Stellettins L and M, Cytotoxic Isomalabaricane-Type Triterpenes, and Sterols from the Marine Sponge *Stelletta tenuis*

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Received February 14, 2007

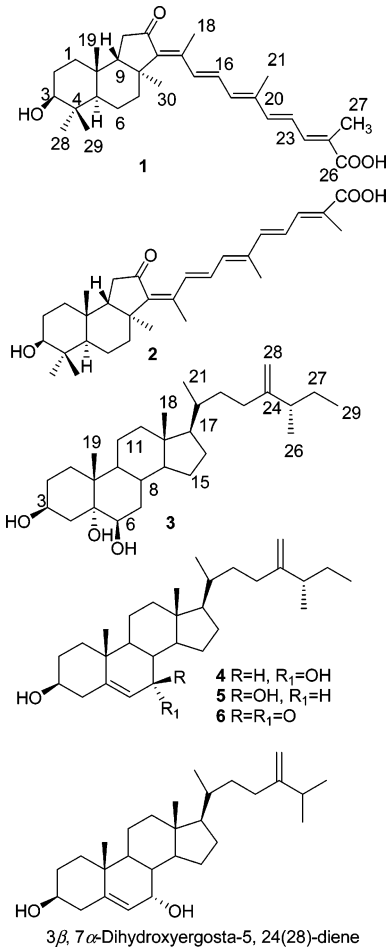
Two new isomalabaricane-type triterpenes, stellettins L (**1**) and M (**2**), and three new sterols (**3**−**5**) were isolated from the marine sponge *Stelletta tenuis* collected in the South China Sea. Chemical structures were established from spectroscopic data and comparison with known compounds. In addition, spectroscopic data reported for the known sterol 24-methylene-27-methylcholest-5-en-3β-ol-7-one (**6**) were revised. Compounds **1** and **2** exhibited significant cytotoxic activity against stomach cancer (AGS) *in vitro*.

Marine sponges have proved to be a particularly fruitful source of new compounds with novel structures and bioactivities. While investigating novel secondary metabolites from marine organisms collected in the South China Sea, we found that a crude extract of the sponge *Stelletta tenuis* (Lindgren), collected off Sanya Bay, Hainan Province, exhibited significant activity against the HL-60 human leukemia cell line (IC_{50} < 0.01 μg/mL). Previous studies of this sponge identified the isomalabaricane-type triterpenoid stellettin A and several sterols.[1,2] Our further investigation on the active constituents of this sponge resulted in the isolation of two new isomalabaricane triterpenes, stellettins L (**1**) and M (**2**), along with nine known isomalabaricane derivatives identified as stellettins A−E,[1,3] rhabdastrellic acid-A,[4] stellettin H,[5] 22,23-dihydrostellettin B,[6] and geoditin A.[6,7] Six sterol derivatives were also obtained, including three new [24-methylene-27-methylcholestane-3β,5α,6β-triol (**3**), 24-methylene-27-methylcholest-5-ene-3β,7α-diol (**4**), and 24-methylene-27-methylcholest-5-ene-3β,7β-diol (**5**)] and three known sterols [24-methylene-27-methylcholesterol,[8] 24,25-dimethylcholestane-2β,3α,3α-triyl trisodium sulfate,[9] and 24-methylene-27-methylcholest-5-en-3β-ol-7-one (**6**)].[7] In this paper, we report the structure determination of the five new compounds and cytotoxic activity of the two new isomalabaricane triterpenes. The spectroscopic data reported in the literature for known sterol **6** were also revised.

Results and Discussion

An EtOH extract of dried *S. tenuis* (Lindgren) was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was submitted to solvent−solvent partitioning to yield three extracts of increasing polarity (petroleum ether, CCl_4, and EtOAc). The more cytotoxic CCl_4 extract was then subjected to repeated silica gel chromatographic separation followed by semipreparative HPLC isolation to afford five new compounds (**1**−**5**), together with 12 known metabolites. Because isomalabaricane triterpenes can readily photoisomerize, isolation required protection from light by wrapping all glassware in aluminum foil.

Stellettin L (**1**) was obtained as yellow crystals. The molecular formula $C_{30}H_{42}O_4$ was established by HREIMS and, together with the NMR data below, indicated a highly conjugated tricyclic structure. The ^{1}H NMR spectrum exhibited seven methyl singlets at δ 0.83, 1.00, 1.04, 1.40, 2.02, 2.04, and 2.33, with the latter three signals attributed to olefinic methyls and the remaining four methyls to sp^3 quaternary carbons. Moreover, six olefinic proton signals were present at δ 7.39, 7.01, 6.73, 6.66, 6.62, and 6.42, as well as an oxymethine proton at δ 3.32.



The ^{13}C NMR spectrum included carbon signals for five double bonds (δ 147.3, 144.37, 140.9, 140.6, 138.2, 135.6, 135.0, 131.5, 126.3, and 124.3), a ketone (δ 207.7), a carboxylic acid (δ 172.6), an oxymethine carbon (δ 79.3), seven methyls, five CH_2, three CH, and three quaternary carbons. These NMR features were characteristic of an isomalabaricane triterpene possessing a carboxylic

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10.1021/np070069l CCC: $37.00
Published on Web 06/30/2007

Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Stellettin L (**1**) and Stellettin M (**2**)

	1				**2**	
position	δ_C mult	δ_H (*J* in Hz)	HMBC[a]	$^1H-^1H$ COSY	δ_C	δ_H (*J* in Hz)
1	33.4 (CH_2)	1.49 m		H-2	33.4	1.49 m
		1.55 m				1.55 m
2	29.1 (CH_2)	1.70 m		H-1, H-3	29.1	1.70 m
		1.83 m				1.83 m
3	79.3 (CH)	3.32 m	28, 29	H-2	79.3	3.32 m
4	39.2 (C)				39.2	
5	46.7 (CH)	1.73 m	4, 7, 10	H-6	46.6	1.68 m
6	18.6 (CH_2)	1.48 m		H-5, H-7	18.4	1.46 m
		1.56 m				1.54 m
7	39.7 (CH_2)	2.17 m		H-6	38.3	2.10 m
8	45.0 (C)				44.8	
9	50.2 (CH)	1.81 m	8, 11, 30	H-11	50.3	1.84 m
10	35.6 (C)	2.19 m			35.6	2.17 m
11	36.7 (CH_2)	2.22 m	9, 12	H-9	36.8	2.20 m
12	207.7 (CO)				206.6	
13	147.3 (C)				147.9	
14	140.9 (C)				141.8	
15	135.0 (CH)	6.73 d (15.0)	13, 17, 18	H-16	135.2	8.14 d (15.2)
16	131.5 (CH)	7.01 dd (15.0, 11.6)	14, 20	H-15, H-17	130.1	6.96 dd (15.2, 1.6)
17	135.6 (CH)	6.42 d (11.6)	15, 21, 22	H-16	136.5	6.47 d (11.6)
18	14.5 (CH_3)	2.33 s	13, 14, 15		15.9	2.04 s
19	22.3 (CH_3)	1.00 s	1, 5, 9, 10		22.3	1.00 s
20	138.2 (C)				137.6	
21	13.0 (CH_3)	2.04 s	17, 20, 22		12.9	2.00 s
22	144.4 (CH)	6.62 d (15.1)	17, 21, 24	H-23	144.9	6.68 d (15.1)
23	124.6 (CH)	6.66 dd (15.1, 9.5)	20, 25	H-22, H-24	123.8	6.58 dd (15.1, 9.5)
24	140.6 (CH)	7.39 d (9.5)	22, 26, 27	H-23	140.8	7.39 d (9.5)
25	126.3 (C)				125.7	
26	172.6 (C)				172.6	
27	12.6 (CH_3)	2.02 s	24, 25, 26		12.6	2.02 s
28	29.0 (CH_3)	1.04 s	3, 4, 5, 29		29.0	1.05 s
29	15.9 (CH_3)	0.83 s	3, 4, 5, 28		15.9	0.83 s
30	26.0 (CH_3)	1.40 s	7, 8, 9, 13		24.7	1.37 s

[a] HMBC correlations, optimized for 6 Hz, are from proton(s) stated to the indicated carbon.

acid functionality, a ketone, and one additional oxygen moiety. The NMR spectroscopic data, including HMBC, COSY, and NOESY data, of **1** were remarkably similar to those reported for stellettin H obtained from the sponge *Rhabdastrella globostellata*,[5] except for the absence of an acetoxy group. Thus, a hydroxy rather than an acetoxy group was assigned at C-3 (δ_H 3.32, 1H, m) in **1**, which was shifted upfield from that in stellettin H (δ_H 4.55, 1H, m). This was confirmed by spectral data of the compound from the sponge *Jaspis stellifera* as (13*Z*,15*E*,17*E*,22*E*)-3β-hydroxymalabarica-13,15,17,22,24-pentaen-12-one.[10] A malabaricane triterpene with the same molecular formula as **1** was also mentioned in the literature above, with a carboxyl group at C-28, while **1** showed a carboxyl group at the terminal of the side chain with correlations between pairs of δ_H 2.02 (27-CH_3)/δ_C 172.6 and δ_H 7.39 (H-24)/δ_C 172.6 in the HMBC experiment. Thus, compound **1** was elucidated as (13*E*,15*E*,17*E*,22*E*,24*E*)-3β-hydroxy-12-oxo-isomalabarica-13,15,17,22,24-pentaen-26-oic acid and has been named stellettin L. The stereochemistry and geometry of the tricyclic nucleus and side chain were established on the basis of 1D and 2D NMR data and correspondence with those found in the literature.[5,10]

Stellettin M (**2**) has the same molecular formula ($C_{30}H_{42}O_4$) and basically the same ^{13}C NMR data as those of **1**. However, as also found in the literature for stellettins H and I,[5] H-15 was shifted significantly downfield in **2** (δ 8.14, d, J = 15.2 Hz) compared with **1** (δ 6.73), while H_3-18 was shifted upfield (δ 2.04 in **2**, 2.33 in **1**). Thus, $\Delta^{13,14}$ in **2** was deduced as *Z*-oriented, rather than *E*-oriented as in **1**. The remaining structural and stereochemical assignments were identical to those made for **1**, establishing the structure of **2** as (13*Z*,15*E*,17*E*,*22E*,24*E*)-3β-hydroxy-12-oxo-isomalabarica-13,15,17,22,24-pentaen-26-oic acid.

Compound **3** was obtained as a white solid. Protonated molecular ion peaks were observed at *m*/*z* 445 $[M - H]^+$ and 891 $[2M - H]^+$ in the ESIMS, consistent with the molecular formula $C_{29}H_{50}O_3$ found by elemental analysis. The IR spectrum showed absorptions for hydroxy (3100−3460 cm^{-1}) and terminal methylene (1640 and 925 cm^{-1}) groups. The NMR spectra exhibited signals characteristic of a 3β,5α,6β-trihydroxysterol, which is a common skeleton in marine polyhydroxy sterols.[11,12] The assignments of the hydroxy groups were based on a one-proton mutiplet at δ 4.07 (H-3) and a broad singlet at δ 3.53 (H-6) in the ^1H NMR spectrum, as well as two secondary [δ 67.7 (C-3), 76.2 (C-6)] and a tertiary [δ 76.3 (C-5)] oxygenated carbon in the ^{13}C NMR spectrum. The partial structure was confirmed by HMBC correlations between H_3-19 (δ_H 1.18)/C-5, H-4 (δ_H 1.61, 2.08)/C-3 and C-5, and H-6 (δ_H 3.53)/C-5, C-8, and C-10. Additionally, in NOESY experiments, both H-3 and H-6 correlated with H-4α (δ 1.61) and H_3-19 showed a cross-peak with H-4β (δ 2.08), further confirming the β-orientation of the hydroxy groups on C-3 and C-6. The ^1H and ^{13}C NMR spectra also showed signals of three additional methyl groups at δ_H 0.94, 1.00, and 0.84, one terminal double bond at δ_H 4.69 (2H, s, H-28), δ_C 107.2 (C-28), 155.4 (C-24), along with three HMBC-correlated methylene signals at δ_H 1.61, 1.84, and 1.28. The affirmative data were almost identical with those of the side chain of the co-occurring known compound 24-methylene-27-methylcholesterol.[8] The above data indicate unambiguously that **3** is 24-methylene-27-methylcholestane-3β,5α,6β-triol.

Compound **4** was obtained as an amorphous, colorless solid. It displayed protonated molecular ion peaks at *m*/*z* 429 $[M + H]^+$, 451 $[M + Na]^+$, and 879 $[2M + Na]^+$ in the ESIMS, consistent with the molecular formula $C_{29}H_{48}O_2$ found by elemental analysis. Comparing the NMR data to those of **3**, compound **4** has one additional double bond signal at δ_C 146.3 and 123.9, and two oxygenated carbons at δ_C 71.4 and 65.4 were found. In comparison with the previous literature, the NMR data of **4** were almost identical to those of known compound 3β,7α-dihydroxyergosta-5,24(28)-

Table 2. NMR Spectroscopic Data ($CDCl_3$) for Sterols **3**−**6**

	3[a]			**4**[b]		**5**[b]		**6**[c]	
position	δ_C mult	δ_H (*J* in Hz)	HMBC[c]	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	32.5 (CH_2)	1.40 m	2, 10	37.0 (CH_2)	1.13 m	36.9	1.04 m	36.4 (CH_2)	1.19 m
		1.55 m			1.86 m		1.87 m		1.97 m
2	31.1 (CH_2)	1.52 m		31.4 (CH_2)	1.51 m	31.6	1.51 m	31.2 (CH_2)	1.64 m
		1.84 m			1.85 m		1.83 m		1.96 m
3	67.7 (CH)	4.07 m		71.3 (CH)	3.58 m	71.4	3.56 m	70.5 (CH)	3.68 m
4	41.0 (CH_2)	1.61 m	3, 5	42.2 (CH_2)	2.30 m	41.7	2.26 m	41.8 (CH_2)	2.41 m
		2.08 m			2.34 m		2.34 m		2.50 m
5	76.3 (C)			146.2 (C)		143.5		165.2 (C)	
6	76.2 (CH)	3.53 s	5, 8, 10	123.9 (CH)	5.61 m	125.5	5.30 d (4.2)	126.1 (CH)	5.69 s
7	34.8 (CH_2)	1.61 m		65.3 (CH)	3.86 d (3.0)	73.3	3.85 d (7.8)	202.3 (CO)	
8	30.4 (CH)	1.72 m		37.5 (CH)	1.46 m	40.9	1.42 m	45.4 (CH)	2.24 t (7.2)
9	46.1 (CH)	1.28 m		42.3 (CH)	1.22 m	48.3	1.02 m	50.0 (CH)	1.48 m
10	38.5 (C)			37.4 (C)		36.4		38.3 (C)	
11	21.3 (CH_2)	1.40 m		20.7 (CH_2)	1.46 m	21.1	1.49 m	21.2 (CH_2)	1.58 m
					1.53 m		1.55 m		
12	40.2 (CH_2)	1.16 m		39.2 (CH_2)	1.15 m	39.6	1.15 m	38.7 (CH_2)	1.13 m
		2.02 m			2.01 m		2.04 m		2.04 m
13	43.0 (C)			42.2 (C)		43.0		43.2 (C)	
14	56.2 (CH)	1.09 m		49.4 (CH)	1.44 m	55.3	1.13 m	50.0 (CH)	1.32 m
15	24.2 (CH_2)	1.10 m		24.3 (CH_2)	1.15 m	26.4	1.43 m	26.3 (CH_2)	1.28 m
		1.56 m			1.72 m		1.81 m		2.42 m
16	28.2 (CH_2)	1.33 m		28.3 (CH_2)	1.17 m	28.3	1.31 m	28.5 (CH_2)	1.23 m
		1.84 m			1.88 m		1.87 m		1.97 m
17	56.4 (CH)	1.13 m		55.7 (CH)	1.19 m	56.0	1.19 m	54.7 (CH)	1.11 m
18	12.2 (CH_3)	0.69 s	12, 13, 14 17	11.8 (CH_3)	0.70 s	11.8	0.71 s	12.0 (CH_3)	0.69 s
19	16.8 (CH_3)	1.18 s	1, 5, 9, 10	18.2 (CH_3)	1.00 s	18.8	1.06 s	17.3 (CH_3)	1.20 s
20	35.9 (CH)	1.45 m		35.7 (CH)	1.45 m	35.7	1.41 m	35.7 (CH)	1.43 m
21	18.8 (CH_3)	0.94 d (6.4)	17, 20, 22	18.8 (CH_3)	0.97 d (6.6)	19.2	0.96 d (6.6)	18.9 (CH_3)	0.95 d (6.5)
22	34.9 (CH_2)	1.61 m		34.6 (CH_2)	1.18 m	34.6	1.17 m	34.7 (CH_2)	1.60 m
					1.57 m		1.51 m		
23	30.8 (CH_2)	1.84 m	24, 28	30.2 (CH_2)	1.83 m	30.4	1.80 m	30.4 (CH_2)	1.85 m
		2.02 m			2.05 m		2.04 m		2.05 m
24	155.4 (C)			155.3 (C)		155.2		155.2 (C)	
25	41.8 (CH)	1.99 m	24, 26, 27 28	41.7 (CH)	2.01 m	41.7	2.02 m	41.7 (CH)	1.99 m
26	19.8 (CH_3)	1.00 d (6.8)	24, 25, 27	19.8 (CH_3)	1.01 d (5.4)	19.8	1.01 d (6.6)	19.8 (CH_3)	1.00 d (6.9)
27	28.4 (CH_2)	1.28 m	25	28.3 (CH_2)	1.32 m	28.5	1.41 m	28.3 (CH_2)	1.31 m
		1.49 m			1.85 m		1.84 m		1.52 m
28	107.2 (CH_2)	4.69 s	23, 24, 25	107.1 (CH_2)	4.70 s	107.1	4.70 s	107.2 (CH_2)	4.70 s
29	11.9 (CH_3)	0.84 t (7.2)	25, 26, 27	12.0 (CH_3)	0.84 t (7.2)	12.0	0.84 t (7.2)	12.0 (CH_3)	0.84 t (7.4)

[a] Recorded on 500 MHz NMR. [b]Recorded on 600 MHz NMR. [c] HMBC correlations, optimized for 6 Hz, are from proton(s) stated to the indicated carbon.

diene,[13] with the exception of an additional methylene signal (δ_C 28.3, C-27) found in **4**. However, the spectroscopic data for the side chains of **3** and **4** were consistent. These observations indicate that the structure of **4** is 24-methylene-27-methylcholest-5-ene-3β,7α-diol.

Compound **5** had the same formula ($C_{29}H_{48}O_2$) as **4** on the basis of ESIMS and elemental analysis. The ^{1}H and ^{13}C NMR data were similar to those of **4**. The principal differences were the upfield shift of H-6 (δ_H 5.60 to 5.29) in the ^{1}H NMR and the downfield shift of C-7 (δ_C 65.4 to 73.4) in the ^{13}C NMR. These data were consistent with the presence of a 7β-OH, rather than 7α-OH, in **5**. This assignment was supported by a careful comparison of the spectroscopic data of **5**, **4**, and the known compound 3β,7β-dihydroxyergosta-5,24(28)-diene in the literature.[13] Thus, compound **5** was identified as 24-methylene-27-methylcholest-5-ene-3β,7β-diol.

Compound **6** was obtained as colorless needle crystals. The protonated molecular ion peaks appeared at *m*/*z* 427 $[M + H]^+$, 449 $[M + Na]^+$, and 875 $[2M + Na]^+$. The IR absorption at 3534 cm^{-1}, combined with ^{1}H NMR δ_H at 3.68, showed the presence of a hydroxyl group. The ^{13}C NMR signal at δ_C 202.4, together with an IR absorption maximum at 1680 cm^{-1}, supported the presence of a conjugated carbonyl group. The ^{1}H and ^{13}C NMR spectra of compound **6** indicated that it was a steroid and shared the same side chain as that of **3**−**5**. Significant differences were observed for nuclei belonging to ring B when the spectroscopic data of **6** were compared with those of **4**. There was a lack of a hydroxyl (δ_C 65.4 in **4**) and presence of a carbonyl in **6**, and the quaternary olefinic carbon was downfield-shifted from δ_C 146.2 to δ_C 165.2, which indicated that the carbonyl replaced the hydroxyl at C-7. This was confirmed by the HMBC spectrum and the data of the known compound.[14] Thus, compound **6** was elucidated as 24-methylene-27-methylcholest-5-en-3β-ol-7-one.

This compound was first reported from the Chinese marine sponge *Geodia japonica* by Zhang et al. in 2001 and identified as 26-methylergosta-5,24(28)-dien-7-on-3β-ol.[7] However, some discrepancies were noted in the assignment of the NMR data, especially the ^{13}C NMR data, between their reported values and ours. The signals for 29-CH_3 (27-CH_3 in the literature), 19-CH_3, and 21-CH_3 in the literature were assigned at δ_C 19.9, 12.2, and 17.4, respectively, while the HMBC spectrum showed correlations between pairs of a methyl triplet at δ_H0.84 (δ_C 12.0) and C-25, C-26, and C-27, pairs of a methyl singlet at δ_H1.20 (δ_C 17.3) and C-1, C-10, C-5, and C-9, and pairs of a methyl doublet at δ_H 0.95 (δ_C 18.9) and C-20, C-17, and C-22, indicating the assignment of δ_C 12.0 (C-29), 17.3 (C-19), and 18.9 (C-21). The values of C-14 and C-17 in their report were reversed according to the HMBC and HMQC experiments. Correlation peaks were observed between pairs of H-18/δ_C 50.0, H-18/δ_C 54.7, and H-21/δ_C 54.7, implying the chemical shift of C-14 at δ_C 50.0 and C-17 at δ_C 54.7. The assignments of C-1, C-9, C-10, C-13, and C-23 were also unclear. We have now carefully reassigned the spectroscopic data for this compound as shown in Table 2 on the basis of HMBC and HMQC experiments.

Table 3. Cytotoxic Activity of **1** and **2** against Four Human Cancer Cell Lines (IC_{50}: μM)

compound	A549	SMMC-7721	AGS	HT-29
vincristine[a]	0.36	<0.12	<0.12	0.21
5-FU[a]	9.2	51	370	540
1	28	120	3.9	33
2	34	90	2.1	42

[a] Positive cytotoxicity control.

The two new isomalabaricane-type triterpenoids **1** and **2** were tested *in vitro* for cytotoxic activity against human lung (A549), liver (SMMC-7721), stomach (AGS), and colon (HT-29) cancer cell lines (Table 3). Both **1** and **2** showed selective cytotoxicity against AGS cells, with IC_{50} values of 3.9 and 2.1 μM, respectively.

Experimental Section

General Experimental Procedures. Melting points were recorded on a Yamato MP-21 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded as KBr pellets on a Nicolet NEXUS 470 spectrophotometer. ^{1}H, ^{13}C, ^{1}H−^{1}H-COSY, HMQC, HMBC, and NOESY NMR spectra were recorded on a Bruker AMX-500 MHz instrument and a Bruker AVANCE^{II}-600 MHz instrument in $CDCl_3$ with TMS as an internal standard. ESIMS data were obtained on JMS-300 spectrometer. Normal-phase HPLC (Waters 1525 liquid chromatography using a PDA 2996 detector at 360 nm and a semipreparative Hypersil SiO_2 column [Dalian Elite Analytical Instruments Co., Ltd.; 10 μm particle size, 10 mm (i.d.) × 25 cm]) was employed for the purification. Commercial Si gel (Yan Tai Zhi Fu Chemical Group Co., 200−300 and 300−400 mesh) was used for column chromatography, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC. Sephadex LH-20 (Pharmacia) was also used for column chromatography.

Animal Material. The specimen of *Stelletta tenuis* (Lindgren) was collected by scuba in Sanya, Hainan Province, China, in 2004. The sponge was identified by Professor Jinhe Li (Institute of Oceanology, Chinese Academy of Science), and a voucher specimen (HN1120-1) is held at the Marine Laboratory, Changzheng Hospital, Second Military Medicinal University.

Extraction and Isolation. The frozen sponge (4.2 kg, wet wt) was washed and chopped and then extracted with 95% EtOH. The concentrated aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 extract (100 g) was partitioned between 90% aqueous MeOH and petroleum ether (24.5 g). The MeOH solution was adjusted to 70% MeOH and extracted with CCl_4. The bulk of activity was concentrated in the CCl_4 fraction (98 g), which was subjected to vacuum-liquid chromatography on a Si gel column using petroleum ether/EtOAc (20:1−1:1) to yield seven fractions (A−E) on the basis of TLC analysis. Fraction A was further chromatographed on Si gel eluted with petroleum ether/EtOAc (5:1−5:2) to afford compounds **4** (5.3 mg), **5** (4.5 mg), and **6** (128.6 mg). Fraction D was gel-filtered on Sephadex LH-20 (*n*-hexane/CH_2Cl_2/MeOH, 4:5:1) and then purified by repetitive Si gel CC (CH_2Cl_2/MeOH, 50:1−20:1) followed by semipreparative normal-phase HPLC (*n*-hexane/2-propanol, 80:20) to yield **1** (3.6 mg) and **2** (2.7 mg).

Stellettin L (1): yellow crystals; mp 237−239 °C; $[\alpha]_D^{24}$ −74 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 410 (4.63), 394 (4.65), 292 (3.55) nm; IR (KBr) ν_{max} 3357 (broad), 2946, 1709, 1698, 1578 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 466.3081 (calcd for $C_{30}H_{42}O_4$, $[M]^+$ 466.3083).

Stellettin M (2): yellow crystals; mp 232−234 °C; $[\alpha]_D^{24}$ −21 (*c* 0.22, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 410 (4.65), 394 (4.66), 292 (3.53) nm; IR (KBr) ν_{max} 3382 (broad), 2934, 1709, 1691, 1578 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 466.3082 (calcd for $C_{30}H_{42}O_4$, $[M]^+$ 466.3083).

24-Methylene-27-methylcholestane-3β,5α,6β-triol (3): white solid; mp 246−248 °C; $[\alpha]_D^{24}$ −4.7 (*c* 0.2, pyridine); λ_{max} (log ϵ) 206 (1.53) nm; IR (KBr) ν_{max} 3431, 3087, 2945, 2864, 1640, 1463, 1450, 1377, 1292, 1095, 1043, 1014, 925, 887 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 445 $[M - H]^+$, 891 $[2M - H]^+$; *anal*. C 77.95%, H 11.21%, calcd for $C_{29}H_{50}O_3$, C 77.98%, H 11.28%.

24-Methylene-27-methylcholest-5-ene-3β,7α-diol (4): white solid; mp 195−197 °C; $[\alpha]_D^{24}$ −80 (*c* 0.21, $CHCl_3$); λ_{max} (log ϵ) 210 (2.34) nm; IR (KBr) ν_{max} 3323, 2935, 2867, 1634, 1540, 1465, 1382, 1193, 1056, 1008, 953, 808 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 429 $[M + H]^+$, 451 $[M + Na]^+$, 879 $[2M + Na]^+$; *anal*. C 81.21%, H 11.32%, calcd for $C_{29}H_{48}O_2$, C 81.26%, H 11.28%.

24-Methylene-27-methylcholest-5-ene-3β,7β-diol (5): white solid; mp 153−155 °C; $[\alpha]_D^{24}$ +21 (*c* 0.13, $CHCl_3$); λ_{max} (log ϵ) 210 (2.34) nm; IR (KBr) ν_{max} 3336, 2935, 2904, 2869, 1650, 1465, 1378, 1301, 1056, 1013, 952 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 429 $[M + H]^+$, 451 $[M + Na]^+$, 879 $[2M + Na]^+$, *anal*. C 81.23%, H 11.30%, calcd for $C_{29}H_{48}O_2$, C 81.26%, H 11.28%.

24-Methylene-27-methylcholest-5-en-3β-ol-7-one (6): white solid; mp 52−53 °C; IR ν_{max} (KBr) 3534, 1680, 1635, 932 cm^{-1}; ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 427 $[M + H]^+$, 449 $[M + Na]^+$, 875 $[2M + Na]^+$.

Cytotoxicity Assay. Cytotoxicity was evaluated as IC_{50} by using the MTT assay as described previously.[15] Compounds **1** and **2** were solubilized in DMSO with the working concentration of test substances ranging from 1 to 100 μg/mL. Cells were inoculated into 96-well plates. After incubation for 24 h, the cells were treated with various concentrations of test substances for 48 h and then were incubated with 1 mg/mL MTT at 37 °C for 4 h, followed by solubilization in DMSO. The formazan dye product was measured by the absorbance at 470 nm on a microplate reader.

Acknowledgment. This work was financially supported by National High-Tech Research and Development Project (863 Project, 2002AA628160), Major Program of Modernization of Chinese Medicine (04DZ19809), and Key Research Project of Biology and Medicine (0543199040) sponsored by STCSM.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP070069L