Stereochemistry of an 18,22-Cyclosterol, Mer-NF8054X, from *Emericella heterothallica* and *Aspergillus ustus*

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Received July 12, 1994; accepted September 20, 1994

An 18,22-cyclosterol, Mer-NF8054X (1), first isolated from a shaken culture of *Aspergillus ustus* as a strong antifungal agent with activity against *Aspergillus fumigatus*, was isolated from the culture filtrate of *Emericella heterothallica*. The absolute structure of 1 was established as 11-oxo-18,22-cycloergosta-6,8(14)-diene-3β,5β,9β,23*S*-tetraol by spectroscopic and chemical investigation, and X-ray crystallographic analysis.

Keywords *Emericella heterothallica*; *Aspergillus heterothallicus*; steroid; 18,22-cycloergostane; Mer-NF8054X

Recently we reported the isolation and structural elucidation of novel epidithiodioxopiperazines, emethallicins A (**2**), B (**3**), C (**4**), and D (**5**), as potent inhibitors of histamine release, from the mycelial extract of the heterothallic fungus, *Emericella heterothallica* (KWON, FENNELL *et* RAPER) MALLOCH *et* CAIN [anamorph: *Aspergillus heterothallicus* KWON, FENNELL *et* RAPER], strains ATCC 16847 (mating type A)[1] and/or ATCC 16824 (mating type a).[2] New sulfur-containing dioxopiperazines, emethacins A (**6**) and B (**7**), were also isolated along with two other dioxopiperazines (**8**, **9**) from the extract of the culture filtrate of *E. heterothallica*, strains ATCC 16824 and 16847.[3] In the course of searching for other biologically active compounds in the extracts of the culture filtrates of the above two strains of *E. heterothallica*, a steroid (**1**) was isolated. This compound was identical based on a comparison of the ^{1}H- and ^{13}C-NMR spectra with Mer-NF8054X, obtained from a newly isolated strain of *Aspergillus ustus* (BAIN.) THOM *et* CHURCH[4] along with Mer-NF8054A, an antifungal antibiotic active against some strains of *Aspergillus fumigatus* FRES. This compound (**1**) is a new type of steroid, *i.e.*, an 18,22-cycloergostane, but its stereochemistry has not been determined yet.[4] The determination of the stereochemistry of **1** is reported in this paper.

The ^{1}H- and ^{13}C-NMR signals in $CDCl_3$ were assigned (Table I) from the detailed analysis of various two dimensional (2D) NMR spectra [^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-bond correlation spectroscopy (HMBC), heteronuclear multiple quantum coherence (HMQC), *etc.*]. The configurations at C-3, C-5, and C-10 in **1** were confirmed by analysis of the nuclear Overhauser effect (NOE) spectroscopy (NOESY) spectrum (Fig. 1) and difference NOE spectrum, but the stereochemistry of the other 7 chiral centers could not be determined. In order to determine the whole stereochemistry of **1**, an X-ray crystallographic analysis of **1** was undertaken. Crystals of **1** acetone solvate were grown as

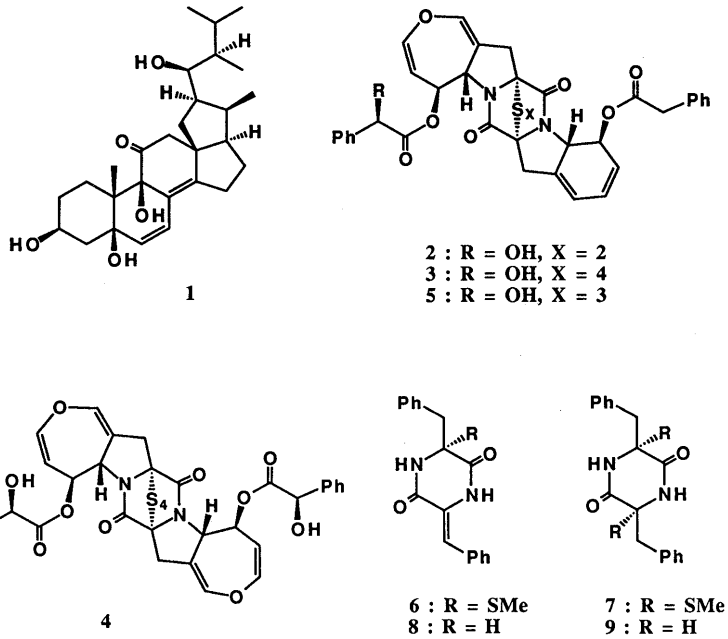

Chart 1

colorless prisms from hexane–acetone solution. The molecular structure of **1** acetone solvate is illustrated in Fig. 2. The molecules of **1** and acetone are connected by hydrogen bonds between O(3) and O(6) (2.764 Å). The molecules of **1** are linked by hydrogen bonds between O(1) and O(5) (2.828 and 2.875 Å). The crystal structure of **1** acetone solvate projected on the C=O bond from O(4) to C(11) is shown in Fig. 3. The positive Cotton effect ($\Delta\varepsilon$+6.8) at 308 nm is explicable in terms of the indicated absolute configuration of Mer-NF8054X as shown in **1**, based on application of the octant rule to the ketone at C-11.

TABLE I. ^{1}H- and ^{13}C-NMR Chemical Shifts of Mer-NF8054X (**1**) in $CDCl_3$

Carbon No.	δ_C	δ_H (Hz)
1	32.47	α 1.502 ddd (10.0, 9.8, 3.6)
		β 1.288 ddd (10.0, 3.3, 3.3)
2	30.64	α 1.788 br d (13.4)
		β 1.409 dddd (13.4, 11.7, 9.8, 3.3)
3	67.18	α 3.529 dddd (12.1, 11.7, 4.0, 4.0)
4	44.32	α 1.888 ddd (12.5, 4.0, 2.2)
		β 1.753 dd (12.5, 12.1)
5	72.80	
5-OH		4.426 s
6	133.49	5.658 d (10.2)
7	123.22	6.257 d (10.2)
8	125.71	
9	80.41	
9-OH		4.137 s
10	43.16	
11	212.49	
12	52.10	2.625 d (11.7)
		2.657 d (11.7)
13	53.10	
14	151.25	
15	29.22	α 2.602 ddd (14.7, 7.5, 3.2)
		β 2.368 ddd (14.7, 7.7, 6.7)
16	25.49	α 1.684 dddd (13.2, 8.5, 7.7, 3.2)
		β 1.860 dddd (13.2, 7.5, 7.5, 3.8)
17	54.82	α 2.385 ddd (8.5, 8.0, 3.8)
18	36.91	α 1.182 dd (13.4, 4.3)
		β 1.229 dd (13.4, 8.8)
19	14.42 (Me)	1.137 s
20	36.91	α 2.500 ddq (8.0, 7.5, 7.3)
21	10.36 (Me)	1.012 d (7.3)
22	46.60	α 2.141 dddd (10.1, 8.8, 7.5, 4.3)
23	72.19	3.745 dd (10.1, 1.7)
24	42.46	1.014 m
25	30.52	1.541 m
26	20.99 (Me)[a]	0.907[a] d (6.6)
27	21.03 (Me)[a]	0.911[a] d (6.6)
28	9.44 (Me)	0.846 d (6.6)

a) The signals may be reversed.

The absolute structure of Mer-NF8054X (**1**) was confirmed as 11-oxo-18,22-cycloergosta-6,8(14)-diene-3β, 5β,9β,23*S*-tetraol. Most of the stereochemistry in **1** is the same as that of ergosterol, which might be a precursor of **1**, but the stereochemistry at C-9 is opposite (9β-hydroxyl group). The stereochemistry of the new chiral centers in **1** is as follows: *R*-configuration at C-22 and *S* at C-23. Mer-NF8054X also has antifungal activities against some strains of *Aspergillus fumigatus*, being almost potent as the 11-deoxo derivative of **1** (Mer-NF8054A).[5]

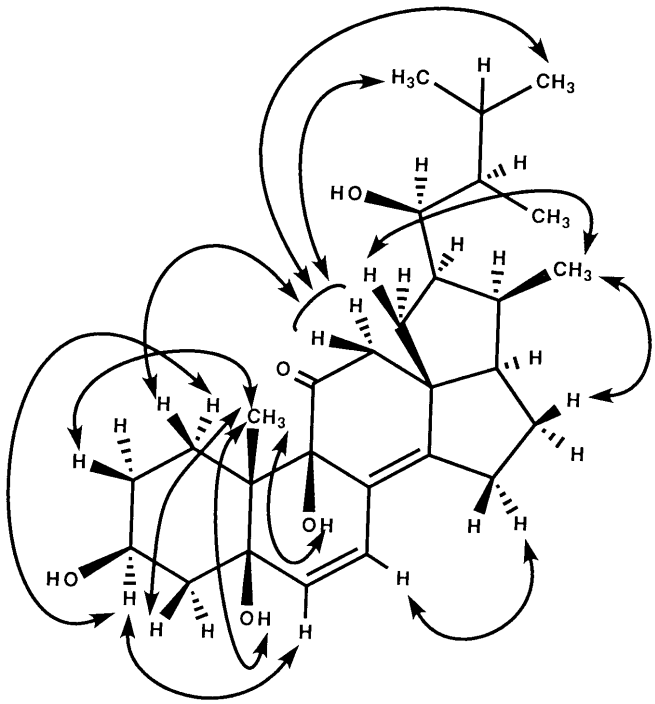

Fig. 1. NOESY Correlations of Mer-NF8054X (**1**)

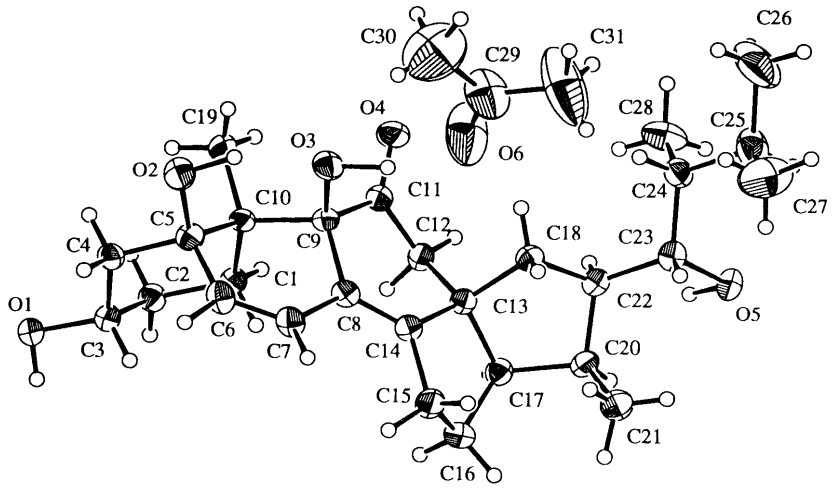

Fig. 2. Perspective View of the Crystal Structure of Mer-NF8054X (**1**) Acetone Solvate with Thermal Ellipsoids at 50% Probability

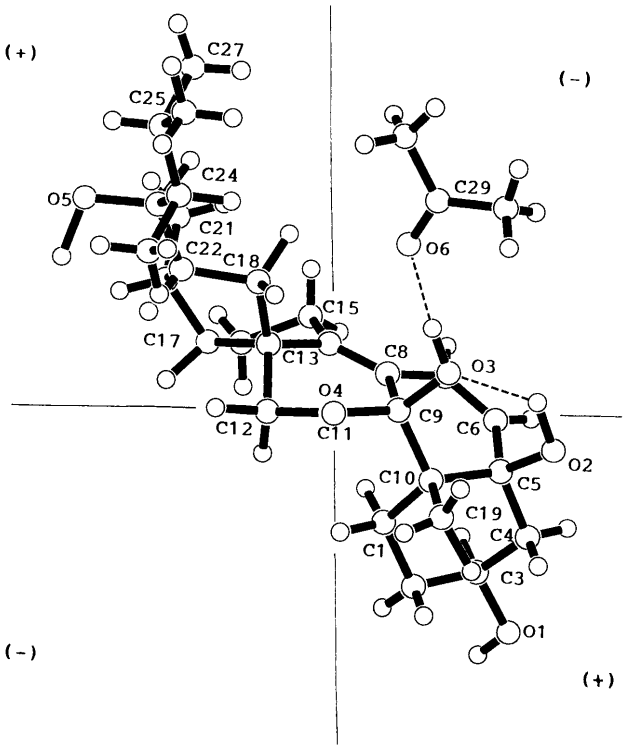

Fig. 3. Crystal Structure of Mer-NF8054X (**1**) Acetone Solvate Projected on the Carbonyl Bond at C-11

Experimental

General Procedures Melting point was determined on a Yanagimoto micro-melting point apparatus without correction. Optical rotation was measured with a JASCO DIP-181 spectrometer. Electron impact (EI)-MS was 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-NMR and ^{13}C-NMR spectra were recorded on a JEOL α-600 spectrometer at 600.05 and at 150.80 MHz, respectively, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = d, quartet = q, multiplet = m, and broad = br. The circular dichroism (CD) curve was determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (LPLC) was performed on a Chemco Low-Prep 81-M-2 pump and glass column (10 i.d. × 200 mm) packed with Silica gel CQ-3 (30—50 μm; Wako). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC plates were detected on the basis of their absorption of UV light and/or by spraying 5% H_2SO_4 followed by heating.

Isolation of Mer-NF8054X (1) from *E. heterothallica* *E. heterothallica*, strain ATCC 16824 (mating type a), and/or strain ATCC 16847 (mating type A), was cultivated at 27 °C for 14 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 [hexane–acetone (2 : 1) and/or benzene–AcOEt (1 : 3)] to give Mer-NF8054X (**1**) (21 mg).

Mer-NF8054X (**1**): Colorless prisms, mp 129—130 °C (from hexane–acetone), Liebermann–Burchard test: +(brown), $[\alpha]_D^{20}$ +105° (c = 1.01, $CHCl_3$). EI-MS m/z (%): 458.3020 (M^+, 458.3032 for $C_{28}H_{42}O_5$, 2), 440.2930 ($M-H_2O$, 440.2927 for $C_{28}H_{40}O_4$, 20), 422 ($M-2H_2O$, 28), 407 ($M-2H_2O-Me$, 13). UV λ_{max}^{MeOH} nm (log ε): 242 (4.31). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715, 1700 (C=O). CD $\Delta\varepsilon$(nm) (MeOH): +6.8 (308), +1.5 sh (262), −8.2 (234). The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table I.

X-Ray Structure Analysis of Mer-NF8054X (1) Acetone Solvate Crystals of **1** were grown from a mixed solution of hexane and acetone to yield **1** acetone solvate as colorless prisms. Crystal data: $C_{28}H_{42}O_5 \cdot C_3H_6O$; M = 516.72; orthorhombic; $P2_12_12_1$; a = 17.320(3), b = 21.912(4), c = 7.912(4) Å; V = 3002(1) Å^3; Z = 4; D_c = 1.143 g·cm^{-3}. The diffraction intensities were collected from a crystal with dimensions 0.40 × 0.10 × 0.05 mm on a Rigaku AFC-7R four-circle diffractometer using Cu$K\alpha$ radiation monochromated by means of a graphite plate. The total of 2589 reflections observed within a 2θ range of 120.1°, 2215 satisfied the criterion $I > 3\sigma(I)$ and only these were used in the solution and refinement of the structure.

Determination of the Structure The structure was solved by the direct method using SHELXS86[6] and refined by the full-matrix least-squares method. Most of the hydrogen atoms were found from the difference Fourier synthesis and the positions of some were calculated. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms, and the parameters for hydrogen atoms were fixed. Final R and R_w values are 0.054 and 0.073.[7]

Acknowledgments We wish to thank Dr. M. Shiro of Rigaku Corporation for the advice on X-ray analysis. We are grateful to the staff of Central Research Laboratories, Mercian Corporation for the data on Mer-NF8054X. We are also grateful to Miss Y. Nagayama of Hoshi University for mass measurements.

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7) Final atomic parameters, bond lengths and angles, and lists of F_O and F_C values are available from one of the authors (K.K.) upon request.