Acanthovagasteroids A−D, Four New 19-Hydroxylated Steroids from the South China Sea Gorgonian *Acanthogorgia vagae* Aurivillius

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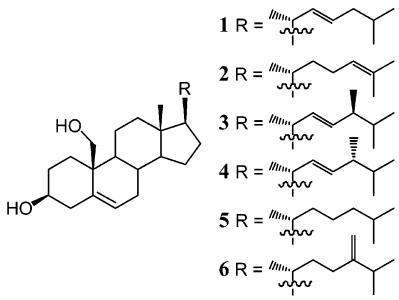
Four new steroids, acanthovagasteroids A−D (**1**−**4**), along with two known analogues (**5**, **6**), were isolated from the gorgonian *Acanthogorgia vagae* Aurivillius from the South China Sea. The structures of the new compounds, characterized with 19-hydroxy groups, were identified on the basis of extensive spectral analysis as well as by comparison with literature data. This is the first report of 19-hydroxy steroids from a gorgonian and suggests a possible biosynthetic relationship between 19-nor and 19-hydroxy steroids.

Unusual steroids have been isolated from many marine organisms. Many of them exhibit intriguing oxygenation patterns and structural modifications of both side chain and the polycyclic core.

19-Hydroxylated steroids have received considerable attention since the middle of the 1970s, when the first natural 19-hydroxylated steroids were reported[1] and helped to explain the biosynthesis of 19-nor steroids. A literature survey revealed that nearly three dozen 19-hydroxylated steroids have been isolated from black coral,[2] soft corals,[3−10] or sponges,[11−15] whereas no record is reported from gorgonians. This paper describes the isolation and structure identification of four new 19-hydroxylated steroids from the gorgonian *Acanthogorgia vagae* Aurivillius (Acanthogorgiidae) collected from the South China Sea. A possible biosynthetic relationship between 19-hydroxy and 19-nor steroids is suggested.

The gorgonian *A. vagae* Aurivillius (dried weight 417.0 g) was kept at −20 °C immediately after collection. The frozen animals were cut into small pieces and subsequently extracted with acetone. The diethyl ether fraction from the acetone extract was fractionated by both silica gel and Sephadex LH-20 column chromatography to afford a steroid mixture. The mixture was fractionated by RP-HPLC, yielding six pure compounds: **1** (3.6 mg), **2** (1.2 mg), **3** (1.0 mg), **4** (0.6 mg), **5** (3.0 mg), and **6** (1.7 mg).



The typical ^{1}H NMR AB system of oxygenated 19-methylene groups easily led to the structures of two known metabolites, cholesta-5-ene-3β,19-diol (**5**) and 24-methylcholesta-5,24(28)-diene-3β,19-diol (litosterol, **6**), by a comparison with reported data. Litosterol, first isolated from the soft coral *Litophyton viridis*,[2] was subsequently reported from two other species of soft coral, *Litophyton arboreum*[7] and *Nephthea erecta*.[8] The structure of **5** was suggested by MS experiment,[16] and an extensive NMR analysis was performed on its 3-acetate derivative,[17] whereas ^{13}C NMR data of **5** have not been reported previously.

Acanthovagasteroid A (**1**) was obtained as a colorless crystal. Its molecular formula $C_{27}H_{44}O_2$ was deduced from the molecular ion at *m/z* 400.3312 in the HREIMS spectrum. The IR spectrum of **1** showed the presence of hydroxyl (3450 cm^{-1}) and trisubstituted olefinic groups (845 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra of compound **1** were closely related to those of **5**, presenting a downfield trisubstituted double bond (δ_H 5.75, 1H, t, J = 2.6 Hz; δ_C 135.6, C; 127.4, CH), a secondary hydroxyl group (δ_H 3.57, 1H, br m; δ_C 71.4, CH), and a primary hydroxyl group (δ_H 3.61, 3.82, AB system, d, J = 11.6 Hz; δ_C 62.8, CH_2). Three of the four ^{1}H NMR methyl resonances of **1** were analogues to those in **5** (δ 0.75, 3H, s; δ 0.86, 6H, d, J = 6.7 Hz). By contrast, H_3-21 was downfield shifted (δ 1.01; δ 0.90 in **5**) due to the deshielding effect of the C-22−C-23 double bond, which presented an AB system at δ 5.21 (1H, dd, J = 15.3, 8.2 Hz) and 5.28 (1H, ddd, J = 15.3, 7.0, 6.6 Hz). The location of the C-22 double bond was supported by both the connectivities of H-17/H-20(/H_3-21)/H-22/H-23/H_2-24/H-25 in the ^{1}H−^{1}H COSY spectrum and the significant long-range correlations of H_3-21/C-17, C-20, C-22 in the HMBC spectrum. The *trans* stereochemistry of the olefinic protons was deduced from their coupling constant (J = 15.3 Hz), as well as the absence of NOE correlation between H-22 and H-23 in the NOESY spectrum. The observation of a NOE between H_3-18 and H-8 and H-20 and between H-17 and H-14 was in agreement with the α-orientation of H-17 and the R* stereochemistry of C-20, features normally exhibited by a steroid skeleton. Assignments of proton and carbon signals for the side chain of **1** were strongly supported by literature data.[2,18−20] These lines of evidence established the structure (22*E*)-cholesta-5,22-diene-3β,19-diol for compound **1**.

Acanthovagasteroid B (**2**) was obtained as an amorphous powder. The molecular formula, $C_{27}H_{44}O_2$, the same as that of compound **1**, was suggested by the HREIMS spectrum. Both the ^{1}H and ^{13}C NMR spectra of **2**, closely related to those of compound **5**, suggested the same polycyclic skel-

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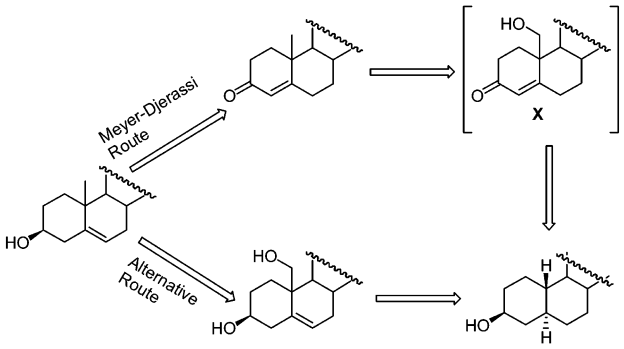
Scheme 1. Probable Biosynthetic Route of 19-Norstanols from 5-Ene-3β-hydroxy Sterols Based on the Meyer-Djerassi Proposal



eton. A difference was observed in the side chain. The methyls of the isopropyl group (δ 0.85, 6H, J = 6.7 Hz, H_3-26, H_3-27) of **5** were replaced by two vinyl methyls (δ 1.60 and 1.68, each 3H, s). The multiplicity of the olefinic proton (δ 5.08, br t, J = 7.1 Hz) linked the trisubstituted double bond to a methylene. These data led **2** to the structure cholesta-5,24-diene-3β,19-diol. All ^{1}H NMR and ^{13}C NMR assignments of the side chain were identical to those of model compounds.[18,21,22]

Acanthovagasteroid C (**3**) was obtained as an amorphous powder. Its molecular formula $C_{28}H_{46}O_2$ was deduced from the molecular ion at *m/z* 414.3507 in the HREIMS spectrum. A comparison of the ^{1}H and ^{13}C NMR spectra between **3** and **5** revealed a different side chain with an additional methyl (δ_H 0.91, 3H, d, J = 6.8 Hz; δ_C 18.0, C) and a *trans* C-22−C-23 double bond (AB system, δ_H 5.16, 1H, dd, J = 15.2, 7.8 Hz; 5.21, 1H, dd, J = 15.2, 7.4 Hz; δ_C 136.1, CH; 131.9, CH). Analogously to **1**, the downfield shift of H_3-21 (δ 1.00; 0.90 in **5**) was observed. The ^{13}C NMR value of C-28 (δ 18.0) was in agreement with an *S* absolute stereochemistry at C-24. In fact, it is reported that the C-28 resonance appears at δ 17.6 ± 0.1 ppm in the 24*R* epimer, while a 0.4 ppm downfield shift should be observed in the 24*S* epimer (δ 18.0 in **3**).[18] This assignment was further supported by the ^{1}H NMR data of H_3-21 (δ 1.00). In fact, H_3-21 should resonate at 0.01 ppm higher field in 24*S* than in its 24*R* epimer (δ 1.01 in **4**).[23] Therefore, the structure of compound **3** is (22*E*,24*S*)-24-methylcholesta-5,22-diene-3β,19-diol. The assignments of proton and carbon signals of the side chain in **3** were strongly supported by those of reported structures possessing the same side chain.[18,20,23−25]

Acanthovagasteroid D (**4**) was obtained as an amorphous powder. Its molecular formula $C_{28}H_{46}O_2$ was the same as compound **3**. The MS and ^{1}H NMR spectra of **4** were completely identical to those of **3** with the exception of the resonance assigned to H_3-21 (δ 1.01; 1.00 in **3**). Hence, compound **4**, (22*E*,24*R*)-24-methylcholesta-5,22-diene-3β,19-diol, is the C-24 epimer of compound **3**.[23]

19-Hydroxy steroids are commonly regarded as key intermediates in the oxidative elimination of the 19-methyl group in animal cells (Scheme 1).[26−30] Since the presence of 19-norstanols has been observed in gorgonians,[31] it is plausible that compounds **1**−**4** may represent the first piece of evidence for this metabolic pathway in these invertebrates. In this line of reasoning, the occurrence of acanthovagasteroids A−D (**1**−**4**) suggests the direct conversion of 19-hydroxy-5-sterols into 19-norstanols (Scheme 1). This would be consistent with the biogenesis proposed for this class of compounds in marine sponges.[30] On the contrary, given the numerous reports of 19-hydroxysteroids in marine organisms, we cannot exclude that **1**−**4** represent the final products of *A. vagae* metabolism, thus implying a still undiscovered eco-physiological role for these metabolites.

Experimental Section

General Exprimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna 750 FT-IR spectrophotometer. The NMR spectra were recorded on a Bruker DRX-400 at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$ solution. ^{13}C NMR and ^{1}H NMR chemical shift values were referenced to $CDCl_3$ (δ 77.0 ppm) and residual $CHCl_3$ signals (δ 7.26 ppm), respectively. EIMS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for column chromatography. Precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Animal Materials. *A. vagae* Aurivillius was collected by hand via scuba along the coast of the South China Sea, Hainan Province, China, in December 2001, at a depth of 20 m. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS.

Extraction and Isolation. The frozen animals (dried weight 417 g) were cut into small pieces and subsequently extracted with acetone at room temperature. The crude extract of *A. vagae* Aurivillius was partitioned between diethyl ether and water. The ether extract was concentrated under rotary evaporation to give a dark green residue (5.1 g), a large portion of which (4.7 g) was fractionated by gradient silica gel column chromatography (0−100% acetone in light petroleum) followed by Sephadex LH-20 purification and repeated normal-phase silica gel column chromatography to afford the steroid mixture. This mixture was subjected to RP-HPLC [semipreparative ODS-HG-5, (5 μm, 250 × 10 mm)], eluted with CH_3CN−MeOH−H_2O (75:10:15), to yield six pure compounds: **1** (3.6 mg), **2** (1.2 mg), **3** (1.0 mg), **4** (0.6 mg), **5** (3.0 mg), and **6** (1.7 mg), respectively.

Acanthovagasteroid A [(22*E*)-cholesta-5,22-diene-3β,-19-diol] (1): colorless crystals, mp 157−159 °C; $[\alpha]^{20}_D$ −21.1° (*c* 0.36, $CHCl_3$); IR ν_{max} (KBr) 3450, 2927, 2866, 845 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (1H, t, J = 2.6 Hz), 5.28 (1H, ddd, J = 15.3, 7.0, 6.6 Hz, H-23), 5.21 (1H, dd, J = 15.3, 8.2 Hz, H-22), 3.82, 3.61 (each 1H, d, J = 11.6 Hz, H-19), 3.57-(1H, br m, H-3), 2.38 (1H, ddd, J = 1.8, 2.4, 12.7, H-4), 2.19 (1H, t, J = 12.7, H-4), 2.03 (1H, m, H-20), 1.83 (1H, m, H-24), 1.11 (1H, m, H-17), 1.01 (3H, s, d, J = 6.6 Hz, H-21), 0.92 (1H, m, H-9), 0.89 (1H, m, H-14), 0.86 (6H, d, J = 6.7 Hz, H-26, 27), 0.75 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS (70 eV) *m/z* 400 (M^+), 382 [$(M - H_2O)^+$], 370 [$(M - CH_2O)^+$], 369 [$(M - CH_2OH)^+$], 352 [100%, $(M - CH_2O - H_2O)^+$], 351 [$(M - CH_2OH - H_2O)^+$], 241 [$(M - CH_2O - H_2O - C_8H_{15})^+$], 111 [$(C_8H_{15})^+$]; HREIMS *m/z* 400.3312 (calcd for $C_{27}H_{44}O_2$, 400.3341).

Acanthovagasteroid B (cholesta-5,24-diene-3β,19-diol) (2): amorphous powder, $[\alpha]^{20}_D$ −47.6° (*c* 0.08, $CHCl_3$); IR ν_{max} (KBr) 3443, 2925, 2857 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (1H, t, J = 2.6 Hz), 5.08 (1H, br t, J = 7.1 Hz, H-24), 3.82, 3.58 (each 1H, d, J = 11.8 Hz, H-19), 3.57 (1H, br m, H-3), 1.60, 1.68 (each 3H, s, H-26, 27), 0.93 (3H, d, J = 6.4 Hz, H-21), 0.73 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS (70 eV) *m/z* 400 (M^+), 382 [$(M - H_2O)^+$], 370 [$(M - CH_2O)^+$], 369 [$(M - CH_2OH)^+$], 352 [$(M - CH_2O - H_2O)^+$], 351 [$(M - CH_2OH - H_2O)^+$], 241 [$(M - CH_2O - H_2O - C_8H_{15})^+$], 111 [$(C_8H_{11})^+$], 57 [100%, $(C_4H_9)^+$]; HREIMS *m/z* 400.3349 (calcd for $C_{27}H_{44}O_2$, 400.3341).

Acanthovagasteroid C [(22*E*,24*S*)-24-methylcholesta-5,22-diene-3β,19-diol] (3): amorphous powder, $[\alpha]^{20}_D$ −47.6° (*c* 0.08, $CHCl_3$); IR ν_{max} (KBr) 3442, 2923, 2849 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (1H, t, J = 2.6 Hz), 5.21 (1H, dd, J = 15.2, 7.4, H-23), 5.16 (1H, dd, J = 15.2, 7.8 Hz, H-22), 3.82, 3.59 (each 1H, d, J = 11.3 Hz, H-19), 3.59 (1H, br m, H-3), 1.00 (3H, d, J = 6.6 Hz, H-21), 0.91 (3H, d, J = 6.8 Hz, H-28), 0.83 (each 3H, d, J = 7.1 Hz, H-26, 27), 0.74 (3H, s, H-18),

Table 1. ^{13}C NMR Data for Compounds **1**−**3** and **5**[a,b]

no.	1	2	3	5
1	33.4, CH_2	33.4, CH_2	33.4, CH_2	33.4, CH_2
2	32.0, CH_2	32.1, CH_2	32.0, CH_2	32.0, CH_2
3	71.4, CH	71.5, CH	71.4, CH	71.4, CH
4	42.4, CH_2	42.4, CH_2	42.4, CH_2	42.4, CH_2
5	135.6, C	135.5, C	135.5, C	135.6, C
6	127.4, CH	127.6, CH	127.4, CH	127.4, CH
7	31.3, CH_2	31.3, CH_2	31.3, CH_2	31.3, CH_2
8	33.5, CH	33.5, CH	33.5, CH	33.5, CH
9	50.5, CH	50.4, CH	50.5, CH	50.5, CH
10	41.6, C	41.6, C	41.6, C	41.6, C
11	21.8, CH_2	21.9, CH_2	21.8, CH_2	21.8, CH_2
12	40.0, CH_2	40.1, CH_2	40.0, CH_2	40.1, CH_2
13	42.5, C	42.6, C	42.5, C	42.6, C
14	57.8, CH	57.7, CH	57.8, CH	57.7, CH
15	24.1, CH_2	24.2, CH_2	24.1, CH_2	24.1, CH_2
16	28.7, CH_2	28.7, CH_2	28.3, CH_2	28.2, CH_2
17	56.0, CH	56.1, CH	56.0, CH	56.2, CH
18	12.4, CH_3	12.3, CH_3	12.4, CH_3	12.2, CH_3
19	62.8, CH_2	62.8, CH_2	62.8, CH_2	62.8, CH_2
20	40.1, CH	35.7, CH	40.2, CH	35.8, CH
21	20.9, CH_3	18.7, CH_3	21.0, CH_3	18.7, CH_3
22	138.1, CH	36.2, CH_2	136.1, CH	36.2, CH_2
23	126.3, CH	24.8, CH_2	131.9, CH	23.8, CH_2
24	42.0, CH_2	125.3, CH	43.1, CH	39.5, CH_2
25	28.6, CH	131.0, C	33.5, CH	28.0, CH
26	22.3, CH_3	17.7,[c] CH_3	19.6,[c] CH_3	22.7,[c] CH_3
27	22.3, CH_3	25.8,[c] CH_3	20.1,[c] CH_3	22.8,[c] CH_3
28			18.0, CH_3	

[a] Bruker DRX 400 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $CDCl_3$ (δ 77.0). [b] By DEPT sequence. [c] Signals may be interchanged.

0.81; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS (70 eV) *m/z* 414 (M^+), 396 [$(M - H_2O)^+$], 384 [$(M - CH_2O)^+$], 366 [$(M - CH_2O - H_2O)^+$], 241 [$(M - CH_2O - H_2O - C_9H_{17})^+$], 57 [100%, $(C_4H_9)^+$]; HREIMS *m/z* 414.3507 (calcd for $C_{28}H_{46}O_2$, 414.3498).

Acanthovagasteroid D [(22*E*,24*R*)-24-methylcholesta-5,22-diene-3β,19-diol] (4): amorphous powder, $[\alpha]^{20}_D$ −21.6° (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3451, 2929, 2858 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.75 (1H, t, J = 2.6 Hz), 5.21 (1H, dd, J = 15.2, 7.4, H-23), 5.16 (1H, dd, J = 15.2, 7.8 Hz, H-22), 3.82, 3.59 (each 1H, d, J = 11.3 Hz, H-19), 3.59 (1H, br m, H-3), 1.01 (3H, d, J = 6.5 Hz, H-21), 0.91 (3H, d, J = 6.7 Hz, H-28), 0.82, 0.83 (each 3H, d, J = 7.1 Hz, H-26, 27), 0.75 (3H, s, H-18); EIMS (70 eV) *m/z* 414 (M^+), 396 [$(M - H_2O)^+$], 384 [$(M - CH_2O)^+$], 366 [$(M - CH_2O - H_2O)^+$], 241 [$(M - CH_2O - H_2O - C_9H_{17})^+$], 57 [100%, $(C_4H_9)^+$]; HREIMS *m/z* 414.3484 (calcd for $C_{28}H_{46}O_2$, 414.3498).

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