7-Nor-ergosterolide, a Pentalactone-Containing Norsteroid and Related Steroids from the Marine-Derived Endophytic *Aspergillus ochraceus* EN-31

Chuan-Ming Cui,[†,‡] Xiao-Ming Li,[†] Li Meng,[§] Chun-Shun Li,[†] Cai-Guo Huang,[§] and Bin-Gui Wang*[,†]

Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266071, People's Republic of China, Department of Biochemistry and Molecular Biology, Second Military Medical University, Xiangyin Road 800, Shanghai 200433, People's Republic of China, and Graduate School of Chinese Academy of Sciences, Yuquan Road 19A, Beijing 100049, People's Republic of China

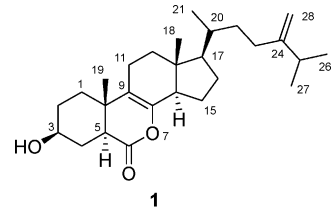
Received June 10, 2010

7-Nor-ergosterolide (**1**), a rare 7-norsteroid with an unusual pentalactone B-ring system, and two new steroid derivatives, 3β,11α-dihydroxyergosta-8,24(28)-dien-7-one (**2**) and 3β-hydroxyergosta-8,24(28)-dien-7-one (**3**), were characterized from the culture extract of *Aspergillus ochraceus* EN-31, an endophytic fungus isolated from the marine brown alga *Sargassum kjellmanianum*. In addition, nine known related steroids were isolated and identified. The structures of these compounds were established on the basis of extensive spectroscopic analysis. The absolute configuration of the new steroids (**1**−**3**) was determined by application of the modified Mosher's method. Compound **1**, which represents the first example of a 7-nor-ergosteroid possessing a pentalactone B-ring system in a naturally occurring steroid, displayed cytotoxicity against NCI-H460, SMMC-7721, and SW1990 cell lines with IC_{50} values of 5.0, 7.0, and 28.0 μg/mL, respectively. Compound **2** also displayed cytotoxicity against the SMMC-7721 cell line with an IC_{50} value of 28.0 μg/mL.

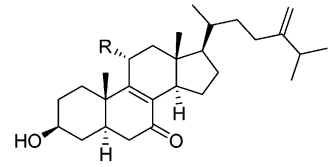
Microorganisms have attracted much attention for their ability to produce diverse secondary metabolites that show promising bioactivities.[1,2] In recent years, marine-derived fungi have proven to be a rich source of structurally unique and biologically active secondary metabolites.[2] From our program on the chemical investigation of marine algal-derived endophytic fungi, a variety of structurally interesting and biologically active compounds were isolated and identified.[3−9] We reported 6-hydroxycircumdatin C, a new alkaloid possessing potent DPPH radical scavenging activity, from the endophytic fungus *Aspergillus ochraceus* EN-31, obtained from the marine brown alga *Sargassum kjellmanianum*.[10] Further investigation of this strain has resulted in the identification of three new (**1**−**3**) and nine known (**4**−**12**) steroid derivatives. Among them, compound **1** (named 7-nor-ergosterolide) represents the first 7-norsteroid possessing a previously unreported γ,δ-unsaturated pentalactone B-ring system in a naturally occurring steroid. The structures of compounds **1**−**12** were established on the basis of spectroscopic analysis, and the absolute configuration of the new steroids **1**−**3** was determined by application of the modified Mosher's method. The cytotoxic activity against several tumor cell lines was determined. This paper describes the isolation, structure determination, stereochemical assignment, and cytotoxic activity of these steroid derivatives.

Results and Discussion

The mycelia and culture broth of *A. ochraceus* EN-31 were separated by filtration and then exhaustively extracted with MeOH and EtOAc, respectively. The combined extracts were purified by repeated column chromatography (CC) on silica gel, reversed-phase silica gel C_{18}, and Sephadex LH-20, to yield 12 steroids (**1**−**12**, see Scheme S1 in the Supporting Information). Compounds **1**−**3** were proven to be new ergosteroids possessing the terminal $\Delta^{24(28)}$-double bond in the side chain, while compounds **4**−**12** were known steroid derivatives having the Δ^{22}-double bond in the side chain (see Scheme S1 in the Supporting Information).



1



2 R=OH
3 R=H

Compound **1** was obtained as a colorless, amorphous powder. The EIMS data exhibited molecular and fragment ion peaks at *m/z* 414 $[M]^+$ and 399 $[M - CH_3]^+$, respectively. The molecular formula was determined as $C_{27}H_{42}O_3$ on the basis of positive HRESIMS (*m/z* 415.3220 $[M + H]^+$, calcd for $C_{27}H_{43}O_3^+$, 415.3212), indicating seven degrees of unsaturation. In examining the ^{1}H NMR spectrum (Table 1), signals for two tertiary (H-18 and H-19) and three secondary (H-20, H-26, and H-27) methyls were observed, as well as one oxymethine resonance (H-3) and two terminal olefinic protons (H-28a and H-28b). The ^{13}C NMR spectroscopic data (Table 1) exhibited the presence of 27 carbon signals, which were clarified by DEPT and HSQC experiments into the categories of five methyls, 10 methylenes (with one olefinic terminal CH_2), six methines (with one oxygenated), and six quaternary (with one ester carbonyl and three olefinic quaternary) carbon atoms. COSY data revealed several spin systems corresponding to substructures C-1 through C-5, C-11 to C-12, C-14 through C-23, and C-25/C-26 and C-27 (Figure 1). The above NMR and COSY data suggested that compound **1** was an ergosteroid derivative possessing the same side chain as that of 24-methylenecholest-4-ene-3β,6β-diol, an ergosteroid identified from the marine soft coral *Alcyonium patagonicum*.[11,12] However, only 27 carbon signals were detected in the ^{13}C NMR spectrum, implying that compound **1** was a nor-ergosteroid. HMBC correlations observed for the terminal olefinic methylene protons H-28a and

* To whom correspondence should be addressed. Tel and Fax: +86-532-82898553. E-mail: wangbg@ms.qdio.ac.cn.
† Institute of Oceanology.
‡ Graduate School of Chinese Academy of Sciences.
§ Second Military Medical University.

10.1021/np100386q
Published on Web 11/02/2010

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Compounds **1**−**3** (in $CDCl_3$)

	1		**2**		**3**	
no.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.70, m	33.0 CH_2	1.93, m	34.0 CH_2	1.82, m	34.4 CH_2
	1.44, m		1.78, m		1.34, m	
2	1.51, m	30.0 CH_2	1.55, m	31.2 CH_2	1.52, m	31.2 CH_2
	1.39, m		1.46, m		1.38, m	
3	3.63, m	70.3 CH	3.67, m	70.0 CH	3.62, m	69.9 CH
4	1.86, m	30.7 CH_2	1.87, m	37.5 CH_2	1.66, m	37.4 CH_2
	1.51, m		1.70, m		1.43, m	
5	2.38, dd (3.3, 12.7)	46.2 CH	2.06, m	41.5 CH	1.93, m	41.0 CH
6		170.8 C	2.43, m	42.6 CH_2	2.38, m	42.5 CH_2
			2.23, dd (3.8, 16.2)		2.15, dd (3.8, 16.2)	
7				199.4 C		198.8 C
8		145.2 C		136.1 C		133.2 C
9		117.8 C		161.4 C		165.1 C
10		34.1 C		38.7 C		38.2 C
11	2.20, m	21.9 CH_2	4.57, dd (4.6, 7.8)	66.7 CH	2.41, m	25.3 CH_2
	2.14, m				2.28, m	
12	2.04, m	35.8 CH_2	2.42, m	49.3 CH_2	2.03, m	35.6 CH_2
	1.43, m		1.66, dd (4.6, 14.0)		1.39, m	
13		44.4 C		46.5 C		42.5 C
14	2.31, m	49.0 CH	2.41, m	46.8 CH	2.10, m	48.2 CH
15	2.04, m	28.9 CH_2	1.96, m	28.8 CH_2	1.97, m	29.2 CH_2
	1.45, m		1.32, m		1.33, m	
16	1.72, m	21.3 CH_2	2.32, m	24.6 CH_2	2.41, m	24.8 CH_2
	1.44, m		1.57, m		1.31, m	
17	1.25, m	54.3 CH	1.32, m	55.0 CH	1.14, m	53.4 CH
18	0.67, s	11.1 CH_3	0.56, s	13.7 CH_3	0.58, s	11.3 CH_3
19	0.96, s	16.9 CH_3	1.18, s	17.5 CH_3	1.16, s	17.2 CH_3
20	1.42, m	36.0 CH	1.39, m	36.0 CH	1.39, m	36.0 CH
21	0.95, d (6.5)	18.7 CH_3	0.96, d (6.3)	18.6 CH_3	0.95, d (6.5)	18.8 CH_3
22	1.54, m	34.7 CH_2	1.56, m	34.4 CH_2	1.54, m	34.6 CH_2
	1.14, m		1.14, m		1.14, m	
23	2.08, m	31.1 CH_2	2.07, m	31.0 CH_2	2.06, m	31.0 CH_2
24		156.6 C		156.6 C		156.7 C
25	2.21, m	33.8 CH	2.22, m	33.8 CH	2.20, m	33.7 CH
26	1.02, d (6.8)	22.0 CH_3	1.03, d (6.9)	22.0 CH_3	1.01, d (6.8)	21.9 CH_3
27	1.01, d (6.8)	21.8 CH_3	1.01, d (6.9)	21.8 CH_3	1.00, d (6.8)	21.8 CH_3
28	4.71, s	106.1 CH_2	4.71, s	106.1 CH_2	4.70, s	106.0 CH_2
	4.65, s		4.65, s		4.64, s	

H-28b to C-23 and C-25 gave further evidence to allocate one of the two double bonds at C-24(28). Further correlations from H-11 and H-14 to the quaternary olefinic carbon C-8 and from H-11, H-12, and H-19 to another quaternary olefinic carbon, C-9, permitted the assignment of the second double bond at C-8 (Figure 1). The existence of rare Δ^8-unsaturation received further support by the characteristic angular methyl signals at δ_H 0.67 (H-18) and 0.96 (H-19), similar to those reported for Δ^8-sterols.[13,14] HMBC correlations were also observed from H-1, H-2, and H-4 to the oxymethine carbon C-3, allowing the placement of an OH group in C-3. The ester carbonyl carbon was assigned at C-6, as supported by the correlation from H-5 to C-6, and the possibility of a C-7 carbonyl carbon could be ruled out by the fact that the chemical shifts for C-5 and C-8 were observed at higher and lower field, respectively.

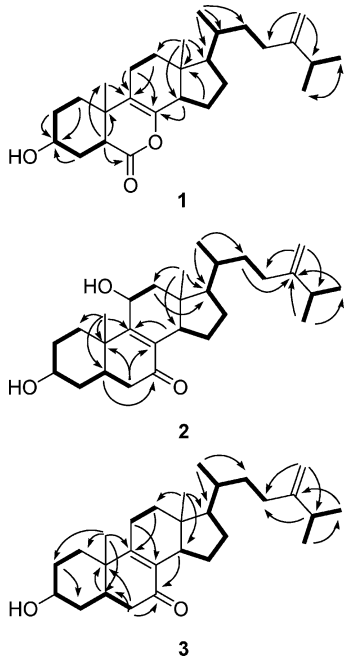


Figure 1. Key HMBC (arrows) and COSY (bold lines) correlations of compounds **1**−**3**.

The relative configuration of **1** was determined by analysis of *J*-values and NOESY data (Figure 2). The large coupling constants for H-5 (J = 12.7 and 3.3 Hz) indicated that this proton was in α (axial) orientation, and the key NOE correlations of H-5 with H-3 and of H-17 with H-14 and H-21 suggested the cofacial orientation of these hydrogens. Similarly, NOE correlations of H-18 with H-19 and H-20 placed these hydrogens on the opposite face. These data enabled assignment of the relative configurations of compound **1**, which was compatible with that offered by molecular modeling (Chem 3D, Figure 2).

The absolute configurations of the chiral centers of **1** were determined using modified Mosher's method. Treatment of **1** with (*R*)-(−)-α- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) gave the (*S*)- and (*R*)-MTPA esters **1s** and **1r**, respectively.[15] The ^{1}H NMR signals of the two MTPA esters were assigned on the basis of their ^{1}H−^{1}H COSY spectra, and the $\Delta\delta_{H(S-R)}$ values were then calculated (Figure 3). The results

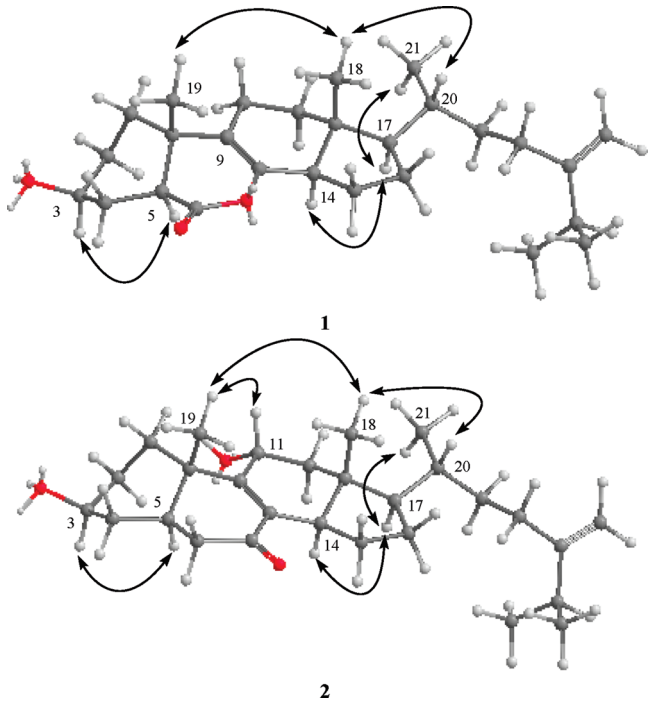


Figure 2. Selected NOESY correlations and the 3D computer modeling of **1** and **2**.

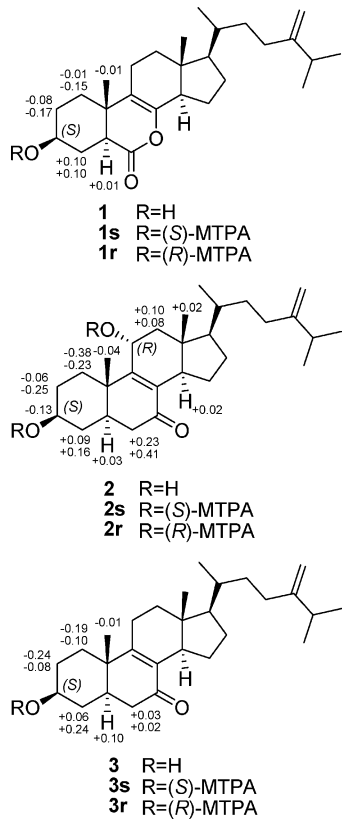


Figure 3. Values of $\Delta\delta_{H(S-R)}$ (measured in $CDCl_3$) of the MTPA esters of compounds **1**−**3**.

indicated that the absolute configuration of C-3 was *S*. Therefore the absolute configurations at C-5, C-10, C-13, C-14, C-17, and C-20 were *S*, *S*, *R*, *R*, *R*, and *R*, respectively. On the basis of the above evidence, the structure of **1** was determined, and the trivial name 7-nor-ergosterolide was assigned to this compound.

Compounds **2** and **3** were also obtained as colorless, amorphous powders. Structure elucidation of **2** and **3** was relatively straightforward due to their close relationships with compound **1** as well as with other known steroids such as 3β-methoxy-24-methyl-5α-cholest-8-en-7-one (**13**, see Scheme S1 in the Supporting Information).[13] Detailed analyses of the NMR (Table 1) and MS data and comparison with reported literature data revealed that compounds **2** and **3** were also ergosteroid derivatives, with each possessing the common 3β-OH group, the rare Δ^8-double bond, and the same side chain as **1**.

Compound **2** was found to have the molecular formula $C_{28}H_{44}O_3$ (seven unsaturations) on the basis of positive HRESIMS data. Its structure was independently assigned by analysis of the 1D and 2D NMR data as well as by comparison with that of **1** and 3β-methoxy-24-methyl-5α-cholest-8-en-7-one (**13**, Scheme S1).[13] The ^{1}H and ^{13}C NMR data of the side chain (from H-20/C-20 through H-28/C-28) were virtually identical to those observed for **1** (Table 1). The ^{13}C NMR data of **2** were similar to those of **13** except the C-3 methoxyl signal at δ_C 55.5 and the C-11 methylene carbon at δ_C 24.9 in **13** were absent in **2**. Instead, the additional oxygenated methine signals at δ_C 66.7 (C-11) and δ_H 4.57 (H-11) were observed in the NMR spectra of **2** (Table 1). This spectroscopic evidence suggested that the C-3 methoxy and the C-11 methylene groups in **13** were replaced by OH and CHOH units, respectively, in **2**. MS data (Experimental Section) as well as COSY and HMBC correlations (Figure 1) supported this deduction.

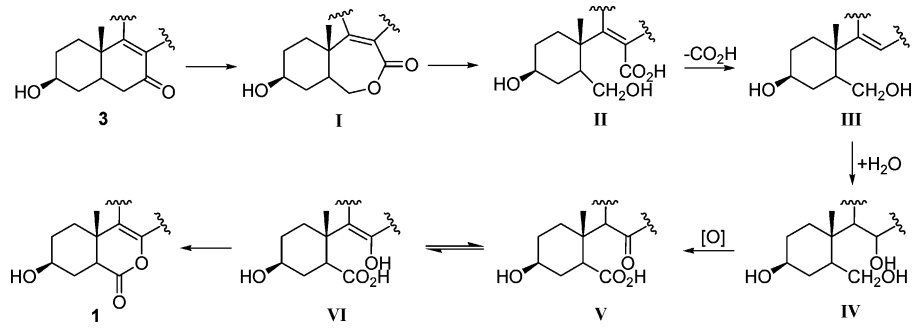
The relative configuration of **2** was assigned on the basis of a NOESY experiment (Figure 2). NOEs observed from H-19 to H-11 and H-18 and from H-18 to H-20 suggested the β-orientation for these hydrogens. Correlations from H-3 to H-5 and the absence of NOE from H-5 to H-19 indicated the α-disposition for H-3 and H-5. The relative configuration assigned by a NOESY experiment of **2** was consistent with those offered by computer modeling (Figure 2). The absolute configuration of **2** was also determined by the modified Mosher's method, which proved the *S*- and *R*-configuration for C-3 and C-11 (Figure 3), respectively. Consequently, the absolute configurations at C-5, C-10, C-13, C-14, C-17, and C-20 were *R*, *S*, *R*, *R*, *R*, and *R*, respectively.

On the basis of the above evidence, the structure for compound **2** was identified as 3β,11α-dihydroxyergosta-8,24(28)-dien-7-one.

Compound **3** was assigned the molecular formula $C_{28}H_{44}O_2$ (seven unsaturations), having one oxygen atom less than **2**, on the basis of positive HRESIMS data. Its NMR spectroscopic data were consistent with the absence of one of the hydroxy groups in **2**. However, each of the ^{1}H and ^{13}C NMR spectra showed the presence of two distinct sets of signals (see S15 and S16 in the Supporting Information). Since the two constituents could not be well separated, analysis was carried out on the mixture, with a focus on the major component. The ^{1}H and ^{13}C NMR chemical shift assignments (Table 1) matched well with those of the corresponding signals for **2** and revealed the same structural features present in **2** except for the absence of the C-11 hydroxy group, which was consistent with the difference in molecular formula. Correspondingly, the oxygenated methine signals at δ_H 4.57 (H-11) and δ_C 66.7 (C-11) in **2** were replaced by nonoxygenated methylene signals, which were observed significantly upfield at δ_H 2.41 and 2.28 and δ_C 25.3, respectively, in **3**. This observation was further supported by the ^{1}H−^{1}H COSY correlation from H-11 to H-12 and by the HMBC correlations from H-11 to C-8 and C-9 (Figure 1). The NOE correlations from H-3 to H-5, from H-17 to H-14 and H-21, and from H-18 to H-19 and H-20 as observed in the NOESY spectrum established the relative configuration of **3**, which is the same as that of **2**. The absolute configuration of C-3 was also determined to be *S* by modified Mosher's method. Consequently, the absolute configurations at C-5, C-10, C-13, C-14, C-17, and C-20 were assigned as *R*, *S*, *R*, *R*, *R*, and *R*, respectively. Therefore, the structure of **3** was identified as 3β-hydroxyergosta-8,24(28)-dien-7-one.

From a biogenetic point of view, compound **1** might be derived from **3** (Scheme 1) through a series of reactions including those

Scheme 1. Possible Biosynthetic Pathway for Compound **1**



catalyzed by enzymes. Briefly, the known ergosteroid **3** could be easily transferred to the key intermediate **I** as catalyzed by some enzymes, e.g., CS 6-oxidase (a kind of brassinolide synthase),[16] followed by ring-opening reaction to yield **II**, which could undergo a decarboxylation as catalyzed by decarboxylase[17] to form the corresponding intermediate **III**. Hydration and oxidation of **III** via intermediate **IV** would produce **V**, which is the keto−enol tautomer of **VI**. Compound **1** could be finally formed from **VI** by intramolecular esterification.

In addition to new steroids **1**−**3**, nine known steroid derivatives, (22*E*,24*R*)-3β,5α,9α-trihydroxyergosta-7,22-dien-6-one (**4**),[18] (22*E*,24*R*)-3β,5α-dihydroxyergosta-7,22-dien-6-one (**5**),[19] ergosterol (**6**),[20] (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**7**),[21] (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (**8**),[22] (22*E*,24*R*)-ergosta-7,22-diene-6β-methoxy-3β,5α-diol (**9**),[18] (22*E*,24*R*)-ergosta-7,22-diene-3β,6β-diol (**10**),[23] (22*E*,24*R*)-ergosta-5α,6α-epoxide-8,22-diene-3β,7α-diol (**11**),[24] and (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**12**),[24] were also isolated and identified.

The new steroids **1**−**3** were assayed for their cytotoxic activities against NCI-H460, SMMC-7721, SW1990, DU145, HepG2, Hela, and MCF-7 tumor cell lines. Compound **1** exhibited selective cytotoxic activitiy against NCI-H460, SMMC-7721, and SW1990 cell lines with IC_{50} values of 5.0, 7.0, and 28.0 μg/mL, while compound **2** displayed selective cytotoxic activity against the SMMC-7721 cell line with an IC_{50} value of 28.0 μg/mL.

Compounds **1**−**3** were also evaluated for antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*, as well as antifungal activity against *Aspergillus niger*, using standard agar diffusion tests. None of the tested compounds displayed any activity.

Experimental Section

General Experimental Procedures. Procedures were the same as recently reported.[3,10]

Fungal Material. The isolation and identification of the fungal material were identical to our previous report.[10]

Fermentation, Extraction, and Isolation. The procedures of fermentation and extraction were identical to our previous report.[10] The extract (75 g) from the fermented cultures was subjected to CC over silica gel eluted with different solvents of increasing polarity (from petroleum ether to MeOH) to yield seven fractions (Frs.1−7) on the basis of TLC analysis. Fr.1 (1.8 g) was further purified by CC on silica gel with a $CHCl_3$−MeOH gradient (from 80:1 to 20:1) and Sephadex LH-20 (MeOH) to afford **1** (30.2 mg), **6** (13.2 mg), **7** (15.3 mg), and **12** (14.1 mg). Fr.2 (2.2 g) was further separated by CC on silica gel eluted with a $CHCl_3$−MeOH gradient (from 80:1 to 20:1), Sephadex LH-20 (MeOH), and preparative TLC (plate: 20 × 20 cm, developing solvents: $CHCl_3$−MeOH, 30:1) to afford **3** (41.6 mg) and **9** (14.5 mg). Fr.3 (5.2 g) was further purified by CC on silica gel eluted with a $CHCl_3$−MeOH gradient (from 80:1 to 20:1) and by preparative TLC ($CHCl_3$−MeOH, 30:1) to afford **2** (9.6 mg) and **5** (31.6 mg). Fr.4 (3.6 g) was further purified by CC on silica gel eluted with a $CHCl_3$−MeOH gradient (from 80:1 to 20:1) and Sephadex LH-20 (MeOH) to afford **4** (6.7 mg), **10** (5.2 mg), **11** (9.2 mg), and **8** (21.4 mg).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compounds 1−3.[15] To a stirred solution of **1** (3.0 mg) in pyridine (400 μL) was added 4-(dimethylamino)pyridine (2 mg) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 10 μL). The mixture was stirred at room temperature for 10 h, and the reaction was stopped by adding 0.2 mL of H_2O. The reaction mixture was then passed through a disposable pipet packed with silica gel and eluted with petroleum ether and EtOAc (5:1) to give the respective (*R*)-Mosher ester **1r**. Treatment of **1** (3.0 mg) with (*R*)-MTPA-Cl (10 μL) as described above yielded the corresponding (*S*)-Mosher ester **1s**. Similarly, compounds **2** and **3** were also reacted with (*S*)- and (*R*)-MTPA-Cl to afford the respective Mosher esters.

7-Nor-ergosterolide (1): colorless, amorphous powder; $[\alpha]_D^{25}$ +16.7 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε) 192 (5.75), 199 (5.67), 228 (6.49) nm; IR (KBr) ν_{max} 3417, 2952, 1746, 1640, 1460, 1374, 1295, 1228, 1182, 1149, 1096 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 414 $[M]^+$ (49), 399 [M - Me]$^+$ (69), 55 (100); HRESIMS *m*/*z* 415.3220 $[M + H]^+$ (calcd for $C_{27}H_{43}O_3^+$, 415.3212).

3β,11α-Dihydroxyergosta-8,24(28)-dien-7-one (2): colorless, amorphous powder; $[\alpha]_D^{25}$ +10.8 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε) 194 (6.28), 206 (6.54), 253 (6.85) nm; IR (KBr) ν_{max} 3462, 2959, 1734, 1659, 1454, 1367, 1029 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 428 $[M]^+$ (16), 410 $[M - H_2O]^+$ (100), 395 (20); HRESIMS *m*/*z* 451.3196 $[M + Na]^+$ (calcd for $C_{28}H_{44}O_3Na^+$, 451.3188).

3β-Hydroxyergosta-8,24(28)-dien-7-one (3): colorless, amorphous powder; $[\alpha]_D^{25}$ −37.8 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 199 (5.39), 208 (5.72) nm, 254 (6.08); IR (KBr) ν_{max} 3357, 2925, 1652, 1637, 1460, 1368, 1036 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 412 $[M]^+$ (53), 397 [M − Me]$^+$ (31), 121 (100); HRESIMS *m*/*z* 435.3247 $[M + Na]^+$ (calcd for $C_{28}H_{44}O_2Na^+$, 435.3239).

Cytotoxicity Assay. The cytotoxic activities against NCI-H460 (human non small cell lung cancer cell line), SMMC-7721 (human hepatoma), SW1990 (human pancreatic cancer), DU145 (human prostate carcinoma), HepG2 (human hepatocellular liver carcinoma cell line), HeLa (human epithelial carcinoma), and MCF-7 (human breast adenocarcinoma) cell lines were determined according to previously reported methods.[25,26]

Antimicrobial Activity. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, as well as antifungal activity against *Aspergillus niger*, was assayed using standard agar diffusion tests.[27]

Acknowledgment. Financial support from the Ministry of Science and Technology of China (2010CB833802 and 2007AA09Z446), from the Research Center for Marine Microbes of the Chinese Academy of Sciences (KSCX2-YW-G-073), and from the National Science Foundation of China (30910103914) is gratefully acknowledged.

Supporting Information Available: Selected 1D and 2D NMR spectra of compounds **1**−**3** and the chemical structures of compounds **1**−**13** (Scheme S1). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bode, H. B.; Bethe, B.; Höfs, R.; Zeeck, A. *ChemBioChem* **2002**, *3*, 619–627.
(2) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143–163.
(3) Cui, C. M.; Li, X. M.; Li, C. S.; Proksch, P.; Wang, B. G. *J. Nat. Prod.* **2010**, *73*, 729–733.
(4) Gao, S. S.; Li, X. M.; Wang, B. G. *Nat. Prod. Commun.* **2009**, *4*, 1477–1480.
(5) Zhang, Y.; Li, X. M.; Wang, B. G. *J. Antibiot.* **2007**, *60*, 204–210.
(6) Zhang, Y.; Wang, S.; Li, X. M.; Cui, C. M.; Feng, C.; Wang, B. G. *Lipids* **2007**, *42*, 759–764.

(7) Zhang, Y.; Li, X. M.; Proksch, P.; Wang, B. G. *Steroids* **2007**, *72*, 723–727.
(8) Zhang, Y.; Li, X. M.; Wang, C. Y.; Wang, B. G. *Chin. Chem. Lett.* **2007**, *18*, 951–953.
(9) Wang, S.; Li, X. M.; Teuscher, F.; Li, D. L.; Diesel, A.; Ebel, R.; Proksch, P.; Wang, B. G. *J. Nat. Prod.* **2006**, *69*, 1622−1625.
(10) Cui, C. M.; Li, X. M.; Li, C. S.; Sun, H. F.; Gao, S. S.; Wang, B. G. *Helv. Chim. Acta* **2009**, *92*, 1366–1370.
(11) Yu, D. Q.; Yang, J. S. *Handbook of Analytical Chemistry: NMR Spectroscopic Analysis*; Chemical Industrial Press: Beijing, 1999; pp 883−900.
(12) Zeng, L.; Li, X.; Su, J.; Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1995**, *58*, 296–298.
(13) D'Auria, M. V.; Paloma, L. G.; Minale, L.; Riccio, R.; Debitus, C.; Lévi, C. *J. Nat. Prod.* **1992**, *55*, 311–320.
(14) Itoh, T.; Sica, D.; Djerassi, C. *J. Chem. Soc., Perkin Trans. 1* **1983**, 147–153.
(15) Wang, B. G.; Ebel, R.; Wang, C. Y.; Wray, V.; Proksch, P. *Tetrahedron Lett.* **2002**, *43*, 5783–5787.
(16) Kim, T. W.; Chang, S. C.; Lee, J. S.; Hwang, B.; Takatsuto, S.; Yokota, T.; Kim, S. K. *Phytochemistry* **2004**, *65*, 679–689.
(17) Chang, Z.; Sitachitta, N.; Rossi, J. V.; Roberts, M. A.; Flatt, P. M.; Jia, J.; Sherman, D. H.; Gerwick, W. H. *J. Nat. Prod.* **2004**, *67*, 1356–1367.
(18) Kawagishi, H.; Katsumi, R.; Sazawa, T.; Mizuno, T.; Hagiwara, T.; Nakamura, T. *Phytochemistry* **1988**, *27*, 2777–2779.
(19) Valisolalao, J.; Luu, B.; Ourisson, G. *Tetrahedron* **1983**, *39*, 2779–2785.
(20) Bok, J. W.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. N. *Phytochemistry* **1999**, *51*, 891–898.
(21) Schulte, K. E.; Rücker, G.; Fachmann, H. *Tetrahedron Lett.* **1968**, *9*, 4763–4764.
(22) Cafieri, F.; Fattorusso, E.; Gavagnin, M.; Santacroce, C. *J. Nat. Prod.* **1985**, *48*, 944–947.
(23) Madaio, A.; Piccialli, V.; Sica, D. *J. Nat. Prod.* **1989**, *52*, 952–961.
(24) Yue, J. M.; Chen, S. N.; Lin, Z. W.; Sun, H. D. *Phytochemistry* **2001**, *56*, 801–806.
(25) Bergeron, R. I.; Cavanaugh, P. F., Jr.; Kline, S. J.; Hughes, R. G., Jr.; Elliott, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 848–854.
(26) Mosmann, T. *J. Immunol. Methods.* **1983**, *65*, 55–63.
(27) Al-Burtamani, S. K. S.; Fatope, M. O.; Marwah, R. G.; Onifade, A. K.; Al-Saidi, S. H. *J. Ethnopharmacol.* **2005**, *96*, 107–112.

NP100386Q