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NOTE

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Trierixin, a Novel Inhibitor of ER Stress-induced XBP1 Activation from *Streptomyces* sp.

II. Structure Elucidation

Yushi Futamura, Etsu Tashiro, Naoka Hironiwa, Jun Kohno, Maki Nishio, Kazutoshi Shindo, Masaya Imoto

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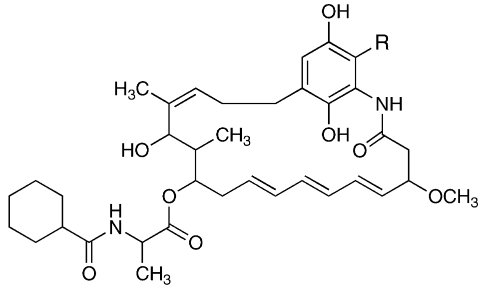
Abstract Trierixin, a new member of the triene-ansamycin group, has been isolated from the fermentation broth of *Streptomyces* sp. AC654 as an inhibitor of ER stress-induced XBP1 activation. The structure of trierixin was determined on the basis of its spectroscopical and chemical properties. Trierixin possessed a 21-membered macrocyclic lactam, which contains a methylthio-benzenediol structure, and a cyclohexanecarbonylalanine moiety. Trierixin is thus elucidated as 21-thiomethylmycotrienin II.

Keywords trierixin, triene-ansamycin, ER stress, XBP1

In the course of screening for an inhibitor of ER stress-induced XBP1 activation in HeLa cells, we isolated trierixin (**1**, Fig. 1), a new member of the triene-ansamycin group, from the fermentation broth of *Streptomyces* sp. AC654. The taxonomy of the producing strain, and the fermentation, isolation, and biological activities of **1** were reported in the preceding paper [1]. In this paper, we describe the physico-chemical properties and structure elucidation of **1**.

The molecular formula of **1** was determined to be $C_{37}H_{52}N_2O_8S$ on the basis of HRESI-MS [$(M-H)^-$, *m*/*z* 683.3372 (+0.60 mmu)]. The UV spectrum of **1** in MeOH exhibited maximum absorption at 261, 271, and 281 nm, indicating that **1** contains a triene moiety in the molecule [2~6]. The other UV absorption at 315 nm shifted to longer wavelengths (+20 nm) by adding a drop of 1 M NaOH. This characteristic shift suggested the presence of phenolic OH(s) in **1**. The IR spectrum revealed that **1** possesses NH/OH (3420 cm^{-1}), ester (1740 cm^{-1}), and amide (1650 cm^{-1} and 1540 cm^{-1}) functionalities. The physico-chemical properties of **1** are summarized in Table 1.

In the isolation process of **1**, we also isolated and



1 R = SCH_3
2 R = H

Fig. 1 Structures of trierixin (**1**) and mycotrienin II (**2**).

E. Tashiro (Corresponding author), **Y. Futamura, N. Hironiwa, M. Imoto:** Department of Bioscience and Informatics, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan,
E-mail: tashiro@bio.keio.ac.jp

K. Shindo: Department of Food and Nutrition, Japan Women's University, 2-8-1, Mejirodai, Bunkyo-ku, Tokyo 112-8681, Japan
J. Kohno, M. Nishio: Discovery Research Laboratories, Tanabe Seiyaku Co., Ltd., 2-2-50, Kawagishi, Toda-shi, Saitama 335-8505, Japan

Table 1 Physico-chemical properties of trierixin (**1**)

Appearance	pale pink powder
Melting point (°C)	121∼122
HRESI-MS (negative)	
found	683.3372 $(M-H)^-$
calcd	683.3366 (for $C_{37}H_{51}N_2O_8S$)
Molecular formula	$C_{37}H_{52}N_2O_8S$
Molecular weight	684
$[\alpha]_D^{20}$	+306.2° (c 0.2, $CHCl_3$)
UV λ_{max} nm (log ε)	
in MeOH	261.5 (4.54), 271.0 (4.66), 281.0 (4.56), 315.0 (3.65)
in 0.1 N HCl-MeOH	261.5 (4.56), 271.0 (4.67), 281.0 (4.57), 315.0 (3.67)
in 0.1 N NaOH-MeOH	261.5 (4.43), 271.0 (4.50), 281.0 (4.40), 335.0 (3.75)
IR ν_{max} (KBr) cm^{-1}	3420, 2930, 2860, 1740, 1650, 1540, 1470, 1380, 1290, 1210, 1160, 1090, 1000, 960, 730
HPLC Rt (minute)[a]	7.9

[a] Column; (CAPCEL PAK C_{18}) UG120 (4.6 mm×250 mm, shiseido), 80% MeOH, 0.7 ml/minute.

identified mycotrienin II (**2**, Fig. 1) [2, 7] as described in the preceding report [1]. Since the UV and ^{1}H-NMR spectra of **1** were quite similar to those of **2**, structural studies on **1** were performed by comparing with **2**.

The ^{1}H- and ^{13}C-NMR for **1** were assigned by analyzing ^{1}H, ^{13}C, COSY, HMQC, and HMBC spectra, and compared with those for **2** (Table 2) [7]. The ^{1}H- and ^{13}C-NMR spectral data for **1** are summarized in Table 2. This comparison proved that the partial structures from C-1 to C-17 and from C-27 to C-36 (cyclohexanecarbonylalaninyl moiety) in **2** were completely preserved in **1**, while one singlet methyl (δ_H 2.17) was observed only in **1**, and two aromatic methines in **2** [2] were decreased to one (δ_H 6.78) in **1**. Considering the difference in the molecular formula between **1** and **2** (CH_2S), the attachment of SCH_3 to C-21 or C-23 in **1** was thus speculated. The attachment of the SCH_3 group at C-21 was determined by the observation of ^{1}H-^{13}C long-range couplings from 1-NH (δ_H 8.53) and SCH_3 to C-21 (δ_C 110.9), and from H-23 (δ_H 6.78) to C-17 (δ_C 33.2) (Fig. 2). The linkage of cyclohexanecarbonylalanine at C-11 was confirmed by the observation of a downfield shift (acylation shift) at H-11 (δ_H 4.88) [8].

The geometries of C-4, C-6, and C-8 were determined to be all E by the coupling constants of $J_{4,5}=15.4$ Hz, $J_{6,7}=15.0$ Hz and $J_{8,9}=15.0$ Hz, respectively. The geometry of C-14 was determined to be Z by the ^{13}C chemical shift

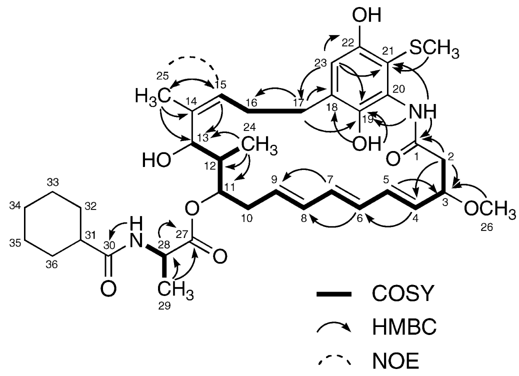


Fig. 2 Selected 2D correlations for **1**.

of C-25 (δ_C 21.2) and NOE observation between H-15 and H-25 (Fig. 2).

From the above findings, the structure of **1** was determined as shown in Fig. 1; Trierixin (**1**) is 21-thiomethylmycotrienin II. Studies on the relative stereochemistry are now underway.

Experimental

Melting points were determined with Yanagimoto micro melting point apparatus and are uncorrected. Mass spectra were measured with a JEOL JMS-T100LC mass spectrometer. Optical rotations were obtained on a JASCO P-1030 polarimeter using a micro-cell (light path 10 cm). UV spectra and IR spectra were recorded on a Hitachi U-2800 spectrophotometer and a Horiba FT-210 spectrometer in KBr disc, respectively. ^{1}H- and ^{13}C-NMR spectra and 2D-NMR were obtained in $CDCl_3$ on a JEOL JMN-AL-300 spectrometer using TMS as internal standard.

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Table 2 [1]H- and [13]C-NMR data for trierixin (**1**) and mycotrienin II (**2**)

No.	**1**		No.	**2**
	δ_C ppm (multiplicity)	δ_H ppm (multiplicity, *J* in Hz)		δ_C ppm (multiplicity)
1	170.8 (s)		1	169.7 (s)
2	43.5 (t)	2.66 (1H, dd, 8.6, 13.6)	2	43.1 (t)
	—	2.97 (1H, dd, 4.5, 13.7)		—
3	80.0 (d)	4.23 (1H, m)	3	79.6 (d)
4	129.8 (d)	5.50 (1H, dd, 7.3, 15.4)	4	129.1 (d)
5	135.8 (d)	6.28 (1H, dd, 10.4, 15.4)	5	134.4 (d)
6	130.3 (d)	6.04 (1H, dd, 10.4, 15.0)	6	129.5 (d)
7	135.5 (d)	6.23 (1H, dd, 10.4, 15.0)	7	134.9 (d)
8	134.7 (d)	6.04 (1H, dd, 10.4, 15.0)	8	133.9 (d)
9	130.4 (d)	5.63 (1H, m)	9	129.6 (d)
10	34.9 (t)	2.31, 2.48 (2H, m)	10	33.7 (t)
11	76.3 (d)	4.88 (1H, m)	11	75.8 (d)
12	39.8 (d)	1.85 (1H, m)	12	39.0 (d)
13	69.4 (d)	4.64 (1H, m)	13	68.7 (d)
14	139.0 (s)		14	137.8 (s)
15	124.8 (d)	5.11 (1H, d, 8.8)	15	124.3 (d)
16	27.1 (t)	2.02, 2.39 (2H, m)	16	26.6 (t)
17	33.2 (t)	2.17, 3.07 (2H, m)	17	31.7 (t)
18	136.9 (s)		18	132.7 (s)
19	143.5 (s)		19	141.1 (s)
20	127.1 (s)		20	125.5 (s)
21	110.9 (s)		21	107.5 (d)
22	150.9 (s)		22	149.2 (s)
23	116.1 (d)	6.78 (1H, s)	23	115.8 (d)
24	10.5 (q)	0.79 (3H, d, 6.8)	24	9.6 (q)
25	21.2 (q)	1.70 (3H, s)	25	20.3 (q)
26	57.6 (q)	3.36 (3H, s)	26	56.6 (q)
27	174.1 (s)		27	173.3 (s)
28	49.5 (d)	4.43 (1H, m)	28	48.7 (d)
29	18.8 (q)	1.42 (3H, d, 7.1)	29	17.7 (q)
30	177.4 (s)		30	176.9 (s)
31	46.0 (d)	2.10 (1H, m)	31	45.1 (d)
32	30.4 (t)	1.23, 1.85 (2H, m)	32	29.4 (t)
33	26.6 (t)		33	25.6 (t)
34	26.6 (t)		34	25.6 (t)
35	26.7 (t)	1.23~1.75 (6H, m)	35	25.7 (t)
36	30.5 (t)	1.23, 1.85 (2H, m)	36	29.4 (t)
1-NH		8.53 (1H, s)		
19-OH		7.68 (1H, s)		
21-SCH_3	19.4 (q)	2.17 (3H, s)		
29-NH		5.94 (1H, d, 6.6)		

Recorded at 300 MHz for ^1H and 75 MHz for ^{13}C in $CDCl_3$.

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