Three New Polyoxygenated Steroids from Two Species of the South China Sea Gorgonian *Muricella flexuosa* and *Menella verrucosa* BRUNDIN

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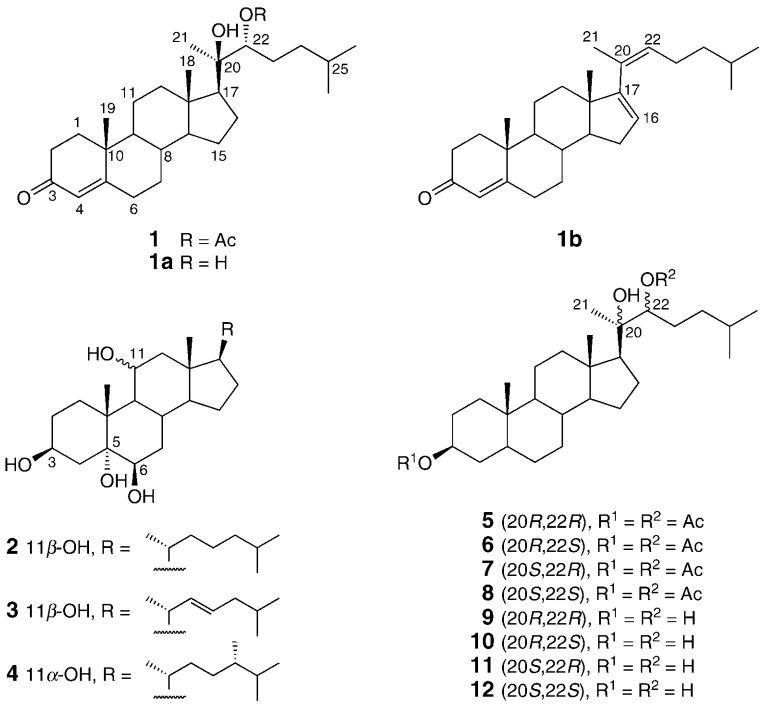
Three new polyoxygenated steroids, muricesteroid (**1**), and menellsteroids A (**2**) and B (**3**), were isolated from two species of the South China Sea gorgonian *Muricella flexuosa* and *Menella verrucosa* BRUNDIN, respectively. The structures of these new compounds were elucidated on the basis of extensive spectroscopic analysis, chemical methods and comparison with known related compounds.

Introduction. – Gorgonians of the genera *Muricella* and *Menella* (both of them belong to the family Paramuriceidae, order Gorgonacea, class Anthozoa) is prolific in the South China Sea. A literature survey revealed that the chemical constituents of the gorgonians of the genus *Muricella* have been intensively investigated. A variety of secondary metabolites with different C-skeletons such as eunicellane diterpenoids [1–3], 9,11-secosteroids [4][5], and a carotenoid [6] were reported from various species of this genus, and some of them exhibited potent cytotoxic activities against several tumor cell lines [2][3][5], and toxic effects to brine-shrimp as well [2–6]. In contrast, chemical studies on the genus *Menella* were relatively rare. In particular, no chemical study has been done on the Hainan gorgonian *Menella verrucosa* BRUNDIN, except for our recent work [7c] reporting five new uncommon guaiane lactones from the species.

Recently, in the course of our systematic studies on the chemical constituents of the South China Sea gorgonians [7], we have made a collection of *Muricella flexuosa* off Sanya, Hainan Province, China. Chemical investigation of the Et_2O-soluble fraction of the Me_2CO extract from *Muricella flexuosa* resulted in the isolation of a new oxygenated steroid, named muricesteroid (**1**). Further, a continuous chemical study on the Et_2O-soluble fraction of the Me_2CO extract from another gorgonian, *Menella verrucosa*, allowed to isolate two new polyhydroxylated steroids, named menellsteroids A (**2**) and B (**3**), respectively. Herein, we describe the isolation and structural elucidation of these new metabolites.

Results and Discussion. – Both gorgonians were collected off the coast of Xiaodong Hai, Hainan Province, China, in December 2001, at a depth of 20 m. The animals were immediately put at $-20°$ and kept frozen until extraction. Frozen materials of *Muricella*



flexuosa were cut into small pieces and subsequently extracted with Me_2CO. The Et_2O-soluble portion from the Me_2CO extract was repeatedly subjected to column chromatographed (silica gel, *Sephadex LH-20*) to afford muricesteroid (**1**). In a similar manner, menellsteroids A (**2**) and B (**3**) were obtained from the gorgonian *Menella verrucosa*.

All three compounds were highly oxygenated steroids possessing a cholestane skeleton. The structures of the new metabolites were elucidated by detailed analysis of their spectroscopic data, chemical correlations, and comparison with those of closely related known compounds, *i.e.*, **4** and **5**–**12**.

Muricesteroid (**1**) was obtained as a colorless glass. Its molecular formula $C_{29}H_{46}O_4$, deduced from the HR-ESI-MS exhibiting the pseudo-molecular ion at *m*/*z* 481.3294 ($[M+Na]^+$), indicated seven degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra (*Table 1*), HMQC, COSY and HMBC data (*Fig. 1*), and comparison with data of known compounds (*Table 2*), established the structure of **1** as (20*R*,22*R*)-22-(acetyloxy)-20-hydroxycholest-4-en-3-one. The absolute configurations (20*R*,22*R*) were confirmed by comparison of the ^{1}H-NMR data of the 22-*O*-deacetyl derivative **1a** with similar compounds.

Analysis of the ^{13}C-NMR and DEPT spectra (*Table 1*) of **1** assigned three of the seven degrees of unsaturation to a C=C (δ 123.8 (*d*), 171.4 (*s*)), one C=O (δ 199.6 (*s*)), and an AcO moiety (δ 21.1 (*q*), 172.5 (*s*)). Consequently, the remaining unsaturations were due to four rings. In addition, ^{13}C-NMR and DEPT spectra also supported the presence of 24 sp^3 C-atoms (5 Me, 10 CH_2, 6 CH, and 3 C), including an oxygenated secondary C-atom (79.1, *d*) and a related tertiary C-atom (77.2, *s*).

Table 1. *NMR Data*[a])[b]) *for Muricesteroid* (**1**). δ in ppm, J in Hz.

	δ(H)	δ(C)[c])		δ(H)	δ(C)[c])
CH_2(1)	1.66–1.71 (*m*), 2.02 (*dt*, *J* = 13.8, 3.6)	35.6 (*t*)	CH_2(15)	1.27–1.34 (*m*), 1.64–1.71 (*m*)	23.8 (*t*)
CH_2(2)	2.31–2.36 (*m*), 2.41–2.46 (*m*)	34.0 (*t*)	CH_2(16)	1.19–1.25 (*m*), 1.82–1.89 (*m*)	22.0 (*t*)
C(3)		199.6 (*s*)	CH(17)	1.43–1.46 (*s*)	55.3 (*d*)
CH(4)	5.73 (*s*)	123.8 (*d*)	Me(18)	0.91 (*s*)	13.6 (*q*)
C(5)		171.4 (*s*)	Me(19)	1.20 (*s*)	17.3 (*q*)
CH_2(6)	2.26 (*dt*, *J* = 13.5, 2.0), 2.37–2.43 (*m*)	32.9 (*t*)	C(20)		77.2 (*s*)
			Me(21)	1.24 (*s*)	20.8 (*q*)
CH_2(7)	1.00–1.05 (*m*), 1.80–1.89 (*m*)	31.9 (*t*)	CH(22)	4.80 (*d*, *J* = 9.0)	79.1 (*d*)
			CH_2(23)	1.18–1.24 (*m*), 1.36–1.44 (*m*)	27.8 (*t*)
CH(8)	1.53–1.63 (*m*)	34.8 (*d*)			
CH(9)	0.87–0.94 (*m*)	53.8 (*d*)	CH_2(24)	1.12–1.19 (*m*), 1.12–1.19 (*m*)	35.7 (*t*)
C(10)		38.6 (*s*)			
CH_2(11)	1.45–1.51 (*m*), 1.43–1.53 (*m*)	20.9 (*t*)	CH(25)	1.45–1.55 (*m*)	27.8 (*d*)
			Me(26)[d])	0.87 (*d*, *J* = 6.6)	22.8 (*q*)
CH_2(12)	1.18–1.25 (*m*), 2.14 (*dt*, *J* = 12.6, 2.4)	40.1 (*t*)	Me(27)[d])	0.88 (*d*, *J* = 6.6)	22.3 (*q*)
			*Me*COO–C(22)	2.10 (*s*)	21.1 (*q*)
C(13)		43.4 (*s*)	MeCOO–C(22)		172.5 (*s*)
CH(14)	0.99–1.04 (*m*)	55.8 (*d*)			

[a]) *Bruker-DRX*-400-MHz spectrometers, $CDCl_3$, chemical shifts referred to $CHCl_3$ (δ(H) 7.26) and to $CDCl_3$ (δ(C) 77.0). [b]) Assignments made by ^{1}H,^{1}H COSY, HSQC, and HMBC. [c]) By DEPT sequence. [d]) Signals may be interchanged.

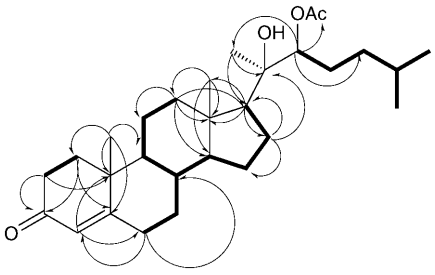


Fig. 1. *^{1}H,^{1}H-COSY* (▬) *and selected HMBC* (⌒) *correlations of* **1**

Two downfield ^{1}H-NMR signals were assigned to the protons of a trisubstituted C=C (δ 5.73 (*s*)) and a CH–O (δ 4.80 (*d*, *J* = 9.0)). Obviously, the Me *s* at δ 2.10 should be assigned to the AcO, while the additional three Me *s* δ 1.24, 1.20, and 0.91 and two *d* of an ^{i}Pr group (δ 0.88 and 0.89 (each *J* = 6.6 Hz, 3 H)) were attributed to the Me groups of the cholestane skeleton. Finally, the *m* integrating for 24 H-atoms between δ 2.48 and 0.98 were due to 10 CH_2 and 4 CH as established by HMQC experiments. ^{1}H,^{1}H-COSY experiments established the proton sequence as depicted in *Fig. 1*. All these data strongly suggested for **1** a cholest-4-enone framework substituted by both OH and AcO. Significant HMBC long-range correlations H–C(17)/C(13), C(16), and C(18), Me(18)/C(12), C(13), and C(14), and Me(19)/C(1), C(5), C(9), and C(10), unambiguously confirmed the suggested skeleton. The presence of a conjugated 4-en-3-one moiety was deduced from the typical δ values of the trisubstituted C=C (δ(H) 5.73 (*s*), δ(C) 123.8 (*d*) and 171.4 (*s*)) and C=O (δ 199.6 (*s*)), and further confirmed by the long-range ^{1}H,^{13}C-correlations CH_2(1)/C(3) and C(5), CH_2(2)/C(3) and C(10), as well as H–C(4)/C(6) and C(10). The obvious correlations H–C(22)/CH_2(23)/CH_2(24)/H–C(25)/Me(26) and Me(27)) in the ^{1}H,^{1}H-COSY allowed to

place the CH–O (δ 4.80) at C(22). The distinguished long-range correlations H–C(22)/C(20), C(21) and C(24) and δ(C) 172.5 not only confirmed the above conclusion but also revealed the linkage between C(22) and AcO. As a consequence, the remaining OH had to be connected to C(20) according to the *s* of Me(21) (δ 1.24) in the ^{1}H-NMR spectrum. This assignment was supported by the long-range correlations Me(21)/C(20), C(17), and C(22) in the HMBC spectrum (*Fig. 1*).

The absolute configuration at the chiral centers C(20) and C(22) of **1** was tentatively assigned as (20*R*) and (22*R*), mainly by comparison of the ^{1}H-NMR data of **1** and its 22-hydrolyzate **1a** with those of the related model compounds **5**–**8** and **9**–**12**, respectively. Some selected ^{1}H-NMR data of **1**, **1a**, and **5**–**12** are listed in *Table 2*. As shown in *Table 2*, both the chemical shifts of Me(21) (δ 1.24) and H–C(22) (δ 4.80) of **1** were in good agreement with those of **5** (δ 1.23, 4.77), the (20*R*,22*R*)-isomer, suggesting that the absolute configuration of C(20) and C(22) of **1** could be the same as that of compound **5**. To confirm this suggestion, compound **1** was hydrolyzed to the expected 22-*O*-deacetyl derivative **1a**. The chemical shifts of Me(21) (δ 1.52) and H–C(22) (δ 3.75) of **1a** were almost identical to those of **9** (δ 1.51, 3.78) but different from those of the other three stereoisomers **10**–**12** (δ 1.60 and 3.70 for **10**, 1.44 and 3.84 for **11**, and 1.32 and 4.02 for **12**) [8], supporting the (20*R*,22*R*) configuration of **1**.

Table 2. *Selected ^{1}H-NMR Data for* **1** *and* **1a** *and Comparison with Those of Model Compounds* **5**–**12**[a]). δ in ppm, *J* in Hz.

	δ(Me(21))	δ(H–C(22))		δ(Me(21))	δ(H–C(22))
1[b])	1.24 (*s*)	4.80 (*d*, *J* = 9.0)	**1a**[c])	1.52 (*s*)	3.75 (*d*, *J* = 8.8)
5[b])	1.23 (*s*)	4.77 (*d*, *J* = 9.0)	**9**[c])	1.51 (*s*)	3.78 (*d*, *J* = 9.0)
6[b])	1.27 (*s*)	4.78 (*d*, *J* = 9.0)	**10**[c])	1.60 (*s*)	3.70 (*d*, *J* = 9.0)
7[b])	1.17 (*s*)	4.71 (*d*, *J* = 9.0)	**11**[c])	1.44 (*s*)	3.84 (*d*, *J* = 9.0)
8[b])	1.06 (*s*)	5.17 (*d*, *J* = 9.0)	**12**[c])	1.32 (*s*)	4.02 (*d*, *J* = 9.0)

[a]) Data reported in [8]. [b]) In $CDCl_3$. [c]) In C_5D_5N.

To our surprise, compound **1** was degraded completely during storage in the NMR tube. Interestingly, the main degradation product **1b** displayed a similarly strong UV absorption as **1** but was a less polar on TLC than **1**. This suggested that the degradation probably occurred at the polar parts of **1**, *i.e.*, in the side chain, while the conjugated 4-en-3-one unit remained intact. The structure of **1b** was corroborated by spectroscopic means.

The appearance of two new olefinic-proton signals at δ 5.60 (br. *s*) and 5.57 (*t*, *J* = 7.0 Hz) in **1b** indicated the formation of a new pair of C=C in the structure, implying elimination of both the OH and the AcO groups. This conclusion was consistent with the absence of the AcO signal in the ^{1}H-NMR spectrum of **1b**, and satisfied the molecular formula of $C_{27}H_{40}O$ established by the pseudo-molecular-ion at *m*/*z* 403.2980 ($[M+Na]^+$) in the HR-ESI-MS. Furthermore, the relatively downfield chemical shift value of the two new olefinic protons (δ 5.60, 5.57) suggested the formation of a conjugated unit which was further confirmed by the strong absorption at λ 229 nm in the UV spectrum. Me(21) was obviously attached to the conjugated C=C moiety as shown by its typical downfield shift at δ 1.76. The signal at δ 5.57 was thus assigned to the olefinic proton H–C(22) due to its coupling pattern (*t*, *J* = 7.0 Hz), and the resonance at δ 5.60 (br. *s*) to H–C(16).

Menellsteroid A (**2**) was obtained as optically active amorphous white powder. The molecular formula $C_{27}H_{48}O_4$ of **2** was established by the molecular ion peak at *m*/*z* 436.3544 (M^+) in the HR-EI-MS, indicating four degrees of unsaturation assignable to four rings of a cholestane skeleton. The structure of **2** was established as

(3β,5α,6β,11β)-cholestane-3,5,6,11-tetrol by the ^{1}H- and ^{13}C-NMR spectra (*Table 3*), COSY, HMBC, and NOESY data (*Fig. 2*), and comparison with the data of (3β,5α,6β)-cholestane-3,5,6-triol [9].

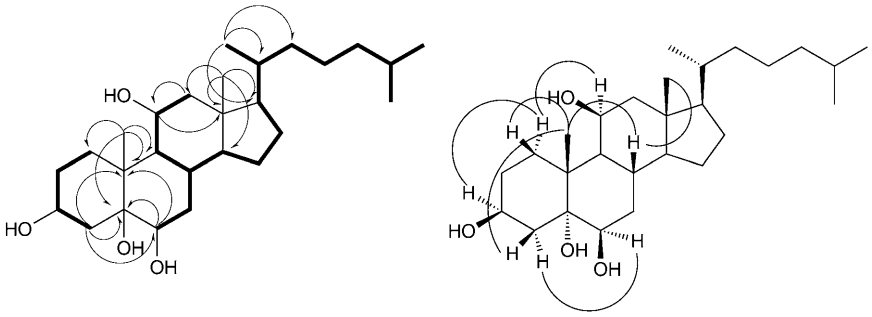


Fig. 2. *^{1}H,^{1}H-COSY (——), selected HMBC (⌒), and key NOESY (⌒) correlations of* **2**

The IR spectrum of **2** implied the presence of OH groups (3443 cm^{-1}), in agreement with the presence of four oxygenated C-atoms at δ 67.5 (*d*), 68.7 (*d*), 76.4 (*d*) and 76.8 (*s*) in the ^{13}C-NMR spectrum and three CH–O signals at δ 4.19, 4.66, and 4.80–4.88 in the ^{1}H-NMR spectrum (*Table 3*). Also, the ^{13}C-NMR and DEPT spectra revealed additional 23 sp^3 C-signals (2 C, 6 CH, 10 CH_2, 5 Me), which were completely assigned to their corresponding proton signals by the HMQC experiment (*Table 3*). Analysis of the ^{1}H,^{1}H-COSY data led to the two separated proton spin systems CH_2(1) to CH_2(4) and H–C(6) to Me(26)/Me(27)), as shown in *Fig. 2*. Two significant HMBC correlations Me(19)/C(1), C(5), C(9), and C(10), and Me(18)/C(12), C(13), C(14), and C(17) allowed to connect the two proton spin systems and to establish the constitution of **2** (*Fig. 2*). The location of HO–C(11) was deduced from the proton correlations H–C(9)/H–C(11)/CH_2(12), and further confirmed by the diagnostic long-range correlations H–C(11)/C(13) and CH_2(12)/C(11). Careful comparison of the ^{13}C-NMR data of **2** with those of cholestane-3β,5α,6β-triol [9] readily revealed that **2** is its 11-hydroxy analog. The α-configuration of H–C(11) was deduced from its coupling pattern (br. *s*) and supported by the observation of a distinct NOE cross-peak between H–C(11) and H_α–C(1). Moreover, the absence of NOE correlations between H–C(11) and both Me(18) and Me(19) further confirmed this conclusion.

Menellsteroid B (**3**) was obtained as optically active amorphous white powder. The HR-EI-MS data of **3** established the molecular formula $C_{27}H_{46}O_4$ (*m/z* 434.3411), resulting in 2 mass units less than **2**. Careful comparison the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** (*Table 3*) revealed that **3** differs from **2** only in the nature of the side chain. Analysis of a couple of new olefinic proton signals and comparison with reported data established the structure of **3** as (3β,5α,6β,11β,22*E*)-cholest-22-ene-3,5,6,11-tetrol.

New olefinic proton signals, an *AB* system at δ 5.34 (*ddd*, *J* = 15.6, 7.1, 6.7 Hz, H–C(23)) and 5.26 (*dd*, *J* = 15.6, 8.2 Hz, H–C(22)), were observed in ^{1}H-NMR spectrum of **3**, as compared to **2**. The corresponding C=C bond was positioned at C(22) due to the downfield shift of Me(21) (δ 1.09 in **3** and 0.99 in **2**). The (*E*)-configuration of this C=C bond was obviously deduced from the coupling constant *J*(22,23) = 15.6 Hz. Assignments of the ^{13}C-NMR signals (*Table 3*) for the side chain of **3** were strongly supported by comparison with reported data [7e][9a].

It may be worth to point out that among the polyhydroxylated steroids, an HO–C(11) group in β-configuration is quite rare. The related compound sarcoldesterol B

Table 3. *^{1}H- and ^{13}C-NMR Data*[a,b]) *for Menellsteroid A* (**2**) *and ^{13}C-NMR Data for Menellsteroid B* (**3**). δ in ppm, J in Hz.

No.	**2**		**3**
	δ(H)	δ(C)[c])	δ(C)[c])
H_α–C(1)	2.13 (*t*, *J* = 11.5)	33.2 (*t*)	33.2 (*t*)
H_β–C(1)	2.34–2.38 (overlapped)		
H_α–C(2)	2.25–2.29 (*m*)	32.6 (*t*)	32.7 (*t*)
H_β–C(2)	2.27–2.37 (*m*)		
H–C(3)	4.80–4.88 (*m*)	67.5 (*d*)	67.5 (*d*)
H_α–C(4)	2.35–2.39 (*m*)	42.6 (*t*)	42.6 (*t*)
H_β–C(4)	3.12 (*dd*, *J* = 12.9, 11.5)		
C(5)		76.8 (*s*)	76.8 (*s*)
H–C(6)	4.19 (br. *s*)	76.4 (*d*)	76.4 (*d*)
H_α–C(7)	2.29–2.35 (*m*)	37.1 (*t*)	37.1 (*t*)
H_β–C(7)	2.17–2.21 (*m*)		
H–C(8)	2.64–2.75 (*m*)	28,3 (*d*)	28.4 (*d*)
H–C(9)	2.18–2.22 (*m*)	49.1 (*d*)	49.2 (*d*)
C(10)		40.2 (*s*)	40.2 (*s*)
H–C(11)	4.66 (br. *s*)	68.7 (*d*)	68.7 (*d*)
H_α–C(12)	1.46–1.51 (overlapped)	50.3 (*t*)	50.2 (*t*)
H_β–C(12)	2.50 (*dd*, *J* = 13.4, 2.4)		
C(13)		42.6 (*s*)	42.5 (*s*)
H–C(14)	1.25–1.30 (*m*)	58.5 (*d*)	58.7 (*d*)
H_α–C(15)	1.72–1.79 (*m*)	24.9 (*t*)	24.8 (*t*)
H_β–C(15)	1.24–1.30 (*m*)		
H_α–C(16)	1.82–1.89 (*m*)	28.5 (*t*)	28.5 (*t*)
H_β–C(16)	1.23–1.30 (*m*)		
H–C(17)	1.10–1.14 (*m*)	57.3 (*d*)	57.0 (*d*)
Me(18)	1.26 (*s*)	15.1 (*q*)	15.2 (*q*)
Me(19)	2.20 (*s*)	20.4 (*q*)	20.4 (*q*)
H–C(20)	1.38–1.44 (*m*)	36.4 (*d*)	40.8 (*d*)
Me(21)	0.99 (*d*, *J* = 6.3)	19.0 (*q*)	21.2 (*q*)
H_a–C(22)	1.00–1.06 (*m*)	36.6 (*t*)	138.9 (*d*)
H_b–C(22)	1.00–1.06 (*m*)		
H_a–C(23)	1.15–1.23 (*m*)	24.3 (*t*)	126.5 (*d*)
H_b–C(23)	1.36–1.44 (*m*)		
H_a–C(24)	1.11–1.18 (*m*)	39.9 (*t*)	42.3 (*t*)
H_b–C(24)	1.11–1.18 (*m*)		
H–C(25)	1.45–1.56 (*m*)	28.4 (*d*)	28.9 (*d*)
Me(26)[d])	0.89 (*d*, *J* = 6.6)	22.8 (*q*)	22.5 (*q*)
Me(27)[d])	0.89 (*d*, *J* = 6.6)	23.0 (*q*)	22.6 (*q*)

[a]) *Bruker DRX*-400-MHz spectrometer, C_5D_5N, chemical shifts referred to C_5H_5N (δ(H) 7.20, 7.57, 8.73) and to C_5D_5N (δ(C) 123.6, 135.8, 150.0). [b]) Assignments made by HMQC and HMBC. [c]) By DEPT sequence. [d]) Signals may be interchanged.

(**4**) [10c] with an α-positioned HO–C(11) exhibited different ^{1}H-NMR data and coupling pattern of H–C(11) (δ 4.35 (*m*)) in comparison with those of **2** and **3** both resonating as br. *s* at δ 4.66.

Polyoxygenated steroids with a 3β,5α,6β-trihydroxy moiety are frequently encountered in marine organisms, such as in sponges [9a][11], anthozoans [9b][10], and starfishes [12]. It was reported that sterols with the 3,5,6-trihydroxy moiety might arise biogenetically from the corresponding sterols with a C(5)=C(6) moiety [9a]. Further studies should be conducted to verify this hypothesis by biosynthetic experiments.

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Experimental Part

General. Column chromatography (CC): silica gel (*Qing Dao Hai Yang Chemical Group Co.*; 200–300 and 400–600 mesh). Anal. TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60 F-254*). Optical rotation: *Perkin-Elmer-341* polarimeter. UV Spectra: *756-CRT* spectrophotometer; λ_{max} in nm. IR Spectra: *Nicolet-Magna-FT-IR-750* spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-DRX-400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); chemical shifts δ in ppm rel. to the residual $CHCl_3$ (δ(H) 7.26) or C_5H_5N (δ(H) 7.20, 7.57, 8.73) signals for ^{1}H, and $CDCl_3$ (δ(C) 77.0) or C_5D_5N (δ(C) 123.6, 135.8, 150.0) for ^{13}C, coupling constant J in Hz; assignments supported by ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY experiments. MS: EI and HR-EI, *Finnigan-MAT-95* mass spectrometer; ESI and HR-ESI, *Q-TOF-Micro-LC-MS-MS* mass spectrometer; in *m/z*.

Animal Material. Both gorgonians *Muricella flexuosa* and *Menella verrucosa* BRUNDIN were collected along the coast of Xiaodong Hai, Hainan Province, China, in December 2001, at a depth of 20 m. The voucher specimens are available for inspection at the Institute of Materia Medica, SIBS-CAS.

Extraction and Purification. The frozen animals (dry weight 28 g for *Muricella flexuosa* and 209 g for *Menella verrucosa*) were cut into small pieces and then extracted with acetone at r.t. The org. extracts were evaporated to give residues which were partitioned between Et_2O and H_2O. The Et_2O solns. were evaporated to give dark green residues: 301 g from *Muricella flexuosa* and 3.3 mg from *Menella verrucosa*). The residues were fractionated by CC (silica gel, light petroleum ether/acetone gradient), yielding fractions containing crude steroids. The crude steroid fraction from *Muricella flexuosa* was subjected to CC (*Sephadex-LH-20*): muricesteroid (**1**; 2.4 mg). The crude steroid fraction from *Menella verrucosa* was purified by CC (*Sephadex-LH-20*), followed by reversed-phase HPLC (semi-prep. *ODS-HG-5* (5 µ, 250×10 mm), MeOH/H_2O 3:1, 2.0 ml/min): menellsteroids A (**2**; 5.3 mg) and B (**3**; 1.2 mg).

Muricesteroid (=*(20*R,*22*R*)-22-(Acetyloxy)-20-hydroxycholest-4-en-3-one*; **1**). Colorless glass. ^{1}H-(400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 1*. HR-ESI-MS: 481.3311 ($[C_{29}H_{46}O_4+Na]^+$; calc. 481.3294).

Hydrolysis of Muricesteroid (**1**). To **1** (0.2 mg) in dry MeOH (1.0 ml), dry Na_2CO_3 (3.0 mg) was added, and the mixture was stirred at r.t. for 72 h. Having adjusted the pH to 7 with dil. HCl soln. (quenching of the reaction), the mixture was extracted with Et_2O: *(20*R,*22*R*)-20,22-dihydroxycholest-4-en-3-one* (**1a**; 0.18 mg). Colorless glass. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.73 (*s*, H–C(4)); 3.38 (*d*, J=8.8, H–C(22)); 1.21 (*s*, Me(21)); 1.19 (*s*, Me(19)); 0.93 (*s*, Me(18)); 0.90, 0.89 (*d*, J=6.8, Me(26), Me(27)). ^{1}H-NMR (400 MHz, C_5D_5N): 5.86 (*s*, H–C(4)); 3.75 (*d*, J=8.8, H–C(22)); 1.52 (*s*, Me(21)); 1.17 (*s*, Me(19)); 1.02 (*s*, Me(18)); 0.93, 0.92 (*d*, J=6.7, Me(26), Me(27)). ESI-MS: 439.167 ($[M+Na]^+$).

Cholesta-4,16,20(22)-trien-3-one (**1b**). Colorless oil. UV (MeOH) 203, 229, 274. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.74 (*s*, H–C(4)); 5.60 (br. *s*, H–C(16)); 5.57 (*t*, J=7.0, H–C(22)); 1.76 (*s*, Me(21)); 1.20 (*s*, Me(19)); 0.98 (*s*, Me(18)); 0.88, 0.87 (*d*, J=6.7, Me(26), Me(27)). HR-ESI-MS: 403.2980 ($[C_{27}H_{40}O+Na]^+$; calc. 403.2977).

Menellsteroid A (=(3β,5α,6β,11β)-Cholestane-3,5,6,11-tetrol; **2**). Amorphous white powder. $[\alpha]_D^{20} = +6.4$ ($c = 0.33$, MeOH). IR (KBr): 3443, 2926, 2857. ^{1}H- (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N): *Table 3*. EI-MS: 436 (M^+), 418, 400, 382, 367, 364, 81, 69, 55. HR-EI-MS: 436.3544 ($C_{27}H_{48}O_4^+$; calc. 436.3535).

Menellsteroid B (=(3β,5α,6β,11β,22E)-Cholest-22-ene-3,5,6,11-tetrol; **3**). Amorphous white powder. $[\alpha]_D^{20} = +11.2$ ($c = 0.11$, MeOH). IR (KBr): 3447, 2931, 2856. ^{1}H-NMR (400 MHz, C_5D_5N): 5.34 (*ddd*, $J = 15.6$, 7.1, 6.7, H–C(23)); 5.26 (*dd*, $J = 15.6$, 8.2, H–C(22)); 4.80–4.88 (*m*, H–C(3)); 4.66 (br. *s*, H–C(11)); 4.19 (br. *s*, H–C(6)); 3.12 (*dd*, $J = 12.9$, 11.5, H_β–C(4)); 2.50 (*dd*, $J = 13.4$, 2.4, H_β–C(12)); 2.20 (*s*, Me(19)); 1.27 (*s*, Me(18)); 1.09 (*d*, $J = 6.6$, Me(21)); 0.89 (*d*, $J = 6.6$, Me(26), Me(27)). ^{13}C-NMR (100 MHz, C_5D_5N): *Table 3*. EI-MS: 434 (M^+), 416, 398, 380, 365, 362, 81, 69, 55. HR-EI-MS: 434.3411 ($C_{27}H_{46}O_4^+$; calc. 434.3396).

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