Structures of Two New 18,22-Cyclosterols, Emesterones A and B, from *Emericella heterothallica*

Tomoo HOSOE,[a] Tomohiro SAMESHIMA,[b] Kazuyuki DOBASHI,[b] and Ken-ichi KAWAI*,[a]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] Ebara 2–4–41, Shinagawa-ku, Tokyo 142, Japan and Central Research Laboratories, Mercian Corporation,[b] Johnan 4–9–1, Fujisawa-shi, Kanagawa 251, Japan.
Received November 6, 1997; accepted December 5, 1997

Two new 18,22-cyclosterols, emesterones A (2) and B (3) , were isolated along with Mer-NF8054X (1) from the culture filtrate of *Emericella heterothallica*. The structures of 2 and 3 were established as 3,11-dioxo-18,22-cycloergosta-6,8(14)-diene-5β,9β,23*S*-triol and 3,11-dioxo-18,22-cycloergosta-4,6,8(14)-triene-9β,23*S*-diol, respectively, on the basis of spectroscopic and chemical investigations. The antifungal activity of emesterone B (3) towards *Aspergillus fumigatus* was slightly weaker than that of Mer-NF8054X (1).

Key words *Emericella heterothallica*; 18,22-cycloergostane; emesterone A; emesterone B; Mer-NF8054X; antifungal activity

Recently we reported the isolation and structural determination of an 18,22-cyclosterol, Mer-NF8054X (**1**), from the culture filtrates of the heterothallic fungus, *Emericella* (*E.*) *heterothallica* (KWON, FENNELL *et* RAPER) MALLOCH *et* CAIN, strains ATCC 16847 (mating type A) and/or ATCC 16824 (mating type a).[1] Compound **1** was originally isolated from *Aspergillus* (*A.*) *ustus* (BAIN.) THOM *et* CHURCH as an antifungal antibiotic against some strains of *A. fumigatus* FRES.[2] In the course of searching for other biologically active sterols from *E. heterothallica*, two new 18,22-cyclosterols designated as emesterones A (**2**) and B (**3**) were isolated. The structural elucidation of **2** and **3** is described in this paper.

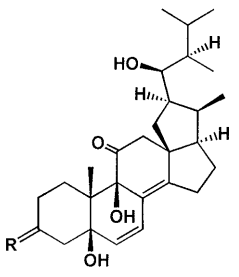
Structure of Emesterone A (2) The molecular formula of emesterone A (**2**) was confirmed to be $C_{28}H_{40}O_5$, which showed the loss of two hydrogen atoms from Mer-NF8054X (**1**), by high-resolution electron-impact ionization mass spectrometry (EI-MS). The ^{1}H-NMR spectrum of **2** was similar to that of **1** (Table 1). The ^{1}H-NMR signal at δ 3.53 in **1**, which was assigned as 3-H, disappeared in **2**, whereas the ^{13}C-NMR signal at δ 67.2 assigned to C-3 in **1** moved downfield in **2** to δ 206.9, which should be assigned to the carbonyl carbon of a ketone. Therefore, **2** was assumed to be a 3-oxo derivative of **1**. In order to confirm the structure, **1** was oxidized with pyridium chlorochromate (PCC) to give **2**, which was identical with naturally occurring emesterone A, based on a comparison of spectroscopic data, including circular dichroism (CD) curves. The structure of emesterone A (**2**) was consequently confirmed to be 3,11-dioxo-18,22-cycloergosta-6,8(14)-diene-5β,9β,23*S*-triol.

Structure of Emesterone B (3) The molecular formula of emesterone B (**3**) was confirmed to be $C_{28}H_{38}O_4$ by high-resolution EI-MS. From a consideration of the molecular formulae, **3** was assumed to be a dehydrate of emesterone A (**2**). The characteristic differences of the ^{1}H- and ^{13}C-NMR spectra between **2** and **3** were as follows (Table 1): two methylene proton signals (δ 2.41 and 2.78), assigned to the methylene at C-4 next to the ketone in **2**, disappeared in **3** and the olefinic proton signal at δ 5.71 (s) newly appeared in **3**, while the signal of one methylene carbon (δ 52.1) and one carbon bearing a hydroxyl group (δ 75.4) in **2** were changed to those of two olefinic carbons (δ 121.9 and 161.6) in **3**. The upfield shift of the carbonyl carbon at C-3 from **2** (δ 206.9) to **3** (δ 198.2) and the bathochromic shift of the absorption maximum from **2** (245 nm) to **3** (337 nm) in the UV spectra suggested that the 5-hydroxyl group was removed and a double bond was introduced between C-4 and C-5. In order to confirm the structure, **2** was treated with *p*-toluenesulfonic acid and calcium chloride to give **3**, which was identical with naturally occurring emesterone B, including the CD curves. The absolute structure of emesterone B (**3**) was consequently confirmed to be 3,11-dioxo-18,22-cycloergosta-4,6,8(14)-triene-9β, 23*S*-diol.

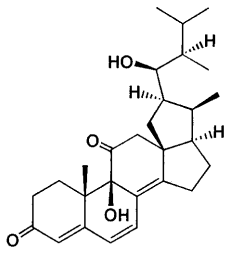
Biological Activity The antifungal activity of Mer-NF8054X (**1**) and emesterones A (**2**) and B (**3**) was examined by the serial micro-broth dilution method,[3] with incubation in Yeast Nitrogen Base (Difco) broth supplemented with glucose at 35 °C for 40 h. The antifungal activity of **3** against *A. fumigatus* strain IFM4942 was slightly weaker than that of Mer-NF8054X (**1**), but that of emesterone A (**2**) was much weaker, as shown in Table 2. The IC_{50} of **1**—**3** for various human tumor cells (K562, HT29, HT180, MCF7, PC6, and MKN28) were more than 100 μg/ml, except that of **3** for K562 (IC_{50} 39 μg/ml).

Discussion

Emesterones A (**2**) and B (**3**) are the second examples of 18,22-cyclosterols isolated from fungi. All of the stereochemistry remained in emesterones A (**2**) and B (**3**) is the same as in ergosterol and Mer-NF8054X (**1**), which might



1: R = β-OH
2:R = O



3

Chart 1

* To whom correspondence should be addressed.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of 18,22-Cyclosterols (**1**—**3**) in $CDCl_3$

Carbon No.	**1**[a]		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H (Hz)	δ_C	δ_H (Hz)
1	32.5	α 1.50 ddd	32.8	α 1.78 ddd (13.7, 8.9, 4.9)	30.9	α 2.17 ddd (11.9, 6.8, 6.1)
		β 1.29 ddd		β 1.59 m		β 1.68 ddd (11.9, 6.4, 5.5)
2	30.6	α 1.79 br d	37.1	α 2.23 ddd (14.3, 7.3, 4.9)	34.1	α 2.44 ddd (12.5, 6.8, 5.5)
		β 1.41 dddd		β 2.42 ddd (14.3, 8.9. 4.2)		β 2.61 ddd (12.5, 6.4, 6.1)
3	67.2	α 3.53 dddd	206.9		198.2	
4	44.3	α 1.89 ddd	52.1	α 2.41 d (14.0)	121.9	5.71 s
		β 1.75 dd		β 2.78 d (14.0)		
5	72.8		75.4		161.6	
5-OH		4.43 s		4.53 s		
6	133.5	5.66 d	131.6	5.63 d (10.1)	128.2	6.41 d (9.8)
7	123.2	6.26 d	123.8	6.26 d (10.1)	130.8	6.57 d (9.8)
8	125.7		125.2		126.2	
9	80.4		79.9		75.9	
9-OH		4.14 s		4.16 s		3.26 br
10	43.2		43.4		44.0	
11	212.5		212.0		211.7	
12	52.1	α 2.63 d	51.3	α 2.63 d (11.8)	51.1	α 2.51 d (12.8)
		β 2.68 d		β 2.70 d (11.8)		β 2.76 d (12.8)
13	53.1		55.3		55.6	
14	151.3		152.7		153.7	
15	29.2	α 2.60 ddd	29.3	α 2.59 ddd (14.3, 8.9, 4.2)	29.5	α 2.57 ddd (13.1, 4.0, 3.7)
		β 2.37 ddd		β 2.41 ddd (14.3, 8.9, 3.9)		β 2.46 ddd (13.1, 9.2, 6.1)
16	25.5	α 1.68 dddd	25.4	α 1.67 ddd (8.9, 4.5, 3.4)	25.7	α 1.71 dddd (13.1, 9.2, 4.2, 3.7)
		β 1.86 dddd		β 1.86 dddd (11.9, 8.4, 7.6, 3.9)		β 1.84 dddd (13.1, 8.5, 6.1, 4.0)
17	54.8	2.39 ddd	54.7	2.38 ddd (8.4, 7.6, 4.5)	55.4	2.37 ddd (8.5, 8.3, 4.2)
18	36.9	α 1.18 dd	36.9	α 1.29 dd (13.6, 3.9)	38.4	α 1.36 dd (10.7, 3.1)
		β 1.23 dd		β 1.19 dd (13.6, 6.4)		β 1.42 dd (10.7, 7.0)
19 (Me)	14.4	1.14 s	14.2	1.34 s	21.0[b]	1.41 s
20	36.9	2.50 ddq	36.9	2.49 ddq (7.6, 7.3, 7.3)	36.6	2.52 m
21 (Me)	10.4	1.01 d	10.3	1.01 d (7.3)	10.7	1.05 d (7.6)
22	46.6	2.14 dddd	46.6	2.15 dddd (10.1, 7.3, 6.4, 3.9)	46.4	2.19 m
23	72.2	3.75 dd	72.1	3.75 dd (10.1, 1.5)	72.4	3.78 dd (10.1, 1.5))
24	42.5	1.01 m	42.5	1.05 m	42.4	0.99 m
25	30.5	1.54 m	30.6	1.55 m	30.6	1.56 dqq (6.7, 6.4, 6.4)
26 (Me)	21.0	0.91 d	21.0	0.91 d (6.7)[b]	21.0[b]	0.93 d (6.4)[b]
27 (Me)	21.0	0.91 d	21.0	0.92 d (6.7)[b]	21.1[b]	0.93 d (6.4)[b]
28 (Me)	9.4	0.85 d	9.4	0.86 d (6.7)	9.5	0.86 d (6.7)

a) See ref. 1. *b*) The signals may be reversed.

Table 2. Antifungal Activity of 18,22-Cyclosterols (**1**—**3**)

Test organism	MIC (μg/ml)		
	1	**2**	**3**
A. fumigatus IFM4942	10	>80	20
A. fumigatus IFM41088	20	>80	80
Candida albicans IFM40009	>80	>80	>80

be a precursor of **2** and **3**. It is interesting that these biologically active 18,22-cyclosterols (**1**—**3**) were obtained from the culture filtrate but not from the mycelium, in view of the role of these compounds.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. EI-MS were taken with a JEOL JMS-D-300 spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL Lambda-500 (^{1}H, 500.00 MHz; ^{13}C, 125.43 MHz) or a JEOL Lambda-600 (^{1}H, 600.05 MHz; ^{13}C, 150.80 MHz) spectrometer, using tetramethylsilane as an internal standard. CD curves were determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). Low-pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (200 × 10 mm) packed with silica gel CQ-3 (30—50 μm, Wako). HPLC was performed with a Senshu SSC-3160 pump (flow rate, 3 ml/min) and a YMC-Pack SIL-06 pre-packed column (300 × 10 mm), equipped with a Shimamura YAD-883 RI detector. TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by spraying with 10% H_2SO_4 and then heating.

Isolation of Emesterones B and C from *E. heterothallica* *E. heterothallica*, strain ATCC 16824, was cultivated at 27 °C for 21 d in 40 Roux flasks containing 250 ml of Czapek medium supplemented with 0.1% yeast extract in each flask. The filtered culture broth (10 l) was acidified with 4 N HCl, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and then evaporated *in vacuo*. The obtained residue (0.8 g) was chromatographed on silica gel with $CHCl_3$–MeOH (100 : 1), followed by repeated LPLC [C_6H_6–EtOAc (3 : 1)] and HPLC [$CHCl_3$–MeOH (200 : 1)] to give emesterone A (**2**) (14 mg) and emesterone B (**3**) (2 mg). Mer-NF8054X (**1**) (21 mg) had been obtained from the above residue. Compounds **1**—**3** were also isolated from the culture filtrate of *E. heterothallica*, strains ATCC 16847.

Emesterone A (**2**): Colorless crystalline powder, mp 108—109 °C (from hexane–acetone), $[\alpha]_D^{20}$ +175° (c=0.14, $CHCl_3$). EI-MS m/z (%): 456.2883 (M^+, 456.2876 for $C_{28}H_{40}O_5$, 2), 438 ($M-H_2O$, 39), 420 ($M-2H_2O$, 55), 405 ($M-2H_2O-Me$, 22), 99 (31), 71 (65), 55 (47), 43 (100). UV λ_{max}^{MeOH} nm (log ε): 245 (4.34). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1710 (C=O). CD (MeOH) Δε (nm): +5.4 (215), −8.2 (234), +5.2 (277), +8.5 (303). The assignments of ^{1}H- and ^{13}C-NMR signals are summarized

in Table 1.

Emesterone B (**3**): Colorless crystalline powder, mp 105—106 °C (from hexane–acetone), $[\alpha]_D^{20}$ +416° (c=0.09, $CHCl_3$). EI-MS m/z (%): 438.2764 (M^+, 438.2769 for $C_{28}H_{38}O_4$, 10), 420.2665 ($M-H_2O$, 420.2665 for $C_{28}H_{36}O_3$, 90), 402 ($M-2H_2O$, 35), 202 (31), 91 (43), 71 (43), 55 (51), 43 (100). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 221 (4.10), 337 (4.36). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1710 (C=O). CD (MeOH) $\Delta\varepsilon$ (nm): −10.3 (218), −3.9 (232), −2.0 (252), +21.8 (305), −8.2 (333), +9.1 (370). The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Oxidation of Mer-NF8054X (1) by PCC A solution of Mer-NF8054X (15 mg) in CH_2Cl_2 (20 ml) was treated with PCC (3 mg) and the reaction mixture was stirred at 0 °C for 1.5 h. After addition of 2-propanol (1 ml) to destroy PCC, the reaction mixture was evaporated. The residue was purified by HPLC [$CHCl_3$–MeOH (200 : 1)] to give **2** (2 mg), which was identical with naturally occurring emesterone A on the basis of comparisons of the ^{1}H-NMR, EI-MS, UV and CD spectra, and TLC and HPLC behavior. Mer-NF8054X (**1**) (11 mg) was also recovered by the above HPLC [$CHCl_3$–MeOH (50 : 1)].

Dehydration of Emesterone A (2) Small amounts of *p*-toluenesulfonic acid and $CaCl_2$ were added to a solution of emesterone A (**2**) (2 mg) in acetone (2 ml) and the mixture was stirred at 40 °C for 30 min. After neutralization with K_2CO_3, the reaction mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and then evaporated *in vacuo*. The resulting residue was purified by HPLC [$CHCl_3$–MeOH (200 : 1)] to give **3** (1.5 mg), which was identical with naturally occurring emesterone B on the basis of comparisons of the ^{1}H-NMR, EIMS, UV and CD spectra, and TLC and HPLC behavior.

Acknowledgements We are grateful to Dr. N. Kawahara of the National Institute of Health Sciences for the NMR measurements. We also thank Mrs. Y. Kawada and Mr. Komiya of Hoshi University for NMR and mass measurements. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 09672169) from the Ministry of Education, Science, Sports and Culture, Japan.

References

1) Mizuno R., Kawahara N., Nozawa K., Yamazaki M., Nakajima S., Kawai K., *Chem. Pharm. Bull.*, **43**, 9—11 (1995).
2) Sakai K., Chiba H., Kaneto R., Sakamoto M., Okamura K., Tone H., *J. Antibiot.*, **47**, 591—594 (1994).
3) Fukazawa Y., Nishikawa T., Uchida K., Shinoda T., Nishimura K., Hamamoto T., *Jpn. J. Med. Mycol.*, **32**, 239—260 (1991).