Unusual Cytotoxic Steroidal Saponins from the Gorgonian *Astrogorgia dumbea*

Authors Ya-Nan Lu[1], Ping Cui[2], Xiao-Qing Tian[1], Li-Guang Lou[3], Cheng-Qi Fan[1]

Affiliations
[1] Key Laboratory of East China Sea & Oceanic Fishery Resources Exploitation and Utilization, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, P. R. China
[2] Changchun University of Chinese Medicine, Changchun, P. R. China
[3] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P. R. China

Key words
- *Astrogorgia dumbea*
- Plexauridae
- steroidal saponin
- astrogorgiosides A–C
- cytotoxic activity

Abstract

Three steroidal saponins, including astrogorgiosides A (**1**) and B (**2**) bearing acetamido-glucose moieties, and astrogorgioside C (**3**) with a 19-nor and bearing an aromatized B ring steroid aglycone, together with a known major saponin dimorphoside A (**4**), were obtained from the gorgonian *Astrogorgia dumbea* collected near Dongshan Island in East China Sea. Structures of these compounds were elucidated by in-depth spectral and chemical methods, including 2D-NMR, HR-ESI-MS spectra, and acidic hydrolysis. For the first time, acetamido-glucose moiety is being reported from a gorgonian. The B-ring aromatized steroid aglycone of compound **3** is also rare in marine natural products. Compounds **1**–**3** exhibited moderate cytotoxic activity with IC_{50} values of 26.8–45.6 µM against human tumor cells Bel-7402 and K562.

Supporting information available online at http://www.thieme-connect.de/products

received Sep. 9, 2015
revised March 11, 2016
accepted March 22, 2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-106168
Planta Med 2016; 82: 882–887

ISSN 0032-0943

Correspondence
Prof. Cheng-Qi Fan
East China Sea Fisheries Research Institute
Chinese Academy of Fishery Sciences
300 Jungong Road
Shanghai 200090
P. R. China
Phone: + 86 21 65 80 77 18
Fax: + 86 21 65 80 77 18
fancq@ecsf.ac.cn

Introduction

As one of the hot topics in the natural products research field, many structural types of compounds, including terpenoids, steroids, alkaloids and lipids, have been reported from gorgonians. These compounds exhibited many kinds of activities, such as anti-TB, anti-inflamation, antitumor, etc. [1–4]. Dongshan Island (23°42′N, 117°26′E, on the south of Fujian province in China) has a rich diversity of gorgonians where the species were estimated to be over 20 [5]. To find more bioactive constituents from these gorgonians, samples of the genera *Astrogorgia, Echinogorgia, Guaiagorgia*, and *Melithaea* were collected from the Dongshan coastal waters. Among them, *Astrogorgia dumbea* Grasshoff (Plexauridae) was the first recorded species of this genus in this district [5]. Gorgonians of the *Astrogorgia* genus are distributed widely in Indonesia, New Caledonia, and Japan, but there are only a few reports about their bioactive secondary metabolites. A secosteroid and a diterpene have been reported by Fusetani et al. from *Astrogorgia* sp. collected from Shikoku, which inhibited cell division of fertilized starfish eggs [6]. The secosteroid astrogorgiadiol also has the potential to treat osteoporosis and autoimmune diseases as an analogue of vitamin D [7]. Lin's group also reported fourteen 9,10-secosteroids and eight analogues from *Astrogorgia* sp. collected off the coast of Beibuwan Bay, South China Sea [8]. Five of them showed significant inhibitory activities against human tumor-related protein kinases. Herein, the isolation, structure elucidation, and bioactivity of three steroidal saponins, astrogorgiosides A–C (**1**–**3**), from the gorgonian *A. dumbea* are reported.

Results and Discussion

After percolation with methanol and extraction with petroleum ether and $CHCl_3$, the aqueous phase of the gorgonian *A. dumbea* was subjected to column chromatography on MCI gel and silica gel to give three novel steroidal saponins, astrogorgiosides A–C (**1**–**3**), with a known one (**4**; **Fig. 1**).

Compound **1**, namely astrogorgioside A, was obtained as a colorless powder, and exhibited a quasi-molecular ion peak at *m/z* 790.4343 ([M + Na]$^+$, calcd. 790.4354) in its HR-ESI MS, compatible with the molecular formula $C_{40}H_{65}NO_{13}$. The IR absorptions at 3425 and 1639 cm^{-1} indicated the presence of OH, NH, and carbonyl groups. By interpretation of the ^{1}H-, ^{13}C-NMR (with DEPT, in

Table 1 ^{1}H-NMR data of compounds **1**–**3** (400 MHz, δ in ppm, J in Hz)[a].

Atom	1	2	3
1	2.52, m	2.79, m	2.75, m
	0.97, m	1.00, m	2.59, m
2	1.57, br d, 14.3	2.21, m	2.16, m
	2.00, br d, 4.4		1.85, m
3	3.62, m	3.94, m	4.12, m
4	1.80, br d, 1.8	2.41, t, 11.0	3.07, dd, 4.0, 16.0
	2.52, m	2.89, d, 10.4	2.80, m
6	5.51, d, 5.5	5.63, m	6.83, d, 8.7
7	3.73, t like, 5.5	2.11, m	6.76, d, 8.1
		1.60, m	
8	2.04, d, 3.7	1.84, m	
9	1.15, m	1.10, m	
11	1.71, br d, 10.7	1.85, m	2.75, m
		1.65, m	2.59, m
12	1.13, m	1.92, m	1.63, m
		1.08, m	2.24, m
14	1.11, m	0.97, m	2.63, m
15	1.85, m	1.50, m	1.42, m
	1.49, m	1.01, m	1.28, m
16	1.30, m	1.67, m	2.07, m
		1.21, m	1.48, m
17	1.09, m	1.07, m	1.32, m
18	0.69, s	0.66, s	0.56, s
20	1.36 t like, 7.7[b]	1.95, m	1.08, m
21	0.93, d, 6.6	0.99, d, 6.6	1.02, d, 6.6
22	1.02, dd, 2.6, 6.2	5.24, m	1.08, m
			1.45, m
23	1.36, t like, 7.7[b]	5.28, m	2.07, m
			1.48, m
24	1.13, m	1.85, m	1.17, m
25	1.52, m		1.54, m
26	0.87, d, 6.6	0.86, d, 6.6	0.89, d, 6.6
27	0.87, d, 6.6	0.86, d, 6.6	0.89, d, 6.6
1′	4.6, d, 7.3	5.35, d, 8.4	4.50, d, 7.7
2′	3.63, m	4.20, m	3.34, m
3′	3.62, m	4.58, m	3.45, dd, 8.8, 19.0
4′	3.4, dd, 9.5, 8.0	4.08, t, 9.0	3.31, m
5′	3.32, m	3.94, m	3.40, m
6′	3.68, d, 5.5	4.24, m	3.63, dd, 4.4, 12.8
	3.65, m	4.44, d like, 9.9	3.74, m
1′′	4.93[b]	5.64, d, 4.0	5.24, d, 4.8
2′′	3.91, dd, 4.8, 8.1	4.60, m	3.99, dd, 8.4, 4.7
3′′	4.11, dd, 7.7, 8.1	5.05, t, 8.1	4.18, t like, 8.0
4′′	3.72, m	4.33, d like, 7.4	3.72, m
5′′	3.86, br d, 11.4	4.14, m	3.87, dd, 1.8,11.7
	3.73, m		3.67, m
$COCH_3$	1.96, s	2.08, s	

[a]Data of **1** and **3** were recorded in CD_3OD, and the data of **2** were recorded in C_5D_5N.
[b]Proton signals were overlapped

CD_3OD; ▶ **Tables 1** and **2**) and HSQC spectra, the presence of a steroid skeleton and two saccharide moieties were observed, which contained five methyls, ten methylenes, six methines, two sp^3 quaternary carbons, two oxygenated methylenes, ten oxygenated methines, one N-linked methine at δ_C 57.3, two olefinic carbons (a quaternary carbon at δ_C 138.0 and a methine at δ_C 131.1), and two carbonyl carbons (δ_C 177.5 and 174.6). These data, especially those of the steroid aglycone, showed a high similarity with those of a known steroidal saponin, dimorphoside A (**4**; ▶ **Table 2**), which was the major metabolite of *A. dumbea* in our study and has been reported as 3-[(3-O-*β*-D-arabinofuranosyl-*β*-D-glucopyranosyl)oxy]-7*β*-hydroxycholest-5-en-19-oic ac-

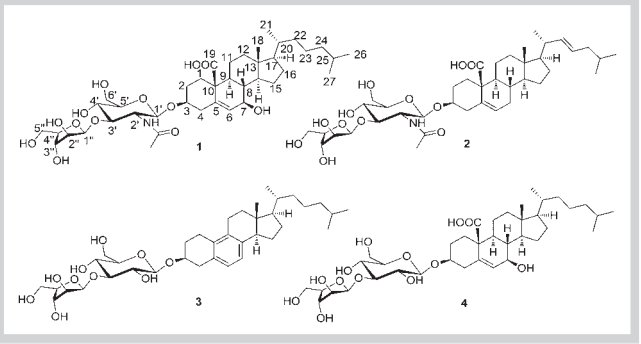

Fig. 1 Structures of compounds **1**–**4**.

Table 2 ^{13}C-NMR data of compounds **1**–**4** (100 MHz, δ in ppm).[a]

C	1	2	2*	3	4
1	35.3	35.5	34.3	26.3[b]	35.3
2	32.7	32.7	32.0	31.6	32.7
3	79.5	79.7	78.0	76.5	79.4
4	42.0	42.5	41.8	37.2	41.7
5	138.0	136.9	136.1	134.9	138.0
6	131.1	126.0	124.3	128.2	131.2
7	73.6	32.7	31.5	125.1	73.6
8	41.7	42.0	42.1	135.3	41.7
9	48.9	50.6	49.1	132.9	48.9
10	52.3	48.9	51.2	139.2	52.3
11	24.8	25.0	23.8	26.3[b]	24.8
12	41.5	41.4	39.7	38.9	41.5
13	44.7	44.0	42.5	43.5	44.7
14	58.0	58.5	56.7	53.5	58.0
15	27.9	25.6	24.4	25.5	27.9
16	30.3	30.3	29.0	30.5	30.2
17	57.4	57.7	55.9	57.2	57.4
18	13.0	13.1	12.2	11.9	13.0
19	177.5	177.9	176.1	–	177.5
20	37.6	33.8	40.4	38.0	37.6
21	19.9	21.9	21.0	19.8	19.8
22	37.9	139.9	138.5	37.9	37.9
23	25.5	128.0	126.4	25.8	25.4
24	41.2	43.6	42.1	41.2	41.2
25	29.6	30.3	28.7	29.7	29.6
26	23.5	23.5	22.3	23.5	23.4
27	23.7	23.2	22.4	23.7	23.7
1′	100.9	100.9	99.7	103.5	102.9
2′	57.3	57.3	57.2	75.0	75.2
3′	85.3	85.5	83.9	86.3	86.1
4′	71.0	71.0	70.3	78.1	78.0
5′	77.8	77.9	78.0	70.5	70.5
6′	62.4	62.4	62.3	62.8	62.8
1′′	104.6	104.6	103.8	104.2	104.1
2′′	79.2	79.2	79.1	79.4	79.3
3′′	74.9	75.0	73.8	75.3	75.0
4′′	84.1	84.2	83.9	84.2	84.2
5′′	63.2	63.2	61.2	63.2	63.2
$\underline{C}OCH_3$	174.6	174.6	171.4		
$CO\underline{C}H_3$	23.5	23.2	23.4		

[a] Data of **1**–**4** were recorded in CD_3OD, and the data of **2*** were recorded in C_5D_5N.
[b] Carbon signals were overlapped

id from another gorgonian, *Anthoplexaura dimorpha* [9]. Compared with the molecular formula $C_{38}H_{62}O_{13}$ of dimorphoside A, compound **1** not only had two additional carbons, including a methyl at δ_H 1.96 (3H, *s*) and δ_C 23.5, and the carbonyl group at δ_C 174.6, which might indicate an acetyl group, but it also contained one N-linked methine at δ_C 57.3 instead of one oxygenated methine in the glycoside moiety of dimorphoside A. Therefore, compound **1** should be a dimorphoside A analogue bearing an acetyl and amino-glucose.

Combined analysis of ^{1}H- and ^{13}C-NMR data, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra (**Fig. 2**) indicated that compound **1** should have the same steroid aglycone of 3*β*,7*β*-dihydroxycholest-5-en-19-oic acid and an arabinose moiety as dimorphoside A. Anomeric protons of the arabinose and another hexose were observed at δ_H 4.93 (1H, overlapped) and 4.61 (1H, *d*, *J* = 7.3 Hz), and corresponded with carbon signals at δ_C 104.6 and 100.9, respectively. The linkage from C-1′ to C-6′ of the undetermined hexose could also be lined out by ^{1}H-^{1}H COSY and HSQC spectra. In the HMBC spectra, both the methyl at δ_H 1.96 and H-2′ at δ_H 3.63 correlated with the carbonyl at δ_C 174.6, which confirmed the linkage of the CH_3CONH- group to the methine at δ_C 57.3 (C-2′). By direct comparison of the two structures, the OH at C-2′ in dimorphoside A was replaced by the acetamido group in compound **1**, so an acetamido-glucose should exist in the structure of compound **1**. In addition, key correlation signals from δ_H 4.61 (H-1′) to δ_C 79.5 (C-3), and from δ_H 4.93 (H-1″) to δ_C 85.3 (C-3′) confirmed that the C-1′ of acetamido-glucose linked to C-3 of the aglycone, and C-1″ of arabinose linked to C-3′ of acetamido-glucose. The couple constant of the anomeric proton (7.3 Hz) of glucose indicated that its relative configuration was *β*-positioned [10]. The cross-peak between the anomeric proton of acetamido-glucose at δ_H 4.60 and H-3′ at δ_H 3.62 in the ROESY spectra confirmed the indication. The couple constant of the arabinose could not be read because the signal in ^{1}H-NMR was overlapped by D_2O. But the data of ^{1}H- and ^{13}C-NMR of arabinose were similar to those of compound **4**. So the relative configuration of C-1″ was evaluated to be *β*-positioned, the same as compound **4**. The cross-peak between the anomeric proton of arabinose at δ_H 4.93 and H-3′ of acetamido-glucose at δ_H 3.62 (**Fig. 3**) in the ROESY spectra supported our evaluation. Thus, the structure of astrogorgioside A (**1**) was firstly elucidated to be 3-[(2-acetamido-3-O-*β*-arabinofuranosyl-*β*-glucopyranosyl)-oxy]-7*β*-hydroxycholest-5-en-19-oic acid as shown in **Fig. 1**, with the absolute configurations of arabinose and acetamido-glucose determined later by comparison with compound **2**.

Compound **2**, namely astrogorgioside B, was obtained as colorless plates. Its molecular formula was deduced as $C_{40}H_{63}NO_{12}$ by HR-ESI MS at *m/z* 772.4254 ([M + Na]$^+$, calcd. 772.4248). The IR absorptions at 3405, 1708, and 1652 cm^{-1} indicated the presence of OH, NH, and carbonyl groups. The ^{1}H- and ^{13}C-NMR data (in pyridine-d_5; **Tables 1** and **2**) of **2** also exhibited the acetamido-glucose and arabinose moieties. The acetamido-glucose showed an anomeric proton at δ_H 5.35 (1H, *d*, *J* = 8.4 Hz), C-1′ at δ_C 99.7, and a -CH(NHCOCH$_3$)- group at δ_H 4.20 (1H, *m*), 2.08 (3 H, *s*), δ_C 57.2, 23.4, and 171.4. The arabinose had the anomeric proton at δ_H 5.64 (1H, *d*, *J* = 4.0 Hz) and C-1″ at δ_C 103.8. The ^{13}C-NMR data of acetamido-glucose and arabinose moieties of compound **2** both in C_5D_5N and CD_3OD showed a high similarity with those of compound **1** in CD_3OD (**Table 2**), which indicated the same structure unit as the 2-acetamido-3-O-*β*-arabinofuranosyl-*β*-glucopyranosyl moiety in **1**. However, compared with the aglycone of 3*β*,7*β*-dihydroxycholest-5-en-19-oic acid in compounds

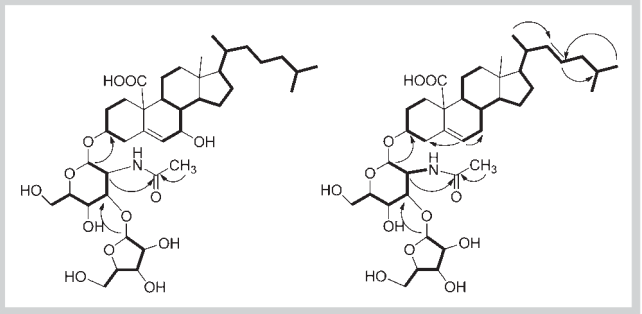

Fig. 2 Key ^{1}H-^{1}H COSY (—) and HMBC (H→C) correlations of compounds **1** and **2**.

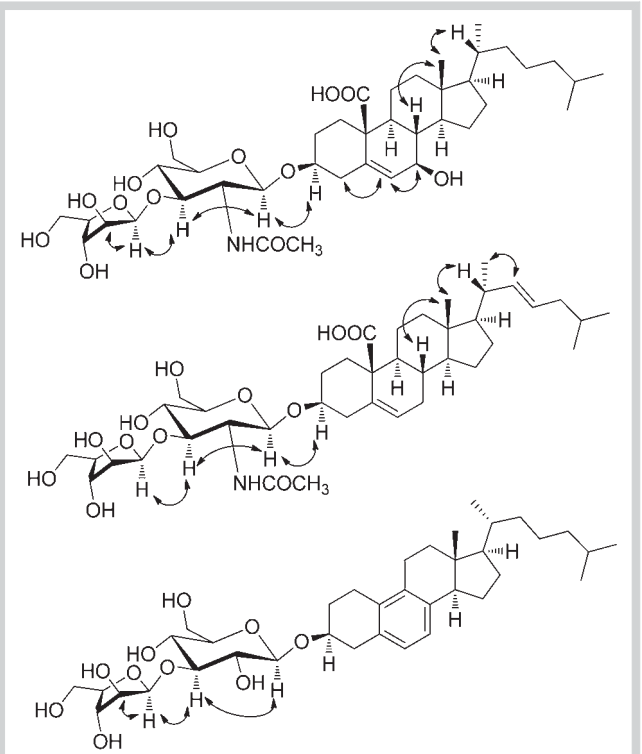

Fig. 3 Key ROESY correlations of compounds **1**–**3**.

1 or **4**, the aglycone of compound **2** showed a few different ^{1}H- or ^{13}C-NMR signals. There were four olefinic carbons at δ_C 136.1 (C), 124.3 (CH), 138.5 (CH), and 126.4 (CH) with three multiple-coupled protons at δ_H 5.63 (1H, *m*), 5.24 (1H, *m*), and 5.28 (1H, *m*) in compound **2**, together with the absence of one methylene and one oxygenated methine. These data indicated the presence of an additional –CH=CH– group and one methylene in compound **2**, taking the place of two methylenes and OH linked 7-methine in compound **1**, respectively. In the HMBC spectra (**Fig. 2**), the key correlations from δ_H 0.99 (H-21) to δ_C 138.5 and from δ_H 5.28 to δ_C 23.4 (C-25) confirmed the presence of the $\Delta^{22,23}$, and the correlation between the olefinic proton at δ_H 5.63 (1H, *m*, H-6) and a methylene at δ_C 32.0 (C-7) was also observed. The coupling constants of glucose (8.4 Hz) and arabinose (4.0 Hz) implied that the relative configuration of these two saccharides were all *β*-positioned [10, 11]. In the ROESY spectra, the cross-

peak between the H-1′ at δ_H 5.35 and H-3′ also showed the β configuration at C-1′ of the acetamido-glucose (**Fig. 3**). On the basis of above evidences and by combined analysis of ^{1}H- and ^{13}C-NMR data, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra, the aglycone of compound **2** was determined as 3β-hydroxycholesta-5,22-dien-19-oic acid.

To determine the absolute configurations of arabinose and acetamido-glucose as the same in compounds **1** and **2**, acidic hydrolysis of compound **2** was carried out. By comparisons of the retention time (t_R) in HPLC with a polyamine column and $[\alpha]_D$ values with those of authentic D-arabinose and 2-N-acetylamino-D-glucose standards, the structure of astrogorgioside B (**2**) was finally elucidated as 3-[(2-acetamido-3-O-β-D-arabinofuranosyl-β-D-glucopyranosyl)oxy]-cholesta-5,22-dien-19-oic acid, and that of astrogorgioside A (**1**) was 3-[(2-acetamido-3-O-β-D-arabinofuranosyl-β-D-glucopyranosyl)oxy]-7β-hydroxycholesta-5-en-19-oic acid. Their NMR data were assigned in **Tables 1** and **2** by total analysis of ^{1}H-^{1}H COSY, HSQC, and HMBC spectra. The ^{13}C-NMR data of **2** in CD_3OD were also obtained for comparison with the other compounds.

Compound **3**, namely astrogorgioside C, was a colorless powder. Its molecular formula $C_{37}H_{58}O_{10}$ was deduced by HR-ESI MS at *m/z* 685.3920 ([M + Na]$^+$, calcd. 685.3928). The IR absorptions at 3415 and 1637 cm^{-1} indicated the presence of OH and carbonyl groups. By interpretation of the ^{1}H-, ^{13}C-NMR (with DEPT, in CD_3OD; **Tables 1** and **2**), and HSQC spectra and by comparison with the NMR data of dimorphoside A (**4**), the anomeric protons at δ_H 4.50 (1H, *d, J* = 7.7 Hz) and 5.24 (1H, *d, J* = 4.8 Hz) with corresponded carbon signals at δ_C 103.5 and 104.2, respectively, together with seven oxygenated methines and two oxygenated methylenes, indicated the presence of glucose, arabinose, and the same linkage as the 3-O-β-arabinofuranosyl-β-glucopyranosyl moiety in compound **4**. The correlations between δ_H 4.50 (H-1′) and δ_C 76.5 (C-3) fixed the linkage site at C-3 of the steroid aglycone.

The signals of the steroid aglycone were observed as four methyls, ten methylenes, five sp3 methines (one oxygenated), and a quaternary carbon, together with six olefinic carbons (four quaternary carbons at δ_C 139.2, 134.9, 135.3, 132.9, and two sp^2 methines at δ_C 128.2 and 125.1), which implied that one of the methyls in the cholesterol skeleton disappeared. In addition, olefinic proton signals at δ_H 6.83 (1H, *d, J* = 8.7 Hz) and 6.76 (1H, *d, J* = 8.7 Hz) showed a correlation in the ^{1}H-^{1}H COSY spectra, indicating the existence of a –CH=CH– moiety. In the HMBC spectra (**Fig. 4**), the proton signals at δ_H 6.83 correlated with δ_C 139.2 and 135.3, and the proton signals at δ_H 6.76 correlated with δ_C 134.9 and 132.9, which revealed that the six aromatic carbons should be an aromatic system. The other HMBC correlations, including δ_H 6.83 to δ_C 37.2 (C-4), δ_H 6.76 to δ_C 53.5(C-14), H-3 to δ_C 134.9, H-11 to δ_C 132.9, and H-14 to δ_C 135.3, confirmed that the B-ring of the aglycone was aromatized, and its C-19 was cut from C-10 to give the aglycone 19-norcholesta-5,7,9-trien-3-β-ol.

Acidic hydrolysis of compound **3** yielded D-arabinose and D-glucose by comparisons of t_R in HPLC and $[\alpha]_D$ values with those of authentic standards, thus the structure of astrogorgioside C (**3**) was elucidated as 19-norcholesta-5,7,9-trien-3-β-ol 3-O-(3-O-β-D-arabinofuranosyl)-β-D-glucopyranoside, and confirmed by further analysis of ^{1}H-^{1}H COSY, HSQC, and HMBC spectra.

This is the first time reporting the chemical compounds of *A. dumbea*. Three new steroidal saponins were isolated with a cholesterol skeleton bearing a 3-O-β-arabinofuranosyl- β-glucopyranosyl moiety at C-3. It is interesting that in compounds **1** and **2**, the 2′-OH of glucose was replaced by the acetamino group, and it is the first report of the acetamido-glucose moieties in the steroidal saponin structures from gorgonian. Compound **3** has an aromatized B-ring. Although the steroids bearing an aromatized B-ring are common in synthesized structures and also have been found as natural products of a fungus [12] and sponge [13], they are found in gorgonians for the first time and are seldom reported in other marine organisms. The structures from gorgonians have been reported mainly as terpenoids, especially diterpenoids. Steroid saponins were seldom founded in gorgonians except for dimorphoside A from *A. dimorpha* [9]. Dimorphoside A and its three analogues from *A. dumbea* showed that these steroid saponins should be one of the chemical characteristics of this genus.

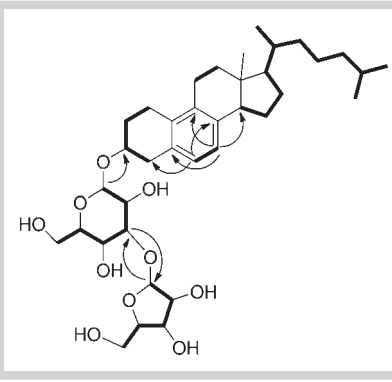

Fig. 4 Key ^{1}H-^{1}H COSY (—) and HMBC (H→C) correlations of compound **3**.

Table 3 Cytotoxic activity (IC_{50} value, μM) of compounds.

	BEL-7402	K562
1	34.8	—[a]
2	26.8	41.1
3	45.6	28.9
ADR[b]	0.08	0.3

[a] The activity of **1** was not evaluated against K562. [b] Positive control

Cytotoxicity of the compounds against two human tumor cell lines, hepatoma Bel-7402 and erythroleukemia K562, was evaluated by means of MTT methods, with ADR as a positive control. All compounds exhibited moderate cytotoxic activity (**Table 3**). Compound **2** exhibited the best activity on Bel-7402 with an IC_{50} value of 26.8 μM, while **3** exhibited better activity on K562 (IC_{50} value of 28.9 μM).

Materials and Methods

Instruments and chemicals

Optical rotations were measured on a Perkin-Elmer 341 polarimeter and Rudolph Autopol VI. IR spectra were obtained on a Perkin-Elmer 577 spectrometer with KBr discs in cm^{-1}. 1D and 2D NMR spectra were recorded on a Bruker AM-400 spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. ESI MS was carried out on a Finnigan LCQ-DECA instrument in *m/z* (rel. %). HPLC analysis and preparation were carried on a system including a Shimadazu LC-20AT pump, SIL-20A autosampler, SPD-M20A PDA and RI (Buchi) detectors, and a YMC-Pack ODS-AQ column (AQ12S05-2510WT, 250 × 10 mm, S-5 μm, 12 nm) or YMC-Pack polyamine II column (PB12S05-2510WT, 250 × 10 mm, S-5 μm, 12 nm). TLC was carried out with precoated silica gel GF_{254} plates (Qingdao Haiyang

Chemical Co. Ltd.). Spots were visualized under UV light and by spraying with 10% H_2SO_4 in EtOH, followed by heating. CC: reversed-phase silica gel (ODS-A, 150–200 mesh; YMC), silica gel H (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.), and Sephadex LH-20 gel (Amersham Biosciences). All solvents used were of analytical grade (Shanghai Chemical Plant). ADR came from Sigma with a purity ≥ 98% (TLC).

Gorgonian material

The gorgonian *A. dumbea* was collected from Dongshan Island of P.R. China and kept frozen at − 20 °C until used. The specimen was identified by Dr. Xiu-Bao Li (South China Sea Institute of Oceanology, Chinese Academy of Sciences). A voucher specimen has been deposited at the East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, P.R. China (Accession number LAD-200804-DS).

Extraction and isolation

The whole samples (2.25 kg) were cut into pieces and were percolated with methanol (4 L × 4). After evaporation of the solvent, the aqueous solution was extracted successively with petroleum ether (PE, 4 × 500 mL) and $CHCl_3$ (4 × 500 mL) to give the PE extracts (16.5 g), $CHCl_3$ extracts (46.5 g), and the aquatic phase. The aquatic solution was evaporated and subjected to MCI gel column chromatography eluted with methanol (MeOH) in water (75 µm, 3 × 50 cm, 20%, 40%, 60%, 100%, v/v, 1000 mL each) to afford four solutions (LAD I–IV).

LAD IV (10.8 g) was subjected to column chromatography on silica gel (3.5 × 50 cm, 300 mesh) eluted with a gradient of CH_3Cl/MeOH (17 : 1 → 1 : 1, each 1000 mL) to give 13 fractions (Fr. 1–13). Fr. 11 (142 mg) was subjected on a Sephadex LH-20 gel column (1.5 × 80 cm) and eluted with 70% ethanol (EtOH) in water to afford compounds **1** (18 mg, ≥ 90% by TLC) and **4** (87 mg). Compound **4** was also a major constituent of Fr. 9–10. Fr. 8 (66 mg) was separated by a reversed-phase ODS-A column eluted with MeOH/H_2O (150 µm, 3 × 40 cm, 60% → 100%) to give compound **2** (25 mg, ≥ 90% by TLC). Fr. 6 (242 mg) was separated by the same reversed-phase ODS-A column eluted with MeOH/H_2O (75% → 100%) to afford 11 fractions (Fr. 601–611). A major constituent in Fr. 605–607 was too difficult to purify. Fr. 609 (32 mg) was subjected to column chromatography on silica gel (1.5 × 30 cm, 300 mesh) and eluted with a gradient of CH_3Cl/MeOH (15 : 1 → 8 : 1, v/v, 200 mL each) to give compound **3** (8 mg, ≥ 90% by TLC).

Astrogorgioside A (**1**): Colorless powder. $[\alpha]_D^{20}$ − 42 (*c* = 0.125, MeOH). IR (KBr) ν_{max}: 3425, 2927, 2867, 1639, 1379, 1078, 1028 cm^{-1}. ^{1}H-NMR data, see **Table 1** and ^{13}C-NMR data, see **Table 2**. Positive HR-ESI-MS(positive mode) *m/z*: 790.4343 ([M + Na]$^+$, $C_{40}H_{65}NO_{13}Na$, calcd. 790.4354).

Astrogorgioside B (2): Colorless powder. $[\alpha]_D^{20}$ − 79 (*c* = 0.125, MeOH). IR (KBr) ν_{max}: 3405, 2950, 2868, 1709, 1653, 1576, 1380, 1068, 1029 cm^{-1}. ^{1}H- and ^{13}C- NMR data, see **Tables 1** and **2**. Positive HR-ESI-MS *m/z*: 772.4254 ([M + Na]$^+$, $C_{40}H_{63}NO_{12}Na$, calcd. 772.4248).

Astrogorgioside C (**3**): Colorless powder. $[\alpha]_D^{20}$ = − 26 (*c* = 0.125, MeOH). IR (KBr) ν_{max}: 3415, 2927, 2869, 1637, 1466, 1382, 1076, 1031 cm^{-1}. ^{1}H-NMR data, see **Table 1** and ^{13}C- NMR, data see **Table 2**. Positive HR-ESI-MS *m/z*: 685.3920 ([M + Na]$^+$, $C_{37}H_{58}O_{10}Na$, calcd. 685.3928).

Acidic hydrolysis of astrogorgiosides B (**2**) *and C* (**3**): To 8 mL solution containing 5% H_2SO_4 and 40% MeOH in H_2O, astrogorgioside B (**2**, 7.8 mg) was added. The solution was kept under reflux for 6 h, and then after evaporation of MeOH, the solution was extracted with $CHCl_3$ (1 mL × 3). The pH value of the water solution was adjusted to 6–7 with $NaHCO_3$, and the mixture was evaporated to give a residue. The sugars were finally purified by HPLC equipped with a polyamine column and refractive index detector, eluted by 2.50 mL/min 75% CH_3CN in H_2O to yield D-arabinose and 2-N-acetylamino-D-glucose, which were identified by comparison of t_R and with authentic samples. D-arabinose: t_R 12.4, $[\alpha]_D^{20}$ 123.6 (*c* = 0.055, H_2O); 2-N-acetylamino-D-glucose: t_R 15.2, $[\alpha]_D^{20}$ 118.2 (*c* = 0.065, H_2O). Then astrogorgioside C (**3**, 4.7 mg) was also hydrolyzed to afford D-arabinose and D-glucose: t_R 13.3, $[\alpha]_D^{20}$ 44.4(*c* = 0.045, H_2O).

Cytotoxicity assay

Cytotoxicity of the compounds against two human tumor cell lines, hepatoma Bel-7402, and erythroleukemia K562 was evaluated by means of the MTT methods as described [14]. Bel-7402 was obtained from Shanghai Institutes for Biological Sciences, CAS, and K562 was obtained from the American Type Culture Collection (ATCC). ADR (Adriamycin) was used as a positive control.

Supporting information

IR, MS, and NMR spectra of compounds **1**–**3** are available as Supporting Information.

Acknowledgements

This project was supported by the Natural Science Foundation of Shanghai (Grant No. 11ZR1 449 800), the foundation of Key Laboratory of Marine Bio-resources Sustainable Utilization (No. LMB101003), and a special research fund for the national nonprofit institutes (East China Sea Fisheries Research Institute, No. 2007M18) of P.R. China. We thank Dr. Xiu-Bao Li from the Key Laboratory of Marine Bio-resources Sustainable Utilization (South China Sea Institute of Oceanology, Chinese Academy of Sciences) for the identification of gorgonian material.

Conflict of Interest

The authors declare no competing financial interest.

References

1 *Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR.* Marine natural products. Nat Prod Rep 2013; 30: 237–323
2 *Cui P, Fan CQ, Lu YN, Shao SN.* [Chemical constituents and their activities from gorgonian in China Sea]. Jilin J Trad Chin Med 2011; 31: 571–575
3 *Berrue F, Kerr RG.* Diterpenes from gorgonian corals. Nat Prod Rep 2009; 26: 681–710
4 *Zhang W, Guo YW.* A pharmaceutical perspective on gorgonian: chemistry and bioactivity. Chin J Nat Med 2003; 1: 69–75
5 *Li XB, Huang H, Lian JS, Yang JH, Huang LM.* Diversity and spatial distribution of gorgonian coral in Dongshan coastal waters in Fujian, China. J Oceanogr Taiwan 2011; 30: 92–96
6 *Fusetani N, Nagata H, Hirota H, Tsuyuki T.* Astrogorgiadiol and astrogorgin, inhibitors of cell division in fertilized starfish eggs, from a gorgonian *Astrogorgia* sp. Tetrahedron Lett 1989; 30: 7079–7082
7 *Taber DF, Farach-Carson MC, Malcolm SC, Xu Y.* Treatment of osteoporosis and autoimmune disease with astrogorgiadiol. US Patent US7345035 B2; 2008
8 *Lai DW, Yu SJ, Ofwegen LV, Totzke F, Proksch P, Lin WH.* 9,10-Secosteroids, protein kinase inhibitors from the Chinese gorgonian *Astrogorgia* sp. Bioorg Med Chem 2011; 19: 6873–6880

9 *Fusetani N, Yasukawa K, Matsunage S, Hashimoto K.* Dimorphosides A and B, novel steroid glycosides from the gorgonian *Anthoplexaura dimorpha*. Tetrahedron Lett 1987; 28: 1187–1190
10 *Horo I, Bedir E, Perrone A, Ozgökçe F, Piacente S, Alankuş-Calişkan O.* Triterpene glycosides from *Astragalus icmadophilus*. Phytochemistry 2010; 71: 956–963
11 *Antonov SA, Afiyatullov SS, Kalinovsky IA, Ponomarenko PL, Dmitrenok SP, Aminin LD, Agafonova GI, Stonik AV.* Mycalosides B–I, eight new spermostatic steroid oligoglycosides from the sponge *Mycale laxissima*. J Nat Prod 2003; 66: 1082–1088
12 *Barrero FA, Oltra JE, Poyatos AJ, Jiménez D, Oliver E.* Phycomysterols and other sterols from the fungus *Phycomyces blakesleeanus*. J Nat Prod 1998; 61: 1491–1496
13 *Luo X, Li F, Shinde BP, Hong J, Lee CO, Im KS, Jung JH.* 26,27-Cyclosterols and other polyoxygenated sterols from a marine sponge *Topsentia* sp. J Nat Prod 2006; 69: 1760–1768
14 *Ni G, Zhang QJ, Zheng ZF, Chen RY, Yu DQ.* 2-Arylbenzofuran derivatives from *Morus cathayana*. J Nat Prod 2009; 72: 966–968