Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, The Chinese Academy of Sciences, Guangzhou, P. R. China

Bebrycoside, a new steroidal glycoside from the Chinese gorgonian coral *Bebryce indica*

JIN YANG, SHU-HUA QI, SI ZHANG, ZHI-HUI XIAO, QING-XIN LI

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Si Zhang, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, The Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, P. R. China
zhsimd@scsio.ac.cn

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A new steroidal glycoside, bebrycoside (**1**), was isolated from the South China Sea gorgonian coral *Bebryce indica*. The structure of **1** was established by extensive spectroscopic analysis, including 1D and 2D NMR data. This was the first time that the steroidal glycoside was isolated from the genus of *Bebryce*.

1. Introduction

Steroidal glycosides have rarely been isolated from gorgonian corals. Previous chemical investigations of this group of interesting marine animals had only yielded less than twenty steroidal glycosides (Cober et al. 1997; Fusetani et al. 1987; Kashman et al. 1991; Qi et al. 2004; Qi et al. 2005; Shi et al. 2002). All these compounds possess a cholesterol carbon skeleton. Searching for novel active compounds from gorgonian corals, we investigated the South China Sea gorgonian coral *Bebryce indica* and obtained a new steroidal glycoside: bebrycoside. This was the first time that a steroidal glycoside was isolated from the genus of *Bebryce*. This paper deals with the isolation and structural elucidation of compound **1**.

2. Investigations, results and discussion

Compound **1** was obtained as white powder, showing the molecular formula of $C_{40}H_{60}O_{12}$ as determined by ESI-MS and NMR spectra. The ^{1}H NMR spectrum exhibited signals for three tertiary methyls at δ_H 0.91, 1.19, and 1.25 (each 3H, s), a secondary methyl group at δ_H 0.93 (3 H, d, J = 6.5 Hz), and a trisubstituted double bond at δ_H 5.73 (1 H, s). The ^{13}C (DEPT)-NMR spectra displayed 40 carbon signals, including twenty-seven basic skeleton carbons possessing a ketone group (δ_C 200.0), a pentose unit [δ_C 96.9 (d), 69.1 (d), 68.9 (d), 67.3 (d), 60.4 (t)] and four acetyl groups [δ_C 170.1 (s), 20.9 (q), 170.4 (s), 20.7 (q), 170.3 (s), 20.8 (q), 172.3 (s), 21.1 (q)]. The signals at δ_C 73.8, 77.0, and 79.8 indicated that three basic skeleton carbons were oxygenated. These data suggested that **1** was a cholest-type monoglycoside.

A comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of 11,21-dihydroxypregn-4-ene-3,20-dione and other analogous derivatives (Yu and Yang 2002) and the existence of the HMBC correlations of H-4 (δ_H 5.73) with C-2 (δ_C 32.9), C-3 (δ_C 200.0), C-10 (δ_C 38.6), Me-19 (δ_H 1.19) with C-1 (δ_C 35.8), C-5 (δ_C 171.4 s) and C-10, and H-7 (δ_H 1.69, 2.03) with both C-3 and C-5 (Fig.) permitted us to assign the signals of C-3, C-4, C-5, and Me-19. HMBC correlations between Me-21 [δ_H 1.25 (3H, s, H-21)] and δ_C 77.0 (s), 79.8 (d), C-17 (δ_C 55.4), H-17 (δ_H 1.46) and δ_C 77.0, 79.8, C-18 (δ_C 20.8), H-22 (δ_H 4.80) and δ_C 77.0, C-17 (δ_C 55.4) indicated the assignment of δ_C 77.0 and 79.8 as C-20 and C-22, respectively. Besides, HMBC correlations of δ_H 4.80 (1 H, d, J = 10.2 Hz, H-22) with δ_C 172.3 (s) suggested that C-22 was acetylated. The relative stereochemistry of C-20 and C-22 was determined to β-configuration and α-configuration, respectively, by comparison with the spectral data of venustone [δ_C 77.6 (C-20β) and 80.8 (C-22α), respectively] (Roth et al. 1995). This was further supported by the NOE correlations of Me-18 with H-β22, and H-α17 with Me-21 in the NOESY spectrum (Fig.).

The HMBC spectrum also showed the correlations of δ_H 0.93 (3 H, d, J = 6.5 Hz, H-26) with δ_C 33.4 (d, C-25), 73.8 (t), and δ_H 1.71 (1 H, m, H-25) with δ_C 17.2 (q, C-26), 73.8 (t), which permitted the assignment of δ_C 73.8 as C-27. Furthermore, comparing with those NMR data of the sugar moiety in junceellosides A-D (Qi et al. 2005) and glycoside-4′-*O*-acetyl-3-*O*-[β-D-arabino-pyranosyl-oxy]-cholest-5-ene-3β,19-diol (Qi et al. 2004), the NMR data of a pentose unit [δ_C 96.9 (d, C-1′), 69.1 (d, C-2′) , 68.9 (d, C-3′), 67.3 (d, C-4′), 60.4 (t, C-5′); δ_H 5.06 (1 H, d, J = 3.6 Hz, H-1′), 5.32 (2 H, m, H-2′, 3′), 5.12 (1 H, dd, J = 3.3, 7.8 Hz, H-4′), 3.92, 3.66 (each 1 H, d, J = 12.8 Hz, H-5′)] in **1** indicated the existence of a β-D-arabinopyranosyl unite. The J value of the anomeric proton (J = 3.6 Hz) suggested the β-arabinose. The down-field chemical shift values of H-2′, H-3′ and H-4′ and the long-range correlations of H-2′, H-3′, H-4′ with δ_C 170.4, 170.3, 170.1 in the HMBC spectrum indicated that C-2′, C-3′ and C-4′ were acetylated. In addition, HMBC correlations of H-27 [3.57, 3.11 (each 1 H, dd, J = 9.2, 3.8 Hz, H-27)] with C-1′, H-1′ with C-27 and C-5′, and H-5′ with C-1′ implied that the β-arabinopyranosyl unit was placed at the aglycone C-27. Consequently, the structure of **1** was elucidated to be 22α,2′,3′,4′-*O*-tetraace-

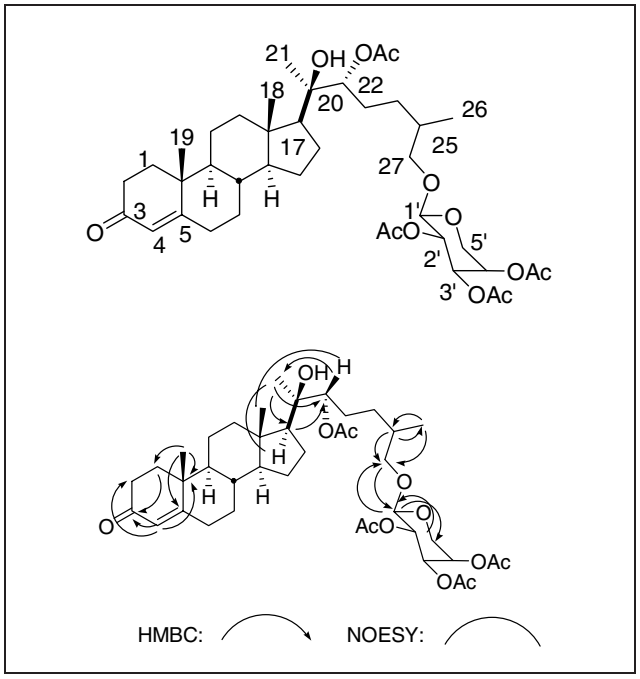

Fig.: Key HMBC and NOESY correlations of compound **1**

tyl-27-*O*-[β-D-arabino-pyranosyl-oxy]-20β-hydroxy-cholest-4-ene-3-one, simply named bebrycoside.

3. Experimental

3.1. General procedures

UV spectra were obtained on a Beckman Du-640 UV spectrophotometer. ^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on an LCQ^{DECA} XP HPLC/MS^n spectrometer for ESIMS. Preparative HPLC was carried out on ODS columns (250 × 10 mm i.d., Phenomenex) with a Waters 996 photodiode array detector. Silica gel (200–300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, the People's Republic of China.

3.2. Biological material

Bebryce indica (Thomson) was collected in Sanya, Hainan province, China in October, 2003 and identified by Zhou Ren-Lin, the South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (No. 0330) was deposited at the South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

3.3. Extraction and isolation

The bodies of *B. indica* were extracted with EtOH/CH_2Cl_2 (2 : 1) three times at room temperature, and the solvent was evaporated *in vacuo*. The residue was partitioned in H_2O and extracted with $CHCl_3$ three times. The $CHCl_3$ extracts was concentrated *in vacuo* to afford 9.1 g of residue. The $CHCl_3$ portion was subjected to column chromatography (CC) on silica, using petroleum ether/EtOAc (19 : 1 to 1 : 1) as eluent. By combining the fractions with TLC (GF_{254}) monitoring, 8 fractions were obtained. Fraction 4 was subjected to repeated column chromatography (Sephadex LH-20, $CHCl_3$-MeOH 1 : 1 and MeOH) and further purification with preparative HPLC (Luna^{TM}C18(2), 250 × 10 mm i.d., acetonitrile-water 64 : 36) to yield **1** (1.5 mg).

3.4. Bebrycoside (*1*)

White amorphous powder: $[\alpha]^{20}_D$ -24.0 (*c* 1.0, $CHCl_3$), UV (MeCN): 203, 215, 248 nm, ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H : 5.73 (1 H, s, H-4), 4.80 (1 H, d, J = 10.2 Hz, H-22), 3.57, 3.11 (each 1 H, dd, J = 9.2, 3.8 Hz, H-27), 2.42, 2.36 (each 1 H, m, H-6), 2.40, 2.29, (each 1 H, m, H-2), 2.12, 1.22 (each 1 H, m, H-12), 2.03, 1.69 (each 1 H, m, H-1), 1.86, 1.04 (each 1 H, m, H-7), 1.83, 1.65 (each 1 H, m, H-16), 1.71 (1 H, m, H-25), 1.69, 1.21 (each 1 H, m, H-23), 1.58 (1 H, m, H-8), 1.56, 1.36 (each 1 H, m, H-15), 1.53, 1.46 (each 1 H, m, H-11), 1.46 (1 H, m, H-17), 1.40, 1.06 (each 1 H, m, H-24), 1.25 (3 H, s, H-21), 1.19 (3 H, s, H-19), 1.02 (1 H, m, H-14), 0.93 (3 H, d, J = 6.5 Hz, H-26), 0.92 (1 H, m, H-9), 0.91 (3 H, s, H-18), 5.06 (1 H, d, J = 3.6 Hz, H-1′), 5.32 (2 H, m, H-2′, 3′), 5.12 (1 H, dd, J = 3.3, 7.8 Hz, H-4′), 3.92, 3.66 (each 1 H, d, J = 12.8 Hz, H-5′), 2.11 (3 H, s, 22-OAc), 2.15, 2.07, 2.01 (each 3 H, s, -OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 35.8 (C-1), 32.9 (C-2), 200.0 (C-3), 123.9 (C-4), 171.4 (C-5), 34.0 (C-6), 31.9 (C-7), 34.9 (C-8), 53.8 (C-9), 38.6 (C-10), 20.9 (C-11), 40.2 (C-12), 43.5 (C-13), 56.0 (C-14), 27.6 (C-15), 22.1 (C-16), 55.4 (C-17), 13.6 (C-18), 17.4 (C-19), 77.0 (C-20), 20.8 (C-21), 79.8 (C-22), 23.9 (C-23), 30.5 (C-24), 33.4 (C-25), 17.2(C-26), 73.8 (C-27), 96.9 (C-1′), 69.1 (C-2′), 68.9 (C-3′), 67.3 (C-4′), 60.4 (C-5′), 172.3, 21.1 (22-OAc), 170.4, 20.7, 170.3, 20.8, 170.1, 20.9 (-OAc). HR-FAB-MS (+) m/z 733.4177 $[M + H]^+$ (calcd for $C_{40}H_{60}O_{12}H^+$, 733.4165).

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