New Steryl Esters of Fatty Acids from the Mangrove Fungus *Aspergillus awamori*

by **Hao Gao**[a])[c]), **Kui Hong**[b]), **Xue Zhang**[a])[c]), **Hong-Wei Liu**[a]), **Nai-Li Wang**[a])[c]), **Ling Zhuang**[b]), and **Xin-Sheng Yao***[a])[c])

[a]) Key Lab for New Drugs Research of TCM, Shenzhen Research Institute of Tsinghua University, Shenzhen 518055, P. R. China (phone/fax: +86-755-26957685; e-mail: yaoxinsheng@vip.tom.com)
[b]) Institute of Tropical Biosciences and Biotechnology, Haikou 571101, P. R. China
[c]) Traditional Chinese Medicine College, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China

The polyhydroxylated ergostane-type sterol **9**, its derivatives **10**–**15**, and the fatty acid esters **1**–**8** were isolated from a fungus strain which was collected from mangrove areas at Wenchang, Hainan Province, P. R. China, exhibited potent cytotoxic activity, and was identified as *Aspergillus awamori*. The structures of **1**–**15** were elucidated by spectroscopic and chemical methods. Among them, the six steryl esters **1**–**6** of fatty acids were new compounds, *i.e.*, (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-palmitate (**1**), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-stearate (**2**), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate (**3**), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate (**4**), (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-palmitate (**5**), and (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-stearate (**6**). The related known fatty acids stearic acid (=octadecanoic acid) and palmitic acid (=octadecanoic acid) were also obtained. A speculative biogenetic relationship of the metabolites is proposed. The known polyhydroxylated sterols and derivatives showed cytotoxic activities, in agreement with earlier reports. The cytotoxic activities against B16 and SMMC-7721 cell lines of the new steryl esters **1**–**6** by the MTT method were weak.

Introduction. – In the searching for new bioactive agents from the sea, thousands of strains were collected from mangrove areas in Hainan Province, P. R. China [1][2]. Cytotoxic screening of the extract of their mycelia presented a fungus strain exhibiting potent inhibitory activity on B16 cell line proliferation by the MTT method (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide), which was identified as *Aspergillus awamori* [3]. By bioactivity-guided isolation of the acetone extract of *A. awamori* mycelia, the polyhydroxylated ergostane-type sterol **9**, its derivatives **10**–**15**, and the fatty acid esters **1**–**8** were isolated from the active fractions. The related fatty acids, stearic acid and palmitic acid, were also obtained. Their structures were elucidated by spectroscopic and chemical methods. Interestingly, fatty acid esters of monosterols are common [4–6], while fatty acid esters of polyhydroxylated sterols are rare. The only reported three polyhydroxylated ergostane-type steryl esters of fatty acids were isolated from fungi: (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate (**7**) and (3*β*,5*α*,6*β*,22*E*)-3,5,6-trihydroxyergost-22-en-7-one 6-oleate from *Tricholomopsis rutilans* [7], and (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate (**8**) from *Catathelasma imperiale* [8]. A speculative biogenetic relationship of the obtained

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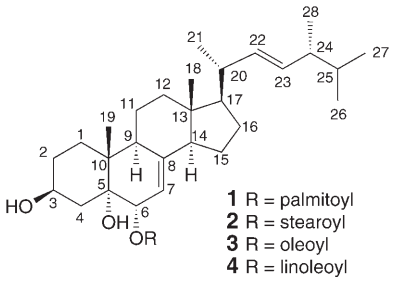
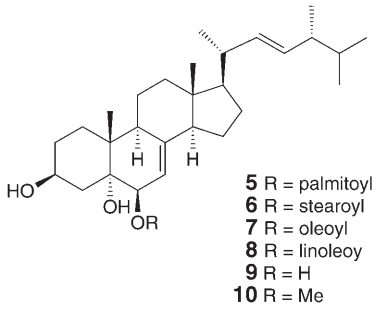
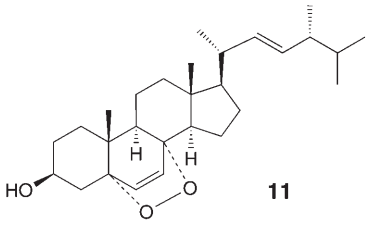
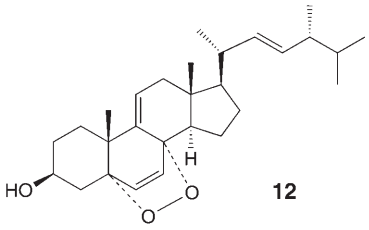
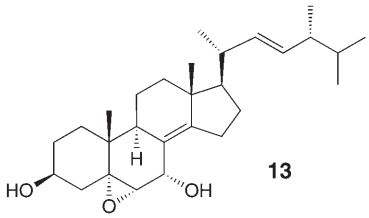
sterols from *A. awamori* is proposed. The known polyhydroxylated sterols showed cytotoxic activities, in agreement with earlier reports [9–15]. We evaluated the cytotoxic activities of the new steryl esters of fatty acids by the MTT method, and the cytotoxicity against B16 and SMMC-7721 cell lines of the new steryl esters was weak.

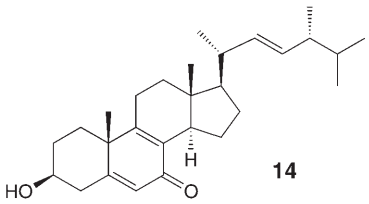


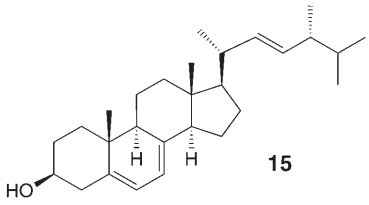












Results and Discussion. – Compound **1** was obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m*/*z* 691 ($[M+Na]^+$), and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **1** was 668. Although in the HR-EI-MS, the molecular ion was not observed, the molecular formula could be determined as $C_{44}H_{76}O_4$ according to the

fragment ion at *m/z* 650.5619 ($[M-H_2O]^+$). The ^{1}H- and ^{13}C-NMR (*Table 1*), HMBC and COSY (*Fig. 1*), and NOESY data (*Fig. 2*) allowed to determine the structure of **1** as (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-palmitate which represents a new natural product.

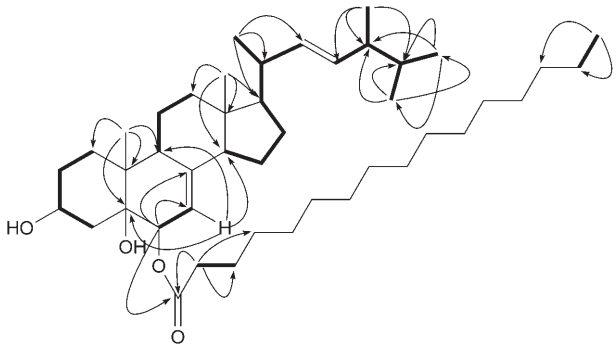


Fig. 1. *Key HMBC* (→) *and COSY* (—) *correlations of* **1**

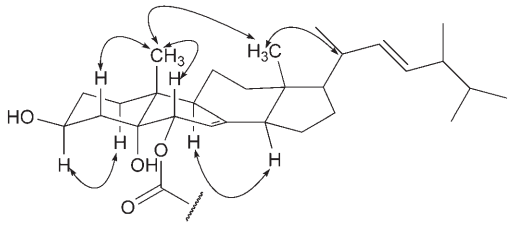


Fig. 2. *Key NOESY* (↔) *correlations of* **1**

The ^{1}H-NMR-spectrum of **1** displayed six characteristic ergostane-type steroidal Me signals at δ 1.02 (*d*, $J = 6.2$ Hz), 1.02 (*s*), 0.91 (*d*, $J = 6.8$ Hz), 0.84 (*d*, $J = 6.4$ Hz), 0.82 (*d*, $J = 6.4$ Hz), and 0.56 (*s*). The *t* at δ 0.88 ($J = 6.6$ Hz, 3 H) and 2.36 ($J = 7.5$ Hz, 2 H) and the huge signal at δ 1.26 suggested the presence of a fatty acid moiety. The ^{13}C-NMR spectrum of **1** combined with the DEPT-135 spectrum confirmed the C_{28}-ergostane-type sterol skeleton and indicated that the fatty acid unit was saturated. In the ESI-IT-MS^n experiments (IT = ion trap), the MS^2 experiment of the ion at *m/z* 691 ($[M+Na]^+$) gave positive fragments at *m/z* 435 ($[M+Na-256]^+$) and 417 ($[M+Na-256-18]^+$), and the loss of 256 mass units matched palmitic acid (= hexadecanoic acid) exactly. The sterol core was determined by a detailed analysis of the COSY and HMBC data (*Fig. 1*), and the configuration of the sterol ring system was deduced from the NOESY correlations (*Fig. 2*). The absolute configurations within the sterol side chain were determined as (20*R*,24*R*) by comparison with known ^{13}C-NMR data [16]. The coupling constant between the protons at δ 5.22 (*dd*, $J = 15.3$, 7.2 Hz, 1 H) and 5.16 (*dd*, $J = 15.3$, 7.8 Hz, 1 H) was consistent with a *trans*-configuration of the C(22)=C(23) bond. Thus, the sterol core was determined as (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol (= 6-epicerevisterol). The key HMBC correlation signal found for the carbonyl C-atom at δ 173.4 and the proton at δ 5.27 indicated that the palmitoyl moiety acid was attached at O–C(6) by an ester bond. The comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of 6-epicerevisterol [17] and palmitic acid showed a reasonable esterification shift (*Tables 1* and *3* (see below)): a downfield shift $\Delta\delta = +1.29$ for H–C(6), a downfield shift $\Delta\delta = +3.3$ for C(6), and an upfield shift $\Delta\delta = -3.4$ for C(1′), which confirmed the above-mentioned conclusion.

Table 1. *NMR Data* (400 MHz, $CDCl_3$) *for* **1**–**4**. δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	31.4	1.50–1.57 (*m*), 1.65–1.75 (*m*)	31.4	1.50–1.57 (*m*), 1.65–1.75 (*m*)	31.4	1.49–1.57 (*m*), 1.65–1.75 (*m*)	31.4	1.49–1.57 (*m*), 1.65–1.75 (*m*)
CH_2(2)	30.8	1.39–1.46 (*m*), 1.82–1.89 (*m*)	30.8	1.40–1.47 (*m*), 1.81–1.89 (*m*)	30.8	1.39–1.47 (*m*), 1.82–1.90 (*m*)	30.8	1.39–1.47 (*m*), 1.82–1.89 (*m*)
H–C(3)	67.3	4.00 (*tt*, J = 11.2, 5.0)	67.3	4.00 (*tt*, J = 11.1, 5.5)	67.3	4.00 (*tt*, J = 11.2, 5.0)	67.3	4.00 (*tt*, J = 11.5, 4.8)
CH_2(4)	39.5	1.47–1.56 (*m*), 1.88–1.96 (*m*)	39.5	1.47–1.56 (*m*), 1.88–1.96 (*m*)	39.5	1.48–1.56 (*m*), 1.88–1.95 (*m*)	39.5	1.47–1.56 (*m*), 1.88–1.96 (*m*)
C(5)	75.2	–	75.2	–	75.2	–	75.2	–
H–C(6)	73.7	5.27 (br. *s*)	73.7	5.27 (br. *s*)	73.7	5.27 (br. *s*)	73.7	5.27 (br. *s*)
H–C(7)	115.2	4.88 (br. *s*)	115.2	4.88 (br. *s*)	115.1	4.88 (br. *s*)	115.1	4.88 (br. *s*)
C(8)	144.3	–	144.3	–	144.3	–	144.3	–
H–C(9)	43.4	2.06–2.14 (*m*)	43.4	2.06–2.14 (*m*)	43.4	2.06–2.13 (*m*)	43.4	2.06–2.14 (*m*)
C(10)	39.0	–	39.0	–	39.0	–	39.0	–
CH_2(11)	21.3	1.50–1.59 (*m*)	21.3	1.49–1.59 (*m*)	21.3	1.49–1.59 (*m*)	21.3	1.49–1.59 (*m*)
CH_2(12)	39.2	1.28–1.35 (*m*), 2.01–2.08 (*m*)	39.2	1.27–1.34 (*m*), 2.01–2.09 (*m*)	39.2	1.26–1.35 (*m*), 2.01–2.08 (*m*)	39.2	1.26–1.35 (*m*), 2.01–2.09 (*m*)
C(13)	43.8	–	43.8	–	43.8	–	43.8	–
H–C(14)	54.8	1.88–1.95 (*m*)	54.8	1.88–1.95 (*m*)	54.8	1.89–1.95 (*m*)	54.8	1.89–1.95 (*m*)
CH_2(15)	22.7	1.38–1.45 (*m*), 1.48–1.54 (*m*)	22.7	1.35–1.43 (*m*), 1.49–1.56 (*m*)	22.7	1.36–1.45 (*m*), 1.48–1.56 (*m*)	22.7	1.36–1.44 (*m*), 1.48–1.56 (*m*)
CH_2(16)	28.0	1.24–1.31 (*m*), 1.69–1.76 (*m*)	28.0	1.24–1.31 (*m*), 1.69–1.77 (*m*)	28.0	1.25–1.32 (*m*), 1.69–1.77 (*m*)	28.0	1.25–1.33 (*m*), 1.69–1.77 (*m*)
H–C(17)	55.9	1.26–1.31 (*m*)	55.9	1.25–1.31 (*m*)	55.9	1.26–1.30 (*m*)	55.9	1.26–1.31 (*m*)
Me(18)	12.2	0.56 (*s*)	12.2	0.56 (*s*)	12.2	0.56 (*s*)	12.2	0.56 (*s*)
Me(19)	17.9	1.02 (*s*)	17.9	1.02 (*s*)	17.9	1.02 (*s*)	17.9	1.02 (*s*)
H–C(20)	40.4	1.98–2.06 (*m*)	40.4	1.98–2.05 (*m*)	40.4	1.99–2.05 (*m*)	40.4	1.98–2.05 (*m*)
Me(21)	21.1	1.02 (*d*, J = 6.2)	21.1	1.02 (*d*, J = 6.5)	21.1	1.02 (*d*, J = 6.5)	21.1	1.02 (*d*, J = 6.5)
H–C(22)	135.4	5.16 (*dd*, J = 15.3, 7.8)	135.4	5.16 (*dd*, J = 15.4, 7.9)	135.4	5.16 (*dd*, J = 15.3, 7.8)	135.4	5.16 (*dd*, J = 15.3, 7.8)
H–C(23)	132.2	5.22 (*dd*, J = 15.3, 7.2)	132.2	5.22 (*dd*, J = 15.2, 7.1)	132.2	5.22 (*dd*, J = 15.3, 7.2)	132.1	5.22 (*dd*, J = 15.2, 7.1)
H–C(24)	42.8	1.81–1.89 (*m*)	42.9	1.81–1.88 (*m*)	42.8	1.82–1.88 (*m*)	42.8	1.81–1.88 (*m*)

Table 1 (cont.)

	1		**2**		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
H–C(25)	33.1	1.44–1.51 (*m*)	33.1	1.43–1.50 (*m*)	33.1	1.43–1.51 (*m*)	33.1	1.43–1.51 (*m*)
Me(26)	20.0	0.84 (*d*, *J* = 6.4)	20.0	0.84 (*d*, *J* = 6.4)	20.0	0.84 (*d*, *J* = 6.4)	20.9	0.84 (*d*, *J* = 6.4)
Me(27)	19.7	0.82 (*d*, *J* = 6.4)	19.7	0.82 (*d*, *J* = 6.4)	19.7	0.82 (*d*, *J* = 6.4)	19.6	0.82 (*d*, *J* = 6.4)
Me(28)	17.6	0.91 (*d*, *J* = 6.8)	17.6	0.91 (*d*, *J* = 6.8)	17.6	0.91 (*d*, *J* = 6.8)	17.6	0.91 (*d*, *J* = 6.8)
C(1′)	173.4	–	173.4	–	173.4	–	173.3	–
CH_2(2′)	34.6	2.36 (*t*, *J* = 7.5)	34.6	2.36 (*t*, *J* = 7.5)	34.6	2.36 (*t*, *J* = 7.5)	34.5	2.36 (*t*, *J* = 7.4)
CH_2(3′)	25.1	1.60–1.69 (*m*)	25.1	1.60–1.69 (*m*)	25.1	1.60–1.70 (*m*)	25.0	1.60–1.70 (*m*)
CH_2(4′) to CH_2(13′)	29.2–29.7	1.23–1.35 (*m*)						
CH_2(4′) to CH_2(15′)			29.2–29.7	1.23–1.35 (*m*)				
CH_2(4′) to CH_2(7′), CH_2(12′) to CH_2(15′)					29.1–29.8	1.23–1.38 (*m*)		
CH_2(4′) to CH_2(7′), CH_2(15′)							29.1–29.7	1.23–1.38 (*m*)
CH_2(8′), CH_2(11′)					27.2	1.97–2.06 (*m*)		
CH_2(8′), CH_2(14′)							27.2	2.05 (*q*, *J* = 7.1)
H–C(9′), H–C(10′)					129.8, 130.0	5.32–5.38 (*m*)	130.0, 130.2	5.29–5.42 (*m*)
CH_2(11′)							25.6	2.77 (*t*, *J* = 6.7)
H–C(12′), H–C(13′)							127.9, 128.1	5.29–5.42 (*m*)
CH_2(14′)	31.9	1.24–1.29 (*m*)						
CH_2(15′)	22.7	1.24–1.33 (*m*)						
Me(16′) or CH_2(16′)	14.1	0.88 (*t*, *J* = 6.6)	31.9	1.24–1.29 (*m*)	31.9	1.23–1.29 (*m*)	31.5	1.24–1.29 (*m*)
CH_2(17′)			22.7	1.24–1.33 (*m*)	22.7	1.25–1.34 (*m*)	22.6	1.24–1.35 (*m*)
Me(18′)			14.1	0.88 (*t*, *J* = 6.7)	14.1	0.88 (*t*, *J* = 6.7)	14.1	0.89 (*t*, *J* = 7.0)

Compound **2** was also obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m/z* 719 ($[M+Na]^+$), and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **2** was 696. Although in the HR-EI-MS the molecular ion was not observed, the molecular formula could be determined as $C_{46}H_{80}O_4$ according to the fragment ion at *m/z* 678.5947 ($[M-H_2O]^+$). The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 1*) were almost the same as those of **1**, indicating that **2** was composed of 6-epicerevisterol and a saturated fatty acid. Considering the molecular formula and the MS^2 experiment of the ion at *m/z* 719 ($[M+Na]^+$), the fatty acid unit was determined as stearic acid (=octadecanoid acid). The COSY, HMBC, and NOESY experiments confirmed the above deduction. The HMBC correlation signal found for the carbonyl C-atom at δ 173.4 and the proton at δ 5.27 and the observed esterification shift indicated that the stearoyl moiety was attached at O–C(6) by an ester bond. Therefore, **2** was determined as (3β,5α,6α,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-stearate which represents a new natural product.

Compound **3** was obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m/z* 717 ($[M+Na]^+$), and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **3** was 694. The molecular formula was determined as $C_{46}H_{78}O_4$ according to the fragment ion at *m/z* 676.5763 ($[M^+-H_2O]^+$) in the HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table 1*) were similar to those of **1** and **2**. The data of the sterol core were the same as those of **1** and **2**, which indicated that **3** was also a 6-epicerevisterol derivative, and the differences in the fatty acid unit suggested the presence of a monounsaturated fatty acid chain in **3**. In the ESI-IT-MS^n experiments, the MS^2 experiment of the ion at *m/z* 717 ($[M+Na]^+$) gave positive fragments at *m/z* 435 ($[M+Na-282]^+$), 417 ($[M+Na-282-18]^+$), and 305 ($[M+Na-282-130]^+$), and the loss of 282 mass units matched the C_{18} monounsaturated fatty acid. After methanolysis of **3**, the GC/MS analysis of the resulting organic phase revealed the presence of methyl oleate. Thus, **3** was composed of 6-epicerevisterol and oleic acid (=(9*Z*)-octadec-9-enoic acid). The COSY, HMBC, and NOESY experiments confirmed the above deduction. The HMBC correlation signal found for the carbonyl C-atom at δ 173.4 and the proton at δ 5.27 and the observed esterification shift indicated that the oleoyl moiety was attached at O–C(6) by an ester bond. Therefore, **3** was determined as (3β,5α,6α,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate which represents a new natural product.

Compound **4** was obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m/z* 715 ($[M+Na]^+$) and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **4** was 692. The molecular formula was determined as $C_{46}H_{76}O_4$ according to the fragment ion at *m/z* 674.5617 ($[M-H_2O]^+$) in the HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of **4** (*Table 1*) were similar to those of **1** and **2**. The data of the sterol core were the same as those of **1** and **2**, which indicated that **4** was also a 6-epicerevisterol derivate, and the differences in the fatty acid unit suggested the presence of a doubly unsaturated fatty acid chain in **4**. In the ESI-IT-MS^n experiments, the MS^2 experiment of the ion at *m/z* 715 ($[M+Na]^+$) gave positive fragments at *m/z* 435 ($[M+Na-280]^+$) and 417 ($[M+Na-$

$280-18]^+$), and the loss of 280 mass units matched the C_{18} diunsaturated fatty acid. After methanolysis of **4**, the GC/MS analysis of the resulting organic phase revealed the presence of methyl linoleate. Thus, **4** was composed of 6-epicerevisterol and linoleic acid (=(9*Z*,12*Z*)-octadeca-9,12-dienoic acid). The COSY, HMBC, and NOESY experiments confirmed the above deduction. The HMBC correlation signal found for the carbonyl C-atom at δ 173.3 and the proton at δ 5.27 and the observed esterification shift indicated that the linoleoyl moiety was attached at O–C(6) by an ester bond. Therefore **4** was determined as (3β,5α,6α,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate which represents a new natural product.

Compound **5** was obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m*/*z* 691 ($[M+Na]^+$), and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **5** was 668. Although in the HR-EI-MS, the molecular ion was not observed, the molecular formula could be determined as $C_{44}H_{76}O_4$ according to the fragment ion at *m*/*z* 650.5621 ($[M-H_2O]^+$). The ^{1}H- and ^{13}C-NMR (*Table 2*), COSY, HMBC, and NOESY data and comparison with cerevisterol (**9**) and palmitic acid established the structure of **5** as (3β,5α,6β,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-palmitate which represents a new natural product.

The ^{1}H-NMR spectrum of **5** also displayed six characteristic ergostane-type steroidal Me signals at δ 1.06 (*s*), 1.03 (*d*, *J* = 6.6 Hz), 0.92 (*d*, *J* = 6.8 Hz), 0.84 (*d*, *J* = 6.5 Hz), 0.82 (*d*, *J* = 6.5 Hz), and 0.58 (*s*). Similar to **1**, the *t* at δ 0.88 (*J* = 6.6 Hz, 3 H) and 2.30 (*J* = 7.4 Hz, 2 H) and the huge signal at δ 1.26 suggested the presence of a fatty acid moiety. The ^{13}C-NMR spectrum of **5** combined with the DEPT-135 spectrum confirmed the C_{28}-ergostane-type sterol skeleton and indicated that the fatty acid unit was saturated. The MS^2 experiment of the ion at *m*/*z* 691 ($[M+Na]^+$) gave positive fragments at *m*/*z* 435 ($[M+Na-256]^+$) and 417 ($[M+Na-256-18]^+$), and the loss of 256 mass units matched palmitic acid exactly. The sterol core was determined by a detailed analysis of the COSY and HMBC data which revealed the same planar structure as that of **1**. The configurations within the sterol side chain of **5** were deduced as described for **1** and were identical to those of **1**, *i.e.*, (20*R*,24*R*,22*E*). The NOESY experiment with **5** revealed almost the same configuration pattern of the sterol ring system as that of **1**, except that no correlation was observed between the Me signal at δ 1.06 (*s*, Me(19)) and the proton at δ 4.83 (*d*, *J* = 5.1 Hz, H–C(6)) in **5**. Thus, the sterol core was determined as (3β,5α,6β,22*E*)-ergosta-7,22-diene-3,5,6-triol (=cerevisterol; **9**), the 6-epimer of 6-epicerevisterol. Though the HMBC correlation signal between the carbonyl C-atom at δ 173.2 (C(1′)) and the proton at δ 4.83 (*d*, *J* = 5.1 Hz, H–C(6)) was not observed, the comparison of the ^{1}H- and ^{13}C-NMR data of **5** with those of cerevisterol (**9**) and palmitic acid showed an obvious esterification shift (*Table 2* and *3*): a downfield shift $\Delta\delta = +1.21$ for H–C(6) and an upfield shift $\Delta\delta = -3.6$ for C(1′), which indicated that the palmitoyl moiety was attached at O–C(6) by an ester bond.

Compound **6** was also obtained as a colorless oily solid, which gave positive results in the *Liebermann–Burchard* reaction. The ESI-IT-MS experiment (positive mode) gave the quasi-molecular ion at *m*/*z* 719 ($[M+Na]^+$), and the ESI-IT-MS experiment (negative mode) responded weakly, indicating that the molecular mass of **6** was 696. The molecular formula was determined as $C_{46}H_{80}O_4$ according to the fragment ion at *m*/*z* 678.5939 ($[M-H_2O]^+$) in the HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of **6** (*Table 2*) were the same as those of **5**, indicating that **6** was composed of cerevisterol and a saturated fatty acid. Considering the molecular formula and the MS^2 experiment of the ion at *m*/*z* 719 ($[M+Na]^+$), the fatty acid unit was determined as stearic acid.

Table 2. *NMR Data* (400 MHz, $CDCl_3$) *for* **5**–**8**. δ in ppm, J in Hz.

	5		**6**		**7**		**8**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	32.5	1.52–1.66 (*m*)	32.5	1.54–1.64 (*m*)	32.5	1.52–1.64 (*m*)	32.5	1.52–1.64 (*m*)
CH_2(2)	30.7	1.42–1.50 (*m*), 1.81–1.89 (*m*)	30.7	1.39–1.47 (*m*), 1.81–1.88 (*m*)	30.7	1.39–1.47 (*m*), 1.81–1.88 (*m*)	30.7	1.39–1.47 (*m*), 1.81–1.88 (*m*)
H–C(3)	67.4	4.08 (*tt*, *J* = 11.3, 6.0)	67.5	4.08 (*tt*, *J* = 11.2, 5.3)	67.4	4.08 (*tt*, *J* = 11.3, 4.4)	67.4	4.08 (*tt*, *J* = 11.2, 5.3)
CH_2(4)	39.3	1.67–1.75 (*m*), 1.84–1.93 (*m*)	39.3	1.66–1.74 (*m*), 1.83–1.92 (*m*)	39.3	1.66–1.75 (*m*), 1.83–1.92 (*m*)	39.2	1.66–1.74 (*m*), 1.83–1.92 (*m*)
C(5)	75.3	–	75.3	–	75.3	–	75.3	–
H–C(6)	73.3	4.83 (*d*, *J* = 5.1)	73.3	4.83 (*d*, *J* = 5.6)	73.3	4.83 (*d*, *J* = 5.1)	73.3	4.83 (*d*, *J* = 5.1)
H–C(7)	114.3	5.26–5.31 (*m*)	114.3	5.25–5.30 (*m*)	114.3	5.25–5.29 (*m*)	114.3	5.25–5.31 (*m*)
C(8)	145.6	–	145.6	–	145.6	–	145.6	–
H–C(9)	43.5	1.94–2.02 (*m*)	43.5	1.93–2.00 (*m*)	43.5	1.93–2.01 (*m*)	43.5	1.93–2.01 (*m*)
C(10)	37.3	–	37.3	–	37.3	–	37.3	–
CH_2(11)	22.1	1.56–1.64 (*m*)	22.1	1.54–1.64 (*m*)	22.1	1.55–1.63 (*m*)	22.1	1.54–1.63 (*m*)
CH_2(12)	39.3	1.28–1.37 (*m*), 2.02–2.10 (*m*)	39.3	1.29–1.37 (*m*), 2.02–2.11 (*m*)	39.3	1.29–1.36 (*m*), 2.01–2.09 (*m*)	39.2	1.29–1.37 (*m*), 2.02–2.10 (*m*)
C(13)	43.9	–	43.9	–	43.9	–	43.9	–
H–C(14)	54.9	1.88–1.95 (*m*)	54.9	1.87–1.95 (*m*)	54.9	1.87–1.95 (*m*)	54.9	1.87–1.95 (*m*)
CH_2(15)	22.9	1.37–1.48 (*m*), 1.50–1.59 (*m*)	22.9	1.37–1.48 (*m*), 1.50–1.58 (*m*)	22.9	1.37–1.46 (*m*), 1.50–1.58 (*m*)	22.8	1.37–1.47 (*m*), 1.50–1.58 (*m*)
CH_2(16)	27.9	1.24–1.30 (*m*), 1.70–1.78 (*m*)	27.9	1.23–1.29 (*m*), 1.71–1.77 (*m*)	27.9	1.24–1.30 (*m*), 1.69–1.78 (*m*)	27.9	1.24–1.30 (*m*), 1.70–1.78 (*m*)
H–C(17)	56.0	1.26–1.33 (*m*)	56.0	1.26–1.31 (*m*)	56.0	1.26–1.31 (*m*)	56.0	1.26–1.31 (*m*)
Me(18)	12.3	0.58 (*s*)	12.3	0.58 (*s*)	12.3	0.58 (*s*)	12.3	0.58 (*s*)
Me(19)	18.4	1.06 (*s*)	18.4	1.06 (*s*)	18.4	1.06 (*s*)	18.4	1.06 (*s*)
H–C(20)	40.4	1.98–2.07 (*m*)	40.4	1.98–2.06 (*m*)	40.4	1.97–2.04 (*m*)	40.3	1.97–2.04 (*m*)
Me(21)	21.1	1.03 (*d*, *J* = 6.6)	21.1	1.02 (*d*, *J* = 6.6)	21.1	1.03 (*d*, *J* = 6.6)	21.1	1.03 (*d*, *J* = 6.5)
H–C(22)	135.4	5.16 (*dd*, *J* = 15.3, 7.7)	135.4	5.16 (*dd*, *J* = 15.1, 7.0)	135.4	5.16 (*dd*, *J* = 15.3, 7.7)	135.4	5.16 (*dd*, *J* = 15.3, 7.7)
H–C(23)	132.2	5.23 (*dd*, *J* = 15.2, 7.1)	132.2	5.22 (*dd*, *J* = 15.3, 6.8)	132.2	5.23 (*dd*, *J* = 15.3, 7.0)	132.2	5.23 (*dd*, *J* = 15.3, 7.0)
H–C(24)	42.9	1.81–1.90 (*m*)	42.9	1.81–1.88 (*m*)	42.9	1.80–1.89 (*m*)	42.9	1.80–1.88 (*m*)
H–C(25)	33.1	1.43–1.52 (*m*)	33.1	1.43–1.50 (*m*)	33.1	1.43–1.51 (*m*)	33.1	1.43–1.50 (*m*)

Table 2 (cont.)

	5		**6**		**7**		**8**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Me(26)	20.0	0.84 (*d*, *J* = 6.5)	20.0	0.84 (*d*, *J* = 6.4)	20.0	0.84 (*d*, *J* = 6.4)	19.9	0.84 (*d*, *J* = 6.4)
Me(27)	19.7	0.82 (*d*, *J* = 6.5)	19.7	0.82 (*d*, *J* = 6.5)	19.7	0.82 (*d*, *J* = 6.6)	19.6	0.82 (*d*, *J* = 6.5)
Me(28)	17.6	0.92 (*d*, *J* = 6.8)	17.6	0.92 (*d*, *J* = 6.8)	17.6	0.92 (*d*, *J* = 6.8)	17.6	0.92 (*d*, *J* = 6.8)
C(1′)	173.2	–	173.2	–	173.1	–	173.1	–
CH_2(2′)	34.7	2.30 (*t*, *J* = 7.4)	34.7	2.30 (*t*, *J* = 6.6)	34.7	2.30 (*t*, *J* = 7.4)	34.7	2.27 (*t*, *J* = 7.4)
CH_2(3′)	25.0	1.56–1.66 (*m*)	25.0	1.56–1.67 (*m*)	25.0	1.56–1.65 (*m*)	25.0	1.56–1.65 (*m*)
CH_2(4′) to CH_2(13′)	29.1–29.7	1.24–1.35 (*m*)						
CH_2(4′) to CH_2(15′)			29.1–29.7	1.22–1.34 (*m*)				
CH_2(4′) to CH_2(7′), CH_2(12′) to CH_2(15′)					29.1–29.8	1.23–1.36 (*m*)		
CH_2(4′) to CH_2(7′), CH_2(15′)							29.1–29.7	1.23–1.38 (*m*)
CH_2(8′), CH_2(11′)					27.2	1.96–2.06 (*m*)		
CH_2(8′), CH_2(14′)							27.2	2.05 (*q*, *J* = 7.1)
H–C(9′), H–C(10′)					129.8, 130.0	5.31–5.36 (*m*)	130.1, 130.2	5.30–5.42 (*m*)
CH_2(11′)							25.6	2.77 (*t*, *J* = 6.3)
H–C(12′), H–C(13′)							127.9, 128.1	5.30–5.42 (*m*)
CH_2(14′)	31.9	1.24–1.29 (*m*)						
CH_2(15′)	22.7	1.24–1.35 (*m*)						
Me(16′) or CH_2(16′)	14.1	0.88 (*t*, *J* = 6.6)	31.9	1.23–1.30 (*m*)	31.9	1.23–1.30 (*m*)	31.9	1.24–1.29 (*m*)
CH_2(17′)			22.7	1.23–1.34 (*m*)	22.7	1.24–1.32 (*m*)	22.7	1.24–1.34 (*m*)
Me(18′)			14.1	0.88 (*t*, *J* = 6.6)	14.1	0.88 (*t*, *J* = 6.7)	14.1	0.89 (*t*, *J* = 6.9)

Table 3. *NMR Data* (400 MHz, $CDCl_3$) *for* **9**, **10**, *and 6-Epicerevisterol*[a]). δ in ppm, J in Hz.

	9		**10**		6-Epicerevisterol[a])
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	33.0	1.53–1.66 (*m*)	32.8	1.50–1.61 (*m*)	32.4
CH_2(2)	30.9	1.40–1.50 (*m*), 1.84–1.92 (*m*)	30.9	1.40–1.50 (*m*), 1.80–1.88 (*m*)	32.2
H–C(3)	67.7	4.08 (*tt*, *J* = 11.3, 4.9)	67.9	4.05 (*tt*, *J* = 11.3, 4.8)	66.9
CH_2(4)	39.5	1.74–1.82 (*m*), 2.11–2.20 (*m*)	39.6	1.72–1.79 (*m*), 2.09–2.18 (*m*)	40.7
C(5)	76.0	–	76.4	–	75.7
H–C(6)	73.7	3.62 (*d*, *J* = 5.1)	82.5	3.17 (*d*, *J* = 5.1)	70.4
H–C(7)	117.6	5.33–5.38 (*m*)	115.0	5.38–5.43 (*m*)	121.4
C(8)	144.0	–	143.7	–	140.8
H–C(9)	43.5	1.93–2.01 (*m*)	43.9	1.86–1.93 (*m*)	43.5
C(10)	37.2	–	37.3	–	39.0
CH_2(11)	22.1	1.54–1.65 (*m*)	22.2	1.52–1.61 (*m*)	21.4
CH_2(12)	39.2	1.29–1.38 (*m*), 2.02–2.11 (*m*)	39.4	1.27–1.36 (*m*), 2.02–1.10 (*m*)	39.6
C(13)	43.8	–	43.9	–	43.6
H–C(14)	54.8	1.88–1.97 (*m*)	55.0	1.86–1.94 (*m*)	55.0
CH_2(15)	22.9	1.39–1.50 (*m*), 1.51–1.59 (*m*)	22.9	1.42–1.54 (*m*)	23.0
CH_2(16)	27.9	1.27–1.34 (*m*), 1.71–1.79 (*m*)	27.9	1.26–1.33 (*m*), 1.70–1.79 (*m*)	28.4
H–C(17)	56.0	1.27–1.34 (*m*)	56.0	1.26–1.33 (*m*)	55.9
Me(18)	12.3	0.60 (*s*)	12.3	0.60 (*s*)	12.3
Me(19)	18.8	1.09 (*s*)	18.4	1.01 (*s*)	17.8
H–C(20)	40.4	1.99–2.08 (*m*)	40.4	1.99–2.07 (*m*)	40.9
Me(21)	21.1	1.03 (*d*, *J* = 6.6)	21.1	1.03 (*d*, *J* = 6.6)	21.3
H–C(22)	135.4	5.16 (*dd*, *J* = 15.3, 7.8)	135.5	5.17 (*dd*, *J* = 15.3, 7.7)	136.2
H–C(23)	132.2	5.23 (*dd*, *J* = 15.2, 7.1)	132.1	5.23 (*dd*, *J* = 15.3, 7.1)	132.0
H–C(24)	42.8	1.81–1.90 (*m*)	42.8	1.81–1.89 (*m*)	42.9
H–C(25)	33.1	1.43–1.52 (*m*)	33.1	1.43–1.51 (*m*)	33.2
Me(26)	20.0	0.84 (*d*, *J* = 6.4)	20.0	0.84 (*d*, *J* = 6.4)	20.0
Me(27)	19.7	0.82 (*d*, *J* = 6.5)	19.7	0.82 (*d*, *J* = 6.5)	19.7
Me(28)	17.6	0.92 (*d*, *J* = 6.8)	17.6	0.92 (*d*, *J* = 6.8)	17.7
*Me*O–C(6)			58.3	3.39 (*s*)	

[a]) ^{13}C-NMR Data for 6-epicerevisterol from [17].

The COSY, HMBC, and NOESY experiments confirmed the above deduction. The observed esterification shift indicated that the stearoyl moiety was attached at O–C(6) by an ester bond. Therefore, **6** was determined as (3β,5α,6β,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-stearate which represents a new natural product.

Compounds **7** and **8** were obtained as colorless oily solids, which gave positive results in the *Liebermann–Burchard* reaction. Their molecular masses were determined as 694 and 692, based on the ESI-IT-MS experiments (positive mode). The ^{1}H- and ^{13}C-NMR spectra of **7** and **8** (*Table 2*) were similar to those of **5** and **6**. The data of the sterol core were the same as those of **5** and **6**; therefore, **7** and **8** were both

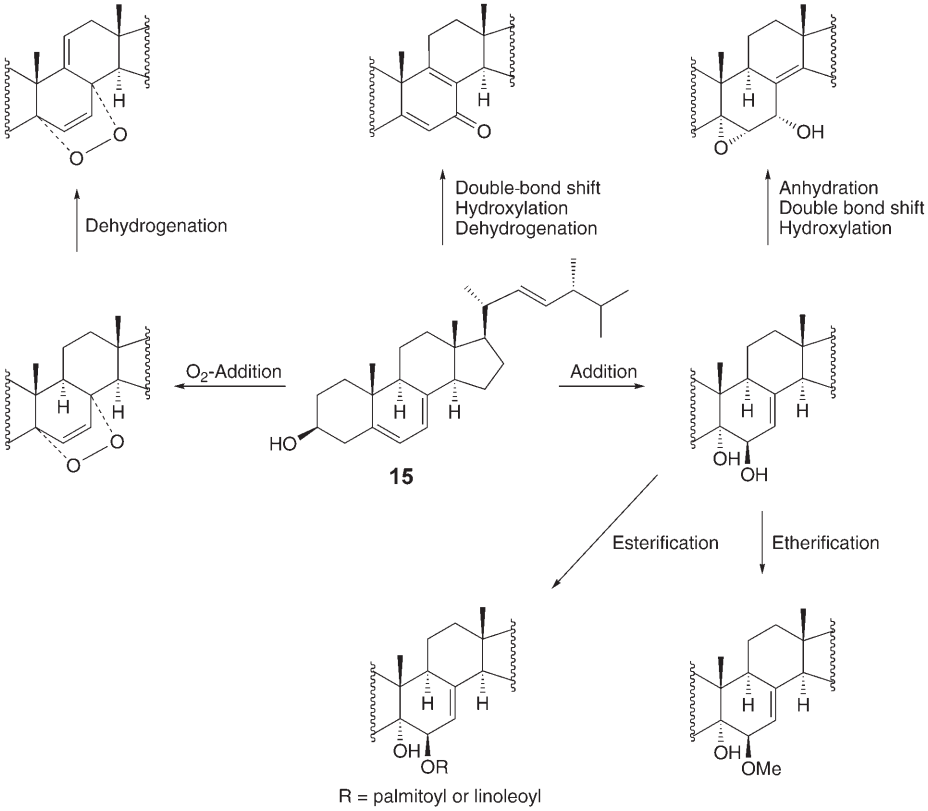
cerevisterol derivatives. The comparison of the ^{1}H- and ^{13}C-NMR data of **7** and **8** with those of **3** and **4** revealed the presence of an oleic acid moiety in **7** and the presence of a linoleic acid moiety in **8**, which were confirmed by the MS^n experiments and the GC/MS analysis of the methanolysis products. Thus, **7** was composed of cerevisterol and oleic acid, and **8** was composed of cerevisterol and linoleic acid, in agreement with the COSY, HMBC, and NOESY experiments. The observed esterification shifts located the oleoyl and linoleoyl moieties at O–C(6) in **7** and **8**, respectively. Therefore, **7** was determined as (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate, and **8** was determined as (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate, which were identical with the corresponding reported esters [7][8].

The structures of the other known compounds were elucidated by spectroscopic analysis and identified by comparison of their physical and spectral properties with references as (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol (=cerevisterol; **9**) [10], (3*β*,5*α*,6*β*,22*E*)-6-methoxyergosta-7,22-diene-3,5-diol (**10**) [10], (3*β*,5*α*,8*α*,22*E*)-5,8-epidioxyergosta-6,22-dien-3-ol (**11**) [18–20], (3*β*,5*α*,8*α*,22*E*)-5,8-epidioxyergosta-6,9,22-trien-3-ol (**12**) [18–20], (3*β*,5*α*,6*α*,7*α*,22*E*)-5,6-epoxyergosta-8(14),22-dien-3,7-diol (**13**) [9], (3*β*,22*E*)-3-hydroxyergosta-5,8,22-trien-7-one (**14**) [21], ergosterol (=(3*β*,22*E*)-ergosta-5,7,22-trien-3-ol; **15**), stearic acid (=octadecanoic acid), and palmitic acid (=hexadecanoic acid).

These known polyhydroxylated sterols bore cytotoxic activities, according to the studies reported before [9–15]. Compound **10** exhibited cytotoxicity against the Hela, A549, SK-OV-3, SK-MEL-2, XF 498, and HCT15 cell lines [9][10]. Epidioxysterol **11** showed cytotoxicity against the L-1210 cell line [11], the MCF-7 and Walker 256 cell lines [12], and the PLC/PRF/5 and KB cell lines [13]. Epidioxysterol **12** was cytotoxic against the Kato III cell line [14] and showed inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase [15]. Epoxydiol **13** exhibited cytotoxicity against the HCT15 cell line [9]. We evaluated the cytotoxic activities of the fatty acid esters **1**–**7** against B16 and smmc-7721 cell lines by the MTT method. Their IC_{50} values were more than 100 μM, and the cytotoxicity against B16 and smmc-7721 cell lines was weak.

The monohydroxylated and polyhydroxylated sterols, especially ergostane-type sterols are common and widely distributed fungal metabolites. Interestingly, fatty acid esters of monosterols are common, while fatty acid esters of polyhydroxylated sterols are rare. There are only three reported polyhydroxylated ergostane-type steryl esters of fatty acids: (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate (**7**) and (3*β*,5*α*,6*β*,22*E*)-3,5-dihydroxyergost-22-en-7-one 6-oleate from *Tricholomopsis rutilans* [7], and (3*β*,5*α*,6*β*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate (**8**) from *Catathelasma imperiale* [8]. Here, we obtained the eight polyhydroxylated ergostane-type steryl esters **1**–**8** of fatty acids from the mangrove fungus *A. awamori*, comprising the six new compounds **1**–**6**. The sterol core of **1**–**8** was cerevisterol or 6-epicerevisterol, in which OH–C(6) is in allylic position. Consequently, the high reactivity of OH–C(6) might explain the location of esterification by biosynthesis in all the steryl esters **1**–**8** of fatty acids. A speculative biogenetic relationship between all obtained sterols is shown in the *Scheme*. These sterols might originate from ergosterol (**15**) by one or a combination of various reactions, involving dehydrogenation, hydroxylation, addition, dehydration, peroxidation, double-bond shift, etherification, or esterification. Interestingly, almost all of the proposed reactions occur in ring B of ergosterol (**15**). The plentiful

Scheme. *The Biogenetic Relation of the Sterols*



endogenous and exogenous (fermentation medium) fatty acids gave rise to steryl esters of fatty acids.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Haiyang Chemical Group Corporation*), *Sephadex LH-20 (Amersham Biosciences AB)*, and *ODS* (60–80 μm; *Merck*). TLC: silica gel GF_{254} (*Qingdao Haiyang Chemical Group Corporation*) and *RP-18* F_{254} (*Merck*). Anal. HPLC: *Shim-pack VP-ODS* column (4.6 × 250 mm); *PDA* detector (*SPD-M10A*). Semi-prep. HPLC: *Zorbax RX-C8* column (9.4 × 250 mm); UV detector (*SPD-10Avp*). Prep. HPLC: *Shim-pack PRC-ODS* column (20 × 250 mm); UV detector (*SPD-10Avp*). Optical rotations: *Jasco P-1020* polarimeter. NMR

Spectra: *Bruker AVANCE-400* NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C); in $CDCl_3$. GC/MS: *Shimadzu GCMS-QP2010* instrument; *DB-5-ms32* column (0.32 mm × 30 m). Electro-spray-ionization (ESI) ion-trap (IT) MS: *Bruker Esquire-2000* mass spectrometer; in *m*/*z*. HR-EI-MS: *Finnigan MAT95* mass spectrometer; in *m*/*z*.

Fungal Material. The fungus strain was isolated from the soils around the mangrove plant *Acrostichum speciosum* at Wenchang, Hainan Province, P. R. China. The species was identified as *A. awamori* by Prof. *Kui Hong*'s lab [3]. A voucher specimen was deposited at the Institute of Tropical Biosciences and Biotechnology, Haikou, P. R. China (No. 094811).

Fermentation, Extraction, and Isolation. An airlift fermentor was employed with 70 l of media (soluble starch (20 g/l), soybean extract (15 g/l), yeast powder (5 g/l), peptone (2 g/l), $CaCO_3$ (4 g/l), NaCl (4 g/l), natural sea water (0.5 l), and water (0.5 l); pH 7.2–7.4) at 28° for 4 d. The mycelia were obtained by filtration from the fermentation broth, spin-dried, and extracted with acetone under r.t. for two times. After evaporation of the acetone of the extract, the residue (*ca.* 90 g) was first subjected to CC (silica gel, $CHCl_3$/MeOH 100:0, 98:2, 95:5, 90:10, 80:20, 70:30, 50:50, and 0:100 (*v*/*v*)): *Fractions 1–18*. The inhibitory activities on B16 cell line proliferation of the fractions, determined by the MTT method, indicated that *Fr. 3* and *Fr. 4* ($CHCl_3$/MeOH 98:2) were bioactive. *Fr. 3* was further applied to CC (silica gel, cyclohexane/AcOEt gradient): *Fr. 3.1–3.16*. Stearic acid (11.4 mg) was obtained from *Fr. 3.3* (cyclohexane/AcOEt 95:5), and palmitic acid (5.6 mg) from *Fr. 3.5* (cyclohexane/AcOEt 95:5). *Fr. 3.9* (cyclohexane/AcOEt 80:20) was further separated by CC (*ODS*, MeOH) and finally by repeated semi-prep. HPLC (*Zorbax RX-C8*, MeOH/H_2O 93:7 and 95:5): **1** (9.8 mg), **2** (11.3 mg), **3** (3.3 mg), and **4** (4.1 mg). *Fr. 3.10* (cyclohexane/AcOEt 70:30) was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50), followed by prep. HPLC (*Shim-pack PRC-ODS*, MeOH/H_2O 90:10): **11** (34.0 mg) and **12** (12.1 mg). *Fr. 3.11* (cyclohexane/AcOEt 70:30) was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50), then by prep. HPLC (*Shim-pack PRC-ODS*, MeOH) and semi-prep. HPLC (*Zorbax RX-C8*, MeOH/H_2O 93:7): **5** (8.0 mg), **6** (9.4 mg), **7** (1.8 mg), and **8** (3.8 mg). Compound **15** (230 mg) was obtained by recrystallization from *Fr. 4*. *Fr. 4* was also applied to CC (silica gel, cyclohexane/AcOEt gradient): *Fr. 4.1–4.17*. *Fr. 4.12* (cyclohexane/AcOEt 70:30) was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 50:50), followed by prep. HPLC (*Shim-pack PRC-ODS*, MeOH/H_2O 85:15): **10** (31.7 mg) and **14** (6.0 mg). *Fr. 4.13* (cyclohexane/AcOEt 50:50) was purified by prep. HPLC (*Shim-pack PRC-ODS*, MeOH/H_2O 85:15): **13** (5.8 mg). *Fr. 4.15* (cyclohexane/AcOEt 0:100) was separated by CC (*ODS*, MeOH/H_2O 80:20), followed by recrystallization: **9** (32.6 mg).

*(3β,5α,6α,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Palmitate* (= *Hexadecanoic Acid (3β,5α,6α,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **1**): Colorless oily solid. $[\alpha]_D^{26} = +33.3$ ($c = 0.33$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-IT-MS (pos.): 691 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 691): 435 ($[M+Na-256]^+$), 417 ($[M+Na-256-18]^+$). ESI-IT-MS^3 (pos.; 691 – 435): 417 ($[(M+Na-256)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 650.5619 ($[M-H_2O]^+$, $C_{44}H_{74}O_3^+$; calc. 650.5638).

*(3β,5α,6α,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Stearate* (= *Octadecanoic Acid (3β,5α,6α,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **2**): Colorless oily solid. $[\alpha]_D^{26} = +23.7$ ($c = 0.17$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-IT-MS (pos.): 719 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 719): 435 ($[M+Na-284]^+$), 417 ($[M+Na-284-18]^+$). ESI-IT-MS^3 (pos.; 719 – 435): 417 ($[(M+Na-284)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 678.5947 ($[M-H_2O]^+$, $C_{46}H_{78}O_3^+$; calc. 678.5951).

*(3β,5α,6α,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Oleate* (= *(9*Z*)-Octadec-9-enoic Acid (3β,5α,6α,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **3**): Colorless oily solid. $[\alpha]_D^{26} = +31.4$ ($c = 0.23$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-IT-MS (pos.): 717 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 717): 435 ($[M+Na-282]^+$), 417 ($[M+Na-282-18]^+$), 305 ($[M+Na-282-130]^+$). ESI-IT-MS^3 (pos.; 717 – 435): 417 ($[(M+Na-282)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 676.5763 ($[M-H_2O]^+$, $C_{46}H_{76}O_3^+$; calc. 676.5794).

*(3β,5α,6α,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Linoleate* (= *(9*Z*,12*Z*)-Octadeca-9,12-dienoic Acid (3β,5α,6α,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **4**): Colorless oily solid. $[\alpha]_D^{25} = +6.6$ ($c = 0.18$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-IT-MS (pos.): 715 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 715): 435 ($[M+Na-280]^+$), 417 ($[M+Na-280-18]^+$). ESI-IT-MS^3 (pos.; 715 – 435): 417 ($[(M+Na-280)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 674.5617 ($[M-H_2O]^+$, $C_{46}H_{74}O_3^+$; calc. 674.5638).

*(3β,5α,6β,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Palmitate* (= *Hexadecanoic Acid (3β,5α,6β,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **5**): Colorless oily solid. $[\alpha]_D^{26} = -63.4$ ($c = 0.19$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-IT-MS (pos.): 691 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 691): 435 ($[M+Na-256]^+$), 417 ($[M+Na-256-18]^+$). ESI-IT-MS^3 (pos.; 691 – 435): 417 ($[(M+Na-256)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 650.5621 ($[M-H_2O]^+$, $C_{44}H_{74}O_3^+$; calc. 650.5638).

*(3β,5α,6β,22*E*)-Ergosta-7,22-diene-3,5,6-triol 6-Stearate* (= *Octadecanoic Acid (3β,5α,6β,22*E*)-3,5-Dihydroxyergosta-7,22-dien-6-yl Ester*; **6**): Colorless oily solid. $[\alpha]_D^{26} = -31.1$ ($c = 0.15$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-IT-MS (pos.): 719 ($[M+Na]^+$). ESI-IT-MS^2 (pos.; 719): 435 ($[M+Na-284]^+$), 417 ($[M+Na-284-18]^+$). ESI-IT-MS^3 (pos.; 719 – 435): 417 ($[(M+Na-284)-18]^+$). ESI-IT-MS (neg.): weak response. HR-EI-MS: 678.5939 ($[M-H_2O]^+$, $C_{46}H_{78}O_3^+$; calc. 678.5951).

Methanolysis of **3**, **4**, **7**, *and* **8** *and GC/MS Analysis.* The fatty acid ester (1 – 3 mg) was refluxed in 1M HCl in MeOH for 24 h. The resulting soln. was extracted with hexane and the combined org. phase dried (Na_2SO_4) for GC/MS analysis. GC: *DB-5-ms32* column (0.32 mm × 30 m); inj. temp. 250°; initial column temp. 80° for 2 min, then increase by 15°/min up to the final temp. of 260°; interface temp. 250°. EI-MS: source temp. 200°, electron energy 70 eV.

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