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Steroids with Aromatic A-Rings from the Hainan Soft Coral *Dendronephthya studeri* Ridley

Xiao-Hong Yan,[‡] Hai-Li Liu,[†] Hui Huang,[§] Xiu-Bao Li,[§] and Yue-Wei Guo*[,†]

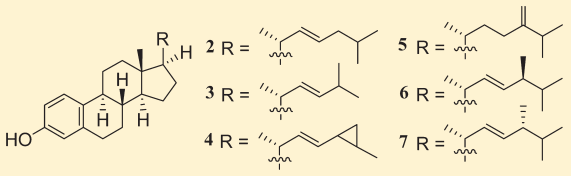
[†]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhang Jiang High-Tech Park, Shanghai, 201203, People's Republic of China

[‡]Guangzhou Institute of Drug Control, Guangzhou 510160, People's Republic of China

[§]South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, People's Republic of China

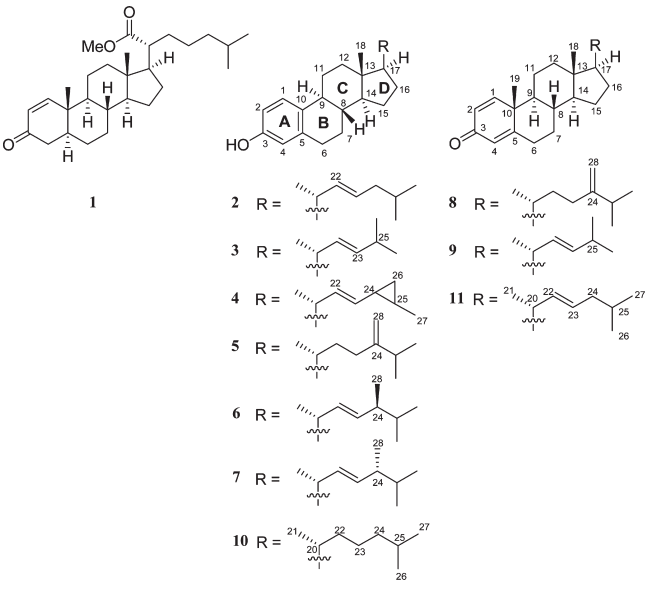
Supporting Information

ABSTRACT: Eight new marine steroids, characterized by either the presence of an aromatic ring or a cross-conjugated dienone system in ring A, were isolated from the Hainan soft coral *Dendronephthya studeri* Ridley. Their structures were elucidated on the basis of detailed spectroscopic analysis and by comparison of their NMR data with those reported in the literature.



Marine organisms have been shown to be an extraordinarily rich source of new steroids displaying unconventional nuclear structures and side chains. Furthermore, many of the structural features of marine steroids have no counterpart among steroidal metabolites from terrestrial plants and animals.[1] The biosynthetic origin of the complex mixtures of steroids often found in marine invertebrates is complicated by the fact that there are diverse contributing sources: *de novo* biosynthesis, assimilation of steroids produced by symbionts, and assimilation and modification of dietary steroids.[2−4]

Soft corals of the genus *Dendronephthya* (order Alcyonacea, family Nephtheidae) are widely distributed throughout tropical coastal waters of the Indo-Pacific Ocean. These animals are also frequently encountered in the South China Sea. The genus *Dendronephthya* is highly prolific and is represented by about 248 species. However, a literature search revealed that only a few species of *Dendronephthya* have been chemically examined so far, and the secondary metabolites isolated from those *Dendronephthya* soft corals are mainly structurally unusual steroids including 13,17-*seco*-steroids,[5] ring A-contracted steroids,[6] norpregnane-type steroids,[7] and polyhydroxylated steroids.[8] In the course of our ongoing program toward the isolation of biologically active substances from Chinese marine invertebrates,[9−14] we recently examined the soft coral *Dendronephthya studeri* Ridley, collected off the coast of Xiaodong Sea, Hainan Province, China, resulting in the discovery of a novel cytotoxic 21-oic acid methyl ester steroid, methyl spongoate (**1**), which exhibited potent cytotoxicity against human hepatoma BEL-7402 tumor cell lines *in vitro*.[15] Our continuing studies on the further constituents of the same extract from which methyl spongoate (**1**)[15] was reported led to the isolation of eight new steroids (**2**−**9**), together with two known related ones, **10** [19-norcholesta-1,3,5(10)-trien-3-ol] and **11** (cholesta-1,4,22-trien-3-one). The present paper deals with the isolation and structure elucidation of these new compounds, all closely related to the co-occurring steroids **10** and **11**, respectively, but exhibiting different side chains.



RESULTS AND DISCUSSION

The usual workup[15] of the Et_2O-soluble fraction of the acetone extract of *D. studeri* yielded, besides the already described methyl spongoate (**1**),[15] the new steroids **2**−**9** as well as two known related ones (**10** and **11**). The known steroids **10** and **11** were

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Table 1. ^{13}C NMR Data of Compounds 2−11[a]

no.	2	3	4	5	6	7	8	9	10	11
1	126.5, CH	126.5, CH	126.5, CH	126.6, CH	126.6, CH	126.5, CH	156.0, CH	156.2, CH	126.3, CH	155.9, CH
2	112.6, CH	112.6, CH	112.6, CH	112.6, CH	112.6, CH	112.6, CH	127.4, CH	127.5, CH	112.4, CH	127.5, CH
3	153.2, C	153.2, C	153.2, C	153.3, C	153.3, C	153.2, C	186.4, C	186.5, C	152.9, C	186.4, C
4	115.2, CH	115.2, CH	115.2, CH	115.3, CH	115.3, CH	115.2, CH	123.8, CH	123.9, CH	115.1, CH	123.8, CH
5	138.4, C	138.4, C	138.4, C	138.5, C	138.5, C	138.4, C	169.5, C	169.6, C	138.2, C	169.4, C
6	29.7, CH_2	29.7, CH_2	29.7, CH_2	29.8, CH_2	29.8, CH_2	29.7, CH_2	33.0, CH_2	33.1, CH_2	29.8, CH_2	32.9, CH_2
7	28.7, CH_2	28.5, CH_2	28.6, CH_2	28.4, CH_2	29.0, CH_2	28.7, CH_2	33.8, CH_2	33.8, CH_2	28.4, CH_2	33.7, CH_2
8	38.8, CH	38.8, CH	38.8, CH	38.9, CH	38.9, CH	38.8, CH	35.7, CH	35.6, CH	38.9, CH	35.6, CH
9	43.8, CH	43.8, CH	43.8, CH_2	43.8, CH	43.9, CH	43.8, CH	52.4, CH	52.6, CH	43.8, CH	52.5, CH
10	133.2, C	133.2, C	133.2, C	133.3, C	133.3, C	133.2, C	43.6, C	43.7, C	133.0, C	43.7, C
11	26.8, CH_2	26.8, CH_2	26.8, CH_2	26.9, CH_2	26.9, CH_2	26.8, CH_2	22.9, CH_2	22.9, CH_2	26.9, CH_2	22.9, CH_2
12	39.9, CH_2	39.89, CH_2	39.8, CH_2	40.1, CH_2	40.0, CH_2	39.9, CH_2	39.5, CH_2	39.5, CH_2	39.6, CH_2	39.4, CH_2
13	42.8, C	42.8, C	42.8, C	43.0, C	42.8, C	42.8, C	42.7, C	42.7, C	42.9, C	42.6, C
14	55.6, CH	55.6, CH	55.6, CH	55.5, CH	55.7, CH	55.6, CH	55.5, CH	55.7, CH	55.5, CH	55.6, CH
15	24.0, CH_2	23.9, CH_2	23.9, CH_2	24.0, CH_2	24.1, CH_2	24.0, CH_2	24.4, CH_2	24.4, CH_2	24.0, CH_2	24.4, CH_2
16	27.7, CH_2	27.7, CH_2	27.7, CH_2	27.7, CH_2	27.7, CH_2	27.7, CH_2	28.1, CH_2	28.4, CH_2	27.8, CH_2	28.6, CH_2
17	56.2, CH	56.3, CH	56.3, CH	56.3, CH	56.3, CH	56.3, CH	55.9, CH	56.0, CH	56.4, CH	55.9, CH
18	12.2, CH_3	12.2, CH_3	12.2, CH_3	12.1, CH_3	12.3, CH_3	12.2, CH_3	12.0, CH_3	12.4, CH_3	12.2, CH_3	12.3, CH_3
19							18.6, CH_3	18.8, CH_3		18.7, CH_3
20	40.2, CH	39.92, CH	39.9, CH	35.9, CH	40.4, CH	40.2, CH	35.7, CH	39.9, CH	35.9, CH	40.1, CH
21	20.8, CH_3	20.8, CH_3	20.7, CH_3	18.8, CH_3	21.1, CH_3	21.0, CH_3	18.7, CH_3	20.8, CH_3	18.9, CH_3	20.8, CH_3
22	138.1, CH	133.6, CH	134.1, CH	34.8, CH_2	136.1, CH	135.9, CH	34.6, CH_2	133.4, CH	36.3, CH_2	137.8, CH
23	126.3, CH	134.9, CH	130.8, CH	31.1, CH_2	132.0, CH	131.8, CH	31.0, CH_2	135.2, CH	24.1, CH_2	126.5, CH
24	42.0, CH_2		22.3, CH	157.0, C	43.2, CH	42.9, CH	156.9, C		40.1, CH_2	41.9, CH_2
25	28.6, CH	31.0, CH	14.7, CH	33.9, CH	33.3, CH	33.1, CH	33.8, CH	31.0, CH	28.2, CH	28.5, CH
26	22.3, CH_3	22.8, CH_3	14.8, CH_2	22.0, CH_3	19.7, CH_3	19.7, CH_3	21.9, CH_3	22.8, CH_3	23.0, CH_3	22.3, CH_3
27	22.3, CH_3	22.8, CH_3	18.6, CH_3	22.1, CH_3	20.2, CH_3	20.0, CH_3	22.0, CH_3	23.0, CH_3	22.7, CH_3	22.3, CH_3
28				106.1, CH_2	18.1, CH_3	17.7, CH_3	106.0, CH_2			

[a] Bruker-DRX-400 spectrometer. The chemical shifts are given in ppm and referenced to $CDCl_3$ (δ 77.0). By DEPT sequence.

identified as 19-norcholesta-1,3,5(10)-trien-3-ol[16,17] and cholesta-1,4,22-trien-3-one,[18] respectively, by comparison of their $[\alpha]_D$ values {**10**: $[\alpha]_D^{20}$ +78.3 (*c* 0.28, EtOH) [lit.[17] $[\alpha]_D$ +82.8 (*c* 0.43, EtOH)]; **11**: $[\alpha]_D^{20}$ +24.5 (*c* 0.12, $CHCl_3$) [lit.[18] $[\alpha]_D^{25}$ +26 (*c* 0.6, $CHCl_3$)]} and their NMR data with those reported in the literature. However, it is worthwhile to point out that compound **10** was isolated as a natural product for the first time.

All of the new compounds demonstrated considerable spectroscopic analogy with the co-occurring steroids **10** and **11**. In particular, compounds **2**−**7** showed IR absorptions indicative of the presence of an aromatic ring (1610, 1498 cm^{-1}), and their NMR spectra were reminiscent of those of co-occurring **10**, whereas **8** and **9** exhibited the same cross-conjugated dienone system in ring A as that of compound **11**.

Compound **2** was obtained as an UV-absorbing [λ_{max} (log ε) nm: 210 (3.91), 221 (3.89), 281 (3.13)], amorphous powder. The HREIMS spectrum of **2** contained an *m*/*z* peak at 366.2909, which allowed the molecular formula to be established as $C_{26}H_{38}O$. The ^{13}C NMR (Table 1) and DEPT spectra revealed the following carbon types: four methyl, seven methylene, 11 methine, and four quaternary carbons. Half of the eight degrees of unsaturation were accounted for by the four double bonds observed in the ^{13}C NMR spectrum. Consequently, the remaining degrees of unsaturation should be attributed to the four rings. In the ^{1}H NMR spectrum, only one typical angular singlet at δ 0.71 (3H, H_3-18) suggested that compound **2** is a 19-norsteroid. The characteristic ^{1}H NMR chemical shifts and splitting pattern associated with three of the four double bonds suggested that a 1,2,4-trisubstituted phenol group [ABX system, δ_H 7.15 (d, 1H, *J* = 8.4 Hz, H-1), 6.62 (dd, 1H, *J* = 8.4, 2.7 Hz, H-2), 6.55 (d, 1H, *J* = 2.7 Hz, H-4)] was present. Independent confirmation of this was provided by HMBC correlations from H-1 to C-3 and from H-2 to C-3, C-4, and C-10. The nature of ring B was established from correlations from H-6 to C-5, C-7, C-8, and C-10 and through correlations from H-9 to C-8 and C-10. The substitution patterns of the C- and D-rings were deduced from ^{13}C NMR chemical shifts and HMBC cross-peaks including those from H-11 to C-9 and C-12; from singlet H_3-18 to C-12, C-13, C-14, and C-17; and from H-15 to C-14, C-16, and C-17. A comparison of the overall ^{1}H and ^{13}C NMR data revealed significant similarities between **2** and the co-occurring **10**, indicating the same 19-norcholesta-1,3,5(10)-trien-3-ol nucleus. The difference between **2** and **10** was the presence in **2** of a disubstituted double bond [δ_H 5.23 (1H, dd, *J* = 15.2, 7.9 Hz, H-22) and 5.31 (1H, ddd, *J* = 15.2, 7.0, 6.6 Hz, H-23)] in the side chain, in agreement with a molecular weight for **2** that was two mass units less than that of **10**. The location of the olefin at Δ^{22} 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 from H_3-21 to C-17, C-20, and C-22 in the HMBC spectrum. The *E* configuration of the Δ^{22} was quickly deduced from the large coupling constant of H-22 (*J* = 15.2 Hz),

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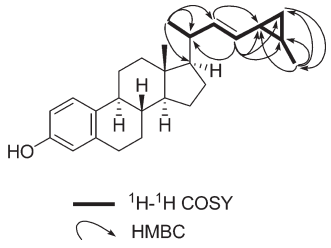

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of compound **4**.

as well as the absence of the NOE correlation between H-22 and H-23 in the ROESY spectrum. The observation of NOE cross-peaks between H_3-18/H-8 H_3-18/H-20, and H-17/H-14 was in agreement with the α-orientation of H-17 and the *R** configuration of C-20, consistent with the features normally exhibited by a steroid skeleton. Assignments of the proton and carbon signals for the side chain of **2** were secured by comparison with literature data.[19−23] These lines of evidence established the structure (22*E*)-19-norcholesta-1,3,5(10),22-tetraen-3-ol for compound **2**.

Compound **3** had a molecular formula of $C_{25}H_{36}O$, as determined by HREIMS (*m*/*z* 352.2767 $[M]^{+\cdot}$), a CH_2 unit less than that of **2**. Its ^{1}H and ^{13}C NMR data (Table 1) closely resembled those of **2**, with the exception that the ^{1}H NMR resonances of an isopropyl group were strongly downfield shifted (H-25 from δ 1.58 to 2.19, H_3-26/H_3-27 from 0.87 to 0.95). In addition, the splitting pattern of H-23 (δ_H 5.28, dd, *J* = 15.2, 6.3 Hz) indicated that the double bond at Δ^{22} was directly connected to the isopropyl group in the side chain. A series of HMBC correlations from H_3-26(/H_3-27) to C-23, from H-23 to C-26(C-27), and from H-22 to C-25, in combination with a diagnostic fragment ion at *m*/*z* 255 $[M^+ - C_7H_{13}]$, further confirmed the side-chain assignment. Thus, the structure of **3** was determined as (22*E*)-19,24-dinorcholesta-1,3,5(10),22-tetraen-3-ol. Compound **3** is an analogue of **2** with a shortened side chain.

Compound **4** was obtained as an amorphous powder. Its molecular formula, deduced from the HREIMS (*m*/*z* 364.2763 $[M]^{+\cdot}$), corresponds to $C_{26}H_{36}O$, indicating nine degrees of unsaturation. Both the ^{1}H and ^{13}C NMR data (Table 1) of **4**, closely related to those of compound **2**, suggested that they shared the same 19-norcholesta-1,3,5(10)-trien-3-ol skeleton. In fact, **4** differs from **2** only at the side chain. The upfield region of the ^{1}H NMR spectrum in **4** presented two signals at δ_H 0.46 (1H, m, H-26a) and 0.38 (1H, m, H-26b), which correlated to a methylene carbon signal at δ_C 14.8 (C-26) in the HMQC spectrum, and two multiplets at δ_H 0.96 (1H, m, H-24) and 0.66 (1H, m, H-25), which correlated to the methine carbon signals at δ_C 22.3 (C-24) and 14.7 (C-25), respectively. These findings were indicative of the presence of a disubstituted cyclopropane ring[24,25] involving C-24−C-26 in **4**, explaining the additional degree of unsaturation compared to **2**, and consistent with the observed molecular weight difference of two mass units between **2** and **4**. Moreover, a series of ^{1}H,^{1}H COSY connectivities (Figure 1) of H_3-21/H-20/H-22/H-23/H-24/H-25(/H_2-26)/H_3-27, strong HMBC correlations (Figure 1) from H-23 (H_3-27) to C-24−C-26, and upfield shifted ^{13}C NMR values of C-24 (δ_C 22.3), C-25 (δ_C 14.7), C-26 (δ_C 14.8), and C-27 (δ_C 18.6) led to the assignment of the planar structure for **4**. Because no detectable NOE enhancements on the adjacent protons (H-24, H-25, and H_3-27) were observed in the NOE difference experiment, the relative configurations at C-24 and C-25 remain undetermined. On the basis of the above evidence, the structure of compound **4** was elucidated as (22*E*)-24,26-cyclo-19-norcholesta-1,3,5(10),22-tetraen-3-ol.

Compound **5** yielded a HREIMS peak at *m*/*z* 380.3094 $[M]^{+\cdot}$, 12 mass units more than that of **10**. The ^{1}H and ^{13}C NMR (Table 1) spectra of **5** revealed a close relationship with those of **10**, except for the presence of an additional exomethylene group in the side chain, as indicated by ^{1}H NMR resonances at δ_H 4.72 (1H, brs, H-28a) and 4.67 (1H, brs, H-28b) and their corresponding ^{13}C NMR resonance at δ_C 106.1 (C-28), as well as an sp^2 quaternary carbon signal at δ_C 157.0 (C-24). Furthermore, analysis of the HMBC cross-peaks from δ 4.72 and 4.67 to δ 31.1 (C-23), 157.0, and 33.9 (C-25) located the exomethylene at $\Delta^{24(28)}$. Moreover, the changes in the side chain of **5** were strongly supported by further analysis of its mass spectrum, which showed diagnostic fragment ions at *m*/*z* 296 $[M^+ - C_6H_{12}]$ and 281 $[M^+ - C_6H_{12} - CH_3]$ derived by a McLafferty rearrangement typical of 24-methylene cholesterol derivatives.[19,23] Compound **5** was therefore established as 24-methylene-19-norcholesta-1,3,5(10),22-tetraen-3-ol.

The NMR spectroscopic data of compound **6** were very compatible with those of **2**, except for the presence of an additional methyl group (δ_H 0.92, 3H, d, *J* = 6.8 Hz, H_3-28; δ_C 18.1, C-28) in the side chain. This assignment was confirmed by the molecular formula of **6** being 14 mass units higher than that of **2**, as established by the HREIMS data (*m*/*z* 380.3097 $[M]^{+\cdot}$). The methyl group was deduced to be connected to the C-24 position on the basis of the HMBC correlations from H_3-28 to C-23−C-25 and from H-23(/H-25) to the methyl carbon (C-28). There is a stereogenic center (C-24) in the side chain. Assuming that these derivatives are in the commonly observed enantiomeric series for sterols, the absolute configuration at C-24 is assigned as *S* on the basis of the ^{13}C NMR value of C-28 (δ_C 18.1). It was reported that the ^{13}C NMR chemical shift of C-28 resonates at δ_C 17.6 ± 0.1 ppm in the 24*R* epimer of sterols with the same side chain, while a relative 0.4 ppm downfield chemical shift for C-28 should be observed in the 24*S* epimer (as in **6**).[20,26] Therefore, the structure of **6** is determined as (22*E*,24*S*)-24-methyl-19-norcholesta-1,3,5(10),22-tetraen-3-ol.

Compound **7** was obtained as an amorphous powder. Its molecular formula $C_{27}H_{40}O$ was the same as compound **6**. The MS and ^{13}C NMR spectra (Table 1) of **7** were almost identical to those of **6** except for the resonance assigned to C-28 (δ_C 17.7 in **7**; 18.1 in **6**).[16,22] Therefore, compound **7** is the C-24 epimer (24*R*) of compound **6**.

A search of the literature revealed that although many C_{21} steroids possessing aromatic A-rings have been isolated from plants[27,28] and animals,[29,30] until now only three normal C_{27} steroids with aromatic A-rings[31,32] have been reported from marine sponges. This is the first report of marine steroids possessing aromatic A-rings from Cnidaria animals.

The molecular formula of compound **8** was determined as $C_{28}H_{42}O$ from HREIMS (*m*/*z* 394.3228 $[M]^{+\cdot}$) and NMR data. The ^{1}H and ^{13}C NMR data (Table 1) of **8** resembled those of co-occurring **11**, which featured two angular methyl groups [δ_H 0.73 (3H, s, H_3-18)/δ_C 12.0 (C-18); δ_H 1.22 (3H, s, H_3-19)/δ_C 18.6 (C-19)]. The protons at δ_H 7.05 (d, *J* = 10.1 Hz, H-1), 6.22 (dd, *J* = 10.1, 1.7 Hz, H-2), and 6.06 (d, *J* = 1.7 Hz, H-4) in association with the carbon resonances at δ_C 156.0 (C-1), 127.4 (C-2), 186.4 (C-3), 123.8 (C-4), and 169.5 (C-5) were attributed to a cross-conjugated dienone system in ring A. In fact, compounds **8** and **11** share the same cholesta-1,4-dien-3-one skeleton and differ

from each other only in the side chain. Furthermore, comparison of its ^{13}C NMR data (Table 1) with those of **5** revealed that the side chain of **8** was the same as that of **5**. Thus, the structure of **8** was elucidated as 24-methylenecholesta-1,4,22-trien-3-one.

The HREIMS and NMR data gave the molecular formula of compound **9** as $C_{26}H_{38}O$. Careful analysis of the ^1H and ^{13}C NMR data (Table 1) of **9** and comparison with those of **3**, **8**, and **11** revealed that **9** had the same steroidal nucleus as **8** and **11**, and the side chain matched well to that of compound **3**. Therefore, compound **9** was identified as (22*E*)-24-cholesta-1,4,22-trien-3-one.

Although the title soft coral has been chemically investigated previously by Su et al.[33] and the specimens were collected from the same body of water, the metabolites found in both collections were quite different. Actually, only several very simple known compounds were isolated and reported in the above-mentioned reference. The discovery of novel steroids **1**−**9** has added to an extremely diverse and complex array of steroids, which is still rapidly expanding.

The promising antitumor activity of methyl spongoate (**1**) inspired us to test the cytotoxicities of compounds **2**−**11**. However, in contrast to **1**, they were all inactive at concentrations up to 20 μg/mL against the growth of several tumor cell lines, including BEL-7402, murine lymphocytic leukemia P388, human promyelocytic leukemia HL-60, and human lung adenocarcinoma A549.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. NMR spectra were measured on a Bruker DRX-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), using the residual $CHCl_3$ signal (δ_H 7.26 ppm) as an internal standard for ^1H NMR and $CDCl_3$ (δ_C 77.0 ppm) for ^{13}C NMR. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. ^1H and ^{13}C NMR assignments were supported by $^1H-^1H$ COSY, HSQC, HMBC, and ROESY experiments. EIMS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 25 cm ×10 mm (i.d.)] column) was also employed. Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for column chromatography (CC), and precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Biological Material. The soft coral *Dendronephthya studeri* Ridley was collected off the coast of Xiaodong Sea, Hainan Province, China, in December 2001, at a depth of 20 m and identified by H.H., X.-B.L., and R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (01-HN-99) is available for inspection at Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen soft coral (456 g dry weight) was processed as previously reported to provide 3.2 g of an Et_2O-soluble extract.[11] This extract was fractionated by gradient silica gel CC eluting with a step gradient (0−100% Et_2O in light petroleum ether) to yield two steroid-containing fractions [A (160 mg) and B (121 mg)]. Fraction A, eluted with light petroleum ether/Et_2O (92:8), was further purified by Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2:1:1), followed by a semipreparative HPLC purification [MeOH/H_2O, 95:5, 3.0 mL/min] to yield **4** (2.5 mg, t_R 29.7 min), **3** (1.0 mg, t_R 34.1 min), **2** (2.8 mg, t_R 43.9 min), **5** (2.6 mg, t_R 46.9 min), **6** (0.9 mg, t_R 50.2 min), **7** (1.0 mg, t_R 53.0 min), and **10** (6.8 mg, t_R 57.0 min), respectively. Fraction B, eluted with light petroleum ether/Et_2O (90:10), was applied to Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2:1:1) to give **1** (2.5 mg)[11] and subfraction B1, which was then further purified by semipreparative HPLC [MeOH/H_2O, 92:8, 3.0 mL/min] to yield **9** (0.8 mg, t_R 43.8 min), **11** (2.7 mg, t_R 58.0 min), and **8** (3.2 mg, t_R 61.8 min), respectively.

(22*E*)-19-Norcholesta-1,3,5(10),22-tetraen-3-ol (2): white powder; $[\alpha]_D^{24}$ +51.6 (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.91), 221 (3.89), 281 (3.13) nm; IR (KBr) ν_{max} 3560, 3358, 2920, 1612, 1581 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.15 (1H, d, J = 8.4 Hz, H-1), 6.62 (1H, dd, J = 8.4, 2.7 Hz, H-2), 6.55 (1H, d, J = 2.7 Hz, H-4), 5.31 (1H, ddd, J = 15.2, 7.0, 6.6 Hz, H-23), 5.23 (1H, dd, J = 15.2, 7.9 Hz, H-22), 4.58 (1H, brs, OH-3), 2.80 (2H, m, H_2-6), 2.15 (1H, m, H-9), 2.05 (1H, m, H-20), 1.83 (2H, m, H_2-24), 1.58 (1H, m, H-25), 1.22 (1H, m, H-17), 1.03 (3H, d, J = 6.7 Hz, H_3-21), 0.71 (3H, s, H_3-18), 0.87 (6H, d, J = 6.7 Hz, H_3-26, H_3-27); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 366 $[M]^{+\cdot}$ (54), 282 (18), 255 (26), 213 (26), 159 (84), 133 (47), 81 (63), 69 (100); HREIMS m/z 366.2909 $[M]^{+\cdot}$ (calcd for $C_{26}H_{38}O$, 366.2923).

(22*E*)-19,24-Dinorcholesta-1,3,5(10),22-tetraen-3-ol (3): white powder; UV (MeOH) λ_{max} (log ε) 211 (3.95), 221 (3.79), 280 (3.18) nm; IR (KBr) ν_{max} 3587, 3360, 2922, 1607, 1570 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.15 (1H, d, J = 8.4 Hz, H-1), 6.62 (1H, dd, J = 8.4, 2.7 Hz, H-2), 6.55 (1H, d, J = 2.7 Hz, H-4), 5.28 (1H, dd, J = 15.2, 6.3 Hz, H-23), 5.18 (1H, dd, J = 15.2, 8.4 Hz, H-22), 4.58 (1H, brs, OH-3), 2.80 (2H, m, H_2-6), 2.19 (1H, m, H-25), 2.15 (1H, m, H-9), 2.02 (1H, m, H-20), 1.21 (1H, m, H-17), 1.03 (3H, d, J = 6.7 Hz, H_3-21), 0.95 (6H, d, J = 6.7 Hz, H_3-26, H_3-27), 0.70 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 352 $[M]^{+\cdot}$ (87), 282 (22), 255 (30), 213 (34), 159 (100), 133 (56), 55 (35); HREIMS m/z 352.2767 $[M]^{+\cdot}$ (calcd for $C_{25}H_{36}O$, 352.2766).

(22*E*)-24,26-Cyclo-19-norcholesta-1,3,5(10),22-tetraen-3-ol (4): white powder; $[\alpha]_D^{20}$ +39.5 (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (3.93), 220 (3.82), 281 (3.12) nm; IR (KBr) ν_{max} 3562, 3356, 2920, 1610, 1583 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.14 (1H, d, J = 8.3 Hz, H-1), 6.61 (1H, dd, J = 8.3, 2.8 Hz, H-2), 6.55 (1H, d, J = 2.8 Hz, H-4), 5.28 (1H, dd, J = 15.2, 8.4 Hz, H-22), 4.92 (1H, dd, J = 15.2, 8.4 Hz, H-23), 4.61 (1H, brs, OH-3), 2.80 (2H, m, H_2-6), 2.20 (1H, m, H-25), 2.14 (1H, m, H-9), 2.02 (1H, m, H-20), 1.21 (1H, m, H-17), 1.04 (3H, d, J = 6.1 Hz, H_3-27), 1.02 (3H, d, J = 6.7 Hz, H_3-21), 0.96 (1H, m, H-24), 0.70 (3H, s, H_3-18), 0.66 (1H, m, H-25), 0.46, 0.38 (each 1H, m, H_2-26); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 364 $[M]^{+\cdot}$ (41), 282 (39), 213 (31), 159 (67), 133 (48), 109 (56), 72 (65), 59 (100); HREIMS m/z 364.2763 $[M]^{+\cdot}$ (calcd for $C_{26}H_{36}O$, 364.2766).

24-Methylene-19-norcholesta-1,3,5(10),22-tetraen-3-ol (5): white powder; $[\alpha]_D^{24}$ +55.1 (c 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.01), 219 (3.85), 282 (3.08) nm; IR (KBr) ν_{max} 3550, 3344, 2938, 1605, 1550 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.15 (1H, d, J = 8.6 Hz, H-1), 6.62 (1H, dd, J = 8.6, 2.7 Hz, H-2), 6.55 (1H, d, J = 2.7 Hz, H-4), 4.72, 4.67 (each 1H, brs, H_2-28), 4.59 (1H, brs, OH-3), 2.80 (2H, m, H_2-6), 2.24 (1H, m, H-25), 2.17 (1H, m, H-9), 2.12, 1.95 (each 1H, m, H_2-23), 1.60, 1.21 (each 1H, m, H_2-22), 1.47 (1H, m, H-20), 1.14 (1H, m, H-17), 1.03 (6H, d, J = 6.7 Hz, H_3-26, H_3-27), 0.98 (3H, d, J = 6.5 Hz, H_3-21), 0.70 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 380 $[M]^{+\cdot}$ (63), 366 (35), 296 (75), 281 (12), 253 (30), 213 (100), 160 (60), 159 (53), 133 (39); HREIMS m/z 380.3094 $[M]^{+\cdot}$ (calcd for $C_{27}H_{40}O$, 380.3079).

(22*E*,24*S*)-24-Methyl-19-norcholesta-1,3,5(10),22-tetraen-3-ol (6): white powder; $[\alpha]_D^{24}$ +50 (c 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (3.99), 219 (3.94), 280 (3.28) nm; IR (KBr) ν_{max} 3571, 3346, 2948, 1617, 1587 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.15 (1H, d, J = 8.4 Hz, H-1), 6.62 (1H, dd, J = 8.4, 2.7 Hz, H-2), 6.55 (1H, d, J = 2.7 Hz, H-4), 5.18 (1H, m, H-22), 5.17 (1H, m, H-23), 4.60 (1H, brs, OH-3), 2.80 (2H, m, H_2-6), 2.14 (1H, m, H-9), 2.03 (1H, m, H-20), 1.83 (1H, m,

H-24), 1.46 (1H, m, H-25), 1.20 (1H, m, H-17), 1.03 (3H, d, J = 6.6 Hz, H_3-21), 0.70 (3H, s, H_3-18), 0.92 (3H, d, J = 6.8 Hz, H_3-28), 0.84 (3H, d, J = 6.7 Hz, H_3-27), 0.83 (3H, d, J = 6.7 Hz, H_3-26); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 380 $[M]^{+\cdot}$ (72), 282 (22), 255 (27), 213 (32), 159 (100), 133 (60), 69 (60), 55 (65); HREIMS m/z 380.3097 $[M]^{+\cdot}$ (calcd for $C_{27}H_{40}O$, 380.3079).

(22*E*,24*R*)-24-Methyl-19-norcholesta-1,3,5(10),22-tetraen-3-ol (7): white powder; $[\alpha]_D^{24}$ +63 (c 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.72), 225 (3.93), 282 (3.29) nm; IR (KBr) ν_{max} 3572, 3346, 2950, 1619, 1587 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 (1H, d, J = 8.4 Hz, H-1), 6.62 (1H, dd, J = 8.4, 2.7 Hz, H-2), 6.55 (1H, d, J = 2.7 Hz, H-4), 5.18 (1H, m, H-22), 5.17 (1H, m, H-23), 2.80 (2H, m, H_2-6), 2.14 (1H, m, H-9), 2.03 (1H, m, H-20), 1.83 (1H, m, H-24), 1.46 (1H, m, H-25), 1.20 (1H, m, H-17), 1.04 (3H, d, J = 6.6 Hz, H_3-21), 0.70 (3H, s, H_3-18), 0.92 (3H, d, J = 6.8 Hz, H_3-28), 0.84 (3H, d, J = 6.7 Hz, H_3-27), 0.83 (3H, d, J = 6.7 Hz, H_3-26); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 380 $[M]^{+\cdot}$ (100), 282 (22), 255 (32), 213 (32), 159 (88), 133 (50), 69 (36), 55 (35); HREIMS m/z 380.3088 $[M]^{+\cdot}$ (calcd for $C_{27}H_{40}O$, 380.3079).

24-Methylenecholesta-1,4,22-trien-3-one (8): white powder; $[\alpha]_D^{20}$ +14.0 (c 0.27, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 246 (3.94) nm; IR (KBr) ν_{max} 2933, 1730, 1687 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.05 (1H, d, J = 10.1 Hz, H-1), 6.22 (1H, dd, J = 10.1, 1.7 Hz, H-2), 6.06 (1H, t, J = 1.7 Hz, H-4), 4.71, 4.64 (each 1H, brs, H_2-28), 2.47 (1H, tdd, J = 12.9, 3.9, 1.5 Hz, H-6a), 2.36 (1H, tt, J = 12.9, 2.4 Hz, H-6b), 2.24 (1H, m, H-25), 2.10, 1.94 (each 1H, m, H_2-23), 1.60, 1.20 (each 1H, m, H_2-22), 1.47 (1H, m, H-20), 1.22 (3H, s, H_3-19), 1.16 (1H, m, H-17), 1.03 (3H, d, J = 6.7 Hz, H_3-27), 1.02 (3H, d, J = 6.7 Hz, H_3-26), 0.93 (3H, d, J = 6.4 Hz, H_3-21), 0.73 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 394 $[M]^{+\cdot}$ (12), 310 (9), 281 (11), 267 (15), 149 (24), 122 (100), 72 (63), 59 (100); HREIMS m/z 394.3228 $[M]^{+\cdot}$ (calcd for $C_{28}H_{42}O$, 394.3236).

(22*E*)-24-Cholesta-1,4,22-trien-3-one (9): white powder; UV (MeOH) λ_{max} (log ε) 245.5 (3.96) nm; IR (KBr) ν_{max} 2928, 1736, 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, J = 10.0 Hz, H-1), 6.23 (1H, dd, J = 10.0, 1.8 Hz, H-2), 6.07 (1H, t, J = 1.8 Hz, H-4), 5.28 (1H, dd, J = 15.5, 6.7 Hz, H-23), 5.16 (1H, dd, J = 15.5, 8.2 Hz, H-22), 2.47 (1H, tdd, J = 12.9, 3.9, 1.5 Hz, H-6a), 2.36 (1H, tt, J = 12.9, 2.4 Hz, H-6b), 2.18 (1H, m, H-25), 2.01 (1H, m, H-20), 1.23 (3H, s, H_3-19), 1.22 (1H, m, H-17), 1.00 (3H, d, J = 6.6 Hz, H_3-21), 0.94 (6H, d, J = 6.7 Hz, H_3-26, H_3-27), 0.75 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS 70 eV m/z (rel int %) 366 $[M]^{+\cdot}$ (16), 340 (10), 270 (15), 122 (100) 97 (32), 55 (33); HREIMS m/z 366.2915 $[M]^{+\cdot}$ (calcd for $C_{26}H_{38}O$, 366.2923).

Cytotoxicity Bioassays. The cytotoxicities of compounds **2**−**11** against BEL-7402, P388, HL-60, and A549 cell lines were evaluated by using the MTT[34] and SRB[35] methods, respectively, according to the protocols described in previous literature.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. 1D and 2D NMR and HREIMS spectra of compounds **2**−**9**. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 86-21-50805813. Fax: 86-21-50805813. E-mail: ywguo@mail.shcnc.ac.cn.

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