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Isomalabaricane-Type Nortriterpenoids and Other Constituents of the Marine Sponge *Geodia japonica*

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Twelve compounds were isolated from the sponge *Geodia japonica* collected from the South China Sea, including two new isomalabaricane-type nortriterpenoids, geoditins A (**1**) and B (**2**), and a new sterol derivative (**4**). All chemical structures were established by interpretation of the spectroscopic data.

In a continuation of our study on the secondary metabolites of marine organisms,[1] we have examined a marine sponge, *Geodia japonica* (Geodidae), collected from the South China Sea. Previous studies on *Geodia* sponges have resulted in the isolation of chemical constituents such as geodiastatins,[2] geodiatoxins,[3] barettin,[4] geodiamolides,[5,6] geodisterol,[7] geodin A,[8] a series of steroids[9] and sterols,[10] histamine,[11] inosine,[11] and nucleosides.[11] Recently stelliferin riboside, together with stellettins A and B, was reported from *Geodia globostellifera*.[12] This paper describes the isolation and characterization of two new nortriterpenoids, geoditins A (**1**) and B (**2**). During the course of isolation, 10 other metabolites were also obtained, among them stellettins A and B, a cyclodipeptide (**3**), and a new sterol derivative (**4**).

Results and Discussion

An EtOH extract of the dried sample of *G. japonica* was partitioned between $CHCl_3$ and H_2O. From the $CHCl_3$ fraction, compounds **1**−**3**, together with stellettins A and B, palmitic acid, and 24-methylcholesta-5,24(28)-dien-3β-ol, were isolated. The aqueous layer was extracted with *n*-BuOH to afford uracil, thymine, 26-methylergosta-5,24-(28)-dien-3β-ol, and compound **4**. The aqueous solution furnished malonic acid.

Geoditin A (**1**), $C_{29}H_{38}O_4$ (elemental analysis), displayed a molecular ion at *m*/*z* 450 [M^+] in the EIMS and 29 carbon signals in its ^{13}C NMR spectrum. The IR spectrum exhibited absorptions due to saturated keto (ν_{max} 1720 cm^{-1}) and conjugated carbonyl functionalities (ν_{max} 1691 cm^{-1}). UV absorption bands at λ_{max} 357 nm (log ϵ 3.26) and 293 nm (log ϵ 2.40) suggested the presence of a highly conjugated system. Interpretation of the HMBC data led to the construction of the side chain shown in **1**. The rest of the NMR data (Table 1) were similar to those of stellettins A and B,[12−15] consistent with an isomalabaricane skeleton.[12−23] In the HMBC spectrum, the methyl proton signals at δ_H 1.13 and 1.06 (CH_3-27 and CH_3-28) exhibited long-range coupling with carbon signals at δ_C 218.6 (C-3), 46.7 (C-4), and 45.3 (C-5). The proton signal at δ_H 0.87 (H-19) coupled with carbons at δ_C 31.3, 45.3, 47.7, 34.8 (C-1, C-5, C-9, and C-10, respectively). In addition, a proton at δ_H 1.44 (CH_3-29) showed long-range correlations with δ_C 44.9 (C-8), 47.7 (C-9), and 148.3 (C-13). The foregoing evidence was consistent with the proposed tricyclic structure of **1**. On the other hand, the proton signal at δ_H 2.10 (CH_3-18) in the side chain was found to couple with δ_C 148.3 (C-13), indicating that the side chain was bonded to the tricyclic skeleton at the C-13 position.

The DQF COSY spectrum of **1** revealed a spin system involving three olefinic protons at δ_H 7.28 (d, J = 10.8 Hz, H-17), 6.94 (dd, J = 10.8, 15.4 Hz, H-16), and 8.37 (d, J = 15.4 Hz, H-15). The large coupling constant between H-15 and H-16 implied an *E* configuration. There were two other

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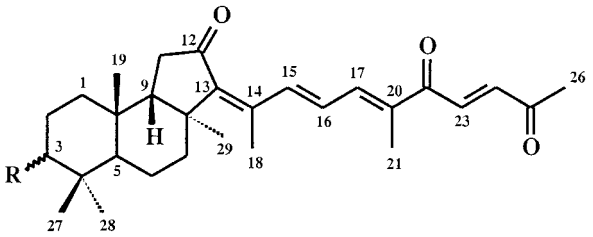

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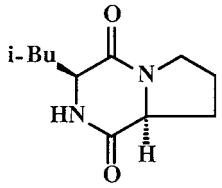
2 R = α-H; β-OAc



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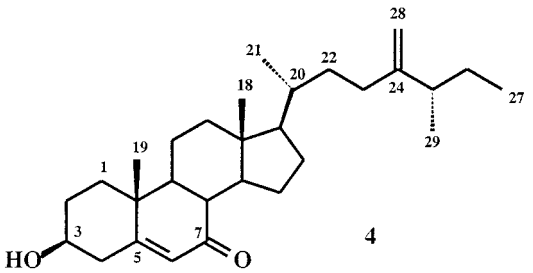

4

olefinic protons [δ_H 7.56 (d, J = 15.7 Hz, H-24) and 6.91 (d, J = 15.7 Hz, H-23)] coupled to each other in an *E*-geometry. The relative stereochemistry of **1** was further determined on the basis of NOE results. Thus, NOE enhancements observed for the pairs H-18/H-16, H-16/H-21, and H-15/H-17 confirmed the *E*-configurations for the 15,17-diene system. The 2D NOESY cross-peaks between H-5/H-28, H-5/H-29, H-9/H-19, and H-19/H-27 indicated a trans−syn−trans stereochemistry of the tricyclic system. Such results are consistent with the proposed isomalabaricane skeleton.[15] Finally, a *Z* geometry at the C-13/C-14 position was assumed on the basis of a deshielding effect observed for H-15 (δ 8.37), presumably being caused by the neighboring C-12 carbonyl group. A similar chemical shift has been observed for H-15 in stellettin B (δ 8.25), whereas the same proton in stellettin A resonated further upfield at δ 6.93. All available evidence led to the determination of the geoditin A structure as depicted in **1**. It is a new norisomalabaricane compound with one carbon atom removed from the side chain of isomalabaricane.

Geoditin B (**2**) was obtained as yellow microcrystals. A molecular ion was observed at *m*/*z* 494 in the EIMS, consistent with a molecular formula $C_{31}H_{42}O_5$. The UV absorption bands at λ_{max} 351 and 293 nm suggested the presence of a highly conjugated system. The IR absorptions at 1734 and 1684 cm^{-1} were consistent with the existence of ester and conjugated ketone groups. A comparison of the NMR data of **2** with those of **1** revealed great similarities, except at C-3. It was obvious that the keto group at C-3 of **1** was replaced by an acetoxy group in **2**. This was supported by the HMBC long-range coupling signals observed between C-3 and H-27/H-28. The stereochemistry at C-3 was then determined to be 3α-H on the basis of an NOE enhancement observed between H-3 and H-28. The structure of geoditin B (**2**) was therefore determined to be a 3β-acetoxy analogue of **1**.

Table 1. ^{13}C NMR Spectral Data of **1**, **2**, and **4**[a]

position	1	2	4
1	31.3 t	33.1 t	31.3 t
2	33.3 t	25.2 t	30.4 t
3	218.6 s	80.7 d	70.5 d
4	46.7 s	38.1 s	41.7 t
5	45.3 d	46.7 d	164.9 s
6	19.6 t	18.4 t	126.0 d
7	36.9 t	38.2 t	202.0 s
8	44.9 s	44.9 s	45.4 d
9	47.7 d	50.0 d	45.5 d
10	34.8 s	35.6 s	43.2 s
11	36.7 t	36.8 t	21.3 t
12	206.0 s	206.5 s	38.8 t
13	148.3 s	149.4 s	29.8 s
14	138.4 s	138.3 s	54.6 d
15	140.2 d	140.5 d	26.3 t
16	128.3 d	128.7 d	28.6 t
17	141.3 s	141.5 d	50.0 d
18	15.9 q	16.0 q	12.1 q
19	23.5 q	22.5 q	12.2 q
20	140.8 s	140.3 s	35.7 d
21	12.1 q	12.2 q	17.4 q
22	190.8 s	190.9 s	36.4 t
23	137.1 d	137.1 d	34.7 t
24	133.6 d	133.8 d	155.0 s
25	197.8 s	197.9 s	41.9 d
26	28.7 q	28.8 q	28.4 t
27	29.2 q	29.1 q	19.9 q
28	19.3 q	17.1 q	107.1 t
29	24.5 q	24.6 q	19.0 q
$OCOCH_3$		170.9 s	
$COCH_3$		21.4 q	

[a] Spectra were obtained in CD_3OD for **1** and **2** and in $CDCl_3$ for **4** at 100 MHz, using TMS as internal standard.

In the literature, only a small number of isomalabaricane structures have been reported, mainly from marine sponge genera such as *Stelleta*,[13−16,19] *Jaspis*,[17,20−21] and *Rhabdastrella*.[18,22] Nortriterpenes based on isomalabaricane are very rare, the only examples being the jaspiferals found in *Jaspis stellifera*.[23]

Compound **3** exhibited characteristic NMR features of a diketopiperazine structure. A comparison with published data led to the identification as *cyclo*(leucyl-prolyl).[24,25] To establish the stereochemistry of the amino acids, **3** was hydrolyzed and subjected to a chiral MS analytical technique recently developed in our laboratory.[26,27] Briefly, the hydrolytic products of the cyclodipeptide were mixed with the chiral selectors, D- and L-BBSer (*N*-*tert*-butoxycarbonyl-*O*-benzylserine), respectively, and subjected to ESIMS/MS. The ions at *m*/*z* 706 and 722, corresponding to the protonated trimers formed between BBSer and proline, and between BBSer and leucine, respectively, were selected for MS/MS analysis. Chiral discrimination was clearly observed in favor of the trimer derived from D-BBSer. It was therefore concluded that both amino acids were in the L-configuration, and compound **3** was determined to be *cyclo*(*S*-leucyl-*S*-prolyl).

Compound **4** displayed a molecular ion at *m*/*z* 426, consistent with the molecular formula $C_{29}H_{46}O_2$ (elemental analysis). Its UV spectrum (λ_{max} 234 nm) suggested the presence of a conjugated ketone system; the IR absorption bands further indicated hydroxyl (ν_{max} 3535 cm^{-1}), α,β-unsaturated keto (ν_{max} 1680 cm^{-1}), and terminal methylene (163 and 932 cm^{-1}) functional groups. On the basis of the characteristic NMR features of a sterol molecule (Table 1) and a positive Liebermann−Burchard reaction, a sterol structure containing 5-ene (δ_H 5.69) and 3β-OH (δ_H 3.70) functionalities was assigned. A comparison of the spectral data of **4** with those of 26-methylergosta-5,24(28)-dien-3β-

Table 2. ^{1}H NMR Spectral Data of **1**, **2**, and **4**[a]

position	1	2	4
1	1.50 m, 2.12 m	1.52 m, 1.38 m	1.98 m[b]
2	2.38m, 2.74 m	1.70 m, 1.80 m	1.58 m, 1.92 m
3		4.57 m	3.70 m
4			2.40 m
5	2.35 m	1.96 m	
6	1.53 m, 1.62 m	1.65 m	5.69 m
7	2.1 m	2.2 m	
8			2.22 m
9	1.90 m	1.92 m	2.22 m
11	2.26 m	2.25 m	1.59 m
12			1.10 m
14			1.16 m
15	8.37 (d, J = 15.4 Hz)	8.35 (d, J = 15.3 Hz)	1.20 m
16	6.94 (dd, J = 10.8, 15.4 Hz)	6.90 (dd, J = 15.3,13.4 Hz)	1.45 m
17	7.28 (d, J = 10.8 Hz)	7.28 (d, J = 13.4 Hz)	1.35 m
18	2.10 (s)	2.06 (s)	0.69 s
19	0.87 (s)	1.04 (s)	1.01 s
20			1.41 m
21	2.03 (s)	2.07 (s)	0.85 (d, J = 7.2 Hz)
22			1.18 m
23	6.91 (d, J = 15.7 Hz)	6.95 (d, J = 15.6 Hz)	1.15 m, 1.56 m
24	7.56 (d, J = 15.7 Hz)	7.56 (d, J = 15.6 Hz)	
25			2.42 m
26	2.40 (s)	2.42 (s)	1.90 m
27	1.13 (s)	0.93 (s)	1.00 (d, J = 5.6 Hz)
28	1.06 (s)	0.90 (s)	4.69 br s
29	1.44 (s)	1.40 (s)	0.94 m
OCH_3		2.07 (s)	

[a] Spectra were obtained in $CDCl_3$ at 400 MHz, using TMS as internal standard. Proton couplings and one-bond ^{1}H−^{13}C correlations were established by COSY and HETCOR experiments, respectively. [b] Overlapped and unresolved signal. Assignments were made on the basis of COSY and HETCOR results.

ol, which was also obtained in the present study, clearly showed that both compounds were almost identical. A major difference was noted, however, at the C-7 position of **4**, where a carbonyl carbon (δ_C 202.0) exhibited long-range HMBC correlation with H-9 (δ_H 2.22) and H-14 (δ_H 1.16). The structure of **4** was thus determined to be 26-methylergosta-5,24(28)-dien-7-one-3β-ol. To the best of our knowledge, it is a new sterol structure.

Other compounds isolated from the sponge extract were identified by interpretation of their spectral data as well as by comparison with published values. They were determined to be 24-methylcholesta-5,24(28)-dien-3β-ol,[28] 26-methylergosta-5,24(28)-dien-3β-ol,[29] palmitic acid,[30] uracil,[31] thymine,[31] and malonic acid.[31]

Experimental Section

General Experimental Procedures. Melting points were recorded on a Leica Galen III melting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL JNM-EX-400-FT-NMR spectrometer. IR spectra were obtained on a Perkin-Elmer 16 PC FT-IR spectrometer, and MS on a Finnigan TSQ 7000 mass spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were measured on a Milton Roy 3000 Array spectrometer.

Sponge Sample. The sponge *Geodia japonica* was collected in Xisha Island, the South China Sea, and identified by Professor Li Chupu (South China Sea Institute of Oceanography, the Chinese Academy of Sciences). Voucher specimens (ZWH95004) were deposited in the Research Center of Organic Natural Products Chemistry, Zhongshan (Sun Yat Sen) University, Guangzhou, China.

Extraction and Isolation. A sample of *G. japonica* (1 kg dry wt) was cut into small species and soaked in EtOH (5 × 3 L) at room temperature overnight. The extract was evaporated under reduced pressure to afford a residue, which was partitioned between H_2O and $CHCl_3$.

The $CHCl_3$-soluble fraction (16 g) was subjected to vacuum liquid chromatography using solvent gradients of hexane−acetone. Five fractions (A−E) were obtained based on their TLC pattern. From fraction A, palmitic acid (56 mg) was obtained following chromatography on Si gel. Fraction B yielded a crop of 24-methylcholesta-5,24(28)-dien-3β-ol (112 mg). Fraction C afforded **3** (10 mg) after chromatography on Si gel using gradient mixtures of hexanes−EtOAc. Fraction D was chromatographed on Si gel (hexanes−EtOAc gradients), followed by RP-18 (CH_3OH−H_2O, 10:2) to afford **2** (8 mg). Fraction E (1.5 g) was subjected to Si gel chromatography (hexane−acetone−CH_3OH) to yield stellettin A (36 mg). Rechromatography of the mother liquors on RP-18 gel (CH_3OH−H_2O, 4:1) resulted in the purification of **1** (18 mg) and stellettin B (23 mg).

The H_2O fraction was partitioned with *n*-BuOH, yielding an *n*-BuOH-soluble fraction (5 g), which was separated on Si gel eluting with $CHCl_3$−CH_3OH gradients. Four compounds, i.e., uracil (30 mg), thymine (25 mg), 26-methylergosta-5,24-(28)-dien-3β-ol (5 mg), and **4** (21 mg), were obtained.

From the H_2O fraction, malonic acid (554 mg) was obtained following ion-exchange chromatography on CCR-3 (eluted with 0.2 N HOAc) and on LH-20 (eluted by CH_3OH).

Geoditin A (1): yellow microcrystals; mp 127−128 °C; $[\alpha]^{25}_D$ −138.9° (*c* 0.011, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 357 (3.26), 293 (2.4) nm; IR (KBr) ν_{max} 2980, 1720, 1691 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m*/*z* 450 [M$^+$] (24), 407 (56), 353 (44), 326 (12), 213 (10), 187 (20), 159 (24), 149 (100), 135 (68); *anal.* C 77.41%, H 8.44%, calcd for $C_{29}H_{38}O_4$, C 77.30%, H 8.50%.

Geoditin B (2): yellow microcrystals; mp 144−145 °C; $[\alpha]^{25}_D$ −271.6° (*c* 0.014, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 293 (1.53), 351 (1.59) nm; IR (KBr) ν_{max} 2985, 1734, 1710, 1684, 1208 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m*/*z* 494 [M]$^+$ (24), 451 (51), 397 (50), 357 (18), 255 (12), 213 (16), 201 (20), 175 (30), 159 (34), 135 (100).

26-Methylergosta-5,24(28)-dien-7-one-3β-ol (4): white solid; mp 50−51 °C; $[\alpha]^{25}_D$ −79° (*c* 0.006, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 234 (3.38) nm; IR (KBr) ν_{max} 3535, 1680, 1635, 932 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m*/*z* 426 [M]$^+$ (40), 408 (12), 389 (18), 328 (76), 287 (10), 285 (60), 205 (100), 187 (68), 161 (56), 91 (54), 55 (68); *anal.* C 81.58%, H 10.78%, calcd for $C_{29}H_{46}O_2$, C 81.63%, H 10.87%.

Hydrolysis and MS/MS Analysis of 3. A solution of **3** (0.5 mg) in 0.2 mL of aqueous sulfuric acid (33%, v/v) was heated overnight in an oven set at 105 °C. Saturated barium hydroxide was added to the reaction mixture to adjust the pH to 2. After removal of the precipitate by centrifugation, portions of the supernatant (25 μL) were mixed with 100 μL of D- or L-BBSer (2 mM in CH_3OH) prior to ESIMS analysis (conditions: syringe pump 3 μL/min, sheath gas 60 psi, spray voltage 4 kV, heated capillary 50 °C), followed by MS/MS (conditions: relative collision energy 8.2%, isolation width 18 u, mass range 350−800 u).

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