coupling partners ($\beta$-configurations). Direct support of the sequence of the sugars and binding sites came from the results of the HMBC experiment, which showed the following cross-peaks: H–C(1) (Xyl$^1$)/C(3), H–C(1) (Qui$^2$)/C(2) (Xyl$^1$), H–C(1) (Xyl$^3$)/C(4) (Qui$^2$), and H–C(1) (MeGlc$^4$)/C(3) (Xyl$^3$). The oligosaccharide moiety of **1** was identical to that of intercedenside B from *M. intercedens* [3]. The positions of the sulfate groups were

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

| | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(C)[a]) | $\delta$(H)[b]) | $\delta$(C)[a]) | $\delta$(H)[b]) |
| Xyl[1](1→C(3)) | | | | |
| H–C(1) | 104.7 (*d*) | 4.79 (*d*, *J*=7.0) | 104.9 (*d*) | 4.78 (*d*, *J*=7.0) |
| H–C(2) | 83.1 (*d*) | 3.94–4.03 (*m*) | 83.3 (*d*) | 3.93–4.04 (*m*) |
| H–C(3) | 75.8 (*d*) | 3.90–4.04 (*m*) | 75.6 (*d*) | 3.89–4.01 (*m*) |
| H–C(4) | 75.3 (*d*) | 5.15–5.21 (*m*) | 75.5 (*d*) | 5.14–5.22 (*m*) |
| $CH_2$(5) | 64.1 (*t*) | 4.65–4.71 (*m*), | 64.3 (*t*) | 4.66–4.71 (*m*), |
| | | 3.63–3.68 (*m*) | | 3.65–3.70 (*m*) |
| Qui[2] (1→2) | | | | |
| H–C(1) | 102.5 (*d*) | 4.99 (*d*, *J*=7.6) | 102.9 (*d*) | 5.01 (*d*, *J*=7.6) |
| H–C(2) | 81.1 (*d*) | 4.04–4.13 (*m*) | 81.4 (*d*) | 4.06–4.14 (*m*) |
| H–C(3) | 74.5 (*d*) | 4.27–4.37 (*m*) | 75.0 (*d*) | 4.26–4.38 (*m*) |
| H–C(4) | 87.7 (*d*) | 3.58–3.63 (*m*) | 87.4 (*d*) | 3.54–3.60 (*m*) |
| H–C(5) | 71.6 (*d*) | 3.66–3.70 (*m*) | 71.9 (*d*) | 3.67–3.72 (*m*) |
| Me(6) | 18.4 (*q*) | 1.68 (*d*, *J*=6.2) | 18.0 (*q*) | 1.64 (*d*, *J*=6.0) |
| Xyl[3](1→4) | | | | |
| H–C(1) | 104.9 (*d*) | 4.87 (*d*, *J*=7.2) | 104.6 (*d*) | 4.86 (*d*, *J*=7.2) |
| H–C(2) | 73.9 (*d*) | 3.84–3.97 (*m*) | 73.8 (*d*) | 3.85–3.94 (*m*) |
| H–C(3) | 87.7 (*d*) | 4.23–4.30 (*m*) | 87.5 (*d*) | 4.21–4.29 (*m*) |
| H–C(4) | 68.9 (*d*) | 4.15–4.21 (*m*) | 68.8 (*d*) | 4.14–4.19 (*m*) |
| $CH_2$(5) | 66.8 (*t*) | 4.37–4.43 (*m*), | 66.5 (*t*) | 4.34–4.41 (*m*), |
| | | 3.72–3.78 (*m*) | | 3.72–3.75 (*m*) |
| MeGlu[4](1→3) | | | | |
| H–C(1) | 105.5 (*d*) | 5.26 (*d*, *J*=7.6) | 105.6 (*d*) | 5.27 (*d*, *J*=7.6) |
| H–C(2) | 75.1 (*d*) | 3.91–3.96 (*m*) | 74.9 (*d*) | 3.89–3.94 (*m*) |
| H–C(3) | 87.9 (*d*) | 3.61–3.67 (*m*) | 87.8 (*d*) | 3.61–3.66 (*m*) |
| H–C(4) | 70.9 (*d*) | 3.95–4.04 (*m*) | 70.6 (*d*) | 3.93–4.02 (*m*) |
| H–C(5) | 77.9 (*d*) | 3.98–4.04 (*m*) | 78.1 (*d*) | 4.00–4.05 (*m*) |
| $CH_2$(6) | 62.0 (*t*) | 4.10–4.16 (*m*), | 62.3 (*t*) | 4.11–4.16 (*m*), |
| | | 4.42 (*dd*, *J*=11.4, 12.0) | | 4.45 (*dd*, *J*=11.6, 12.0) |
| MeO | 60.8 (*q*) | 3.85 (*s*) | 61.1 (*q*) | 3.83 (*s*) |

[a]) Measured at 150 MHz in $C_5D_5N/D_2O$ 4 : 1. [b]) Measured at 600 MHz in $C_5D_5N/D_2O$ 4 : 1.

determined by comparing the $^{13}$C-NMR data of **1** with those of the didesulfated derivative **1a** (*Table 3*); esterification shifts (from 70.9 to 75.3 and 76.6 to 81.1) were observed for the signals of C(4) (Xyl[1]) and C(2) (Qui[2]), indicating that two sulfate groups were located at C(4) of Xyl[1] and C(2) of Qui[2], respectively. Therefore, the structure of compound **1** was deduced as disodium (3$\beta$,9$\beta$,16$\beta$)-16-(acetyloxy)-18-oxo-18,20-epoxylanosta-7,24-dien-3-yl 3-*O*-methyl-$\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-xylopyranosyl-(1→4)-6-deoxy-2-*O*-sulfonato-$\beta$-D-glucopyranosyl-(1→2)-4-*O*-sulfonato-$\beta$-D-xylopyranoside (**1**).

Pentactaside C (**2**), colorless amorphous powder, was analyzed by HR-ESI-MS as $C_{55}H_{84}Na_2O_{28}S_2$, identical to the molecular formula of **1**. The NMR spectral features of **2** (*Tables 2* and *4*) and of didesulfopentactaside C (**2a**) (*Tables 3* and *5*) are similar to

Table 3. *$^1$H- and $^{13}$C-NMR Data for the Sugar Moiety of Didesulfopentactasides B* (**1a**) *and C* (**2a**) ($\delta$ in ppm, $J$ in Hz)

| | **1a** | | **2a** | |
|---|---|---|---|---|
| | $\delta$(C)[a]) | $\delta$(H)[b]) | $\delta$(C)[a]) | $\delta$(H)[b]) |
| Xyl[1](1→C(3)) | | | | |
| H–C(1) | 105.8 (*d*) | 4.75 (*d*, *J*=7.2) | 104.9 (*d*) | 4.74 (*d*, *J*=7.2) |
| H–C(2) | 84.4 (*d*) | 3.98–4.05 (*m*) | 83.8 (*d*) | 3.97–4.05 (*m*) |
| H–C(3) | 78.3 (*d*) | 4.01–4.09 (*m*) | 76.5 (*d*) | 3.91–4.02 (*m*) |
| H–C(4) | 70.9 (*d*) | 5.12–5.19 (*m*) | 70.4 (*d*) | 5.14–5.20 (*m*) |
| $CH_2$(5) | 66.8 (*t*) | 4.65–4.72 (*m*), 3.63–3.67 (*m*) | 65.3 (*t*) | 4.64–4.71 (*m*), 3.64–3.70 (*m*) |
| Qui[2](1→2) | | | | |
| H–C(1) | 105.7 (*d*) | 4.93 (*d*, *J*=7.6) | 103.2 (*d*) | 5.04 (*d*, *J*=7.6) |
| H–C(2) | 76.6 (*d*) | 4.03–4.12 (*m*) | 76.1 (*d*) | 4.05–4.15 (*m*) |
| H–C(3) | 75.6 (*d*) | 4.25–4.34 (*m*) | 75.3 (*d*) | 4.21–4.35 (*m*) |
| H–C(4) | 86.0 (*d*) | 3.59–3.65 (*m*) | 86.9 (*d*) | 3.52–3.59 (*m*) |
| H–C(5) | 71.9 (*d*) | 3.64–3.71 (*m*) | 71.8 (*d*) | 3.67–3.73 (*m*) |
| Me(6) | 17.7 (*q*) | 1.67 (*d*, *J*=6.2) | 17.9 (*q*) | 1.66 (*d*, *J*=6.0) |
| Xyl[3](1→4) | | | | |
| H–C(1) | 105.6 (*d*) | 4.90 (*d*, *J*=7.6) | 104.9 (*d*) | 4.89 (*d*, *J*=7.6) |
| H–C(2) | 73.5 (*d*) | 3.86–4.02 (*m*) | 73.7 (*d*) | 3.85–3.96 (*m*) |
| H–C(3) | 87.5 (*d*) | 4.24–4.30 (*m*) | 87.4 (*d*) | 4.21–4.28 (*m*) |
| H–C(4) | 69.1 (*d*) | 4.14–4.21 (*m*) | 68.9 (*d*) | 4.15–4.20 (*m*) |
| $CH_2$(5) | 66.5 (*t*) | 4.38–4.43 (*m*), 3.71–3.78 (*m*) | 66.5 (*t*) | 4.37–4.43 (*m*), 3.72–3.77 (*m*) |
| MeGlc[4](1→3) | | | | |
| H–C(1) | 105.3 (*d*) | 5.27 (*d*, *J*=7.6) | 105.4 (*d*) | 5.25 (*d*, *J*=7.8) |
| H–C(2) | 75.0 (*d*) | 3.90–3.97 (*m*) | 74.9 (*d*) | 3.89–3.95 (*m*) |
| H–C(3) | 88.0 (*d*) | 3.62–3.69 (*m*) | 87.9 (*d*) | 3.61–3.67 (*m*) |
| H–C(4) | 70.7 (*d*) | 3.95–4.05 (*m*) | 70.6 (*d*) | 3.94–4.04 (*m*) |
| H–C(5) | 78.6 (*d*) | 3.95–4.03 (*m*) | 78.4 (*d*) | 3.99–4.05 (*m*) |
| $CH_2$(6) | 62.3 (*t*) | 4.08–4.14 (*m*), 4.32–4.40 (*m*) | 62.1 (*t*) | 4.11–4.17 (*m*), 4.32–4.45 (*m*) |
| MeO | 60.8 (*q*) | 3.85 (*s*) | 61.0 (*q*) | 3.82 (*s*) |

[a]) Measured at 150 MHz in $C_5D_5N/D_2O$ 4 : 1. [b]) Measured at 600 MHz in $C_5D_5N/D_2O$ 4 : 1.

those of **1** and **1a**, respectively, except for signals due to the side chain, and thus indicated that **2** and **1** possess the same tetrasaccharide chain and holotane ring system. The full assignment of the $^1$H- and $^{13}$C-NMR signals by 2D-NMR and MS experiments resulted in the establishment of the structure of **2** as disodium (3*β*,9*β*,16*β*)-16-(acetyloxy)-18-oxo-18,20-epoxylanosta-7,25-dien-3-yl 3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-6-deoxy-2-*O*-sulfonato-*β*-D-glucopyranosyl-(1→2)-4-*O*-sulfonato-*β*-D-xylopyranoside (**2**).

The $^1$H- and $^{13}$C-NMR spectra of **2** showed resonances for a trisubstituted C(7)=C(8) bond ($\delta$(C) 120.1 (C(7)) and 145.5 (C(8)); 5.64 (br. *s*, H–C(7))), and a disubstituted terminal C=C bond ($\delta$(C) 145.6 (C(25)) and 110.6 (C(26)); 4.77 (*s*, $CH_2$(26))). The $^1$H-NMR spectrum also showed an olefinic Me signal at $\delta$(H) 1.67 (*s*, Me(27)) and of five Me groups characteristic of a holostane skeleton at $\delta$(H) 1.05 (*s*,

Table 4. *$^1$H- and $^{13}$C-NMR Data for the Aglycone Moiety of Pentactaside C* (**2**) ($\delta$ in ppm, $J$ in Hz)

| | $\delta$(C)[a]) | $\delta$(H)[b]) | NOESY | HMBC |
|---|---|---|---|---|
| $CH_2$(1) | 35.6 (*t*) | 1.34–1.86 (*m*) | | |
| $CH_2$(2) | 26.4 (*t*) | 1.83–2.05 (*m*) | | |
| H–C(3) | 88.3 (*d*) | 3.18 (*dd*, *J* = 4.2, 11.4) | $H_\alpha$–C(1) (Xyl$^1$), $H_\alpha$–C(5), Me(30) | C(1)(Xyl$^1$) |
| C(4) | 39.5 (*s*) | | | |
| H–C(5) | 47.5 (*d*) | 0.99 (*t*, *J* = 7.8) | $H_\alpha$–C(3), Me(30) | C(4), C(7) C(30), C(31) |
| $CH_2$(6) | 22.4 (*t*) | 1.95–2.09 (*m*) | | |
| H–C(7) | 120.1 (*d*) | 5.64 (br. *s*) | | |
| C(8) | 145.5 (*s*) | | | |
| H–C(9) | 46.9 (*d*) | 3.45 (br. *d*, *J* = 14.4) | | |
| C(10) | 36.0 (*s*) | | | |
| $CH_2$(11) | 22.5 (*t*) | 1.51–1.76 (*m*) | H–C(9) | |
| $CH_2$(12) | 30.8 (*t*) | 1.96–2.15 (*m*) | $H_\alpha$–C(11), $H_\beta$–C(11) | |
| C(13) | 59.3 (*s*) | | | |
| C(14) | 47.5 (*s*) | | | |
| $CH_2$(15) | 43.2 (*t*) | 1.74–1.86 (*m*, $H_\beta$), 2.56 (*dd*, *J* = 8.1, 12.0, $H_\alpha$) | $H_\alpha$–C(16) | |
| H–C(16) | 74.2 (*d*) | 5.88 (*ddd*, *J* = 7.8, 8.4, 9.0) | $H_\alpha$–C(17), $H_\alpha$–C(15) | C(17), Me*C*O |
| H–C(17) | 54.0 (*d*) | 2.63 (*d*, *J* = 9.0) | $H_\alpha$–C(16) | C(13), C(21) |
| C(18) | 179.6 (*s*) | | | |
| Me(19) | 24.1 (*q*) | 1.21 (*s*) | $H_\beta$–C(2), Me(31), $H_\beta$–C(11) | C(1), C(5), C(9), C(10) |
| C(20) | 85.1 (*s*) | | | |
| Me(21) | 28.3 (*q*) | 1.46 (*s*) | | C(17), C(20), C(22) |
| $CH_2$(22) | 38.4 (*t*) | 1.85–2.33 (*m*) | | |
| $CH_2$(23) | 23.8 (*t*) | 1.95–2.04 (*m*) | | |
| $CH_2$(24) | 38.2 (*t*) | 1.97–2.06 (*m*) | | C(26), C(27) |
| C(25) | 145.6 (*s*) | | | |
| $CH_2$(26) | 110.6 (*t*) | 4.77 (*s*) | | |
| Me(27) | 22.0 (*q*) | 1.67 (*s*) | | |
| Me(30) | 17.2 (*q*) | 1.05 (*s*) | $H_\alpha$–C(1) (Xyl$^1$), $H_\alpha$–C(3), $H_\alpha$–C(5) | C(3), C(5), C(31) |
| Me(31) | 28.7 (*q*) | 1.11 (*s*) | Me(19) | C(3), C(5), C(30) |
| Me(32) | 31.8 (*q*) | 1.14 (*s*) | $H_\alpha$–C(16) | C(8), C(13), C(14) |
| Me*C*O | 170.2 (*s*) | | | |
| *Me*CO | 21.0 (*q*) | 2.01 (*s*) | | Me*C*O |

[a]) Measured at 150 MHz in $C_5D_5N/D_2O$ 4 : 1. [b]) Measured at 600 MHz in $C_5D_5N/D_2O$ 4 : 1.

Me(30)), 1.11 (*s*, Me(31)), 1.21 (*s*, Me(19)), 1.14 (*s*, Me(32)), and 1.46 (*s*, Me(21)). The positions of two C=C bonds as C(7)=C(8) and C(25)=C(26) were corroborated by the HMBCs Me(32)/C(8), H–C(5)/C(7), H–C(9)/C(7), and Me(27)/C(26), Me(27)/C(24), $CH_2$(26)/C(24), $CH_2$(23)/C(25), respectively. The NOESY spectrum of **2** allowed to established the relative configuration of all stereogenic centers of the aglycone.

Pentactasides B and C (**1** and **2**, resp.) differ from philinopsides A and B, previously isolated from this species [4], only in the location of the second sulfate group on the

Table 5. *$^1$H- and $^{13}$C-NMR Data for the Aglycone Moiety of Didesulfopentactasides B* (**1a**) *and C* (**2a**) ($\delta$ in ppm, $J$ in Hz)

| | **1a** | | **2a** | |
|---|---|---|---|---|
| | $\delta$(C)[a]) | $\delta$(H)[b]) | $\delta$(C)[a]) | $\delta$(H)[b]) |
| 1 | 36.2 (*t*) | 1.43–1.86 (*m*) | 36.1 (*t*) | 1.42–1.87 (*m*) |
| 2 | 27.3 (*t*) | 1.89–2.10 (*m*) | 27.2 (*t*) | 1.78–1.99 (*m*) |
| 3 | 89.3 (*d*) | 3.27 (*dd*, $J$=4.2, 11.9) | 89.1 (*d*) | 3.26 (*d*, $J$=8.8) |
| 4 | 39.7 (*s*) | | 39.4 (*s*) | |
| 5 | 48.1 (*d*) | 0.99–1.06 (*m*) | 48.3 (*d*) | 0.94–2.02 (*m*) |
| 6 | 23.4 (*t*) | 1.96–2.07 (*m*) | 23.1 (*t*) | 1.95–2.04 (*m*) |
| 7 | 120.5 (*d*) | 5.63 (br. *s*) | 120.2 (*d*) | 5.64 (br. *s*) |
| 8 | 145.7 (*s*) | | 145.4 (*s*) | |
| 9 | 47.2 (*d*) | 3.75 (*m*) | 47.1 (*d*) | 3.46 (br. *d*, $J$=14.4) |
| 10 | 35.7 (*s*) | | 35.9 (*s*) | |
| 11 | 22.7 (*t*) | 1.54–1.77 (*m*) | 22.6 (*t*) | 1.53–1.79 (*m*) |
| 12 | 31.4 (*t*) | 1.98–2.27 (*m*) | 31.2 (*t*) | 1.96–2.17 (*m*) |
| 13 | 59.3 (*s*) | | 59.2 (*s*) | |
| 14 | 47.6 (*s*) | | 47.5 (*s*) | |
| 15 | 43.7 (*t*) | 2.61 (*m*, $H_\alpha$), 1.63–1.72 (*m*, $H_\beta$) | 43.4 (*t*) | 2.58 (*dd*, $J$=8.1, 12.0, $H_\alpha$), 1.74 (*m*, $H_\beta$) |
| 16 | 75.1 (*d*) | 5.89 (*m*) | 75.2 (*d*) | 5.88 (*m*) |
| 17 | 54.6 (*d*) | 2.68 (*m*) | 54.1 (*d*) | 2.69 (*d*, $J$=9.0) |
| 18 | 179.5 (*s*) | | 179.2 (*s*) | |
| 19 | 24.1 (*q*) | 1.21 (*s*) | 23.8 (*q*) | 1.19 (*s*) |
| 20 | 85.0 (*s*) | | 85.3 (*s*) | |
| 21 | 28.4 (*q*) | 1.44 (*s*) | 28.3 (*q*) | 1.48 (*s*) |
| 22 | 38.8 (*t*) | 1.60–1.89 (*m*) | 38.7 (*t*) | 1.97–2.53 (*m*) |
| 23 | 23.8 (*t*) | 1.77–1.90 (*m*) | 23.5 (*t*) | 1.97–2.04 (*m*) |
| 24 | 124.6 (*d*) | 5.05–5.13 (*m*) | 38.3 (*t*) | 1.95–2.03 (*m*) |
| 25 | 132.7 (*s*) | | 145.6 (*s*) | |
| 26 | 26.2 (*q*) | 1.64 (*s*) | 110.3 (*t*) | 4.79 (*s*) |
| 27 | 22.1 (*q*) | 1.67 (*s*) | 22.2 (*q*) | 1.66 (*s*) |
| 30 | 17.3 (*q*) | 1.11 (*s*) | 17.4 (*q*) | 1.08 (*s*) |
| 31 | 28.6 (*q*) | 1.28 (*s*) | 28.6 (*q*) | 1.21 (*s*) |
| 32 | 32.2 (*q*) | 1.17 (*s*) | 31.9 (*q*) | 1.18 (*s*) |
| Me*C*O | 169.9 (*s*) | | 170.1 (*s*) | |
| *Me*CO | 21.4 (*q*) | 1.02 (*s*) | 21.2 (*q*) | 2.00 (*s*) |

[a]) Measured at 150 MHz in $C_5D_5N/D_2O$ 4 : 1. [b]) Measured at 600 MHz in $C_5D_5N/D_2O$ 4 : 1.

quinovose residue instead of xylose[3]. However, the structures assigned to philinopsides A and B in *Chemical Abstracts* [11] correspond to pentactasides B and C (**1** and **2**, resp.).

Compounds **1** and **2** were tested *in vitro* for cytotoxicity against five human tumor cell lines (P-388, A-549, HCT-116, MCF-7, and MKN-28) by using the sulforhodamine (SRB) method [12], and 10-hydroxycamptothecine (HCP) as a positive control. The $IC_{50}$ values were determined on the basis of the observed cell viability after 72 h of drug exposure. The results are listed in *Table 6*. Compound **1** and **2** showed significant activity against all tumor cell lines. Notably, the activity of **1** against HCT-116 ($IC_{50}$=

0.09 μM) and **2** against A-549 ($IC_{50}$ = 0.58 μM) was significantly higher than that of the positive control ($IC_{50}$ of 0.14 and 0.74 μM, resp.). Based on these promising results, pentactasides B (**1**) and C (**2**) certainly deserve further study as potential antitumor agents. Also, more extensive studies are needed before a clear structure–activity relationship can be put forward.

Table 6. $IC_{50}$ *Values* [μM][a]) *of Pentactasides B* (**1**) *and C* (**2**) *against Human Tumor Cells* in vitro

| Cell line | P-388 | A-549 | HCT-116 | MCF-7 | MKN-28 |
|---|---|---|---|---|---|
| **1** | 0.87 ± 0.08 | 1.30 ± 0.13 | 0.09 ± 0.02 | 1.56 ± 0.22 | 0.82 ± 0.09 |
| **2** | 0.75 ± 0.06 | 0.58 ± 0.05 | 0.15 ± 0.07 | 2.30 ± 0.20 | 0.81 ± 0.07 |
| HCP[b]) | 0.23 ± 0.02 | 0.74 ± 0.04 | 0.14 ± 0.01 | 0.90 ± 0.04 | 0.48 ± 0.04 |

[a]) $IC_{50}$ Values are means from three independent experiments (mean ± SD). [b]) 10-Hydroxycamptothecine (pos. control).

## Experimental Part

*General*. Column chromatography (CC): silica gel ($SiO_2$: 200–300 mesh, 10–40 μm; *Yantai*, P. R. China) and *Sephadex LH-20* (20–150 μm; *Merck*). TLC: precoated silica-gel $GF_{254}$ plates (10–40 μm; *Yantai*, P. R. China), detection by spraying with 15% aq. $H_2SO_4$ followed by heating. HPLC: *Agilent-1100* liquid chromatograph equipped with a refractive-index detector and *Zorbax 300 SB-C18* column (5 μm; 250 × 9.4 mm i.d., *Agilent*). M.p.: *XT5-XMT* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-341* polarimeter. IR: *Bruker Vector-22* apparatus; in $cm^{-1}$. NMR: *Bruker Avance-II-600* apparatus; at 600 MHz ($^1H$) and 150 MHz ($^{13}C$); $\delta$ in ppm rel. to $Me_4Si$, *J* in Hz. HR-ESI-MS and ESI-MS: *Q-TOF Micro* LC-MS-MS mass spectrometer; in *m/z*. GC/MS: *Finnigan Voyager* GC/MS apparatus, with *ULTRA-2* column (50 m × 0.2 mm i.d.).

*Animal Material*. Specimens of *P. quadrangularis* were collected from the South China Sea near Guangdong Province, P. R. China, in May 2000. The organism was identified by Prof. *J. R. Fang* of the Fujian Institute of Oceanic Research, P. R. China. A voucher specimen (No. SA200042) was deposited with the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

*Extraction and Isolation.* The sea cucumbers (3.4 kg, dry weight) were cut into pieces and extracted four times with 70% EtOH (4 × 5 l, each for 1 h) under reflux. The combined EtOH extracts were evaporated, and the residue was dissolved in $H_2O$, and then passed through a *DA101* resin column (60 × 30 cm; Nankai University, Tianjin, P. R. China), eluting with $H_2O$ and 50% aq. EtOH. The combined 50% EtOH eluate was evaporated and subjected to CC (*Sephadex LH-20* (3 × 50 cm); MeOH/$H_2O$ 2:1). The fraction containing saponins was resubjected to CC (dry column (2 × 50 cm) of $SiO_2$; $CHCl_3$/MeOH/$H_2O$ 7:3:1, lower phase). Each subfraction containing saponins was purified by reversed-phase HPLC (*Zorbax SBC-18*; 60% MeOH, 1.5 ml/min) to afford **1** (38 mg; $t_R$ 22.1 min) and **2** (25 mg; $t_R$ 24.5 min).

*Pentactaside B* (= *Disodium (3β,9β,16β)-16-(Acetyloxy)-18-oxo-18,20-epoxylanosta-7,24-dien-3-yl 3-*O*-Methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-6-deoxy-2-*O*-sulfonato-β-*D*-glucopyranosyl-(1→2)-4-*O*-sulfonato-β-*D*-xylopyranoside*; **1**). Colorless amorphous powder. M.p. 209–211°. $[\alpha]_D^{20} = -26.7$ ($c = 0.5$, pyridine). IR (KBr): 3456 (OH), 1742 (C=O), 1643 (C=C), 1237, 1210 ($SO_3Na$). $^1H$- and $^{13}C$-NMR: *Tables 1* and *2*. ESI-MS (pos.): 1325 ($[M+Na]^+$), 1205 ($[M+Na-NaHSO_4]^+$), 1085 ($[M+Na-2\,NaHSO_4]^+$). ESI-MS (neg.): 1279 ($[M-Na]^-$). HR-ESI-MS (pos.): 1325.6125 ($[M+Na]^+$, $C_{55}H_{84}Na_3O_{28}S_2^+$; calc.1325.6128).

*Pentactaside C* (= *Disodium (3β,9β,16β)-16-(Acetyloxy)-18-oxo-18,20-epoxylanosta-7,25-dien-3-yl 3-*O*-Methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-6-deoxy-2-*O*-sulfonato-β-*D*-glucopyranosyl-(1→2)-4-*O*-sulfonato-β-*D*-xylopyranoside*; **2**). Colorless amorphous powder. M.p. 218–220°.

$[\alpha]_D^{20} = -13.4$ ($c = 0.5$, pyridine). IR (KBr): 3426 (OH), 1738 (C=O), 1631 (C=C), 1266, 1215 ($SO_3Na$). $^1$H- and $^{13}$C-NMR: *Tables 4* and *2*. ESI-MS (pos.): 1325 ($[M+Na]^+$). ESI-MS (neg.): 1279 ($[M-Na]^-$). HR-ESI-MS (pos.): 1325.6123 ($[M+Na]^+$, $C_{55}H_{84}Na_3O_{28}S_2^+$; calc. 1325.6128).

*Acid Hydrolysis.* The glycoside **1** or **2** (1 mg) was heated in 2M $CF_3COOH$ (1 ml) at 120° for 2 h. The mixture was evaporated to dryness, and the residue was partitioned between $CH_2Cl_2$ and $H_2O$. The aq. phase was concentrated *in vacuo*. Then, pyridine (1 ml) and $NH_2OH \cdot HCl$ (2 mg) were added to the dried residue, and the mixture was stirred at 90° for 30 min. After that time, 1 ml of $Ac_2O$ was added, and the heating at 90° was continued for another hour. The soln. was concentrated, and the resulting aldononitrile peracetates were analyzed by GC/MS. The carbohydrates were determined by comparing the retention times and MS behavior with standard aldononitrile peracetates prepared from authentic sugars by the same procedure performed for the sample. Xylose, quinovose, and 3-*O*-methyl-glucose were identified in a ratio of 2:1:1.

*Desulfation of Pentactaside B* (**1**) *and Pentactaside C* (**2**). Glycoside **1** or **2** (10 mg) was dissolved in pyridine/dioxane 1:1 (3.0 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 HPLC eluting with 80% MeOH at a rate of 1.5 ml/min to yield the pure **1a** (8 mg) or **2a** (7.5 mg).

*Didesulfopentactaside B* (=*(3β,9β,16β)-3-{[3-*O*-Methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-6-deoxy-β-*D*-glucopyranosyl-(1→2)-β-*D*-xylopyranosyl]oxy}-18-oxo-18,20-epoxylanosta-7,24-dien-16-yl Acetate*; **1a**). White amorphous powder. M.p. 214–216°. $[\alpha]_D^{20} = -30.8$ ($c = 0.5$, pyridine). $^1$H- and $^{13}$C-NMR: *Tables 3* and *5*. ESI-MS (pos.): 1121 ($[M+Na]^+$). ESI-MS (neg.): 1097 ($[M-H]^-$).

*Didesulfopentactaside C* (=*(3β,9β,16β)-3-{[3-*O*-Methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-xylopyranosyl-(1→4)-6-deoxy-β-*D*-glucopyranosyl-(1→2)-β-*D*-xylopyranosyl]oxy}-18-oxo-18,20-epoxylanosta-7,25-dien-16-yl Acetate*; **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 *5*. ESI-MS (pos.): 1121 ($[M+Na]^+$). ESI-MS (neg.): 1097 ($[M-H]^-$).

*Cytotoxicity Tests.* The cytotoxicities of **1** and **2** against leukemic cells (P388), human colon cancer (HCT-116), human breast cancer (MCF-7), human gastric cancer (MKN-28), and human lung cancer (A-549) cells were evaluated by the sulforhodamine-B (SRB) assay, as described in [12], with the anticancer agent 10-hydroxycamptothecin as a positive control. Briefly, the target tumor cells were grown to log phase in *RPMI-1640* medium containing 10% fetal bovine serum (FBS). After dilution to $4 \times 10^4$ cells/ml with complete medium, 90 µl of the obtained cell suspension was added to each well of a 96-well culture plate. Cultures were pre-incubated for 24 h in a humidified 5% $CO_2$ atmosphere at 37°. Then, control or test soln. (DMSO as solvent; 10 µl) was put into each well, and the plate was incubated for additional 72 h. At the end of exposure, the cells were fixed by addition of 50 µl of cold 50% $CCl_3COOH$ at 48° for 1 h, and the plates were washed with tap water (5×), and air-dried. Then, 0.4% SRB soln. (50 µl) in 1% AcOH was added, and staining was allowed to proceed for 30 min. The residual dye was washed out with 1% AcOH (4×), and the plates were air-dried. To each well, 10 mM non-buffered *Tris* soln. (150 µl) was added, and the optical density of each well was measured with a microplate reader at 520 nm. The activities of **1**, **2**, and the positive control were determined at 100, 10, 1, 0.1, and 0.01 µM (each concentration being tested in triplicate). Data were calculated as percent inhibition of $I$ according to the formula: $I = (100 - (OD_t/OD_s) \times 100)$, where $OD_t$ and $OD_s$ are the mean optical densities of the test compounds and the solvent control, resp. The concentration inducing 50% inhibition of cell growth ($IC_{50}$) was determined graphically for each experiment by curve fitting using the Prism 4.0 software (*GraphPad*) and the equation derived by *DeLean et al.* [13]. $IC_{50}$ Values represent the mean of three independent experiments, and are expressed as mean ± S.D. using *Student*'s *t*-test.

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