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Studies on Fungal Products. XIV.¹⁾ Emestrin B, a New Epitrithiodioxopiperazine, from *Emericella striata*

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In the course of a search for metabolites related to emestrin (**2**) from *Emericella striata*, a new compound designated emestrin B (**1**) was isolated, together with shamixanthone, from the mycelial acetone extract. Shamixanthone is a compound related to emericellin, which had previously been isolated from the mycelium of the above fungus. The structure of emestrin B (**1**), including its absolute configuration, was established on the basis of the spectroscopic investigations. Emestrin B (**1**) is an epitrithio derivative corresponding to the antifungal macrocyclic epidithiodioxopiperazine, emestrin (**2**).

Keywords—*Emericella striata*; epitrithiodioxopiperazine; epidithiodioxopiperazine; emestrin B; emestrin; shamixanthone; antibacterial activity

Recently we reported^{2,3)} the isolation and structural elucidation of the characteristic macrocyclic epidithiodioxopiperazine, emestrin (**2**), from the mycelium and its degradation product, violaceic acid (**3**), from the culture filtrate of *Emericella striata* (RAI, TEWARI *et* MUKERJI) MALLOCH *et* CAIN (strain 80-NE-22). In the course of a search for metabolites related to **2**, dethiosecoemestrin (**4**)⁴⁾ and aurantioemestrin (**5**)^{5,6)} were isolated from the culture filtrate of the above fungus. From the structures and chemical reactions of **4** and **5**, we postulated^{5,6)} that **2** was biologically degraded to **5** at first, then to **4**, and finally to **3**. In order to find key intermediates in the transformation from **2** to **3**, **4**, and/or **5**, further investigation of the mycelial acetone extract was performed, and two compounds were isolated: a new compound designated as emestrin B (**1**), from the fraction slightly more polar than emestrin (**2**), and shamixanthone, from the fraction slightly less polar than emericellin. Shamixanthone, accompanied with emericellin, was also obtained from *E. varicolor* BERK. *et* BR.⁷⁾ and *E.*

Chart 1

ruglosa (THOM *et RAPER*) C. R. BENJAMIN,⁸⁾ whose anamorphs are belonging to the section *Nidulantes* in the genus *Aspergillus* as same as *E. striata*. The structural elucidation of **1** is reported in this paper.

Emestrin B (**1**), mp 230—238 °C (dec.), gave a molecular ion at m/z 630 in field desorption (FD) mass spectrometry, and the elemental analysis, including sulfur, confirmed the molecular formula as $C_{27}H_{22}N_2O_{10}S_3$, which showed that **1** had only one more sulfur atom than **2**. The proton nuclear magnetic resonance (1H -NMR) spectrum of **1** is similar to that of **2** (Table I), and the carbon-13 nuclear magnetic resonance (^{13}C -NMR) spectrum of **1** is also closely similar to that of **2** (Table II). Positive silver nitrate test (dark brown)⁹⁾ and the ion at m/z 566 (M-S₂) and 534 (M-S₃) in the FD mass spectrum suggested the presence of a trithio bond in emestrin B (**1**). From the above results, the relative structure of emestrin B was determined as **1**. The 1H -NMR chemical shifts of the signals assigned to the protons of the dihydrooxepine ring and to the aromatic protons in **1** are close to those of triacetyldidethiobis(methylthio)emestrin (**6**), which was derived from **2** by acetylation and reductive methylation,³⁾ rather than **1**, as shown in Table I. This may be because of a change of conformation in going from **2** to **1**.

The circular dichroism (CD) spectrum of **1**, whose absolute stereochemistry is known,³⁾

TABLE I. 1H -NMR Chemical Shifts of Emestrin B (**1**) and Related Compounds in $CDCl_3$

Proton No.	1	2^{a)}	6	Proton No.	1	2^{a)}	6
2-NMe	3.536	3.255	3.15	6'-H	6.933 ^{d)}	7.166	7.25 ^{d)}
5a-H	5.402	5.672	5.35	7'-H	4.809	4.967	5.93
6-H	5.328	4.672	5.35	2''-OMe	4.065	3.944	3.98
7-H	5.001	4.910	4.98	3''-H	7.026 ^{c)}	7.211	7.05 ^{c)}
8-H	6.358	6.412	6.33	4''-H	7.862 ^{d)}	7.576	7.76 ^{d)}
10-H	6.828	7.063	7.04	6''-H	8.351 ^{b)}	7.377	8.08 ^{b)}
11-H	5.226	5.466	6.51	OAc			2.18, 2.31, 2.34
2'-H	8.718 ^{b)}	7.767	8.80 ^{b)}	SMe			1.50, 1.98
5'-H	6.805 ^{c)}	6.883	6.96 ^{c)}				

a) The spectrum was measured in $DMSO-d_6$. b—d) Assignments may be reversed.

TABLE II. ^{13}C -NMR Chemical Shifts of Emestrin B (**1**) and Emestrin (**2**)

Carbon No.	1^{a)}	2^{b)}	Carbon No.	1^{a)}	2^{b)}
1	165.36 (Sq) ^{c)}	164.28 (Sm)	1'	127.85 (Sdd)	127.08 (Sm)
2-NMe	28.32 (Q)	27.32 (Q)	2'	121.16 (Dbrd)	120.35 (Dbrd)
3	83.41 (Sd)	75.55 (Sd)	3'	146.87 (Sm) ^{e)}	145.31 (Sdd) ^{d)}
4	163.28 (Sd)	160.55 (Sd)	4'	154.11 (Sm)	153.26 (Sm)
5a	58.27 (Dm)	59.96 (Dm)	5'	114.80 (D)	115.42 (Dbrs)
6	74.61 (Dm) ^{d)}	72.88 (Dm)	6'	126.34 (Dm)	122.66 (Dm)
7	109.73 (Ddd)	107.15 (Ddd)	7'	78.39 (Dd)	74.76 (Dd)
8	138.34 (Dm)	137.18 (Ddd)	1''	144.54 (Sm) ^{e)}	143.44 (Sdd) ^{d)}
10	141.10 (Dm)	141.68 (Dm)	2''-OMe	56.27 (Q)	55.84 (Q)
10a	110.38 (Sdd)	112.39 (Sdd)	3''	112.19 (D)	112.34 (D)
11	75.47 (Dm) ^{d)}	74.76 (Dm)	4''	128.87 (Dm)	124.92 (Ddd)
11a	78.78 (Sm)	81.00 (Sm)	5''	122.51 (Sd)	122.15 (Sdd)
			6''	125.94 (Dd)	123.95 (Dd)
			7''	166.38 (Sdd)	164.43 (Sdd)

a) The spectrum was measured in a mixture of $CDCl_3$ and $DMSO-d_6$. b) The spectrum was measured in $DMSO-d_6$. c) Capital and small letters refer to $^1J_{C,H}$ and $^{\gamma}J_{C,H}$, respectively. d, e) Assignments may be reversed.

showed maxima at 228 (negative), 257 (positive), and 293 nm (positive), whereas that of **2** showed maxima at 233 (negative), 266 (positive), and 301 nm (positive). Thus, it is clear that **1** and **2** have 3*R*, 11*aR* configurations and consequently the absolute structure of emestrin B is as depicted in **1**.¹⁰⁾

It is interesting that emestrin B (**1**), an epitritiodioxopiperazine, was isolated along with emestrin (**2**), an epidithiodioxopiperazine, from the same fungus, *E. striata*, in relation to biosynthesis of dethiosecoemestrin (**4**) and aurantioemestrin (**5**). In the previous paper,^{3,4)} we reported that emestrin (**2**) had antifungal and antibacterial activity, and that the activities of **2** against bacteria seemed to be slightly less than those against fungi. Recently we reported¹¹⁾ that **2** showed effective inhibition at 0.78–1.56 $\mu\text{g/ml}$ against *Tricophyton* spp. and at 3.125–6.25 $\mu\text{g/ml}$ against *Microsporium* spp. (*in vitro*). In a preliminary test,⁴⁾ emestrin B (**1**) also showed inhibitory activity at 25 μg per disc against *Bacillus subtilis* and *Escherichia coli*. Further studies on the antibacterial and antifungal activity are planned.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Infrared (IR) and ultraviolet (UV) spectra were recorded on a Hitachi 215 spectrophotometer and a Hitachi 124 spectrophotometer, respectively. FD mass spectra (MS) were obtained on a JEOL JMS-D 300 spectrometer. ¹H- and ¹³C-NMR spectra were measured with a JEOL JNM-GX 400 spectrometer at 399.78 MHz for proton and at 100.43 MHz for carbon-13, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet=S or s, doublet=D or d, triplet=t, quartet=Q or q, multiplet=m, and broad=br. CD curves were determined on a JASCO J-40 spectrophotometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (LPLC) was performed on a Chemco Low-Prep pump (81-M-2) and a glass column (200 $\times$ 10 mm) packed with silica gel CQ-3 (30–50 μ ; Wako). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 F₂₅₄ plates (Art. 5715; Merck). Spots on TLC were detected under UV light, and/or by spraying silver nitrate solution.

Isolation of Emestrin B (1)—*Emericella striata*, strain 80-NE-22, was cultivated at 30 °C for 3 weeks in Czapek-Dox medium (50 l). The dried mycelia (660 g) were pulverized and extracted with acetone at room temperature. After the chromatographic separation of the acetone extract (26 g) with benzene–acetone (10 : 1, v/v), the fraction slightly more polar than emestrin (**2**) was purified by repeated LPLC [benzene–acetone (20 : 1, v/v)] to give emestrin B (**1**) (100 mg).

Emestrin B (1): Colorless crystalline powder, mp 230–238 °C (dec.) from MeOH. $[\alpha]_D^{20} + 245^\circ$ ($c=0.19$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450 (OH), 1715 (–COO–), 1690, 1680, 1670 (–CON–). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 204 (4.66), 255 sh (4.32), 285 sh (3.92). FD-MS m/z (%): 630 (82, M⁺), 629 (80, M–1), 566 (58, M–S₂), 534 (73, M–S₃), 533 (69), 532 (78), 288 (100). *Anal.* Calcd for C₂₇H₂₂N₂O₁₀S₃: C, 51.42; H, 3.52; N, 4.44; S, 15.25. Found: C, 51.16; H, 3.41; N, 4.43; S, 14.89. ¹H-NMR (CDCl_3) δ : 3.536 (3H, s, NMe), 4.065 (3H, s, OMe), 4.809 (1H, s, 7'-H), 5.001 (1H, dd, $J=7.8$, 2.4 Hz, 7-H), 5.226 (1H, br s, 11-H), 5.328 (1H, ddd, $J=8.4$, 2.4, 2.2 Hz, 6-H), 5.402 (1H, dd, $J=8.4$, 2.3 Hz, 5a-H), 6.358 (1H, dd, $J=7.8$, 2.2 Hz, 8-H), 6.805 (1H, d, $J=8.3$ Hz), 6.828 (1H, d, $J=2.3$ Hz, 10-H), 6.933 (1H, dd, $J=8.3$, 2.2 Hz), 7.016 (1H, s, 4'-OH), 7.026 (1H, d, $J=8.5$ Hz), 7.862 (1H, dd, $J=8.5$, 2.2 Hz), 8.351 (1H, d, $J=2.2$ Hz), 8.718 (1H, d, $J=2.2$ Hz). CD $[\theta]$ (nm): –5700 (228), +6900 (257), +20100 (293). The ¹³C-NMR signals are summarized in Table II.

Isolation of Shamixanthone—The fraction slightly less polar than emericellin from the mycelial acetone extract was chromatographed on silica gel with benzene–ethyl acetate (98 : 2, v/v) to give a yellow crystalline powder (23 mg), mp 152–154 °C. This compound was identical with shamixanthone on the basis of IR, ¹H-NMR, and mass spectral comparisons.

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