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Philinopgenin A, B, and C, Three New Triterpenoid Aglycones from the Sea Cucumber *Pentacta quadrangulasis*

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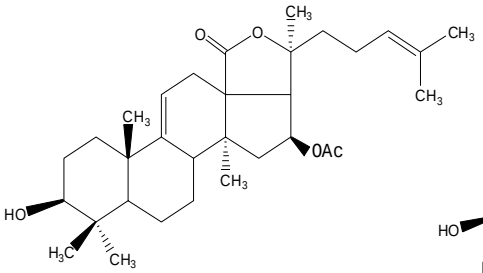
Abstract: Three new triterpenoid aglycones named Philinopgenin A (**1**), B (**2**), and C (**3**) were isolated from the acid hydrolysate of the crude glycoside mixture prepared from the whole sea cucumber *Pentacta quadrangulasis* Lesson. The corresponding structures were determined as 16β-acetoxyholosta-8(9), 24(25)-diene-3β-ol (**1**), 20, 25-epoxy-lanosta-9(11)-ene-3β-ol 18(16)–lactone (**2**) and 16β-acetoxyholosta-9(11), 24(25)-diene-3β-ol (**3**), respectively, on the basis of spectral evidence.

Keywords: *Pentacta quadrangulasis* Lesson, Philinopgenin A, Philinopgenin B, Philinopgenin C, triterpenoid aglycone.

Introduction

Investigations of triterpene glycosides in sea cucumbers (Holothuroidea, Echinodermata) have a long history. In the search for new biologically active substances from marine organisms, we have been investigating antitumour constituents in the sea cucumber *Pentacta quadrangulasis* Lesson, which is widely distributed throughout the South China Sea, especially in the area near Guangdong. Before examining the components of this sea cucumber to elucidate the structure of the biologically active glycosides, we investigated the aglycones. We report herein the isolation, purification, and structural elucidation of three new aglycones named Philinopgenin A (**1**), B (**2**), and C (**3**) from the acid hydrolysate of the crude glycoside fraction.



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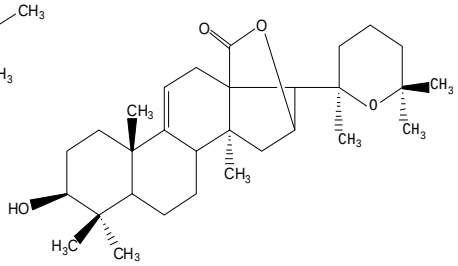

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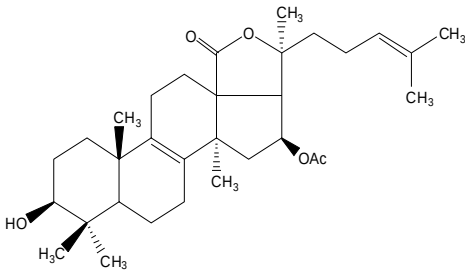

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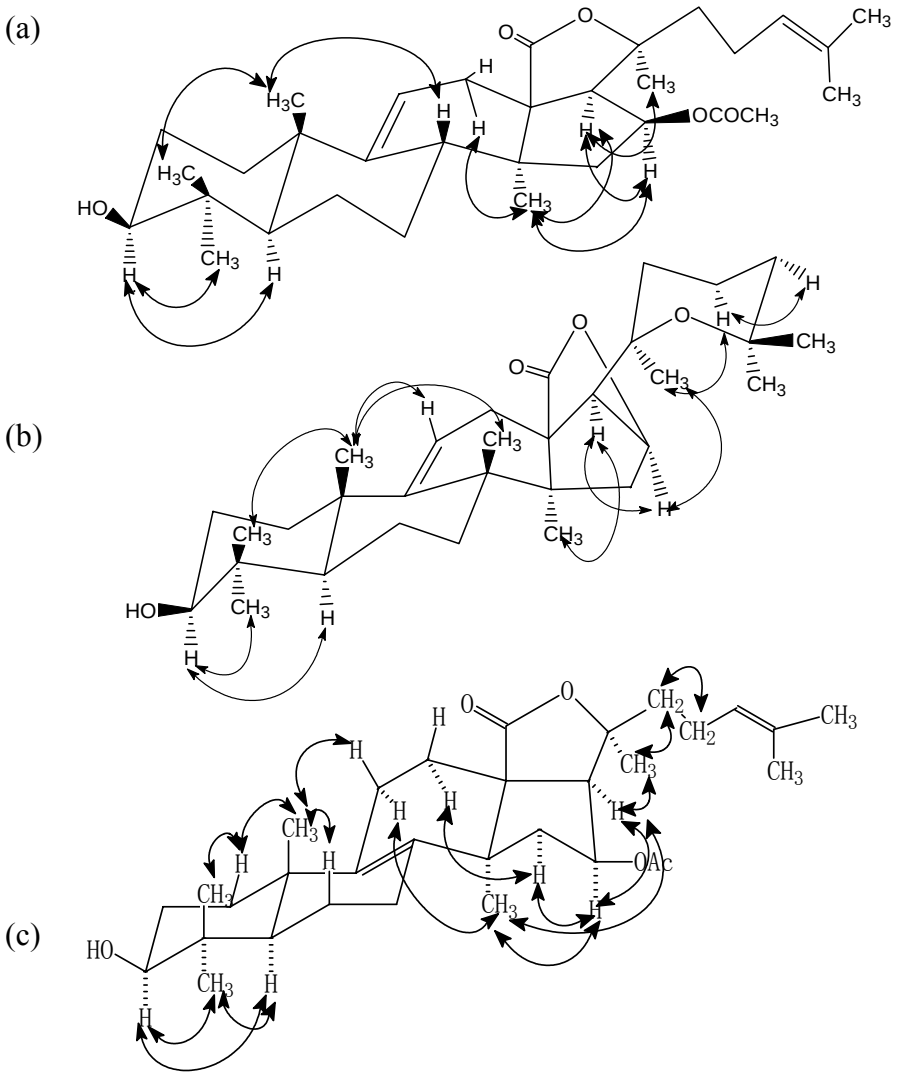
Results and Discussion

The molecular formula of Philinopgenin A (**1**) was determined as $C_{32}H_{48}O_5$ (m/z 512) by the pseudomolecular ion peaks at *m/z* 512 ($[M]^+$) and 535 ($[M + Na]^+$) in the HRESI-MS (positive-ion mode). The IR spectrum showed absorptions due to hydroxyl (3443 cm^{-1}), γ-lactone (1770 cm^{-1}) and carbonyl (1748 cm^{-1}) functions. The structure of compound **1** was deduced from extensive NMR spectroscopy studies (^{1}H- and ^{13}C-NMR, DQCOSY, HMQC, and HMBC, Table 1). These spectra indicated that compound **1** is a triterpenoid compound with two olefinic bonds, one ester, and one lactone carbonyl group. These features showed a close similarity to those reported for the triterpenoid lactone aglycone stichopogenin A_2, isolated from the acid hydrolysate of holotoxin A [1]. The ^{13}C-NMR and DEPT spectra exhibited 32 carbon signals (8×CH_3, 8× CH_2, 7×CH, 9×C). The ^{1}H-, ^{13}C- and DEPT spectra of compound **1** showed resonances for a 9(11)-double bond [δ_C 110.8 (s, C-11) and 151.2 (S, C-9)] and those due to an acetoxy group [δ_C 170.0(s) and 21.4(q); δ_H 1.93(3H, s)]. The location of the acetoxy group at C-16 was deduced from the chemical shift of the H-16 signal (δ 5.76), which showed coupling to signals at δ 2.57 (H-17α), 2.19 (H-15α), and 1.43 (H-15β) in the DQCOSY spectrum. This conclusion was also confirmed by the cross peak at δ 5.76/170.0 (H-16/CH_3CO) in HMBC spectrum. The 16β configuration of the acetoxy group was confirmed by NOESY experiments and by coupling constant analysis for the C-16 proton with the C-17α proton (see Figure 1a). The calculated coupling constant value of 9.6Hz ($J_{16\alpha,\ 17\alpha}$) for the most stable conformation of 16β-acetoxy-holosta-9,24-diene-3β-ol obtained by Molecular Mechanics (Hyperchem) coincided with the experimental and reported values [2] and differed considerably from those calculated for 16α-isomer [1.2Hz ($J_{16\beta,\ 17\alpha}$)]. The ^{1}H-NMR spectrum showed two vinyl methyl signals at δ 1.50 and 1.57 and one olefinic proton signal at δ 5.04, assigned to the terminal isopropenyl group of the side chain. The ^{13}C-NMR spectrum confirmed the presence of this group: two methyl groups at δ 25.5 (C-26) and 17.7 (C-27) attached to olefinic carbons at δ 131.8 (C-25) and 124.4 (C-24). This conclusion was also confirmed in the HMBC spectrum. Therefore the structure of compound **1** was determined as 16β-acetoxyholosta-9(11), 24(25)-diene-3β-ol.

The molecular formula of Philinopgenin B (**2**) was determined as $C_{30}H_{46}O_4$ (m/z 470) by pseudomolecular ion peaks at *m/z* 471 ($[M+H]^+$) and 493 ($[M+Na]^+$) in the HRESI-MS (positive-ion mode). The IR spectrum showed absorptions due to hydroxyl (3446cm^{-1}) and γ-lactone (1770cm^{-1}) groups. The ^{13}C-NMR and DEPT spectrum exhibited 30 carbon signals (7×CH_3, 9× CH_2,

6×CH, 8×C). The ^{1}H- and ^{13}C-NMR spectra of compound **2** (Table 1) showed some similarities to those of compound **1** and to those of the aglycone moieties of cucumarioside G_2 [3]. The main difference from compound **1** being the replacement of the 16 OAc-substitutuent (δ_C 75.0, δ_H 5.63) by one oxygen-bearing methine group (δ_C 79.2, δ_H 4.77) forming the 18(16)-lactone with the C-18 carbonyl group, in agreement with the chemical shifts for C_{15} and C_{17}, and the replacement of the side chain by a 2,2-dimethyl-6-pyranyl moiety. The quaternary carbons at δ_C 73.0 and 71.8 suggested that the side chain is in a pyran form. The correct assignments for the side chain are based the fact that the carbon peaks at δ_C 73.0 (C-20) and 28.2 (C-22) ppm correlate with the methyl protons H-21 and the carbon peaks at δ_C 71.8 (C-25) and 36.5 (C-24) ppm correlate with the methyl protons H-26 and H-27 in the HMBC spectrum. Furthermore, the HMQC spectrum of compound **2** shows that H-22, H-23 and H-24 correlate with the carbon peaks at δ_C 33.8 ppm, 36.5 and 71.8 ppm respectively. The chemical shifts of the H-22 signals (δ 1.48) and H-24 signals (δ 1.36, 1.51) showed coupling to signals at δ 1.58 and 1.76 (H-23) in the DQCOSY spectrum. This conclusion was also confirmed in the NOESY spectrum (see Figure 1b). Thus, compound **2** was determined as 20,25-epoxylanosta-8(9)-ene-3β-ol 18(16)-lactone.

Figure 1: Key NOESY connections in compounds **1** (a), **2** (b) and **3** (c)



The molecular formula of Philinopgenin C (**3**) was determined as $C_{32}H_{48}O_5$ (m/z 512) by pseudomolecular ion peaks at *m/z* 535 ($[M+Na]^+$) in the HRESI-MS (positive-ion mode). Compound **3** shows spectral features (Table 1) similar to those of compound **1**, except for the signals due to the carbon atoms at the B/C-ring junction. These findings suggest that compound **3** is an olefinic function regioisomer of compound **1**. The two olefinic carbon signals at δ=130.2 (s) and 135.6 (s) in the ^{13}C-NMR spectrum of compound **3** indicate that compound **3** is the $\Delta^{8(9)}$ isomer of **1**. Thus, compound **3** can be identified as 16β-acetoxyholosta-8(9), 24(25)-diene-3β-ol.

About 40 genuine and artifact triterpenoid aglycones have been obtained by acidic or enzymatic hydrolysis of crude sea cucumber glycosides [4]. The newly obtained aglycones, described in this paper, differ from previously reported ones in their substitution patterns. It should be noted, however, that compounds **1**, **2**, and **3** may be artifacts arising from the extraction treatment, because the coexistence of minute amounts of other not identified regioisomers was noted in the acid hydrolysate of the glycoside mixture, and signals due to a trisubstituted olefin group ($\Delta^{7(8)}$) were observed in the NMR spectra of the parent oligoglycoside sulfates of this sea cucumber.

Acknowledgments

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Experimental

General

Melting points were determined with a XT5-XMT micro melting point apparatus and are uncorrected. IR spectrum was measured on a BRUKER VECTOR-22 spectrophotometer. The ESI-MS (positive and negative ion modes) was obtained on Micromass Quattro mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ (at 100 MHz and 400 MHz for ^{13}C- and ^{1}H-NMR, respectively) with an Inova-400 spectrometer. Chemical shifts are given in δ values with tetramethylsilane (TMS) as internal standard in ^{1}H-NMR. Silica gel (Yantai, 200-300 mesh) was used for column chromatography. Pre-coated silica gel plates (Yantai, HSG, 0.15-0.20mm) were used for analytical TLC. HPLC was carried out with Zorbax 300 SB-C18 reverse phase.columns (9.4×250mm, Zorbax).

Animal material

Specimens of *P. quadrangnlaris* were collected at different locations around the South China Sea (near Guangdong Province, China) and identified by Prof. J. R. Fang of Fujian Institute of Oceanic Research, China in May 2002. A voucher specimen (No. SA200042) is deposited at the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, China.

Extraction and Purification

Air-dried body walls of the sea cucumber *Pentacta quadrangulasis* (10kg) were extracted with 50% EtOH (50 L). The EtOH was evaporated *in vacuo* to give a crude extract (23.1g), which was partitioned between water (15 L) and chloroform (15 L). The water layer was extracted with n-butanol (15 L) and the organic layer was evaporated *in vacuo* to give the n-butanol extract (16.3g). A portion of this extract (6.0g) was heated at reflux in aqueous 15% H_2SO_4 (2000 mL) and the cooled reaction mixture was then extracted with three portions of chloroform (800 mL, 600 mL and 600 mL), the combined chloroform extracts were washed with water (300 mL), dried over Na_2SO_4 and concentrated to give an aglycone mixture (1.890g). This mixture was separated by flash chromatography on silica gel (3x40cm column, eluent: 4:1 n-hexane/EtOAc, flow rate: 1.5 mL/min) with detection of eluates by TLC (SiO_2, 2:1 n-hexane/EtOAc; SCRC reagent) to yield a main fraction (600.9 mg, v_R: 80mL-160mL). This fraction was further separated by HPLC (85% MeOH/H_2O, flow rate 1.5 mL/min) to afford Philinopgenin A (**1**) (54.4 mg, t_R: 15.91min), Philinopgenin B (**2**) (63 mg, t_R: 16.86min) and Philinopgenin C (**3**) (77.7 tR: 17.50min).

Spectral Data

Philinopgenin A (**1**): a white powder, mp 208.5-208.5 °C; IR (KBr): ν = 3443, 2970, 2937, 2869, 1770, 1748, 1378, 1240 cm^{-1}; EIMS: m/z = 512 $[M]^+$, 493[M-H_2O, A], 479 [A-CH_3, B], 435, 69 [C_5H_9, base peak]; ^{13}C-NMR and ^{1}H-NMR: see Table 1.

Philinopgenin B (**2**): a white powder, mp 212.5-213.5°C; IR (KBr): ν = 3446, 2968, 2934, 2869, 1770, 1649 cm^{-1}; EIMS: m/z = 470 $[M^+]$, 452 [m^+-H_2O], 391, 109, 69 [C_5H_9, base peak]; ^{13}C-NMR and ^{1}H- NMR: see Table 1.

Philinopgenin C (**3**): a white powder, mp 216.5-217.5 °C; IR (KBr): ν = 3466, 2968, 2935, 1869, 1770, 1748, 1649, 1030 cm^{-1}; EIMS: m/z = 513 [M+1], 494 [M+1-H_2O, A], 479 [A-CH_3, B], 435, 69 [C_5H_9, base peak]. ^{13}C-NMR and ^{1}H-NMR: see Table 1.

Table 1. ^{13}C- and ^{1}H NMR data for compounds **1, 2,** and **3**

Compound		**1**		**2**		**3**
Position	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]	δ_H mult[b] (*J* in Hz)
1	36.6t	H_α 1.44 m H_β 1.80	35.9t	H_α 1.43 m H_β 1.87 m	36.0t	H_α 1.45 m H_β 1.84 m
2	28.0t	H_α H_β	27.8t	H_α1.68 m H_β 1.72 m	27.8t	H_α H_β
3	79.1d	3.34, dd (5.2, 11.6)	78.3d	3.20 dd, (4.6, 11.6)	79.0d	3.21 dd, (5.2, 11.6)
4	40.0s		39.2s		38.5s	

Table 1. Cont.

Compound	**1**		**2**		**3**	
Position	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]	δ_H mult[b] (*J* in Hz)
5	52.6d	0.90, d, 11.0	52.6d	0.86 tr	52.5d	0.90 m
6	21.4t	H_α 1.58 m	21.1t	H_α 1.46 m	21.0t	H_α 2.07 m
		H_β 1.75 m		H_β 1.70 m		H_β 2.07 m
7	28.0t	H_α	28.3t	H_α 1.78 m	27.8t	H_α 1.36 m
		H_β		H_β 1.78 m		H_β 1.62 m
8	39.6d	3.22, dd (2.4, 13.2)	43.4d		130.2s	
9	151.2s		151.3s		135.6s	
10	40.5s		39.4s		39.3s	
11	110.8d	5.16s	113.4d	5.40 d, (6.0)	27.8t	
12	34.0t	H_α 2.36 m	24.9t	H_α 2.33 m	32.3t	H_α 2.40 m
		H_β 2.36 m		H_β 2.40 m		H_β 2.45 m
13	58.6s		54.8s		59.6s	
14	43.2s		40.4s		44.7s	
15	44.1t	H_α 2.19 m	42.0t	H_α1.56 m	41.1t	H_α 2.22 m
		H_β 1.43m		H_β 1.74 m		H_β 1.42m
16	75.0d	5.76 m	79.2d	4.77 tr	75.0d	5.63 m
17	52.9d	2.57 d (9.6)	63.5d	2.25 m	52.3d	2.51 d, (9.6)
18	176.7s		178.1s		176.8s	
19	22.3q	1.30 s	21.5q	1.25 s	21.8q	1.19 s
20	84.8s		73.0s		84.4s	
21	28.8q	1.36 s	26.0s	1.25 s	28.2q	1.50 s
22	38.5t	H_α 1.96 m	33.8t	H_α1.48 m	38.4t	H_α 1.76 m
		H_β 2.30m		H_β 1.48 m		H_β 2.42 m
23	23.8t	H_α 1.98 m	16.3t	H_α 1.58 m	23.5t	H_α 1.92 m
		H_β 2.02 m		H_β 1.76 m		H_β 2.11 m
24	124.4d	5.04 d (11.2)	36.5t	H_α 1.36 m)	123.7d	5.05 d, (11.2)
25	131.8s		71.8s		132.1s	
26	25.5q	1.57 s	32.7q	1.13 s	25.5q	1.69 s
27	17.7q	1.50 s	28.4q	1.20 s	18.6q	1.60 s
30	16.4q	0.98 s	15.2q	0.80 s	15.4q	0.85 s
31	28.6q	1.10 s	28.3q	0.98 s	28.1q	1.02 s
32	21.4q	0.80 s	21.7q	1.07 s	27.0q	1.10 s
$CH_3\underline{C}OO$	170.0s				170.3s	
$\underline{CH_3}COO$	21.4q	1.93 s			21.0q	2.02 s

[a] Recorded at 100MHz in $CDCl_3$; multiplicity by DEPT.
[b] Recorded at 400MHz in $CDCl_3$

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Sample Availability: Samples are available from the authors.