Oculatol, Oculatolide, and A-nor Sterols from the Sponge *Haliclona oculata*

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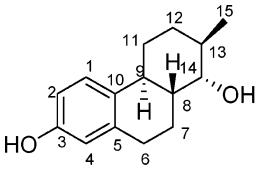
Received February 1, 2006

Chemical investigation of the marine sponge *Haliclona oculata* resulted in the isolation of eight new compounds including oculatol (**1**) and oculatolide (**2**) and six unusual A-nor steroids, 2-ethoxycarbonyl-2β-hydroxy-A-nor-ergosta-5,24(28)-dien-4-one (**3**), 2-ethoxycarbonyl-24-ethyl-2β-hydroxy-A-nor-cholesta-5-en-4-one (**4**), 2-ethoxycarbonyl-2β,7β-dihydroxy-A-nor-ergosta-5,24(28)-dien-4-one (**5**), 2-ethoxycarbonyl-2β,7β-dihydroxy-A-nor-cholesta-5-en-4-one (**6**), 2-ethoxycarbonyl-2β,7β-dihydroxy-24-methyl-A-nor-cholesta-5,22(*E*)-dien-4-one (**7**), and 2-ethoxycarbonyl-2β,7β-dihydroxy-A-nor-cholesta-5,22(*E*)-dien-4-one (**8**), along with 16 known steroids and indole derivatives. Their structures were unambiguously determined on the basis of extensive spectroscopic analyses.

Marine sponges are known to be a prolific and chemically diverse source of terpenoids and steroids. The marine sponge genus *Haliclona* (Demospongiae) has been extensively examined, and at least 190 metabolites including steroids, terpenoids, alkaloids, cyclic peptides, and unsaturated fatty acids have been reported. Several of these compounds showed interesting biological activities such as the cytotoxic haliclonacyclamines,[1] the antifungal pentacyclic alkaloids[2] and haliclonadiamine,[3] and the antimicrobial cytotoxic haliclamines and manzamines.[4−6] Previous chemical study of the finger sponge *Haliclona oculata* from the Black Sea resulted in unusual high levels of C24−C30 fatty acids[7] and a number of sterols.[8,9] As part of our continued investigation on the chemical diversity of marine organisms from the South China Sea, the sponge *H. oculata* was collected from an inner coral reef. A preliminary bioassay of the fractions from a MeOH extract obtained via flash chromatography revealed that the petroleum ether fraction showed moderate inhibition against human tumor cell lines A-549 and HL-60. This fraction was subsequently subjected to a detailed chromatographic separation, which resulted in the isolation and characterization of 24 metabolites involving oculatol (**1**) and oculatolide (**2**) as well as six new unusual A-nor-steroids (**3**−**8**).



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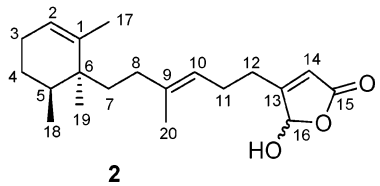

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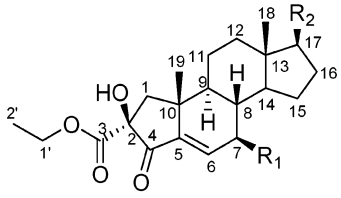
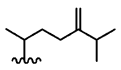
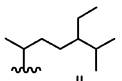
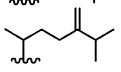
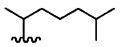
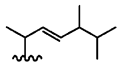


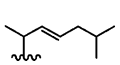
3. R_1 = H, R_2 = 

4. R_1 = H, R_2 = 

5. R_1 = OH, R_2 = 

6. R_1 = OH, R_2 = 

7. R_1 = OH, R_2 = 

8. R_1 = OH, R_2 = 

Results and Discussion

The known steroids could be classified as A-nor-steroids and cholesterol-type steroids, of which 2-ethoxycarbonyl-2β-hydroxy-A-nor-cholesta-5-ene-4-one, 2-ethoxycarbonyl-2β-hydroxy-A-nor-cholesta-5,22(*E*)-diene-4-one, and 2-ethoxycarbonyl-2β-hydroxy-24-methyl-A-nor-cholesta-5,22(*E*)-dien-4-one were previously reported from a Chinese soft coral *Dendronephthya* sp.,[10] while 6β-hydroxyergosta-4,24(28)-dien-3-one,[11] 6β-hydroxycholesta-4-en-3-one,[11] ergosta-5,24(28)-dien-3β-ol,[12] ergosta-4,24(28)-dien-3-one,[10] cholesta-5,22,24-trien-3β-ol,[13] and 6β-hydroxyergosta-4,22-dien-3-one[11] were isolated from this sponge for the first time. In addition, seven indole derivatives were isolated and identified as 6-bromo-3-indolecarboxaldehyde,[14] 6-bromoindole-3-carboxylic acid,[14] 6-hydroxyindole-3-carboxaldehyde,[15] indole-3-carboxylic acid,[16] indole-3-carboxaldehyde,[16] indole-3-methanol,[17] and indol-3-ol.[18]

Oculatol (**1**) was obtained as a white, amorphous powder, and its molecular formula was determined to be $C_{15}H_{20}O_2$ through HREIMS (*m/z* 232.1486, calcd 232.1463) and NMR data, indicating 6 degrees of unsaturation. The IR absorptions at 3317, 1609, and 1585 cm^{-1} suggested the presence of hydroxyl and aromatic groups. ^{13}C NMR and DEPT spectra exhibited 15 carbons involving a methyl, four methylenes, seven methines, and three quaternary carbons, of which the signals at δ 127.6 (CH), 113.3 (CH), 154.5 (C), 115.5 (CH), 139.5 9 (C), and 132.0 (C) were attributable to a trisubstituted aromatic ring. Thus, the remaining degrees of unsaturation could be accounted for by the formation of two aliphatic rings. The HMQC spectrum allowed the assignment of the protons

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10.1021/np0600494 CCC: $33.50
Published on Web 08/26/2006

Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) and Principle HMBC Correlations for Oculatol (**1**) and Oculatolide (**2**)

	oculatol (**1**)			oculatolide (**2**)		
no.	δ_C	δ_H	HMBC (H→C)	δ_C	δ_H	HMBC (H→C)
1	127.6 CH	7.21 d (8.0)	C-3, C-5, C-9	139.3 qC		
2	113.3 CH	6.67 dd (2.0, 8.0)	C-4, C-10	122.9 CH	5.41 m	C-17, C-3, C-6
3	154.5 qC			24.0 CH_2	1.62 m, 1.94 m	C-1, C-2, C-4
4	115.5 CH	6.58 d (2.0)	C-2, C-6, C-10	27.5 CH_2	1.40 m, 1.60 m	C-2, C-3, C-5, C-18
5	139.5 qC			37.7 CH	1.57 m	C-3, C-4, C-6, C-18, C-19
6	30.7 CH_2	α 2.86 dd (6.5, 16.5) *β* 2.89 ddd (7.0, 12.0, 16.5)	C-4, C-8, C-10	39.5 qC		
7	27.1 CH_2	1.72 dd (7.0, 17.5)		34.6 CH_2	1.34 ddd (3.6, 13.3, 13.3)	C-1, C-5, C-6, C-9, C-19
		1.82 dddd (6.5, 11.5, 12.0, 17.5)			1.48 ddd (5.3, 12.5, 13.3)	
8	39.2 CH	1.66 dd (11.5, 11.5)		35.7 CH_2	1.85 ddd (3.6, 12.5, 13.3) 1.98 ddd (5.3, 13.3, 13.3)	C-6, C-9, C-10, C-20
9	35.9 CH	2.75 ddd (4.0, 11.5, 12.0)	C-1, C-5, C-11, C-8	138.9 qC		
10	132.0 qC			121.4 CH	5.09 dd (3.5, 3.5)	C-8, C-20, C-11, C-12
11	26.0 CH_2	1.38 dddd (3.5, 12.0, 12.0, 13.5)		25.2 CH_2	2.31 m	C-9, C-10, C-13
		2.20 dd (5.0, 13.5)			2.32 m	
12	26.7 CH_2	1.57 m		27.7 CH_2	2.41 ddd (6.5, 14.0, 16.5)	C-10, C-14, C-16
		2.12 ddd (5.0, 5.0, 13.5)			2.54 ddd (7.5, 16.5, 16.5)	
13	35.5 CH	2.05 m	C-12,C-13, C-8, C-15	168.8 qC		
14	77.2 CH	3.70 brs	C-8, C-12, C-13	117.9 CH	5.86 s	C-12, C-15, C-16
15	17.0 CH_3	1.03 d (7.5)	C-12, C-13, C-14	170.8 qC		
16				98.7 CH	5.98 d 7.0	C-12, C-14, C-15
17				19.6 CH_3	1.63 s	
18				15.9 CH_3	0.95 d (7.0)	C-4, C-5, C-6
19				26.4 CH_3	1.03 s	C-1, C-5, C-6, C-7
20				16.3 CH_3	1.63 s	C-8, C-9, C-10
16-OH					3.67 d (7.0)	C-13, C-16

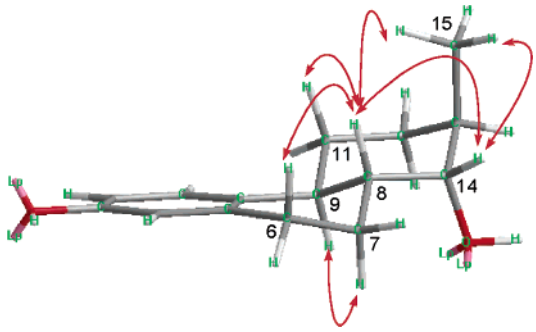

Figure 1. Key NOE correlations of **1**.

and their corresponding carbons. An ABX coupling system at δ_H 7.21 (d, J = 8.0 Hz, H-1), 6.67 (dd, J = 2.0, 8.0 Hz, H-2), and 6.58 (d, J = 2.0 Hz, H-4) in association with HMBC correlations (Table 1) suggested a hydroxyl group to be substituted at C-3 of the aromatic ring. The DQFCOSY correlations between H-9 (δ_H 2.75, ddd)/H-8 (δ_H 1.66, dd), H-9/H_2-11 (δ_H 1.38, dddd; 2.20, dd), H_2-11/H_2-12 (δ_H 1.57, m; 2.12, ddd), H-13/H_2-12, H-13/H_3-15 (δ_H 1.03, d), H-13/H-14 (δ_H 3.70, br), H-14/H-8, H-8/H_2-7 (δ_H 1.72, dd; 1.82, dddd), and H_2-7/H_2-6 (δ_H 2.86, dd; 2.89, ddd), respectively, entirely assigned all protons around the aliphatic six-membered rings B and C with the substitution of a methyl group (δ_H 1.03, d, J = 7.5 Hz, H_3-15) at C-13 and a hydroxyl group at C-14. The assignments of ring fusion and position of substituents were further supported by HMBC correlations from H-1 to C-9 (δ_C 35.9), from H-4 to C-6 (δ_C 30.7), from H_3-15 to C-13 (δ_C 35.5), C-12 (δ_C 26.7), and C-14 (δ_C 77.2), and from H-14 (δ_H 3.70, brs) to C-12, C-13, C-15 (δ_C 17.0), C-8 (δ_C 39.2), and C-9. The relative stereochemistry of **1** was determined on the basis of coupling constants and NOE correlations. The $J_{H-9/H-8}$ value (11.5 Hz) was indicative of *trans* orientation between H-9 and H-8, while the NOESY correlation between H-8 and H_3-15 indicated the methyl group to be in *β*-form (axial). Thus, the broad signal of H-14 (δ_H 3.70, brs) was assignable to an equatorial (*β*-form) position in the chair conformation of ring C.

Oculatolide (**2**) was isolated as a yellow oil. Its molecular formula was established as $C_{20}H_{30}O_3$ on the basis of ESIMS (*m/z* 317 [M − 1]$^-$) and NMR data. The ^{13}C NMR and DEPT data showed 20 carbons, which included four methyls, six methylenes, five methines, and five quaternary carbons (Table 1). 2D NMR data analysis and comparison of its NMR data with those of subersin, a diterpenoid originated from the sponge *Jaspis splendens*,[19] revealed the partial structure of **2** to be identical with that of subersin. Compound **2** differed from the latter solely at the right end, where the furan ring of subersin had been replaced by a *γ*-hydroxybutenolide moiety, as indicated by the presence of IR absorptions at 3307, 1759, 1741, and 1646 cm^{-1} and the carbon resonances at δ_C 168.8 (C-13), 117.9 (C-14), 170.8 (C-15), and 98.7 (C-16). The HMBC correlations between H-14 (δ_H 5.86, s) and C-15, C-16, and C-12 (δ_C 27.7), between H-16 (δ_H 5.98, d, J = 7.0 Hz) and C-14, C-15, and C-12, and between a D_2O exchangeable proton at δ_H 3.67 (d, J = 7.0 Hz) and C-16 and C-13 defined the connection between C-12 and C-13 of the heterocyclic region. The relative stereochemistry was determined through NOESY correlations between H_3-18 (δ_H 0.95, J = 7.0 Hz) and H_2-7 (δ_H 1.34 and 1.48) and between H_3-19 (δ_H 1.03, s) and H-5 (δ_H 1.57 m), indicating the *trans* relationship between H_3-18 and H_3-19. Further NOESY correlation between H_3-20 (δ_H 1.63, s) and H_2-11 (δ_H 2.32, m) indicated an *E*-form of the double bond.Oculatol (**1**) is speculated to be derived from estrone via oxidation and hydration. Estrone is an estrogen with broad hormonal activity that is important for regulation of reproduction in vertebrates including echinoderms, mollusks, and cnidarians.[20] Although estrone has not been isolated from this sponge, the discovery of oculatol implies that it may contain estrogens. Subersin-type diterpenes are a small group previously found in the marine sponge genera *Suberea*, *Smenospongia*, and *Agelas*.[19,21,22] This is the first example of subersin derivatives from the genus *Haliclona*.

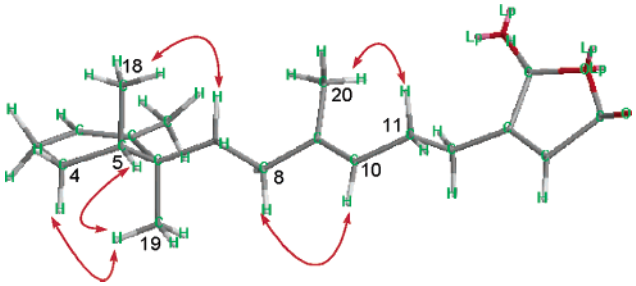

Figure 2. Key NOE correlations of **2**.

The NMR data of **3** to **9** were characteristic of a unique ring A-contracted steroid nucleus, closely related to those reported in the literature.[10,23] The core ^{13}C NMR resonances (C-1 to C-19) of **3** and **4** were virtually identical for each compound (Table 2) and were well comparable with those of anthosterones A and B and phorbasterones A−D from the sponge *Phorbas amaranthus*.[23]

A comparison of the ^1H and ^{13}C NMR data of **3** (Table 2) with those of anthosterone B revealed the identical ring A-contracted steroid backbone and side chain for both compounds, with the exception of an additional ethoxycarbonyl group (δ 1.26, t, J = 7.0 Hz, 3H; 4.25, q, J = 7.0 Hz, 2H) in **3** to replace the methoxycarbonyl group of anthosterone B. This fact was supported by HMBC correlations from the oxymethylene protons at δ 4.25 to the carbonyl carbon at δ 172.8 (C-3).

The structure of **4** was determined to be an ethoxycarbonyl derivative of phorbasterone D. This was based on a direct comparison of its NMR data (Table 2) with those of **3** for the ring A-contracted skeleton and those of phorbasterone D for the side chain.

The molecular formula of **5** was determined to be $C_{30}H_{46}O_5$ by HREIMS and NMR data, with 16 amu more than that of **3**. The ^1H and ^{13}C NMR data of **5** (Table 2) indicated the presence of a ring A-contracted steroid nucleus with the same side chain as that of **3**. However, the NMR spectra of **5** present an additional hydroxylated methine group [H-7 (δ_H 4.12, dd, J = 3.5, 7.5 Hz, 1H) and C-7 (δ_C 73.1)]. The DQFCOSY correlation between this proton and the olefinic proton H-6 (δ_H 6.50, d, J = 3.5 Hz, 1H) and the HMBC correlations between H-6 and the carbons C-7 and C-8 (δ_C 42.1) and in turn between the proton H-7 and C-5 (δ_C 146.6) and C-6 (δ_C 133.6) allowed the assignment of a hydroxyl group at C-7. With respect to the relative configuration of H-7, the presence of NOE correlations between H-7/H-9 (δ_H 1.32, m), H-8 (δ_H 1.68, ddd)/H_3-19, and H-8/H_3-18 (δ_H 0.76, s) and absence of NOE correlation between H-7/H-19 indicated α-configurations of H-7 and H-9 and β-configurations of H-8, H_3-18, and H_3-19. The remaining relative stereochemistry of **5** was assumed to be identical to that of anthosterone B due to the similar NOE correlations and virtually corresponding NMR data.

The ^1H and ^{13}C NMR spectroscopic data of **6** (Table 2) closely resembled those of **5**, showing the A-nor nucleus pattern, with the exception of its molecular weight being 12 amu less than that of **5** and the absence of the olefinic signals at C-24 of **5**, but with the presence of an additional CH_2 (δ_H 1.21, m; δ_C 39.5). The NMR chemical shifts for the side chain of **6** were identified to be the same as that of cholesterol on the basis of well-comparable NMR data between **6** and phorbasterone B.[23] The relative stereochemistry of **6** was in agreement with that of **5** due to the similar NOESY correlations. Accordingly, the structure of **6** was consistent with 2-ethoxycarbonyl-2β,7β-dihydroxy-A-nor-cholesta-5-en-4-one.

Compounds **7** and **8** were isolated only as minor components. Although we were unable to obtain their ^{13}C NMR spectra, the well-resolved ^1H NMR and COSY spectra in association with their molecular formula as determined by HREIMS enabled the determination of their structures. The ^1H NMR spectrum of **7** displayed seven methyl signals at δ_H 0.79 (s, H_3-18), 1.28 (s, s, H_3-19), 1.29 (t, J = 7.0 Hz, H_3-2′), 1.05 (d, J = 6.5 Hz, H_3-21), 0.85 (d, J = 7.0 Hz, H_3-26), 0.87 (d, J = 7.0 Hz, H_3-27), and 0.95 (d, J = 6.5 Hz, H_3-28). The typical olefinic proton at δ_H 6.53 (d, J = 4.0 Hz) was attributable to H-6, which correlated with a proton at δ_H 4.14 (dd, J = 4.0, 8.0 Hz, H-7) in the COSY spectrum, indicating the presence of a hydroxyl group at C-7. A comparison of the ^1H NMR data of **7** with those of **5** and **6** indicated that they are sharing the same nucleus with the exception of the side chain. Two olefinic protons resonating at δ_H 5.22 (dd, J = 6.5, 16.0 Hz) and 5.19 (dd, J = 7.5, 16.0 Hz) were assignable to H-22 and H-23 of the side chain and indicated C-24 to be substituted by a methyl group. The side chain of **7** was consistent with that of phorbasterone A[23] on the basis of comparable ^1H NMR data for both compounds and DQF COSY correlations. Thus, the structure of **7** was elucidated as 2-ethoxycarbonyl-2β,7β-dihydroxy-24-methyl-A-nor-cholesta-5,22(*E*)-dien-4-one.

The ^1H NMR spectrum of **8** displayed six methyl signals, indicating that **8** had lost one methyl group compared to **7**. The signals resonating at δ_H 0.79 (s), 1.28 (s), and 1.29 (t, J = 7.0 Hz) were consistent with H_3-18, H_3-19, and H_3-2′, and the olefinic proton at δ_H 6.53 (d, J = 3.5 Hz, H-6) showing a COSY correlation at δ 4.15 (dd, J = 3.5, 11.0 Hz, H-7) was indicative of a hydroxyl group at C-7. The ^1H NMR data of the side chain included three methyl signals at δ_H 1.06 (d, J = 6.5 Hz, H_3-21), 0.89 (d, J = 7.0 Hz, H_3-26), and 0.91 (d, J = 7.0 Hz, H_3-27) and two olefinic protons at δ_H 5.32 (dt, J = 7.0, 16.0 Hz, H-23) and 5.27 (dd, J = 8.0, 16.0 Hz, H-22), thus being comparable to those of 2-ethoxy-carbonyl-2β-hydroxy-A-nor-cholesta-5,22 (*E*)-dien-4-one.[10] The ^1H NMR data and COSY spectrum analysis in association with HREIMS led to the assignment of **8** as 2-ethoxycarbonyl-2β,7β-dihydroxy-A-nor-cholesta-5,22(*E*)-dien-4-one.

Since the extraction and isolation procedure for the described compounds did not include ethanol, the ethylated sterols are assumed to be true natural products. It is worth noting that the indole derivatives from this sponge were also discovered in the sponge *Iotrochoto birotulata*[11] from the same coral reef on Hainan Island. This evidence suggested that they shared a similar biogenetic pathway to produce the indole metabolites for predator-defense or antifouling.

The bioassay results indicated that **3** showed no activity against target proteins PTP1B (diabetes-II), AChE, and Cox-2 (IC_{50} > 10 μg/mL), but exhibited remarkable activity against human tumor cell lines HL-60 (IC_{50} = 0.32 μg/mL), A-549 (IC_{50} = 0.47 μg/mL), and BEL-7402 (IC_{50} = 0.73 μg/mL), while no activity toward Hela (IC_{50} > 10 μg/mL) was observed.

Experimental Section

General Experimental Procedures. Melting points were measured on a XT-4A micromelting point apparatus and are reported without correction. Optical rotations were measured with a Perkin-Elmer 243B polarimeter using a 1 dm microcell. The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE-500 FT NMR spectrometer using TMS as an internal standard. EIMS was performed with a Bruker APEX II mass spectrometer, and ESIMS was recorded on a PE Q-STAR ESI-TOF-MS/MS spectrometer. Column chromatography was carried out on Si gel (200−300 mesh), and HF254 Si gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, People's Republic of China. Sephadex LH-20 (18−110 μm) was provided by Pharmacia Co.

Marine Sponge. The marine sponge *H. oculata* was collected off the inner coral reef at a depth of 15 m, near the coastline of southern Sanya, Hainan Island, People's Republic of China, in June 2002. The sample was frozen immediately after collection and kept frozen until extraction. The species was identified by Dr. R. van Soest (Institute of Systematic Population Biology, Amsterdam University, The Netherlands). A voucher specimen (HSC-19) is deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **3**−**6**

no.	**3**		**4**		**5**		**6**	
1	46.7 CH_2	2.09 d (14.0) 2.16 d (14.0)	46.3 CH_2	2.10 d 13.5 2.20 d 13.5	46.6 t	2.10 d (14.0) 2.18 d (14.0)	46.6 CH_2	2.12 d (13.9) 2.20 d (13.9)
2	79.8 qC		80.0 qC		79.6 s		79.5 qC	
3	172.8 qC		173.1 qC		172.5 s		172.6 qC	
4	201.0 qC		201.4 qC		201.6 s		201. 4 qC	
5	145.3 qC		145.5 qC		146.6 s		146.7 qC	
6	135.9 CH	6.73 dd (3.0, 4.5)	135.8 CH	6.75 dd 3.5, 3.5	133.6 d	6.50 d (3.5)	133.7 CH	6.52 d (3.4)
7	32.3 CH_2	2.40 ddd (3.4, 6.5, 21.0) 1.86 ddd (3.5, 10.0, 21.0)	32.5 CH_2	2.42 ddd 3.6, 5.8, 22.0 1.89 m	73.1 d	4.12 dd (3.5, 7.5)	73.1 CH	4.15 dd (3.4, 7.3)
8	32.3 CH	1.69 m	33.4 CH	1.70 m	42.1d	1.68 ddd (7.5, 11.5, 12.0)	42.0 CH	1.67 ddd (7.3, 12.0, 12.0)
9	49.7 CH	0.97 m	50.1 CH	1.02 m	50.6 d	1.32 ddd (4.8, 12.0, 12.0)	50.5 CH	1.32 m
10	39.9 qC		40.1 qC		39.6 s		39.5 qC	
11	21.8 CH_2	1.50 m 1.54 m	20.1 CH_2	1.45 m 1.48 m	22.3 t	1.50 m 1.60 ddd (3.5, 13.0, 13.0)	22.2 CH_2	1.51 m 1.61 m
12	39.4 CH_2	1.20 m 2.05 m	40.2 CH_2	1.23 m 2.05 m	39.2 t	1.21 m 2.08 ddd (3.5, 3.5, 13.0)	39.2 CH_2	1.20 m 2.09 ddd (3.4, 3.5, 12.5)
13	43.0 qC		43.2 qC		43.5 s		43.4 qC	
14	56.3 CH	1.15 m	56.4 CH	1.17 m	55.5 d	1.25 m	55.6 CH	1.24 m
15	24.3 CH_2	1.48 m 1.58 m	24.5 CH_2	1.50 m 1.54 m	26.0 t	1.78 m	26.0 CH_2	1.79 m
16	28.1 CH_2	1.31 m 1.90 m	28.2 CH_2	1.31 m 1.91 m	28.3 t	1.37 m 1.94 m	28.3 CH_2	1.36 m 1.95 m
17	56.0 CH	1.09 m	56.4 CH	1.10 m	55.5 d	1.16 m	55.4 CH	1.11 m
18	12.0 CH_3	0.74 s	12.3 CH_3	0.75 s	12.0 q	0.76 s	12.0 CH_3	0.77 s
19	22.1 CH_3	1.21 s	22.3 CH_3	1.23 s	21.9 q	1.26 s	22.0 CH_3	1.27 s
20	35.7 CH	1.44 m	36.4 CH	1.45 m	35.7 d	1.47 m	36.2 CH	1.49 m
21	18.7 CH_3	0.97 d (6.5)	18.8 CH_3	0.97 d 7.0	18.8 q	0.97 d (7.0)	18.8 CH_3	0.96 d (6.5)
22	34.7 CH_2	1.16 m 1.56 m	34.6 CH_2	1.40 m	34.8 t	1.19 m 1.58 m	35.7 CH_2	1.20 m 1.30 m
23	31.0 CH_2	1.84 m	26.5 CH_2	1.34 m	31.1 t	1.90 m 2.10 m	23.9 CH_2	1.39 m 1.40 m
24	156.8 qC	1.25 m	46.1 CH	1.83 m	156.8 s		39.5 CH_2	1.21 m
25	33.8 CH	2.23 dq (7.0, 7.0)	28.6 CH	1.48 m	33.9 d	2.23 dq (7.0, 7.0)	28.0 CH	1.56 m
26	22.1 CH_3	1.03 d (7.0)	19.8 CH_3	0.87 d 6.5	22.02 q	1.03 d (7.0)	22.8 CH_3	0.90 d (6.5)
27	21.8 CH_3	1.04 d (7.0)	20.5 CH_3	0.85 d 6.5	22.04 q	1.04 d (7.0)	22.6 CH_3	0.89 d (6.5)
28	106.0 CH_2	4.27 br 4.66 br	23.1 CH_2	1.62 m	106.1 t	4.67 brs 4.72 brs		
29			12.8 CH_3	0.89 t 7.0				
1′	62.7 CH_2	4.25 q (7.0)	62.9 CH_2	4.26 q 7.0	62.8 t	4.25 q (7.5)	62.8 CH_2	4.28 q (7.0)
2′	14.1 CH_3	1.26 t (7.0)	13.0 CH_3	1.27 t 7.0	14.1 q	1.25 t (7.5)	14.1 CH_3	1.28 t (7.0)

Extraction and Isolation. The sponge (500 g) was homogenized and extracted with MeOH to yield a residue (39.1 g) after concentration under vacuum. The residue was separated into four fractions (A−D) by Si gel CC by using a gradient of petroleum ether−acetone and then MeOH. Fraction A (1.2 g) was subjected to Sephadex LH-20 CC eluting with MeOH−H_2O (95:5) to yield seven fractions (F1−F7). F2 (32 mg) showed one spot on TLC but exhibited a mixture of sterol signals in the ^{1}H NMR spectrum. This fraction was further separated on semipreparative HPLC (ODS, MeOH−H_2O, 89:11) to afford compounds **3** (8.0 mg), **4** (3.1 mg), **5** (3.4 mg), **6** (3.6 mg), **1** (3.2 mg), and **2** (5.0 mg). F3 (8.2 mg) also showed one spot on TLC but appeared to be a mixture of two components by ^{1}H NMR. This fraction was separated on semipreparative HPLC (ODS, MeOH−H_2O, 85:15) to obtain compounds **7** (1.0 mg) and **8** (1.1 mg). Fraction B (80.5 mg) was chromatographed on Sephadex LH-20 by eluting with MeOH to remove lipids and subsequently purified on Si gel CC to afford 2-ethoxycarbonyl-2β-hydroxy-A-nor-cholesta-5-en-4-one (11.0 mg), 2-ethoxycarbonyl-2β-hydroxy-A-nor-cholesta-5,22-dien-4-one (8.7 mg), 2-ethoxycarbonyl-2β-hydroxy-24-methyl-A-nor-cholesta-5,22-dien-4-one (4.2 mg), ergosta-6β-hydroxy-4,24(28)-dien-3-one (4.5 mg), cholesta-6β-hydroxy-4-en-3-one (3.7 mg), ergosta-5,24(28)-dien-3β-ol (4.8 mg), ergosta-4,24(28)-dien-3-one (5.2 mg), cholesta-5,22,24-trien-3β-ol (4.4 mg), and ergosta-6β-hydroxy-4,22-dien-3-one (2.1 mg). Seven indole derivatives were separated from fraction C (1.1 g) by using Si gel CC with a gradient of CH_2Cl_2−MeOH (from 98:2 to 90:10) to obtain 6-bromo-3-indolecarboxaldehyde (3.0 mg), 6-bromoindole-3-carboxylic acid (2.5 mg), 6-hydroxyindole-3-carboxaldehyde (7.3 mg), indole-3-carboxylic acid (4.2 mg), indole-3-carboxaldehyde (3.3 mg), indole-3-methanol (6.8 mg), and indole-3-ol (2.1 mg).

Oculatol (1): white, amorphous powder; $[\alpha]^{25}_D$ +30 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 3317 (br), 2920, 2852, 1609, 1585, 1502, 1459, 1364, 1288, 1230, 1021 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 232 $[M]^+$, 214, 199, 185, 172, 157, 145, 115, 91; HREIMS *m*/*z* 232.1468 (calcd for $C_{15}H_{20}O_2$, 232.1463).

Oculatolide (2): yellow oil; $[\alpha]_D^{25}$ +11.5 (*c* 0.39, $CHCl_3$); IR (KBr) ν_{max} 3307 (br), 2930, 1759, 1741,1646, 1548, 1450, 1423, 1379, 1128, 952, 875 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 319 $[M + H]^+$, 301, 283, 260, 251, 215, 185.

2-Ethoxycarbonyl-2β-hydroxy-A-nor-ergosta-5,24(28)-dien-4-one (3): white, amorphous powder; $[\alpha]_D^{25}$ +2.25 (*c* 0.02, $CHCl_3$); IR (Nujol) ν_{max} 3467 (br), 2960, 2869, 1742, 1721, 1650, 1462, 1379, 1265, 1231, 1180, 1103, 891 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 470 $[M]^+$, 397, 286, 371, 354, 312, 284, 269, 255, 227, 185, 159, 145, 131, 119; HREIMS *m*/*z* 470.3377 (calcd for $C_{30}H_{46}O_4$, 470.3396).

2-Ethoxycarbonyl-24-ethyl-2β-hydroxy-A-nor-cholesta-5-en-4-one (4): white, amorphous powder; $[\alpha]_D^{25}$ −19.9 (*c* 0.08, $CHCl_3$); IR (Nujor) ν_{max} 3440 (br), 2965, 2871, 1740, 1723, 1653, 1460, 1371, 1260, 1185, 1110, 892 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 486 $[M]^+$, 441, 427, 400, 358, 285, 275, 227, 199, 161, 135, 107, 95; HREIMS *m*/*z* 486.3707 (calcd for $C_{31}H_{50}O_4$, 486.3709).

2-Ethoxycarbonyl-2β,6β-dihydroxy-A-nor-ergosta-5,24(28)-dien-4-one (5): white, amorphous powder; $[\alpha]_D^{25}$ −5.75 (*c* 0.01, $CHCl_3$); IR (Nujor) ν_{max} 3338 (br), 2921, 2851, 1746, 1721, 1661, 1594, 1460,

1379, 1102, 801 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 486 $[M]^+$, 403, 402, 368, 359, 285, 239, 225, 198, 169, 149, 123, 109, 95; HREIMS *m*/*z* 486.3331 (calcd for $C_{30}H_{46}O_5$, 486.3345).

2-Ethoxycarbonyl-2β,6β-dihydroxy-A-nor-cholesta-5-en-4-one (6): white, amorphous powder; $[\alpha]_D^{25}$ +8.33 (*c* 0.01, $CHCl_3$); IR (Nujor) ν_{max} 3368 (br), 2921, 2851, 1748, 1725, 1656, 1465, 1375, 1262, 1104, 1028, 749 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 474 $[M]^+$, 456, 400, 367, 339, 277, 261, 198, 165, 135, 121, 107, 95; HREIMS *m*/*z* 474.3337 (calcd for $C_{29}H_{46}O_5$, 474.3345).

2-Ethoxycarbonyl-2β,6β-dihydroxy-24-methyl-A-nor-cholesta-5,-22(*E*)-dien-4-one (7): white, amorphous powder; ^1H NMR (500 MHz) δ 0.79 (s, H_3-18), 1.28 (s, H_3-19), 1.29 (t, $J = 7.0$ Hz, H_3-2′), 1.05 (d, $J = 6.5$ Hz, H_3-21), 0.85 (d, $J = 7.0$ Hz, H_3-26), 0.87 (d, $J = 7.0$ Hz, H_3-27), 0.95 (d, $J = 6.5$ Hz, H_3-28), 6.53 (d, $J = 4.0$ Hz, H-6), 4.14 (dd, $J = 4.0$, 8.0 Hz, H-7), 4.28 (q, $J = 7.0$ Hz, H_2-1′), 5.22 (dd, $J = 6.5$, 16.0 Hz, H-22), 5.19 (dd, $J = 7.5$, 16.0 Hz, H-23); EIMS *m*/*z* 486 $[M]^+$; HREIMS *m*/*z* 486.3342 (calcd for $C_{30}H_{46}O_5$, 486.3345).

2-Ethoxycarbonyl-2β,6β-dihydroxy-A-nor-cholesta-5,22 (*E*)-dien-4-one (8): white, amorphous powder; ^1H NMR (500 MHz) δ 0.79 (s, H_3-18), 1.28 (s, H_3-19), 1.29 (t, $J = 7.0$ Hz, H_3-2′), 6.53 (d, $J = 3.5$ Hz, H-6), 4.15 (dd, $J = 3.5$, 8.0 Hz, H-7), 1.06 (d, $J = 6.5$ Hz, H_3-21), 0.89 (d, $J = 7.0$ Hz, H_3-26), 0.91 (d, $J = 7.0$ Hz, H_3-27), 5.32 (dt, $J = 7.0$, 16.0 Hz, H-23), 5.27 (dd, $J = 8.0$, 16.0 Hz, H-22); EIMS *m*/*z* 472 $[M]^+$; HREIMS *m*/*z* 472.3186 (calcd for $C_{29}H_{44}O_5$, 472.3189).

Acknowledgment. This project was supported by grants from the National High Technology Development Project (863 project) (No. 2003AA620403 and 2002AA217081), NSFC (No. 40176038, 30171106), and the International Cooperation Projects of BMBF-MOST (2+2).

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NP0600494