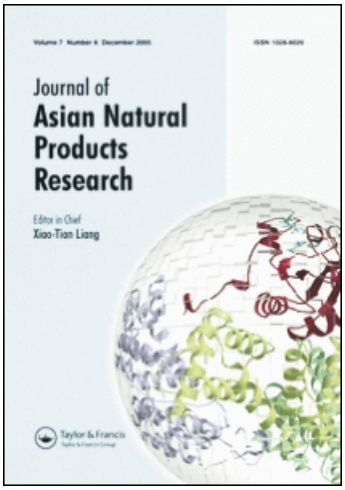

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Zhang, Li-Xin , Fan, Xiao and Shi, Jian-Gong(2005) 'A novel polyhydroxyl sterol from Asterina pectinifera', Journal of Asian Natural Products Research, 7: 1, 25 — 29
To link to this Article: DOI: 10.1080/1028602310001608994
URL: http://dx.doi.org/10.1080/1028602310001608994

Journal of Asian Natural Products Research, Vol. 7, No. 1, February 2005, 25–29

A novel polyhydroxyl sterol from *Asterina pectinifera*

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(Received 8 April 2003; revised 19 May 2003; in final form 26 May 2003)

A novel polyhydroxyl sterol (**1**) along with one known polyhydroxyl sterol (**2**), and two known monoglycosides, asterosaponin P_1 (**3**) and its desulfated monoglycoside (**4**), have been isolated from the whole bodies of a common Pacific starfish *Asterina pectinifera*. The structure of the new polyhydroxyl sterol was determined as 15β,16β-isopropylidenedioxy-5α-cholestane-3β,4β,6α,7α,8,26-hexaol by spectroscopic methods, including FABMS, HR-FABMS, 1D and 2D NMR techniques.

Keywords: *Asterina pectinifera*; Starfish; Polyhydroxyl sterol

1. Introduction

Asterina pectinifera Müller et Troschel (Hai Yan in Chinese), belonging to the Echinodermata phylum, Asteroidea class, is a starfish commonly found in the North Pacific Ocean [1]. It has been used as a crude drug to treat rheumatism and as a tonic in traditional Chinese medicine [2]. From its whole bodies nine steroid oligoglycoside sulfates, including asterosaponin P_1 [1], asterosaponin P_2 [3] and pectiniosides A–G [4–7], and several polyhydroxyl sterols [7,8], have been isolated. The structures and biological activities, such as hemolytic, cytotoxic, antibacterial, antiviral and antiinflammatory activities, have been reviewed [9]. In our chemical investigation of this invertebrate collected in Qingdao, China, a novel polyhydroxyl sterol (**1**) along with one known polyhydroxyl sterol (**2**), and two known monoglycosides, asterosaponin P_1 (**3**) and its desulfated monoglycoside (**4**), have been isolated. On the basis of spectral analyses, the structure of **1** was elucidated as 15β,16β-isopropylidenedioxy-5α-cholestane-3β,4β,6α,7α,8,26-hexaol. This paper reports the isolation and structural elucidation of compound **1**.

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020310001608994

2. Results and discussion

The fresh starfish was extracted with 95% EtOH at room temperature and the concentrated extract was then suspended in water and extracted with light petroleum, EtOAc and n-BuOH successively. The n-BuOH extract was chromatographed over HPD-100 macroporous resin, normal-phase silica gel and Sephadex LH-20 to yield compounds **1**–**4** (figure 1).

Compound **1** was obtained as a light yellow amorphous powder and showed a strong broadened absorption band at 3415 cm^{-1} in the IR spectrum due to hydroxyl groups. The positive FABMS exhibited a quasi-molecular ion peak at *m/z* 563 $[M + Na]^+$ and the molecular formula of **1** was established as $C_{30}H_{52}O_8$ by HR-FABMS. The ^1H NMR (DMSO-d_6) spectrum of **1** showed signals of six methyls at δ 1.25 (s, Me-18), 1.04 (s, Me-19), 0.86 (d, $J = 6.5$ Hz, Me-21), 0.81 (d, $J = 6.5$ Hz, Me-27), 1.25 (s, Me-2′) and 1.41 (s, Me-3′), six oxymethines at δ 3.21 (m, H-3), 3.96 (brs, H-4), 4.01 (dd, $J = 11.5$, 2.5 Hz, H-6), 3.62 (d, $J = 2.5$ Hz, H-7), 4.71 (t, $J = 6.0$ Hz, H-15), and 4.52 (t, $J = 6.0$ Hz, H-16), and one oxymethylene at δ 3.13 (m, 23-H_a) and 3.22 (m, 23-H_b). The presence of these groups was confirmed by ^{13}C NMR (DMSO-d_6) and DEPT spectral data (table 1). In addition, the ^{13}C NMR and DEPT displayed one oxygenated quaternary carbon (δ 77.3, C-8), one dioxygenated quaternary carbon (δ 109.0, C-1′) and two quaternary sp^3 carbons (δ 36.1, C-10; 42.5, C-13) as well as seven methylene and six methine carbons (table 1).

The ^{13}C NMR spectral data of compound **1** were very similar to those of 5α-cholestane-3β,4β,6α,7α,8,15β,16β,26-octol [8], except for the downfield shifts of C-15 ($\Delta\delta$ 6.3 ppm) and C-16 ($\Delta\delta$ 8.2 ppm) signals, and additional signals at δ 109.0, 23.1 and 25.2, which are attributed to an isopropylidenedioxy unit in the ^{13}C NMR of **1**. Therefore, the structure of **1** was suggested to be 15β,16β-isopropylidenedioxy-5α-cholestane-3β,4β,6α,7α,8,26-hexaol.

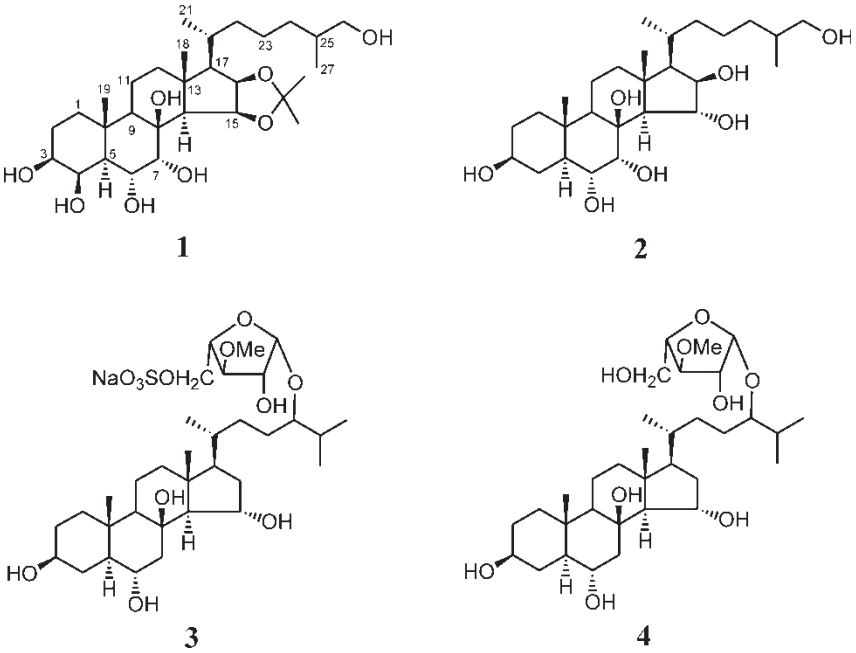

Figure 1. Structures of compounds **1**–**4**.

Table 1. ^{13}C and ^{1}H NMR data for compound **1**.

No.	δ_C	δ_H	*No.*	δ_C	δ_H
1	38.5t	(a) 0.83 (1H, m)	16	80.9d	4.52 (1H, t, $J = 6.0$ Hz)
		(b) 1.48 (1H, m)	17	58.9d	0.90 (1H, m)
2	25.4t	(a) 1.38 (1H, m)	18	16.3q	1.25 (3H, s)
		(b) 1.63 (1H, m)	19	16.2q	1.04 (3H, s)
3	71.7d	3.21 (1H, m)	20	28.9d	1.16 (1H, m)
4	67.4d	3.96 (1H, brs)	21	17.8q	0.86 (3H, d, $J = 6.5$ Hz)
5	46.0d	1.31 (1H, m)	22	35.0t	(a) 1.13 (1H, m)
6	64.2d	4.01 (1H, dd, $J = 11.5$, 2.5 Hz)			(b) 1.54 (1H, m)
7	75.3d	3.62 (1H, d, $J = 2.5$ Hz)	23	22.8t	(a) 1.22 (1H, m)
8	77.3s				(b) 1.60 (1H, m)
9	49.1d	1.09 (1H, m)	24	33.4t	(a) 0.94 (1H, m)
10	36.1s				(b) 1.39 (1H, m)
11	17.2t	(a) 1.31 (1H, m)	25	35.4d	1.51 (1H, m)
		(b) 1.66 (1H, m)	26	66.1t	(a) 3.13 (1H, m)
12	42.4t	(a) 1.05 (1H, m)			(b) 3.22 (1H, m)
		(b) 1.77 (1H, m)	27	17.0q	0.81 (3H, d, $J = 6.5$ Hz)
13	42.5s		1′	109.0s	
14	53.0d	1.40 (1H, m)	2′	23.1q	1.24 (3H, s)
15	77.3d	4.71 (1H, t, $J = 6.0$ Hz)	3′	25.2q	1.41 (3H, s)

To confirm the structural assignment of **1**, $^{1}H-^{1}H$ COSY, phase sensitive $^{1}H-^{1}H$ COSY, HMQC, HMBC and NOESY experiments were carried out. Detailed analyses of the $^{1}H-^{1}H$ COSY, phase sensitive $^{1}H-^{1}H$ COSY and HMQC led to unambiguous assignments of ^{1}H and ^{13}C signals in the spectra (table 1). A comprehensive interpretation of the HMBC spectrum (figure 2) confirmed the cholestane nucleus and the locations of the six hydroxyl and one isopropylidenedioxy groups in the structure of **1**. In combination with the chemical shift values of the protons and carbons, the HMBC correlations from H-3 to C-1 and C-5, H-4 to C-2 and C-10, H-6 to C-8 and C-10, H-7 to C-5 and C-9 confirmed the locations of hydroxyl groups at C-3, C-4, C-6, C-7 and C-8, while the HMBC correlations from H-15, H-16, H-2′ and H-3′ to C-1′ revealed the location of the isopropylidenedioxy at C-15 and C-16. The relative stereochemistry of compound **1** was confirmed by the NOESY spectrum (figure 3). Accordingly, the structure of **1** is 15β,16β-isopropylidenedioxy-5α-cholestane-3β,4β,6α,7α,8,26-hexaol. Compound **1** was proposed to be a natural polyhydroxyl isopropylidenedioxy sterol since the extraction and isolation procedure was carried out in the absence of acetone.

Compounds **2**–**4** were characterized by comparing their FABMS, ^{1}H and ^{13}C NMR spectral data with those in the literature [1,8], and were confirmed by $^{1}H-^{1}H$ COSY, HMQC, HMBC and NOESY experiments.

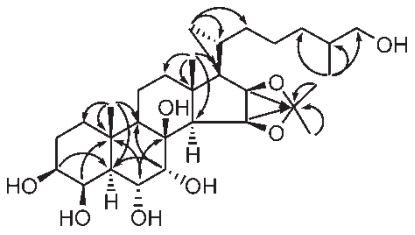

Figure 2. Key HMBC correlations of compound **1**.

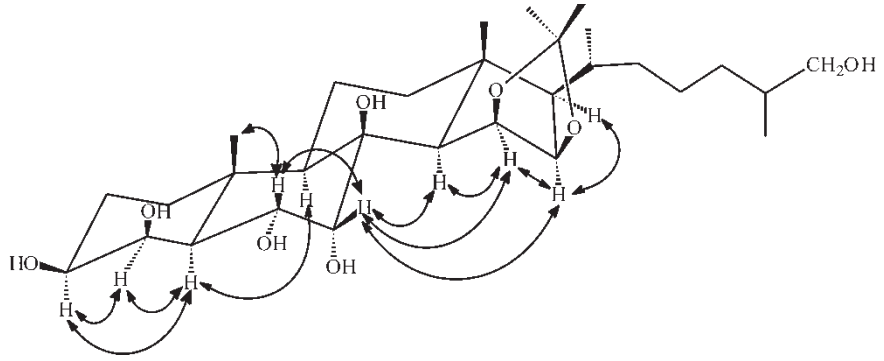

Figure 3. NOESY correlations for compound **1**.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet IMPACT 400 FT-IR instrument. NMR spectra were recorded on a Varian INOVA-500 spectrometer in DMSO-d_6. Positive-ion FABMS and HRFABMS were obtained using a glycerol matrix on a Micromass Autospec-Ultima ETOF spectrometer. HPD-100 (corresponding to XAD-2) macroporous resin was produced by Hebei Cangzhou Bon Chemical Co., Ltd. Adsorption column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China). TLC was carried out with glass silica gel GF254 plates (Qingdao Marine Chemical Inc., China). Spots were visualized under UV by first spraying with 5% H_2SO_4 in 95% EtOH followed by heating. All solvents used were either spectral grade or analytical reagents.

3.2 Animal material

The fresh animals of *Asterina pectinifera* were collected near DaMaiDao of Qingdao, Shandong Province, China in April 2002. Their identification was verified by Professor Yulin Liao (Department of Invertebrates Taxology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China).

3.3 Extraction and isolation

The fresh animals (20 kg) were cut into small pieces and extracted with 95% EtOH at room temperature (3 ×, 7 days each time). The solvent was then removed under reduced pressure at <40°C to give a residue (560 g) that was chromatographed on a macroporous resin column and eluted gradiently with H_2O–MeOH (100% $H_2O \rightarrow$ 100% MeOH). The fraction (15 g) eluted with 50% MeOH was repeatedly chromatographed on silica gel columns (solvent: $CHCl_3$–MeOH–H_2O), and then Sephadex LH-20 columns

(solvent: MeOH–H_2O, 50–100%) to yield compounds **1** (15 mg), **2** (43 mg), **3** (150 mg) and **4** (8 mg).

3.4 Compound 1

A light yellow, amorphous powder, mp 140–145°C; $[\alpha]_D^{18} = +8.5$ (*c* 1.06, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3415, 2939, 2870, 1456, 1383, 1282, 1203, 1026, 976, 879, 760, 677, 633; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see table 1; positive FABMS *m/z* 563 $[M + Na]^+$ (75), positive HR-FABMS *m/z* 563.3547 (calcd. for $C_{30}H_{52}O_8Na$, 563.3560).

Acknowledgements

We thank the staff of the analytical group of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College for recording the HFABMS and NMR spectra. This work was financially supported by the National "863" Program of P. R. China (Grant No. 2001AA620403).

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