# Structures of Four New Triterpenoid Oligoglycosides: DS-Penaustrosides A, B, C, and D from the Sea Cucumber Pentacta australis

Tomofumi Miyamoto, Kenichi Togawa, Ryuichi Higuchi, Tetsuya Komori, and Takuma Sasaki

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# STRUCTURES OF FOUR NEW TRITERPENOID OLIGOGLYCOSIDES: DS-PENAUSTROSIDES A, B, C, AND D FROM THE SEA CUCUMBER *PENTACTA AUSTRALIS*

TOMOFUMI MIYAMOTO, KENICHI TOGAWA, RYUICHI HIGUCHI, TETSUYA KOMORI,*

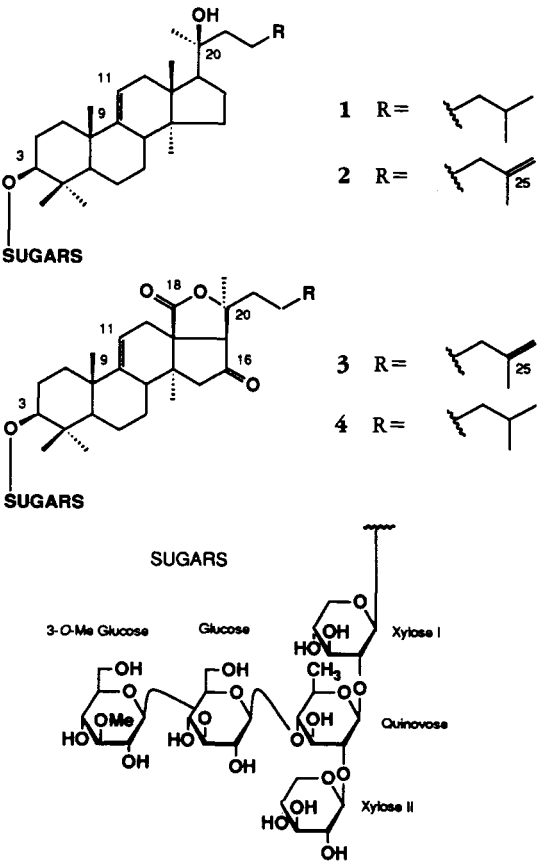
*Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, 812 Fukuoka, Japan*

and TAKUMA SASAKI

*Cancer Research Institute, Kanazawa University, Takaramachi 13-1, Kanazawa, 920, Japan*

ABSTRACT.—Two new non-holostane-type triterpenoid oligoglycosides, DS-penaustrosides A [**1**] and B [**2**], were isolated from the solvolysate of a crude glycoside fraction obtained from a sea cucumber *Pentacta australis*, together with two holostane-type glycosides, DS-penaustrosides C [**3**] and D [**4**]. The structure of **1**–**4** have been elucidated on the basis of spectral and chemical evidence.

Continuing with a previous study (1), the isolation and characterization of biologically active compounds from a sea cucumber of the family Cucumariidae have been conducted. In this paper, we report the structures of four new triterpenoid oligoglycosides, DS-penaustrosides A [**1**], B [**2**], C [**3**], and D [**4**], obtained from the whole bodies of the sea cucumber, *Pentacta australis* Ludwig. The prefix DS indicates that the compound is a desulfated derivative of the parent sulfated glycoside. This is the first isolation of lanosterol-type triterpenoid oligoglycosides from a sea cucumber.



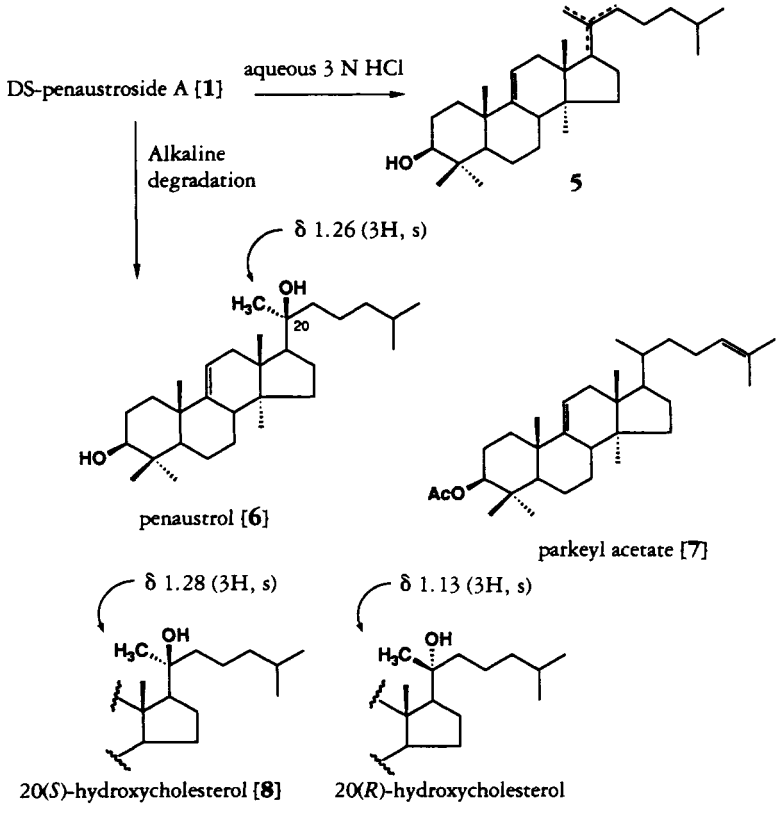
## RESULTS AND DISCUSSION

The *n*-BuOH-soluble part of the MeOH extract obtained from the whole bodies of *P. australis* (5.1 kg) was subjected to Si gel cc to furnish a crude glycoside fraction. Since this fraction showed a positive potassium rhodizonate test (1), indicating the existence of sulfated substances, the fraction was treated with 1,4-dioxane and pyridine, followed by purification by reversed-phase cc on Lobar to give four triterpenoid oligoglycosides, DS-penaustrosides A [**1**], B [**2**], C [**3**], and D [**4**].

DS-penaustroside A [**1**], $C_{59}H_{100}O_{24}$, showed ir absorptions due to a hydroxy (3430 $cm^{-1}$) and a glycosidic linkage (1100, 1080 $cm^{-1}$). Acidic hydrolysis (aqueous 3 N HCl) of **1** provided an artifact aglycone **5** consisting of double bond isomers formed by elimination of the tertiary hydroxy group of **1**, 2 mol of xylose (Xyl), and 1 mol each of quinovose (Qui), glucose (Glc), and 3-*O*-methyl Glc (3-*O*-Me Glc).

On the other hand, alkaline degradation (2) of **1** provided the genuine aglycone penaustrol [**6**] (Scheme 1).

Compound **6** was assigned the molecular formula $C_{30}H_{52}O_2$ from the hreims spectrum. The $^1H$- and $^{13}C$-nmr spectra of **6** suggested the presence of 2 secondary methyls, 6 tertiary methyls, 10 methylenes, 4 methines, 4 quaternary carbons, 1 tertiary alcohol, 1 secondary alcohol, and a trisubstituted double bond. These data indicated that **6** is a tetracyclic triterpenoid possessing 2 hydroxyl groups and 1 double bond. The $^{13}C$-nmr spectrum of **6** was similar to that of parkeyl acetate [3β-acetoxylanosta-9(11),24-diene [**7**] (3)], except for the signals due to the side chain moiety; on the other hand the signals ascribable to the side chain group resembled those of




SCHEME 1

20(*S*)-hydroxycholesterol [**8**]. The *S* configuration of C-20 was also suggested by comparison of the C-21 methyl proton signal of **6** [δ 1.26 (3H, s)] with that of 20(*S*)- and 20(*R*)-hydroxycholesterol [δ 1.28 (3H, s) and 1.13 (3H, s)] (4–6) in their $^1$H-nmr spectra. Accordingly, the structure of **6** must be 20(*S*)-3β,20-dihydroxylanost-9(11)-ene.

Comparison of the $^{13}$C-nmr spectra of compounds **1** and **6** indicated that the oligosaccharide moiety of **1** was linked to the C-3 hydroxyl group of **6** (Table 1), and the structure of the pentasaccharide moiety was determined as follows. Partial hydrolysis of **1** with aqueous 0.4 N HCl and hot $H_2O$/1,4-dioxane, provided two prosapogenins, **9** and **10**, respectively, which were characterized as a monoxyloside and a tetrasaccharide of **5** on the basis of spectral data (Scheme 2).

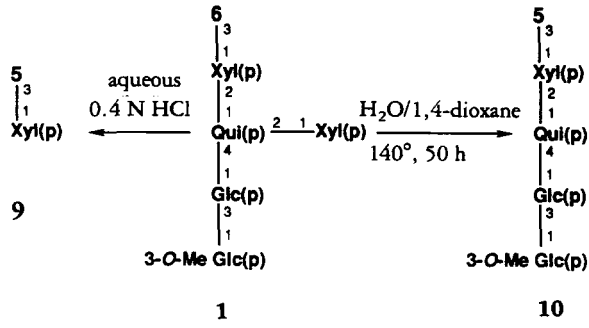
By taking the gc-ms analysis of the partially methylated alditol acetates prepared from the permethylated derivative of **10** (methylation analysis) and the negative fabms fragmentation of **10** into account, the sugar moiety of **10** could be determined as 3-*O*-Me-Glc(p)-(1→3)-Glc(p)-(1→4)-Qui(p)-(1→2)-Xyl(p). Based on the above facts and chemical (methylation analysis) and spectral (negative fabms data), the oligosaccharide moiety of **1** is regarded as 3-*O*-Me-Glc(p)-(1→3)-Glc(p)-(1→4)-[Xyl(p)-(1→2)]-

TABLE 1. Comparison of $^{13}$C nmr Data (67.5 MHz) of DS-penaustrosides A [**1**], B [**2**], C [**3**], and D [**4**], Penaustrol [**6**], Parkeyl acetate [**7**], 20(*S*)-Hydroxycholesterol [**8**], and DS-psoluthurin A.

| Carbon | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **6** | **7** and **8**[a] | **3** | **4** | DS-psoluthurin A |
| C-1 . . . | 36.6 (t) | 36.7 (t) | 36.1 (t) | 35.8 (t) | 36.4 (t) | 36.4 (t) | 36.5 |
| C-2 . . . | 27.3 (t) | 27.3 (t) | 27.8 (t) | 24.2 (t) | 27.2 (t) | 27.2 (t) | 27.4 |
| C-3 . . . | 88.9 (d) | 88.9 (d) | 78.9 (d) | 80.9 (d) | 88.8 (d) | 88.8 (d) | 89.0 |
| C-4 . . . | 39.4 (s)[b] | 39.5 (s)[b] | 39.1 (s)[b] | 38.0 (s) | 39.8 (s) | 39.8 (s) | 39.9 |
| C-5 . . . | 53.2 (d) | 53.2 (d) | 52.5 (d) | 52.6 (d) | 53.0 (d) | 53.0 (d) | 53.1 |
| C-6 . . . | 21.6 (t) | 21.6 (t) | 21.4 (t) | 21.1 (t) | 21.2 (t) | 21.2 (t) | 22.3 |
| C-7 . . . | 28.5 (t) | 28.5 (t) | 28.1 (t) | 28.0 (t) | 28.6 (t) | 28.6 (t) | 28.7 |
| C-8 . . . | 41.7 (d) | 41.7 (d) | 41.4 (d) | 41.7 (d) | 38.8 (d) | 38.8 (d) | 38.9 |
| C-9 . . . | 149.0 (s) | 149.0 (s) | 148.7 (s) | 148.1 (s) | 151.4 (s) | 151.4 (s) | 151.5 |
| C-10 . . . | 40.0 (s)[b] | 40.0 (s)[b] | 39.4 (s)[b] | 39.2 (s) | 40.0 (s) | 40.0 (s) | 40.2 |
| C-11 . . . | 115.3 (d) | 115.3 (d) | 114.8 (d) | 115.2 (d) | 111.1 (d) | 111.1 (d) | 111.2 |
| C-12 . . . | 38.1 (t) | 38.1 (t) | 37.5 (t) | 36.4 (t) | 32.2 (t) | 32.2 (t) | 32.3 |
| C-13 . . . | 45.2 (s) | 45.2 (s) | 44.2 (s) | 44.3 (s) | 55.8 (s) | 55.8 (s) | 55.8 |
| C-14 . . . | 47.6 (s) | 47.6 (s) | 47.3 (s) | 47.0 (s) | 42.1 (s) | 42.1 (s) | 42.2 |
| C-15 . . . | 33.9 (t) | 33.9 (t) | 33.4 (t) | 33.9 (t) | 52.1 (t) | 52.1 (t) | 52.1 |
| C-16 . . . | 22.7 (t) | 22.8 (t) | 22.1 (t) | 28.0 (t) | 213.1 (s) | 213.1 (s) | 213.1 |
| C-17 . . . | 53.3 (d) | 53.4 (d) | 52.4 (d) | 50.9 (d) | 61.4 (d) | 61.5 (d) | 61.5 |
| C-18 . . . | 16.6 (q) | 16.6 (q) | 16.1 (q) | 14.4 (q) | 176.0 (s) | 175.7 (s) | 176.1 |
| C-19 . . . | 23.0 (q) | 22.6 (q) | 22.3 (q) | 22.3 (q) | 22.1 (q) | 22.2 (q) | 21.3 |
| C-20 . . . | 74.2 (s) | 74.1 (s) | 75.3 (s) | 75.3 (s) | 83.1 (s) | 83.2 (s) | 83.2 |
| C-21 . . . | 26.3 (q) | 26.2 (q) | 25.8 (q) | 26.4 (q) | 22.4 (q) | 22.7 (q) | 22.6 |
| C-22 . . . | 45.7 (t) | 45.0 (t) | 44.7 (t) | 44.2 (t) | 38.6 (t) | 39.2 (t) | 38.6 |
| C-23 . . . | 22.6 (t) | 22.8 (t) | 22.0 (t) | 22.0 (t) | 22.5 (t) | 22.7 (t) | 22.4 |
| C-24 . . . | 40.1 (t) | 38.8 (t) | 39.6 (t) | 39.6 (t) | 38.1 (t) | 39.4 (t) | 38.2 |
| C-25 . . . | 28.3 (d) | 146.2 (s) | 27.9 (d) | 27.9 (d) | 145.6 (s) | 30.0 (d) | 145.7 |
| C-26 . . . | 22.5 (q) | 110.4 (d) | 22.6 (q) | 22.6 (q) | 110.6 (t) | 22.6 (q) | 110.7 |
| C-27 . . . | 22.6 (q) | 28.3 (q)[c] | 22.7 (q) | 22.7 (q) | 28.2 (q) | 22.8 (q) | 28.2 |
| C-28 . . . | 19.1 (q) | 19.1 (q) | 18.7 (q) | 18.5 (q) | 20.7 (q) | 20.8 (q) | 20.8 |
| C-29 . . . | 28.2 (q) | 28.2 (q)[c] | 28.3 (q) | 28.2 (q) | 26.9 (q) | 26.9 (q) | 26.9 |
| C-30 . . . | 17.0 (q) | 17.0 (q) | 15.7 (q) | 16.8 (q) | 16.9 (q) | 16.9 (q) | 16.8 |

[a]The signals of **8** are underlined.

[b,c]Assignments may be reversed in each column.




SCHEME 2

Qui(p)-(1↦2)-Xyl(p). The configurations of each monosaccharide were considered to be β, on the basis of their anomeric proton signals [δ = 4.86 (d, $J$ = 7.3 Hz), 4.93 (d, $J$ = 7.6 Hz), 5.22 (δ, $J$ = 7.3 Hz), 5.30 (δ, $J$ = 7.6 Hz), 5.41 (δ, $J$ = 6.8 Hz)] in the $^1$H-nmr spectrum of **1**. Accordingly, if Xyl, Qui, Glc, and 3-*O*-Me Glc are assumed to be of the commonly found D series, DS-penaustroside A [**1**] is determined to be 20(*S*)-hydroxy-3-*O*-{β-D-3-*O*-methylglucopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[β-D-xylopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦2)-β-D-xylopyranosyl}lanost-9(11)-ene.

Penaustroside B [**2**], $C_{59}H_{98}O_{24}$, exhibited spectral features quite similar to those of **1** except for signals due to a terminal isobutyl functionality in the aglycone moiety [$^1$H nmr δ 4.84 (2H, br s), 1.54 (3H, s), $^{13}$C nmr see Table 1]. Thus the aglycone moiety of **2** was assignable to 20(*S*)-3β,20-dihydroxylanost-9(11),25-diene, which was supported by the conversion of **2** into **1** on catalytic hydrogenation.

Consequently, if each monosaccharide is assumed to belong to the D series, the structure of DS-penaustroside B is considered to be 20(*S*)-hydroxy-3-*O*-{β-D-3-*O*-methylglucopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[β-D-xylopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦2)-β-D-xylopyranosyl}lanosta-9(11),25-diene.

Acid hydrolysis of DS-penaustroside C [**3**], $C_{59}H_{92}O_{26}$, provides the same aglycone mixture as holotoxin A (7), together with 2 mol of Xyl, and 1 mol each of Qui, Glc, and 3-*O*-Me Glc. The carbon signals in the aglycone moiety of **3** resemble those of DS-psoluthurin A (8), which contains the same aglycone as holotoxin A. These data suggested that **3** is the 3-*O*-pentaglycoside of holotoxigenin [3β-hydroxyholosta-9(11),25-dien-16-one]. The signals of the sugar moiety of **3** observed in the $^1$H-nmr and $^{13}$C-nmr spectra agree well with those observed in the corresponding spectra of **1**. Consequently, DS-penaustroside C must be 3-*O*-{β-D-3-*O*-methylglucopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[β-D-xylopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦2)-β-D-xylopyranosyl}holosta-9(11),25-dien-16-one.

The spectral data reveal that DS-penaustroside D [**4**], $C_{59}H_{94}O_{26}$, is a compound related to **3**, differing from the latter by the structure of the aglycone moiety. Comparison between $^{13}$C-nmr spectra of **4** and **3** shows that the aglycone moiety of **4** was assignable to the $\Delta^{25}$-dihydro derivative of **3**, because the signals due to the terminal isopropenyl functionality [δ 145.6 (s), 110.6 (t), 28.3 (q)] in **3** changed to those due to a terminal isopropyl functionality [δ 30.0 (d), 22.6 (q), 22.8 (q)] in **4**. This has been confirmed by the chemical conversion of **3** into **4** on catalytic hydrogenation. Thus, the structure of DS-penaustroside D has been determined as 3-*O*- { β-D-3-*O*-methylglucopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[β-D-xylopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦2)-β-D-xylopyranosyl } holost-9(11)-en-16-one.

Although a variety of holostane-type triterpenoid oligoglycosides have been obtained from Holothuroidea (9,10), DS-penaustrosides A [**1**] and B [**2**] are the first examples of non-holostane-type glycosides. Compounds **1** and **2** are also interesting because they are presumed to be precursors of DS-penaustrosides C [**3**] and D [**4**].

DS-penaustrosides A and B revealed cytotoxic activities against murine lymphoma L1210 and human epidermoid carcinoma KB cell lines [$IC_{50}$ = 0.12 (L1210) and 0.62 (KB) μg/ml for **1**, 2.1 (L1210) and 4.6 (KB) μg/ml for **2**].

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Except for those described below, experimental procedures were as described previously (1).

COLLECTION AND ISOLATION.—Whole specimens (5.1 kg, wet wt) of mature *P. australis*, collected in February 1987 from the Ariake Sea in Saga Prefecture (a voucher specimen of the animal is maintained in our laboratory, Kyushu University), were homogenized and extracted with MeOH (72 liters), and the extract was filtered. The MeOH layer was evaporated in vacuo and the residue partitioned between $H_2O$ (3 liters) and $CHCl_3$ (5 liters). The $H_2O$ layer was extracted with *n*-BuOH (5 liters) and the organic layer evaporated in vacuo to give 5.5 g extractive. Part of this (2.03 g) was chromatographed on a Si gel column. Elution was performed with $CHCl_3$-MeOH-$H_2O$ (2:1:0.1) to yield 6 fractions: fractions 1 (567 mg), 2 (131 mg), 3 (71 mg), 4 (537 mg), 5 (167 mg), and 6 (36 mg). Fraction 4 (135 mg) was refluxed in pyridine-dioxane (1:1) (12 ml) for 3 h. After cooling, the reaction mixture was diluted with $H_2O$, extracted with *n*-BuOH, and evaporated in vacuo. The *n*-BuOH extract (100.5 mg) was chromatographed on Lobar Lichroprep RP-8 (Grosse B, Merck). Elution with 80% MeOH yielded compounds **1** (21.8 mg), **2** (6.6 mg), **3** (16.1 mg), and **4** (3.1 mg).

CARBOHYDRATE ANALYSIS.—The glycoside (ca. 2 mg) was heated in a screw-cap vial with aqueous 3 N HCl (1 ml) at 100°for 3 h, and the precipitated aglycone was removed by filtration. The filtrate, containing sugars, was evaporated to dryness under reduced pressure. The sugar mixture was dissolved in pyridine (0.05 ml), and 1-(trimethylsilyl)imidazole (0.05 ml) was added and the reaction mixture heated at 60° in a stoppered test tube for 15 min before being analyzed by glc (3% Silicone OV-17 on Chromosorb W; column 2.5 mm × 1 m; gas pressure 1.0 $kg/cm^{-2}$ $N_2$, 1.5 $kg \cdot cm^{-2}$ $H_2$, 0.9 $kg \cdot cm^{-2}$ air; column temperature 120°, injector temperature 240°, detector temperature 210°, FID mode). By means of carbohydrate analysis, the following compounds were identified: Xyl (Rt 4.5, 5.5, 7.5 min), Qui (Rt 6.8, 8.5 min), Glc (Rt 14.8, 32.5 min), and 3-*O*-Me Glc (Rt 8.5, 12.0 min).

METHYLATION ANALYSIS.—Methylation analysis was conducted as reported previously (1). By this means the following carbohydrates could be detected: 2-linked Xyl(p) ([1D]-1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylxylitol, Rt 5.8 min, *m/z* 190, 130, 117, 101, 88); 2,4-linked Qui(p) ([1D]-1,2,4,5-tetra-*O*-acetyl-3-*O*-Me quinovitol, Rt 7.4 min, *m/z* 203, 190, 143, 130, 117, 87); 4-linked Qui(p) ([1D]-1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylquinovitol, Rt 5.8 min, *m/z* 203, 143, 130, 118, 102, 101); terminal Xyl(p) ([1D]-1,5-di-*O*-acetyl-2,3,4-tri-*O*-methlyxylitol, Rt 4.0 min, *m/z* 162, 161, 118, 117, 102, 101); 3-linked Glc(p) ([1D]-1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucitol, Rt 8.3 min, *m/z* 234, 202, 161, 129, 118, 101, 87); terminal 3-*O*-methyl Glc(p) ([1D]-1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, Rt 6.2 min, *m/z* 205, 162, 161, 145, 129, 118, 102).

DS-PENAUSTROSIDE A [**1**].—White powder: mp 292–296°; $[\alpha]^{24}D$ −2.3° (*c* = 0.5, pyridine); hr fabms (negative) found $[M-H]^-$ 1191.6527 ($C_{59}H_{99}O_{24}$ requires 1191.6526); ir (KBr, $cm^{-1}$) 3430, 1110, 1080; fabms (negative) *m/z* $[M-H]^-$ 1191, $[M-133]^-$ 1059, $[M-177]^-$ 1015, 853, 721; $^1H$ nmr (270 MHz, pyridine-$d_5$ + $D_2O$) 0.88 (3H, d, *J* = 6.6 Hz), 0.89 (3H, d, *J* = 6.6 Hz), 0.94 (3H, s), 1.13 (6H, s), 1.20 (3H, s), 1.30 (3H, s), 1.55 (3H, s), 1.72 (3H, d, *J* = 5.3 Hz), 3.89 (3H, s), 4.86 (1H, d, *J* = 7.3 Hz), 4.93 (1H, d, *J* = 7.6 Hz), 5.22 (1H, d, *J* = 7.3 Hz), 5.30 (1H, d, *J* = 7.6 Hz), 5.41 (1H, d, *J* = 6.8 Hz); $^{13}C$ nmr (67.5 MHz, pyridine-$d_5$ + $D_2O$) 105.4 [d, C-1 of Xyl(I)], 83.5 [d, C-2 of Xyl(I)], 78.0 [d, C-3 of Xyl(I)], 70.4 [d, C-4 of Xyl (I)], 66.7 [t, C-5 of Xyl(I)], 103.2 (d, C-1 of Qui), 82.8 (d, C-2 of Qui), 75.9 (d, C-3 of Qui), 86.8 (d, C-4 of Qui), 71.2 (d, C-5 of Qui), 18.2 (q, C-6 of Qui), 106.0 [d, C-1 of Xyl(II)], 75.8 [d, C-2 of Xyl(II)], 77.3 [d, C-3 of Xyl(II), 70.6 [d, C-4 of Xyl(II)], 67.1 [d, C-5 of Xyl(II)], 105.0 (d, C-1 of Glc), 73.7 (d, C-2 of Glc), 88.1 (d, C-3 of Glc), 69.8 (d, C-4 of Glc), 78.0 (d, C-5 of Glc), 62.2 (t, C-6 of Glc), 105.7 (d, C-1 of 3-*O*-Me Glc), 75.1 (d, C-2 of 3-*O*-Me Glc), 88.1 (d, C-3 of 3-*O*-Me Glc), 70.6 (d, C-4 of 3-*O*-Me Glc), 78.4 (d, C-5 of 3-*O*-Me Glc), 62.2 (t, C-6 of 3-*O*-Me Glc), 60.9 (q, *O-Me*).,

*Aglycone* **5**.—Eims *m/z* $[M]^+$ 426, $[M-H_2O]^+$ 406; $^{13}C$ nmr (67.5 MHz, $CDCl_3$) 149.7 (s, C-9), 142.4 (s), 126.3 (s), 125.5 (s), 115.0 (d, C-11), 109.7 (t).

ALKALINE DEGRADATION OF **1**.—DS-Penaustroside A [**1**] (3.7 mg) was dissolved in *n*-BuOH (0.74 ml) and added to NaOH (145 mg). The reaction mixture was heated for 6 h at 80° and filtered. The filtrate was quenched by addition of $H_2O$ (2 ml), neutralized with aqueous HCl, and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated in vacuo to yield 7.1 mg extracts, which was treated by chromatography on Si gel using $CHCl_3$ as eluent to yield the genuine aglycone, penaustrol [**6**] (1.7 mg).

*Penaustrol* [**6**].—Colorless needles: mp 142–144°; $[\alpha]^{25}D$ +18.7° ($c$ = 0.075, $CHCl_3$); hreims found $[M]^+$ 444.3958 ($C_{30}H_{52}O_2$ requires 444.3952); ir ($CHCl_3$, $cm^{-1}$) 3500–3400 (OH), 2925, 2850; fdms *m/z* $[M]^+$ 444 (base peak), 427, 359; $^1H$ nmr (270 MHz, $CDCl_3$), 0.76 (3H, s), 0.82 (3H, s), 0.84 (3H, s), 0.88 (6H, d, $J$ = 6.6 Hz), 0.99 (3H, s), 1.04 (3H, s), 1.26 (3H, s), 3.21 (1H, dd, $J$ = 4.3, 11.2 Hz), 5.23 (1H, br d, $J$ = 5.0 Hz); $^{13}C$ nmr see Table 1.

*Prosapogenin* **9**.—White powder: fdms *m/z* $[M]^+$ 558 (base peak), 409; $^{13}C$ nmr ($CDCl_3$) 104.7 (d, C-1 of Xyl), 74.0 (d, C-3 of Xyl), 72.5 (d, C-2 of Xyl), 70.0 (d, C-4 of Xyl), 63.8 (t, C-5 of Xyl), 90.0 (d, C-3); $^1H$ nmr ($CDCl_3$), 3.0–4.2 (5H, m), 4.49 (1H, d, $J$ = 5.0 Hz).

HYDROTHERMOLYSIS OF **1**.—Compound **1** (13 mg) was dissolved in $H_2O$ (1.5 ml) and 1,4-dioaxane (1.5 ml) and heated in a screw-capped tube at 140° for 50 h. The hydrothermolysate was evaporated in vacuo and chromatographed on an RP-8 column. Elution was performed with a discontinuous gradient of MeOH (50%–100%). The 100% MeOH eluent, when chromatographed on a Si gel cc with $CHCl_3$-MeOH-$H_2O$ (8:2:0.2) as the eluent, yielded prosapogenin **10** (5.4 mg).

*Prosapogenin* **10**.—Fabms (negative, *m/z*) 1041 $[M-H]^-$, 865 $[M-177; A]^-$, 703 $[A-162]^-$.

DS-PENAUSTROSIDE B [**2**].—White powder: mp 220–225°; $[\alpha]^{23}D$ −3.0° ($c$ = 0.6, pyridine); hrfabms (negative) found $[M-H]^-$ 1189.6368 ($C_{59}H_{97}O_{24}$ requires 1189.6367); ir (KBr, $cm^{-1}$) 3430, 1110, 1080; fabms (negative) *m/z* $[M-H]^-$ 1189, $[M-133]^-$ 1057, $[M-177]^-$ 1013, 851, 719; $^1H$ nmr (270 MHz, pyridine-$d_5$ + $D_2O$) 0.93 (3H, s), 1.13 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.31 (3H, s), 1.54 (3H, s), 1.73 (3H, d, $J$ = 5.3 Hz), 3.90 (3H, s), 4.86 (2H, br s), 4.87 (1H, d, $J$ = 7.9 Hz), 4.94 (1H, d, $J$ = 7.9 Hz), 5.23 (1H, d, $J$ = 7.3 Hz), 5.31 (1H, d, $J$ = 7.9 Hz), 5.42 (1H, d, $J$ = 6.8 Hz); $^{13}C$ nmr (67.5 MHz, pyridine-$d_5$ + $D_2O$) 105.4 [d, C-1 of Xyl(I)], 83.5 [d, C-2 of Xyl(I)], 78.0 [d, C-3 of Xyl(I)], 70.5 [d, C-4 of Xyl(I)], 66.7 [t, C-5 of Xyl(I)], 103.2 (d, C-1 of Qui), 82.8 (d, C-2 of Qui), 75.9 (d, C-3 of Qui), 86.9 (d, C-4 of Qui), 71.2 (d, C-5 of Qui), 18.2 (q, C-6 of Qui), 106.1 [d, C-1 of Xyl(II)], 75.9 [d, C-2 of Xyl(II)], 77.3 [d, C-3 of Xyl(II)], 70.7 [d, C-4 of Xyl(II)], 67.2 [d, C-5 of Xyl(II)], 105.0 (d, C-1 of Glc), 73.8 (d, C-2 of Glc), 88.1 (d, C-3 of Glc), 69.8 (d, C-4 of Glc), 78.0 (d, C-5 of Glc), 62.3 (t, C-6 of Glc), 105.8 (d, C-1 of 3-*O*-Me Glc), 75.2 (d, C-2 of 3-*O*-Me Glc), 88.1 (d, C-3 of 3-*O*-Me Glc), 70.5 (d, C-4 of 3-*O*-Me Glc), 78.2 (d, C-5 of 3-*O*-Me Glc), 62.3 (t, C-6 of 3-*O*-Me Glc), 60.9 (q, *O-Me*).

REDUCTION OF **2**.—Compound **2** (2.0 mg) was dissolved in dry EtOH (2 ml) and added to Pd-C (10%, 1 mg). The reaction mixture was stirred at room temperature in the presence of hydrogen at an initial pressure of 2 atm for 4 h. After filtration, the filtrate was evaporated, in vacuo, to yield DS-penaustroside A (0.9 mg), which was analyzed by reversed-phase hplc [ERC-ODS 2151 (Erma Co Ltd.), flow rate 1.5 ml/min, solvent 92% MeOH] with an authentic sample of **1**. The Rt values were 10.2 min for **2**, and 11.7 min for **1**.

DS-PENAUSTROSIDE C [**3**].—White powder: mp 254–260°; $[\alpha]^{23}D$ −77.4° ($c$ = 0.4, pyridine); hrfabms (negative) found $[M-H]^-$ 1215.5779 ($C_{59}H_{92}O_{26}$ requires 1215.5781); ir (KBr, $cm^{-1}$) 3430, 1760, 1740, 1630, 1160, 1070, 1040; fabms (negative) *m/z* $[M-H]^-$ 1215, $[M-133]^-$ 1083, $[M-177]^-$ 1039, 877, 745; cd ($c = 1.0 \times 10^{-4}$, 1,4-dioxane), $[\vartheta]_{228}$ −32000 (negative max), $[\vartheta]_{266}$ +2900 (positive max), $[\vartheta]_{313}$ −17500; $^1H$ nmr (270 MHz, pyridine-$d_5$ + $D_2O$) 0.86 (3H, s), 0.94 (3H, s), 1.13 (3H, s), 1.30 (3H, s), 1.42 (3H, s), 1.45 (3H, s), 1.69 (3H, d, $J$ = 6.3 Hz), 3.90 (3H, s), 4.79 (2H, br s), 4.87 (1H, d, $J$ = 7.3 Hz), 4.95 (1H, d, $J$ = 7.6 Hz), 5.23 (1H, d, $J$ = 7.0 Hz), 5.33 (1H, d, $J$ = 7.9 Hz), 5.36 (1H, d, $J$ = 6.8 Hz); $^{13}C$ nmr (67.5 MHz, pyridine-$d_5$ + $D_2O$) 105.4 [d, C-1 of Xyl(I)], 83.5 [d, C-2 of Xyl(I)], 78.0 [d, C-3 of Xyl(I)], 70.4 [d, C-4 of Xyl(I)], 66.7 [t, C-5 of Xyl(I)], 103.2 (d, C-1 of Qui), 82.7 (d, C-2 of Qui), 75.9 (d, C-3 of Qui), 86.9 (d, C-4 of Qui), 71.2 (d, C-5 of Qui), 18.2 (q, C-6 of Qui), 106.0 [d, C-1 of Xyl(II)], 75.8 [d, C-2 of Xyl(II)], 77.3 [d, C-3 of Xyl(II)], 70.6 [d, C-4 of Xyl(II)], 67.2 [d, C-5 of Xyl(II)], 105.0 (d, C-1 of Glc), 73.8 (d, C-2 of Glc), 88.1 (d, C-3 of Glc), 69.8 (d, C-4 of Glc), 78.1 (d, C-5 of Glc), 62.1 (t, C-6 of Glc), 105.8 (d, C-1 of 3-*O*-Me Glc), 75.1 (d, C-2 of 3-*O*-Me Glc), 88.1 (d, C-3 of 3-*O*-Me Glc), 70.6 (d, C-4 of 3-*O*-Me Glc), 78.4 (d, C-5 of 3-*O*-Me Glc), 62.2 (t, C-6 of 3-*O*-Me Glc), 60.8 (q, *O-Me*).

DS-PENAUSTROSIDE D [**4**].—White powder: mp 278–281°; $[\alpha]^{22}D$ −64.6° ($c$ = 0.12, pyridine); hrfabms (negative) found $[M-H]^-$ 1217.5935 ($C_{59}H_{94}O_{26}$ requires 1217.5955); ir (KBr, $cm^{-1}$) 3420, 1750, 1730, 1680, 1160, 1130, 1070; fabms (negative) *m/z* $[M-H]^-$ 1217, $[M-133]^-$ 1085, $[M-177]^-$ 1041, 879, 747; cd ($c = 2.0 \times 10^{-4}$, 1,4-dioxane) $[\vartheta]_{242}$ −8600, $[\vartheta]_{261}$ +1600 (positive max),

$[\vartheta]_{304}$ −25600 (negative max); $^1$H nmr (270 MHz, pyridine-$d_5$ + $D_2O$), 0.83 (3H, d, $J$ = 6.6 Hz), 0.85 (3H, d, $J$ = 6.6 Hz), 0.95 (3H, s), 1.13 (3H, s), 1.31 (3H, s), 1.42 (3H, s), 1.45 (3H, s), 1.72 (3H, d, $J$ = 5.0 Hz), 3.88 (3H, s), 4.86 (1H, d, $J$ = 7.0 Hz), 4.95 (1H, d, $J$ = 7.3 Hz), 5.23 (1H, d, $J$ = 7.0 Hz), 5.33 (1H, d, $J$ = 7.6 Hz), 5.37 (1H, d, $J$ = 7.0 Hz); $^{13}$C nmr (67.5 MHz, pyridine-$d_5$ + $D_2O$), 105.4 [d, C-1 of Xyl(I)], 83.5 [d, C-2 of Xyl(I)], 78.0 [d, C-3 of Xyl(I)], 70.4 [d, C-4 of Xyl(I)], 66.7 [t, C-5 of Xyl(I)], 103.2 (d, C-1 of Qui), 82.8 (d, C-2 of Qui), 75.9 (d, C-3 of Qui), 86.9 (d, C-4 of Qui), 71.2 (d, C-5 of Qui), 18.2 (q, C-6 of Qui), 106.0 [d, C-1 of Xyl(II)], 75.8 [d, C-2 of Xyl(II)], 77.3 [d, C-3 of Xyl(II)], 70.6 [d, C-4 of Xyl(II)], 67.2 [d, C-5 of Xyl(II)], 105.0 (d, C-1 of Glc), 73.7 (d, C-2 of Glc), 88.1 (d, C-3 of Glc), 69.8 (d, C-4 of Glc), 78.1 (d, C-5 of Glc), 62.1 (t, C-6, of Glc), 105.8 (d, C-1 of 3-*O*-Me Glc), 75.1 (d, C-2 of 3-*O*-Me Glc), 88.1 (d, C-3 of 3-*O*-Me Glc), 70.6 (d, C-4 of 3-*O*-Me Glc), 78.4 (d, C-5 of 3-*O*-Me Glc), 62.2 (t, C-6 of 3-*O*-Me Glc), 60.9 (q, *O-Me*).

CHEMICAL CONVERSION OF **3** INTO **4**.—Compound **3** (3.0 mg) was reduced in a similar manner as compound **2**, to yield DS-penaustroside D (1.3 mg), which was analyzed by reversed-phase hplc [ERC-ODS 2151 (Erma Co Ltd.), flow rate 1.5 ml/min., solvent 85% MeOH] with an authentic sample [**4**]. The Rt values were 8.8 min for **3**, and 9.6 min for **4**.

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