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STEROIDS FROM THE MARINE BRYOZOAN *Bugula neritina*

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*One new compound, 3*β*-hydroxy-25-methoxy-(23*E*)-cholesta-5,23-diene (**1**), together with five known steroids, cholesteryl myristate (**2**), cholest-4-en-3-one (**3**), cholesterol (**4**), 3*β*,5*α*,9*α*-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**5**), and 3*β*,5*α*,6*β*-trihydroxy-(22*E*,24*R*)-ergosta-7,22-diene (**6**), were isolated and identified from the marine bryozoan* Bugula neritina.

Keywords: marine bryozoan, *Bugula neritina*, 3*β*-hydroxy-25-methoxy-(23*E*)-cholesta-5,23-dien, sterols.

The marine bryozoan *Bugula neritina* L. is one of the most prominent bryozoans among the common fouling organisms, with a broad geographic range in the Atlantic, Pacific, and other areas. Since the discovery of their medical potential in 1968, a series of macrocyclic lactones termed bryostatins 1–20 and neristatins 1–2 [1–11] have been isolated from this bryozoan, while only a few reports on the steroids exist.

We previously reported the isolation of bryostatins from this animal and discovered a new antineoplastic macrolide, bryostatin 19 [10]. A further investigation on the steroids from the petroleum ether extract is reported in this paper.

The petroleum ether extract was repeatedly chromatographed over SiO_2 and Sephadex LH-20 using different solvent systems. A new steroid (**1**) and five known compounds, cholesteryl myristate (**2**), cholest-4-en-3-one (**3**), cholesterol (**4**), 3*β*,5*α*,9*α*-trihydroxy-(22*E*, 24*R*)-ergosta-7,22-dien-6-one (**5**), and 3*β*,5*α*,6*β*-trihydroxy-(22*E*,24*R*)-ergosta-7,22-diene (**6**) were isolated. The structures were identified using spectral methods, including 1D, 2D NMR spectroscopies and mass spectrometry.

Compound **1** was isolated as a white crystal. The ^1H and ^{13}C NMR spectra exhibited the presence of six methyls (four of which were tertiary methyls, one of which was oxygenated), nine methylenes, six methines (one of which was oxygenated), three characterstics quaternary carbons at δ_C 42.40, 36.53, and 74.88, and four olefinic carbons with corresponding proton signals at δ_H 5.53 (m), 5.41 (d, J = 16.1 Hz), and 5.35 (t). These data show that **1** possessed a cholesta skeleton having two double bonds, one hydroxyl and one methoxyl. Two double bonds were assigned to be located between C-5 and C-6, as well as C-23 and C-24, by comparison of chemical shifts and coupling constants of three olefinic proton signals with that of a related compound [12, 13]. The assignment was further confirmed by HMBC experiment (Fig. 1), in which long-range coupling for the olefinic proton δ_H 5.35 (H-6) to δ_C 140.80 (C-5), H-6 to 36.53 (C-10), H-6 to 31.90 (C-7), indicated the position of an olenific linkage between C-5 and C-6. The other olefinic protons [δ_H 5.53 (H-23), 5.41(H-24)] showing cross peaks to δ_C 39.15 (C-22) and 74.88 (C-25), respectively, not only indicated another olefinic linkage between C-23 and C-24 but also confirmed the methoxyl at C-25 [a correlation between δ_H 3.14 (OCH_3) and δ_C 74.88 (C-25)]. The other hydroxyl was placed at C-3, based on cross peaks between δ_H 2.26 (H-4) and δ_C 71.82 (C-3) in the HMBC spectrum.

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TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR Data of **1** (125 MHz) ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	37.28 (CH_2)	1.86 m	14	56.78 (CH)	0.99 m
		1.05 m	15	24.33 (CH_2)	1.07 m
2	31.69 (CH_2)	1.87 m			1.59 m
			16	28.28 (CH_2)	1.26 m
3	71.82 (CH)	3.50 m			1.84 m
4	42.33 (CH_2)	2.26 m	17	55.86 (CH)	1.13 m
		2.31 m	18	11.93 (CH_3)	0.69 s
5	140.80 (C)		19	18.75 (CH_3)	0.93 (d, J = 6.5)
6	121.68 (CH_2)	5.35 t	20	36.08 (CH)	1.47 m
7	31.90 (CH_2)	1.46 m	21	19.41 (CH_3)	1.01 s
		1.97 m	22	39.15 (CH_2)	1.84 m
8	31.94 (CH)	1.44 m			2.16 m
			23	128.65 (CH)	5.53 m
9	50.15 (CH)	0.92 m	24	136.67 (CH)	5.41 (d, J = 16.1)
10	36.53 (C)		25	74.88 (C)	
11	21.10 (CH_2)	1.45 m	26	25.81 (CH_3)	1.25 s
12	39.75 (CH_2)	2.00 m	27	26.15 (CH_3)	1.25 s
		1.14 m	OCH_3	50.24	3.14 s
13	42.40 (C)				

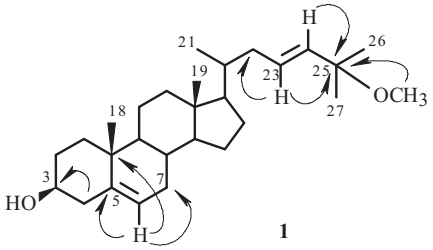

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Fig. 1. Key HMBC correlations of **1**.

The structure of **1** was elucidated by a combination of ^{1}H–^{1}H COSY, HMQC, and HMBC experiments as 3β-hydroxy-25-methoxy-(23*E*)-cholesta-5,23-diene. Compounds **2** to **6** were isolated from *Bugula neritina* L. for the first time.

EXPERIMENTAL

Uncorrected melting points were determined on a Yamato MP-21 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Inova-400 and Bruker Avance-500 using $CDCl_3$ as a solvent. Mass spectra were recorded on a MAT-212 and Quattro spectrometer. Column chromatography was performed over silica gel 60 (200–300 mesh) supplied by Qindao Marine Chemical Factory. Sephadex LH-20 (25–100 μm) used for gel permeation and partition chromatography was obtained from Pharmacia Co., Ltd.

About 100 kg (wet weight) *Bugula neritina* were collected off Daya Bay in May 2004 at a depth of 0–0.5 m. Taxonomic identification was performed by Professor Chuan-yan Li (Third Institute of Oceanography, National Bureau of Oceanography). Specimens were air-dried and extracted with 95% ethanol five times, and each extraction took one week. The resulting extract solution was removed and concentrated under reduced pressure to give aqueous extracts. The aqueous extracts were extracted with dichloromethane. The dichloromethane phase was concentrated in vacuo, suspended in a 9:1 methanol–water solution, and partitioned with petroleum ether. The petroleum ether phase was separated and concentrated in vacuo to give 856 g petroleum ether extract. The petroleum ether extract was subjected to silica gel flash column chromatography using a gradient solvent system (petroleum ether–ethyl acetate) to give five fractions. Fraction 1 gave a white crystal when it was eluted, and was repeatedly crystallized to give compound **2** (78.6 mg). Fraction 3 was worked up by silica gel chromatography repeatedly using different solvent systems, and compounds **3** (49.3 mg) and **4** (59.7 mg) were isolated. Fraction 5 was chromatographed over Sephadex LH-20 (CH_2Cl_2–MeOH, 1:1) and silica gel in different solvent systems to afford compounds **1** (12 mg), **5** (23.6 mg), and **6** (30.5 mg).

3β-Hydroxy-25-methoxy-(23*E*)-cholesta-5,23-diene (1), $C_{28}H_{46}O_2$, white crystal, mp 121–123°C. APCI-MS *m/z*: 384 $[M - OCH_3]^+$, 282, 256, 132. ^1H NMR and ^{13}C NMR data: Table 1.

Cholesteryl myristate (2), $C_{40}H_{68}O_2$, white solid, mp 74–76°C. EI-MS *m/z*: 386, 368, 255, 124, 55. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.35 (1H, t, H-6), 4.61 (1H, m, H-3), 1.02 (3H, s, H-19), 0.96 (3H, d, J = 6.5, H-21), 0.88 (3H, d, J = 6.6, H-26), 0.87 (3H, d, J = 6.6, H-27), 0.69 (3H, s, H-18). The ^{13}C NMR spectrum agrees with that published [14].

Cholest-4-en-3-one (3), $C_{27}H_{44}O$, white solid, mp 80–82°C. EI-MS *m/z*: 384 $[M]^+$, 342, 261, 229, 124, 55. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.72 (1H, s, H-4), 1.18 (3H, s, H-19), 0.93 (3H, d, J = 6.5, H-21), 0.88 (3H, d, J = 6.6, H-26), 0.87 (3H, d, J = 6.6, H-27), 0.72 (3H, s, H-18). The ^{13}C NMR spectrum agrees with that published [15].

Cholesterol (4), $C_{27}H_{46}O$, colorless crystal, mp 142–144°C. EI-MS *m/z*: 386 $[M]^+$, 368, 255, 213, 145, 107, 55. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.35 (1H, t, H-6), 3.50 (1H, m, H-3), 1.01 (3H, s, H-19), 0.93 (3H, d, J = 6.5, H-21), 0.88 (3H, d, J = 6.6, H-26), 0.87 (3H, d, J = 6.6, H-27), 0.69 (3H, s, H-18). The ^{13}C NMR spectrum agrees with that published [16].

3β,5α,9α-Trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (5), $C_{28}H_{44}O_4$, white powder, mp 195–197°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.65 (1H, s, H-7), 5.19 (2H, m, H-22,23), 4.06 (1H, m, H-3), 1.03 (3H, s, H-19), 1.00 (3H, d, J = 6.6, H-28), 0.92 (3H, d, J = 6.5, H-21), 0.85 (3H, d, J = 6.6, H-26), 0.82 (3H, d, J = 6.6, H-27), 0.68 (3H, s, H-18). The ^{13}C NMR spectrum agrees with that published [17].

3β,5α,6β-Trihydroxy-(22*E*,24*R*)-ergosta-7,22-diene (6), $C_{28}H_{46}O_3$, white powder, mp 182–184°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.30 (1H, s, H-7), 5.19 (1H, m, H-23), 5.04 (1H, m, H-22), 3.99 (1H, m, H-3), 1.06 (H, s, H-19), 1.03 (3H, d, J = 6.6, H-28), 0.92 (3H, d, J = 6.5, H-21), 0.86 (3H, d, J = 6.6, H-26), 0.84 (3H, d, J = 6.6, H -27), 0.62 (3H, s, H-18). The ^{13}C NMR spetrum agrees with that published [18].

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REFERENCES

1. G. R. Pettit, C. L. Herald, D. L. Doubek, and B. H. Wood, *J. Am. Chem. Soc.*, **104**, 6846 (1982).
2. G. R. Pettit, C. L. Herald, Y. Kamano, D. Gust, and A. Reiko, *J. Nat. Prod.*, **46**, 528 (1983).
3. G. R. Pettit, C. L. Herald, and Y. Kamano, *J. Org. Chem.*, **48**, 5354 (1983).
4. G. R. Pettit, Y. Kamano, C. L. Herald, and T. Machiko, *J. Am. Chem. Soc.*, **106**, 6768 (1984).
5. G. R. Pettit, Y. Kamano, C. L. Herald, and T. Machiko, *Can. J. Chem.*, **63**, 1204 (1985).
6. G. R. Pettit, Y. Kamano, and C. L. Herald, *J. Nat. Prod.*, **49**, 661 (1986).
7. G. R. Pettit, Y. Kamano, and C. L. Herald, *J. Org. Chem.,* **52**, 2848 (1987).
8. G. R. Pettit, J. E. Leet, C. L. Herald, Y. Kamano, F. E. Boettner, and R. A. Nieman, *J. Org. Chem.,* **52**, 2854 (1987).
9. G. R. Pettit, F. Gao, P. M. Blumberg, C. L. Herald, C. L. Coll, N. E. Lewin, Y. Kamano, J. M. Schmidt, and J. C. Chapuis, *J. Nat. Prod.,* **59**, 286 (1996).
10. H. W. Lin, Y. H. Yi, W. L. Li, and X. S. Yao, *Chin. J. Mar. Drugs*, **65** (1), 1 (1998).
11. N. Lopanik, K. R. Gustafson, and N. Lindquist, *J. Nat. Prod.,* **67**, 1412 (2004).
12. J. A. Findlay and A. D. Patil, *Can. J. Chem.*, **63**, 2406 (1985).
13. L. K. Shubina, T. N. Makar′eva, and V. A. Stonik, *Chem. Nat. Comp.,* **20**, 438 (1984).
14. D. H. Croll, D. M. Small, and J. A. Hamilton, *J. Chem. Phys.*, **85**, 12, 7381 (1986).
15. X. Jiang and F. D. Covey, *J. Org. Chem.*, **67**, 4893 (2002).
16. D. J. Xiao, S. Z. Deng, and L. M. Zeng, *Chin. J. Mar. Drugs*, **21** (2), 1 (2002).
17. Y. Yaoita, M. Endo, Y. Tani, K. Machida, K. Amemiya, K. Furumura, and M. KiKuchi, *Chem. Pharm. Bull.*, **47** (6), 847 (1999).
18. A. Madaio, V. Piccialli, and D. Sica, *J. Nat. Prod.,* **52** (5), 952 (1989).