Suberoretisteroids A – E, Five New Uncommon Polyoxygenated Steroid 24-Ketals from the Hainan Gorgonian *Suberogorgia reticulata*

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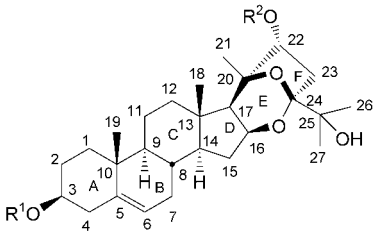
Five new uncommon polyoxygenated steroids, namely suberoretisteroids A – E (**1** – **5**), were isolated from the gorgonian *Suberogorgia reticulata*, never studied so far. The structures and relative configurations of these new compounds were elucidated on the basis of extensive spectral analysis. This work led to the structural revision of some steroids previously found in the Indian gorgonian *Gorgonella umbraculum*.

Introduction. – A large number of unusual steroids have been described from marine sources, many of which exhibit ketal functions. In particular, steroid 22-spiroketals, such as hippurins [1 – 5], are the most common in this group, whereas steroid 24-ketals are infrequent. To the best of our knowledge, only two compounds possessing the 24-ketal function have been reported so far [6][7].

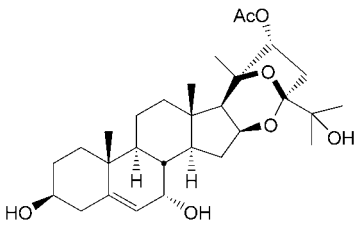
Among gorgonians, the genus of *Suberogorgia* has been extensively investigated, resulting in the isolation of several sesquiterpenes, which exhibit different structures, including subergane [8 – 10], quadrone [11], and β-caryophyllene [11][12] carbon skeletons, along with 9,11-secosteroids [13][14]. We report here the results of our chemical investigation of the Et_2O extract of *Suberogorgia reticulata* (Coelenterata, Gorgonaea, Suberogorgiidae), collected along the coasts of the South China Sea, that led to the isolation of five new uncommon polyoxygenated steroids, named suberoretisteroids A – E (**1** – **5**), all displaying the rare 24-ketal function. The structure and the relative configuration of these compounds were elucidated on the basis of extensive spectroscopic analysis and by comparison with reported data.

Results and Discussion. – Freshly collected animals (dry weight 461 g) from the South China Sea were immediately stored at $-20°$ and kept frozen until the extraction. Frozen material was cut into small pieces and subsequently extracted with acetone. The extract was evaporated, and the residue was partitioned between Et_2O and H_2O. On evaporation, the Et_2O extract yielded a dark green crude residue (4.7 g), which was fractionated by column chromatography (silica gel, light petroleum ether/acetone gradient) followed by *Sephadex LH-20* purification and repeated normal-phase and reversed-phase column chromatography (silica gel) to afford five pure compounds: **1** (11.9 mg), **2** (13.7 mg), **3** (7.5 mg), **4** (5.1 mg), and **5** (1.7 mg).

Compound **1** was obtained as an optically active colorless glass. Its molecular formula $C_{29}H_{44}O_6$, deduced from the HR-ESI-MS (*m/z* 511.3031 $[M+Na]^+$), indicated



1 R^1 = Ac, R^2 = H
2 R^1 = H, R^2 = Ac
3 R^1 = R^2 = Ac



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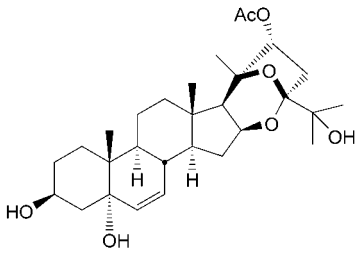

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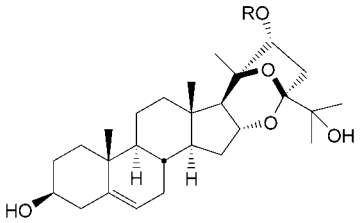

6 R = Ac
7 R = H

eight degrees of unsaturation. The IR spectrum of **1** showed two intense bands at 3444 and 1736 cm^{-1} that suggested the presence of OH and ester functions in the molecule. The structure and relative configuration of **1** were elucidated by extensive spectroscopic analysis (*Table 1*) as well as by comparison with related compounds. Suberoretisteroid A (**1**) is, therefore, (3*S*,16*S*,20*R*,22*R*,24*S*)-16,24 : 20,24-diepoxycholest-5-ene-3,22,25-triol 3-acetate[1]).

The ^{1}H-NMR spectrum of **1** showed signals attributable to an olefinic proton (δ 5.38, *d*, *J* = 4.8 Hz), an AcO group (δ(H) 2.04, *s*), three CH–O protons at δ 4.59 (br. *m*, 1 H), 4.51 (*m*, 1 H), and 3.86 (*dd*, *J* = 11.0, 4.9 Hz, 1 H), and five tertiary Me groups at δ 1.04, 1.06, 1.15, 1.24, and 1.37 (each *s*). Analysis of the ^{13}C-NMR spectrum allowed us to assign two of eight unsaturation degrees to a trisubstituted C=C bond (δ(C) 139.7 (*s*) and 122.3 (*d*)) and an AcO function (δ(C) 170.7 (*s*) and 21.4 (*q*)). The remaining unsaturations were due to six rings. In addition, the ^{13}C-NMR spectrum showed down-field resonances assigned to a ketal C-atom (δ(C) 107.6, *s*) and to five C-atoms linked to O-atoms at δ 83.3 (*s*), 79.2 (*d*), 73.9 (*d*), 72.6 (*d*), and 72.4 (*s*). The remaining signals between δ 54.8 and 14.4 were attributed to 19 sp^3 C-atoms (5 Me, 8 CH_2, 4 CH, and 2 C as deduced by the DEPT sequence). These data suggested the presence of a highly oxygenated sterol framework. In particular, a structure derived from a 3β-(acetyloxy)-cholest-5-ene skeleton with additional oxygenated features, including a ketal function involving both ring D and the side chain, was clearly indicated by detailed analysis of the 2D NMR data (^{1}H,^{1}H COSY, HMQC, and HMBC). From the ^{1}H,^{1}H COSY experiment, separate spin systems revealing the proton connectivities of the steroid skeleton were easily recognized, as well as the isolated *ABX* system in the side chain due to H–C(22) (δ(H) 3.86, *dd*, *J* = 11.0, 4.9 Hz) coupled to CH_2(23) (δ(H) 1.76, *dd*, *J* = 13.9, 4.9 Hz; 2.73, *dd*, *J* = 13.9, 11.0 Hz). Comparison of both ^{1}H- and ^{13}C-NMR values of compound **1** with those of several steroids exhibiting ketal functions, and in particular with those of 22-ketals [1–5] and 24-ketals [6][7], led to the location of the ketal function at C(24) and to connect this to C(16) and C(20) of a cholest-5-ene

[1]) The proposed absolute configurations of **1**–**5** are the same as those of other natural sterols of this kind.

Table 1. *NMR Data*[a])[b]) *of Suberoretisteroid A* (**1**). δ in ppm, J in Hz.

	δ(H)	δ(C)[c])	H,C-HMBC
H_α–C(1)	1.14 (*m*)	36.9 (*t*)	
H_β–C(1)	1.86 (*m*)	–	C(5), C(10)
H_α–C(2)	1.60 (*m*)	27.7 (*t*)	
H_β–C(2)	1.87 (*m*)	–	C(3), C(5)
H–C(3)	4.59 (br. *m*)	73.9 (*d*)	
H_α–C(4)	2.32 (*m*)	38.0 (*t*)	C(2), C(3), C(5), C(6), C(10)
H_β–C(4)	2.32 (*m*)	–	C(2), C(3), C(5), C(6), C(10)
C(5)	–	139.7 (*s*)	
H–C(6)	5.38 (*d*, J = 4.8)	122.3 (*d*)	C(10)
H_α–C(7)	1.98 (*m*)	31.7 (*t*)	C(5), C(6), C(8), C(9)
H_β–C(7)	1.98 (*m*)	–	C(5), C(6), C(8), C(9)
H–C(8)	1.59 (*m*)	31.0 (*d*)	C(7)
H–C(9)	0.86 (*m*)	50.0 (*d*)	
C(10)	–	36.6 (*s*)	
H_α–C(11)	1.53 (*m*)	20.4 (*t*)	C(8)
H_β–C(11)	1.53 (*m*)	–	C(8)
H_α–C(12)	1.20 (*m*)	39.5 (*t*)	C(17)
H_β–C(12)	2.02 (*m*)	–	
C(13)	–	41.6 (*s*)	
H–C(14)	0.99 (*m*)	54.8 (*d*)	
H_α–C(15)	1.38 (*m*)	33.4 (*t*)	C(13), C(17)
H_β–C(15)	2.14 (*m*)	–	C(13), C(17)
H–C(16)	4.51 (*m*)	72.6 (*d*)	C(13)
H–C(17)	1.39 (*d*, J = 5.5)	49.4 (*d*)	C(13), C(16), C(18)
Me(18)	1.06 (*s*)	14.4 (*q*)	C(12), C(13), C(14), C(17)
Me(19)	1.04 (*s*)	19.3 (*q*)	C(1), C(5), C(9), C(10)
C(20)	–	83.3 (*s*)	
Me(21)	1.37 (*s*)	23.4 (*q*)	C(17), C(20), C(22)
H–C(22)	3.86 (*dd*, J = 11.0, 4.9)	79.2 (*d*)	C(17), C(21)
H_α–C(23)	1.76 (*dd*, J = 13.9, 4.9)	37.2 (*t*)	C(22), C(24)
H_β–C(23)	2.73 (*dd*, J = 13.9, 11.0)	–	C(22)
C(24)	–	107.6 (*s*)	
C(25)	–	72.4 (*s*)	
Me(26)[d])	1.15 (*s*)	23.4 (*q*)	C(24), C(25), C(27)
Me(27)[d])	1.24 (*s*)	24.0 (*q*)	C(24), C(25), C(26)
*Me*COO	2.04 (*s*)	21.4 (*q*)	Me*C*OO
Me*C*OO	–	170.7 (*s*)	

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

structure, exhibiting a side chain involved in a bicyclic framework. Two additional OH groups were positioned at C(22) and C(25). Diagnostic HMBC correlations between C(24) (δ(C) 107.6) and CH_2(23) (δ(H) 1.76 and 2.73), Me(26) (δ(H) 1.15), and Me(27) (δ(H) 1.24), and between C(20) (δ(C) 83.3) and Me(21) (δ(H) 1.37) supported the proposed structure **1**. All NMR resonances were assigned by careful analysis of 2D-NMR spectra (see *Table 1*).

The relative configurations of the stereogenic centers of both ring D and the side chain of **1** were suggested by the NOESY data and analysis of molecular models. Significant cross-peaks between H–C(16) and both H–C(14) and H–C(17) clearly indicated that H–C(16) and H–C(17) are α-oriented, consequently implying a *cis*-junction between ring D and the cyclic ketal framework. A further diagnostic NOE was H–C(16)/H_α–C(23), implying the β-configuration of the O-bridge between C(20) and C(24). The suggested

configuration was confirmed by the additional NOEs Me(18)/Me(27) and Me(21)/Me(18). Finally, the α-orientation of OH–C(22) was supported by the NOE Me(21)/H–C(22).

Compound **2** was an optically active colorless glass. Its molecular formula $C_{29}H_{44}O_6$, deduced from HR-ESI-MS, was the same as that of compound **1**. The IR spectrum of **2** also showed the presence of OH and ester functions in the molecule with the absorption bands at 3433 and 1734 cm^{-1}, respectively. The ^{1}H- and ^{13}C-NMR data of **2** (*Table 2*) closely resembled those of **1,** indicating the same framework and similar functional groups in the molecule. Compound **2** was confirmed as (3*S*,16*S*,20*R*,22*R*,24*S*)-16,24 : 20,24-diepoxycholest-5-ene-3,22,25-triol 22-acetate[1]).

In the ^{1}H-NMR spectrum of **2**, H–C(3) (δ 3.51) was upfield shifted with respect to the corresponding proton in **1** (δ 4.59), while H–C(22) resonated at lower field at δ 4.66 (δ 3.86 in **1**). These data clearly indicated that compound **2** differs from **1** in the acetylation pattern at C(3) and C(22), which was further confirmed unambiguously by the identical spectra of the 22-acetate of **1** and the 3-acetate of **2**.

Apparently, the proposed structure of **2** is a C(16) epimer of **6** [6], which was isolated from the Indian gorgonian *Gorgonella umbraculum*. However, careful comparison of ^{1}H- and ^{13}C-NMR data of **2** and **6** did not show the expected differences due to the different junction between rings D and E (*cis* in **2**, *trans* in **6**). In fact, the ^{13}C-NMR data of **2** and **6** were almost identical, with the exception of the resonance assigned to C(23) (δ(C) 35.2 in **2**, 29.7 in **6**). Moreover, the expected upfield shift (2 ppm) of the C(23) signal due to the 22-acetate moiety (as compared with **1**) was observed in **2**, whereas, in both **6** and its 22-*O*-deacetyl derivative **7** [7], the chemical shift of C(23) was attributed to the same signal at δ(C) 29.7. Probably, this latter signal is due to the isolated CH_2 signals of a minor lipid impurity, instead of the real resonance of C(23). As a consequence, compound **6** should be identical to **2**, and the configuration

Table 2. *^{13}C-NMR Data*[a]) [b]) *of Suberoretisteroids B* (**2**), *C* (**3**), *and E* (**5**). δ in ppm.

	2	**3**	**5**		**2**	**3**	**5**
C(1)	37.1 (*t*)	36.9 (*t*)	28.4 (*t*)	C(17)	50.1 (*d*)	50.1 (*d*)	50.5 (*d*)
C(2)	31.6 (*t*)	27.7 (*t*)	30.5 (*t*)	C(18)	14.4 (*q*)	14.4 (*q*)	14.6 (*q*)
C(3)	71.6 (*d*)	73.8 (*d*)	67.1 (*d*)	C(19)	19.3 (*q*)	19.3 (*q*)	14.7 (*q*)
C(4)	42.2 (*t*)	38.0 (*t*)	40.9 (*t*)	C(20)	82.4 (*s*)	82.4 (*s*)	82.4 (*s*)
C(5)	140.9 (*s*)	139.8 (*s*)	73.9 (*s*)	C(21)	23.7 (*q*)	23.6 (*q*)	23.7 (*q*)
C(6)	121.3 (*d*)	122.2 (*d*)	132.4 (*d*)	C(22)	79.5 (*d*)	79.5 (*d*)	79.6 (*d*)
C(7)	31.5 (*t*)	31.6 (*t*)	133.7 (*d*)	C(23)	35.2 (*t*)	35.3 (*t*)	35.3 (*t*)
C(8)	31.0 (*d*)	30.9 (*d*)	37.5 (*d*)	C(24)	107.8 (*s*)	107.8 (*s*)	107.8 (*s*)
C(9)	50.6 (*d*)	50.6 (*d*)	45.1 (*d*)	C(25)	72.4 (*s*)	72.3 (*s*)	72.4 (*s*)
C(10)	36.5 (*s*)	36.6 (*s*)	38.0(*s*)	C(26)[c])	23.6 (*q*)	23.4 (*q*)	23.4 (*q*)
C(11)	20.5 (*t*)	20.4 (*t*)	20.6 (*t*)	C(27)[c])	24.0 (*q*)	24.0 (*q*)	24.1 (*q*)
C(12)	39.5 (*t*)	39.4 (*t*)	39.8 (*t*)	*Me*COO–C(3)	–	21.4 (*q*)	–
C(13)	41.7 (*s*)	41.7 (*s*)	43.1 (*s*)	MeCOO–C(3)	–	170.5 (*s*)	–
C(14)	54.9 (*d*)	54.8 (*d*)	52.2 (*d*)	*Me*COO–C(22)	21.1 (*q*)	21.1 (*q*)	21.1 (*q*)
C(15)	33.4 (*t*)	33.4 (*t*)	32.9 (*t*)	MeCOO–C(22)	170.5 (*s*)	170.5 (*s*)	170.3 (*s*)
C(16)	72.6 (*d*)	72.6 (*d*)	72.6 (*d*)				

[a]) *Bruker DRX*-400-MHz spectrometer; $CDCl_3$; δ referred to $CDCl_3$ (δ 77.0). [b]) By DEPT sequence. [c]) Signals may be interchanged.

at C(16) of **6** has to be revised and to be the same as that of **2**. Furthermore, the NMR resonances of Me(18), Me(19), and Me(21) of **6** should also be reassigned according to our 2D NMR experiments. In light of the above observations, the configuration at C(16) and the NMR assignments of Me(18), Me(19), and Me(21) of compound **7**, isolated from the same Indian gorgonian as that of **6**, should also be revised.

Compound **3** was obtained as an optically active colorless glass. The HR-ESI-MS of **3** displayed a pseudomolecular ion at *m/z* 553.3139 ($[M+Na]^+$), indicating a molecular formula of $C_{31}H_{46}O_7$. Analogously to compounds **1** and **2**, the IR spectrum of **3** supported the presence of OH (3450 cm^{-1}) and ester (1739 cm^{-1}) groups. Comparison of the NMR data of **3** with those of both **1** and **2** revealed their close relationship and also showed the presence of two AcO functions in **3** (2 *s* at δ(H) 2.03 and 2.10), *i.e.*, esterification of both secondary OH groups (H–C(3) at δ(H) 4.60 (br. *m*) and H–C(22) at 4.65 (*dd*, $J = 11.1, 4.8$ Hz)). Thus, compound **3** is (3*S*,16S,*20*R,22*R*,24*S*)-16,24 : 20,24-diepoxycholest-5-ene-3,22,25-triol 3,22-diacetate[1]), the AcO derivative of **1** and **2**. This was confirmed by comparing the spectral data of **3** with those of the acetylation products of both **1** and **2**, which were identical in all respect.

Compound **4**, an optically active colorless glass, had a molecular formula $C_{29}H_{44}O_7$, as deduced from the pseudomolecular ion at *m/z* 527.2999 ($[M+Na]^+$) in the HR-ESI-MS, indicating an additional O-atom with respect to compounds **1** and **2**. Analogously to **1**–**3**, bands at 3425 and 1743 cm^{-1}, due to OH and ester functions, were observed in the IR spectrum. The ^{1}H-NMR spectrum of **4** was very similar to that of compound **2**. Extensive 2D NMR studies (^{1}H,^{1}H-COSY, HMQC, HMBC) allowed complete NMR assignments (*Table 3*). The relative configurations of all stereogenic centers were assigned by NOESY experiments and was the same as in the related compounds **1**–**3**. Compound **4** was, therefore, (3*S*,7*S*,16*S*,20*R*,22*R*,24*S*)-16,24 : 20,24-diepoxycholest-5-ene-3,7,22,25-tetrol 22-acetate[1]).

The main difference between the ^{1}H-NMR spectra of **4** and **2** was the presence of a signal at δ(H) 3.88 (br. *s*, $w_{1/2} = 11.1$ Hz) due to a CH–O proton in the case of **4**. In the ^{13}C-NMR and DEPT spectra of **4**, a signal at δ(C) 65.3 (*d*) was observed in the place of a CH, further supporting the presence of an additional secondary OH group. Analysis of the ^{1}H,^{1}H-COSY data clearly showed that the signal at δ(H) 3.88 had cross-peaks with signals at δ(H) 5.62 and 1.64, attributed to an olefinic (H–C(5)) and an angular CH (H–C(8)) proton, respectively, whereas all spin systems of rings C–F were the same as those of **2**. This allowed to locate the additional OH group in ring B at C(7). The axial orientation of this substituent was suggested by both the multiplicity of H–C(7) ($w_{1/2} = 11.1$ Hz) in the ^{1}H-NMR spectrum and the remarkable upfield shift of both C(9) ($\Delta\delta = -8.2$) and C(14) ($\Delta\delta = -6.9$) due to the γ-gauche effect of the axial OH–C(7). NOE Interaction between H–C(7) and H–C(8) confirmed the suggested relative configuration.

Compound **5** was obtained as an optically active colorless glass. The molecular formula $C_{29}H_{44}O_7$, derived from HR-ESI-MS, was the same as that of **4**. IR-Bands at 3436 and 1744 cm^{-1} indicated the presence of OH and ester functions, analogously to the above described steroids. Compound **5** was determined as (3*S*,5*S*,16*S*,20*R*,22*R*,24*S*)-16,24 : 20,24-diepoxycholest-6-ene-3,5,22,25-tetrol 22-acetate[1]).

The presence of a disubstituted C=C in **5** was suggested by a *m* at δ(H) 5.62 (2 H) as well as by two sp^2 CH groups at δ(C) 132.4 and 133.7 in the spectra. The ^{1}H-NMR spectrum of **5** showed some similarities with that of its isomer **4**, exhibiting identical signals at δ(H) 4.67 (*dd*, $J = 11.0, 4.7$ Hz, H–C(22)) and 4.48 (*m*, H–C(16)), whereas the *m* assigned to H–C(3) was found at lower field (δ(H) 4.11, br. *m*). In the ^{13}C-NMR spectrum, an

Table 3. *NMR Data*[a])[b]) *of Suberoretisteroid D* (**4**). δ in ppm, J in Hz.

	δ (H)	δ (C)[c])	H,C-HMBC
H_α–C(1)	1.11 (*m*)	37.0 (*t*)	C(10)
H_β–C(1)	1.88 (*m*)	–	C(2), C(10)
H_α–C(2)	1.56 (*m*)	31.4 (*t*)	
H_β–C(2)	1.87 (*m*)	–	
H–C(3)	3.59 (br. *m*)	71.3 (*d*)	C(10)
H_α–C(4)	2.33 (*m*)	42.0 (*t*)	
H_β–C(4)	2.33 (*m*)	–	
C(5)	–	146.7 (*s*)	
H–C(6)	5.62 (*dd*, J = 5.4, 1.4)	123.7 (*d*)	C(7), C(8)
H_α–C(7)	–	65.3 (d)	
H_β–C(7)	3.88 (br. *s*, $w_{1/2}$ = 11.1 Hz)	–	
H–C(8)	1.64 (*m*)	36.8 (*d*)	
H–C(9)	1.25 (*m*)	42.4 (*d*)	
C(10)	–	37.5 (*s*)	
H_α–C(11)	1.62 (*m*)	20.2 (*t*)	C(8)
H_β–C(11)	1.62 (*m*)	–	C(8)
H_α–C(12)	1.21 (*m*)	39.1 (*t*)	
H_β–C(12)	2.00 (*m*)	–	C(13)
C(13)	–	41.6 (*s*)	
H–C(14)	1.45 (*m*)	48.0 (*d*)	C(8), C(13)
H_α–C(15)	1.43 (*m*)	33.4 (*t*)	C(14), C(16), C(17)
H_β–C(15)	2.32 (*m*)	–	C(13), C(17)
H–C(16)	4.50 (*m*)	72.8 (*d*)	C(13)
H–C(17)	1.32 (*d*, J = 6.3)	50.4 (*d*)	C(12), C(13), C(16), C(20)
Me(18)	1.07 (*s*)	14.2 (*q*)	C(12), C(13), C(14), C(170
Me(19)	1.02 (*s*)	18.2 (*q*)	C(5), C(9), C(10)
C(20)	–	82.5 (*s*)	
Me(21)	1.40 (*s*)	23.7 (*q*)	C(17), C(20), C(22)
H–C(22)	4.67 (*dd*, J = 11.0, 4.8)	79.6 (*d*)	C(17), C(20), C(21), C(24), C(δ 170.5)
H_α–C(23)	1.76 (*dd*, J = 14.4, 4.8)	35.3 (*t*)	C(22), C(24)
H_β–C(23)	2.87 (*dd*, J = 14.4, 11.0)	–	C(20), C(22), C(24), C(25)
C(24)	–	107.9 (*s*)	
C(25)	–	72.4 (*s*)	
Me(26)[d])	1.16 (*s*)	23.4 (*q*)	C(24), C(25), C(27)
Me(27)[d])	1.24 (*s*)	24.1 (*q*)	C(24), C(25), C(26)
*Me*COO	2.10 (*s*)	21.2 (*q*)	Me*C*OO
Me*C*OO	–	170.5 (*s*)	

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

additional signal at δ 73.9 (C(5), *s*) due to a quaternary O-bearing C-atom was detected, supporting the presence of a tertiary OH group. However, comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** and **5** suggested that the structural difference between them should be limited to the rings A and B. Analysis of the ^{1}H,^{1}H COSY data showed that H–C(3) of **5** correlated with two CH_2 groups at δ 2.34 (CH_2(4)) and at 1.52 and 1.88 (CH_2(2)), the latter being further coupled with another CH_2 at δ 1.18 and 1.88 (CH_2(1)). Thus, the C=C had to be placed in ring B between C(6) and C(7) and, accordingly, the tertiary OH substituent at C(5). The relative *trans* configuration of the A/B ring junction implying α-orientation of OH–C(5) was suggested by comparing the δ(C)s of ring A with those reported for sterols exhibiting 3β,5α-diol functionality [15]. The remarkable upfield

shifts of the C(1) ($\Delta\delta = -8.7$), C(3) ($\Delta\delta = -4.5$), and C(10) ($\Delta\delta = -5.5$) signals of **5** with respect to those of **4** were due to the γ-gauche effects of the axial OH–C(5) and further supported its α-orientation [15].

In summary, five novel polyoxygenated steroids, each exhibiting a 24-ketal moiety, have been characterized. This is the first chemical work on gorgonian *S. reticulata*.

This research work was financially supported by the *National Marine 863 Project* (No. 2001AA620403 and 2003AA624030), the *National Foundation for Outstanding Chinese Youths* (No. 30125044), and the *CNR/CAS Joint Projects 2001/2004*. We are indebted to Prof. *Ren-Lin Zhou*, South China Sea Institute of Oceanology , Chinese Academy of Sciences, for identification of the specimen.

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* polarimeter *341*. IR Spectra: *Nicolet Magna-FT-IR-750* spectrometer; ν_{max} in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker DRX-400* spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C); chemical shifts δ in ppm rel. to the residual $CHCl_3$ signals (δ(H) 7.26, δ(C) 77.0), coupling constant J in Hz; assignments supported by $^1H,^1H$-COSY, HMQC, HMBC, and NOESY experiments. ESI-MS and HR-ESI-MS: *Q-TOF Micro-LC/MS/MS* mass spectrometer.

Animal Material. The gorgonian *S. reticulata* was collected along the coast of Xiaodong Hai, Hainan Province, China, in December 2001, at a depth of 20 m, and identified by *Ren-Lin Zhou*. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS.

Extraction and Purification. The frozen animals (dry weight 460.7 g) were cut into small pieces and then extracted with acetone at r.t. The crude extract of *S. reticulata* was partitioned between Et_2O and H_2O after filtration. The Et_2O extract was evaporated and the dark green residue (4.7 g) was fractionated by CC (silica gel, light petroleum ether/acetone gradient) followed by *Sephadex LH-20* purification and repeated normal- and reversed-phase CC (silica gel): pure **1** (11.9 mg), **2** (13.7 mg), **3** (7.5 mg), **4** (5.1 mg), and **5** (1.7 mg).

Suberoretisteroid A (=*(3S,16S,20R,22R,24S)-16,24:20,24-Diepoxycholest-5-ene-3,22,25-triol 3-Acetate*[1]); **1**). Colorless glass. $[\alpha]_D^{20} = -22.5$ ($c = 0.250$, $CHCl_3$). IR (KBr): 3444, 2958, 1736, 802. ^1H- (400 MHz) and ^{13}C-NMR (100 MHz): *Table 1*. HR-ESI-MS: 511.3031 ($[C_{29}H_{44}O_6 + Na]^+$; calc. 511.3036).

Suberoretisteroid B (=*(3S,16S,20R,22R,24S)-16,24:20,24-Diepoxycholest-5-ene-3,22,25-triol 22-Acetate*[1]); **2**). Colorless glass. $[\alpha]_D^{20} = -44.4$ ($c = 0.380$, $CHCl_3$). IR (KBr): 3433, 2931, 1743. ^1H-NMR (400 MHz, $CDCl_3$): 1.02 (*s*, Me(19)); 1.06 (*s*, Me(18)); 1.15 (*s*, Me(26)); 1.24 (*s*, Me(27)); 1.38 (*s*, Me(21)); 2.10 (*s*, MeCOO); 1.75 (*dd*, $J = 14.4, 4.8$, H_α–C(23)); 2.86 (*dd*, $J = 14.4, 11.1$, H_β–C(23)); 3.51 (br. *m*, H–C(3)); 4.44 (*m*, H–C(16)); 4.66 (*dd*, $J = 11.1, 4.8$, H–C(22)); 5.35 (*d*, $J = 5.2$, H–C(6)). ^{13}C-NMR (100 MHz): *Table 2*. HR-ESI-MS: 511.3041 ($[C_{29}H_{44}O_6 + Na]^+$; calc. 511.3036).

Suberoretisteroid C (=*(3S,16S,20R,22R,24S)-6,24:20,24-Diepoxycholest-5-ene-3,22,25-trial 3,22-Diacetate*[1]); **3**). Colorless glass. $[\alpha]_D^{20} = -33.0$ ($c = 0.375$, $CHCl_3$). IR (KBr): 3450, 2935, 1739. ^1H-NMR (400 MHz, $CDCl_3$): 1.03 (*s*, Me(19)); 1.05 (*s*, Me(18)); 1.14 (*s*, Me(26)); 1.24 (*s*, Me(27)); 1.38 (*s*, Me(21)); 2.03 (*s*, MeCOO–C(3)); 2.10 (*s*, MeCOO–C(22)); 1.75 (*dd*, $J = 14.3$, 4.8, H_α–C(23)); 2.86 (*dd*, $J = 14.3$, 11.1, H_β–C(23)); 4.44 (*m*, H–C(16)); 4.60 (br. *m*, H–C(3)); 4.65 (*dd*, $J = 11.1$, 4.8, H–C(22)); 5.37 (*d*, $J = 4.8$, H–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 2*. HR-ESI-MS: 553.3139 ($[C_{31}H_{46}O_7 + Na]^+$, calc. 553.3141).

Suberoretisteroid D (=*(3S,7S,16S,20R,22R,24S)-16,24:20,24-Diepoxycholest-5-ene-3,7,22,25-tetrol 22-Acetate*[1]); **4**). Colorless glass. $[\alpha]_D^{20} = -73$ ($c = 0.056$, $CHCl_3$). IR (KBr): 3425, 2929, 1743. ^1H- (400 MHz) and ^{13}C-NMR (100 MHz): *Table 3*. HR-ESI-MS: 527.2999 ($[C_{29}H_{44}O_7 + Na]^+$; calc. 527.2985).

Suberoretisteroid E (=*(3S,5S,16S,20R,22R,24S)-16,24:20,24-Diepoxycholest-6-ene-3,5,22,25-tetrol 22-Acetate*[1]); **5**). Colorless glass. $[\alpha]_D^{20} = -26$ ($c = 0.142$, $CHCl_3$). IR (KBr): 3436, 2931, 1744. ^1H-NMR (400 MHz, $CDCl_3$): 0.94 (*s*, Me(19)); 1.09 (*s*, Me(18)); 1.15 (*s*, Me(26)); 1.24 (*s*, Me(27)); 1.40 (*s*, Me(21)); 2.08 (*s*, MeCOO); 1.20 (*m*, H–C(1)); 1.23 (*m*, H–C(12)); 1.52 (*m*, H–C(2)); 1.62 (*m*, CH_2(11)); 1.76 (*dd*, $J = 14.0, 4.7$, H_α–C(23)); 1.88 (*m*, H–C(1), H–C(2)); 2.02 (*m*, H–C(12)); 2.34 (*m*, CH_2(4)); 2.86 (*dd*, $J = 14.0, 11.0$, H_β–C(23)); 4.11 (br. *m*, H–C(3)); 4.48 (*m*, H–C(16)); 4.67 (*dd*, $J = 11.0, 4.7$, H–C(22)); 5.62 (*m*, H–C(6), H–C(7)). ^{13}C-NMR (100 MHz): *Table 2*. HR-ESI-MS: 527.2986 ($[C_{29}H_{44}O_7 + Na]^+$, calc. 527.2985).

Acetylation of **1**. To **1** (1.0 mg) in pyridine (0.5 ml), 5 drops of Ac_2O were added, and the mixture was stirred at r.t. overnigth. After evaporation, mini-CC (silica gel (300 mesh)) afforded 0.8 mg of 22-acetate of **1** (= **3**). ^{1}H-NMR (400 MHz, $CDCl_3$): see **3**. ESI-MS: 553.4 ($[M+Na]^+$, $C_{31}H_{46}NaO^+$); 529.3 ($[M-H]^-$, $C_{31}H_{45}O^-$).

Acetylation of **2**. As described above, with **2** (1.0 mg): 0.7 mg of 3-acetate of **2** (= **3**). ^{1}H-NMR (400 MHz, $CDCl_3$): see **3**. ESI-MS: 553.4 ($[M+Na]^+$, $C_{31}H_{46}NaO^+$).

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Received, August 6, 2004