NOVEL EPIDITHIODIOXOPIPERAZINES, EMETHALLICINS E AND F, FROM EMERICELLA HETEROTHALLICA[1]

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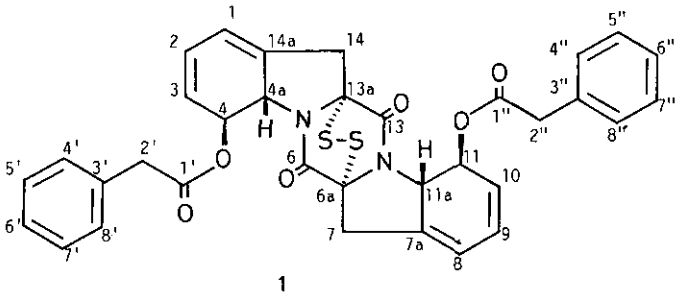
Abstract ——— Novel epidithiodioxopiperazines, emethallicins E (**1**) and F (**2**), were isolated from the mycelial extract of Emericella heterothallica (mating type A). The structures of emethallicins E (**1**) and F (**2**) were determined on the basis of spectroscopic and chemical investigation, compared with other emethallicins isolated from the above fungus and the other strain. Emethallicin E (**1**) is an epidithiodioxopiperazine having a novel carbon skeleton related to emethallicins A (**3**), B (**4**), C (**5**), and D (**6**), whereas emethallicin F (**2**) has the same carbon skeleton as emethallicins A (**3**), B (**4**), and D (**6**). Emethallicins E (**1**) and F (**2**) have a potent inhibitory activity for histamine release as well as the other emethallicins.

Recently a considerable amount of emethallicin A (**3**), an epidithiodioxopiperazine derivative, was isolated from the mycelial chloroform extract of Emericella heterothallica (Kwon, Fennell & Raper) Malloch & Cain, strain ATCC 16847 (mating type A).[2] We also reported the isolation of epitetrathiodioxopiperazines, emethallicins B (**4**) and C (**5**), and a epitrithiodioxopiperazine, emethallicin D (**6**) as its acetate (**7**), from the mycelial chloroform extract of the other mating type strain (strain ATCC 16824; mating type a) of E. heterothallica, along with small amount of **3**.[3] In the course of search for minor epipolythiodioxopiperazines in the



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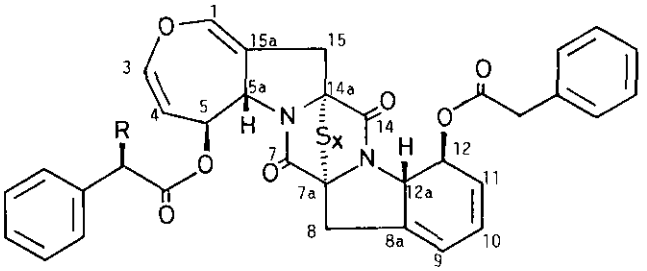


2 : R = H, x = 2
3 : R = OH, x = 2
4 : R = OH, x = 4
6 : R = OH, x = 3
7 : R = OAc, x = 3

mycelial extraxt of E. heterothallica (strain ATCC 16824), new compounds designated emethallicins E (1) and F (2) were isolated. The structures of compounds 1 and 2 are reported in this paper.

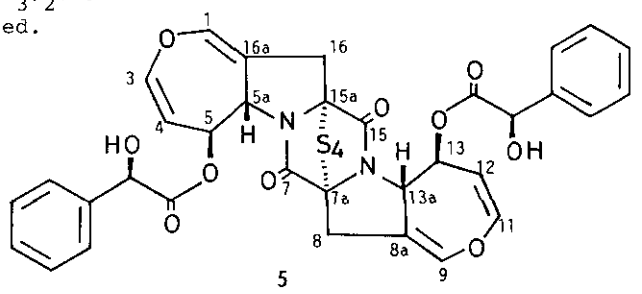
Emethallicin E (1), $[\alpha]_D$ -104° (chloroform), and F (2), $[\alpha]_D$ -221° (chloroform), gave molecular ions at m/z 625 $(M+1)^+$ and 641 $(M+1)^+$, respectively, by fast-atom bombardment mass spectrometry, and their elemental analyses confirmed their molecular formulae as $C_{34}H_{28}N_2O_6S_2$ and $C_{34}H_{28}N_2O_7S_2$, respectively. A positive coloration with silver nitrate (dark brown-black)[2,4] suggested the presence of the disulfide bond in **1** and **2**. The ir absorption maxima at 1740 and 1700 cm^{-1} in **1** and **2** suggested the presence of both ester and amide in each compound.

The ^1H nmr and ^{13}C nmr (Table) spectra of emethallicin E (**1**) showed the presence of 14 protons and 17 carbons, which were exactly half the number of the protons and carbons, respectively, as those confirmed from the molecular formula. Therefore **1** should have a symmetrical structure. The ^{13}C nmr signal of two amide carbonyl carbons at δ 162.75 was obseved. The cd curve of **1** was closely similar to those of epidithiodioxopiperazines, as described later. The ^1H nmr signals at δ 5.02 (2H), 5.50 (2H), 5.90-6.05 (4H), and 6.05 (2H) were assigned to the protons of two sets of dihydrobenzene moiety, and the ^1H nmr signals at δ 3.74 (4H) and 7.25-7.35 (10H) suggested the presence of two phenylacetate

Table. ^{13}C Nmr chemical shifts of emethallicins in $CDCl_3$

Carbon No.	2	3	4[a]	Carbon No.	1	Carbon No.	5[a]
1	139.31	139.39	137.97	1	119.84	1	138.01
3	141.21	141.45	140.24	2	124.50	3	140.33
4	105.23	104.20	104.66	3	127.64	4	104.71
5	70.08	71.55	70.71	4	74.56	5	70.78
5a	62.88	62.74	60.22	4a	64.23	5a	60.36
7	163.00	163.45	165.29	6	162.75	7	165.38
7a	78.15	78.34	80.04	6a	78.28	7a	76.22
8	36.15	36.08	40.05	7	36.40	8	40.41
8a	132.33	132.07	133.50	7a	132.50	8a	109.58
9	119.90	119.99	119.79	8	119.84	9	138.01
10	124.42	124.45	125.38	9	124.50	11	140.33
11	127.70	127.64	127.68	10	127.64	12	104.71
12	74.45	74.37	74.26	11	74.56	13	70.78
12a	64.36	64.40	63.74	11a	64.23	13a	60.36
14	162.23	162.06	166.16	13	162.75	15	165.38
14a	75.86	75.73	75.97	13a	78.28	15a	76.22
15	34.47	34.53	40.49	14	36.40	16	40.41
15a	113.43	112.73	109.50	14a	132.50	16a	109.58
1'	170.38	171.70	171.55	1'	170.93	1'	171.66
2'	40.87	72.86	72.44	2'	41.31	2'	72.51
3'	133.69	138.10	139.19	3'	134.16	3'	139.24
4'(8')	129.54[b]	126.54	126.73	4'(8')	129.55	4'(8')	126.81
5'(7')	128.40[c]	128.35[b]	128.09[b]	5'(7')	128.40	5'(7')	128.18
6'	126.91[d]	128.20	128.18	6'	126.91	6'	127.80
1"	170.91	170.90	170.33	1"	170.93	1"	171.66
2"	41.30	41.28	40.37	2"	41.31	2"	72.51
3"	134.13	134.09	134.18	3"	134.16	3"	139.24
4"(8")	129.63[b]	129.52	129.47	4"(8")	129.55	4"(8")	126.81
5"(7")	128.43[c]	128.40[b]	128.14[b]	5"(7")	128.40	5"(7")	128.18
6"	127.03[d]	126.92	126.65	6"	126.91	6"	127.80

[a] The spectra were measured in $(CD_3)_2SO$.
[b-d] The assignments may be reversed.



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moieties. The remaining ^{1}H nmr signals at δ 2.87 (2H) and 3.72 (2H) were assigned to two sets of methylene protons. The homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C shift correlation spectra of emethallicin E (1) confirmed its structure.

In order to determine the relative stereochemistry of emethallicin E (**1**), the homonuclear ^{1}H-^{1}H nuclear Overhauser enhancement correlation (^{1}H-^{1}H NOESY) spectrum and the heteronuclear ^{1}H-^{13}C long-range shift correlation (^{1}H-^{13}C COLOC) spectrum of **1** were undertaken. In the ^{1}H-^{1}H NOESY spectrum (Figure 1), the strong correlation peak between the downfield methylene proton signal at C-7 and C-14 (δ 3.72) and the proton signal at C-4a and C-11a (δ 5.02) was observed, whereas the upfield methylene proton signal (δ 2.87) was merely weakly correlated with the proton signal observed at δ 5.02. Furthermore, though the correlation peak was observed between the amide carbonyl carbon signal at δ 162.75 (C-6 and C-13) and the downfield methylene proton signal at δ 3.72 in the ^{1}H-^{13}C COLOC spectrum (Figure 2), no cross peak was observed between the above amide carbonyl carbon signal and the upfield methylene proton signal at δ 2.87. In the same spectrum, the carbon signals at δ 64.23 (C-4a and C-11a) and 119.84 (C-1 and C-9) were correlated only to the upfield signal of the methylene protons at C-7 and C-14. Therefore the ^{1}H nmr signals at δ 3.72 and 2.87 were assigned to 7β-H (14β-H) and 7α-H (14α-H), respectively, and consequently the relative structure of emethallicin E was confirmed as **1**.

The comparison of the cd curve of emethallicin E (**1**) [217 (negative), 237 (negative), 273 (positive), and 328 nm (negative)] with those of emethalicin A (**3**) [219 (negative), 268 (positive), and 338 nm (negative)][2] and acetylaranotin [233 (negative), 270 (positive), and 340 nm (negative)][5] confirmed that these compounds had the same configuration about the epidithiodioxopiperazine ring. Emethallicin E must therefore have the 6a<u>R</u>,13a<u>R</u> configuration and consequently the abosolute structure was determined as shown in **1**.

Emethallicin F (**2**) had only one more oxygen atom than emethallicin E (**1**) and only one less oxygen atom than emethallicin A (**3**). The ^{1}H nmr spectrum of **2** was similar to that of **3**, except that two signals assigned to a methylene protons were observed at δ 3.56 (1H) and 3.63 (1H) in **2** instead of the signal at δ 6.08 in **3**, assigned to the proton attached to the carbon bearing hydroxyl group (2'-H). The corresponding ^{13}C nmr signal at δ 72.86 (Dt) in **3** shifted upfield to δ 40.86 (Tt) in **2**, and the other signals of **2** were well corresponded to those of

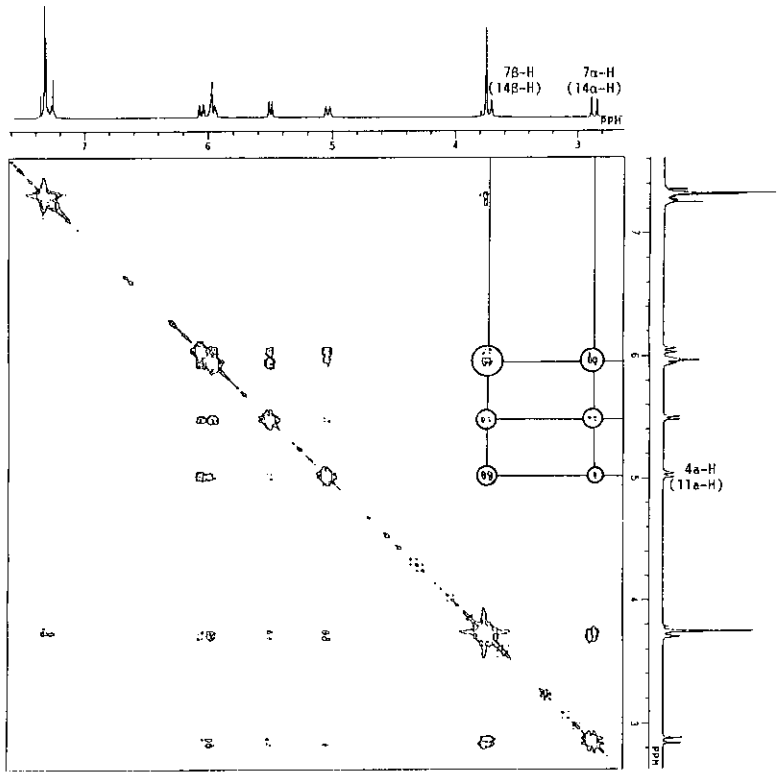

Figure 1. ^{1}H-^{1}H NOESY spectrum of emethallicin E (1).

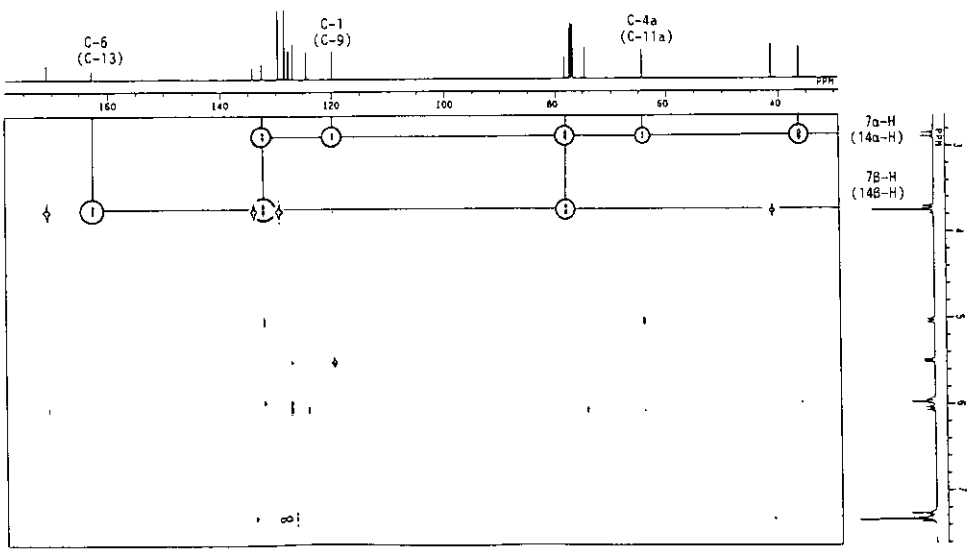

Figure 2. ^{1}H-^{13}C COLOC spectrum of emethallicin E (1).

3 (Table). The above results indicated that emethallicin F (2) does not contain the mandelate unlike as in emethallicin A (3) but contains the phenylacetate. Emthallicin F (2) was obtained by hydrolysis of emethallicin A (3) with sodium hydroxide followed by phenylacetylation with phenylacetic anhydride.[6] The comparison of cd curve of 2 [224 (negative), 234 (negative), 272 (positive), and 340 nm (negative)] with that of 3 [219 (negative), 268 (positive), and 338 nm (negative]^{2} confirmed that emethallicins F (1) and A (3) had the same absolute configuration about the epidithiodioxopiperazine ring.[5] The above results confirmed the absolute structure of emehallicin F (2).

Emethallicin E (1) is an epidithiodioxopiperazine derivative having a novel skeleton related to emethallicins A (3), B (4), C(5), and D (6), whereas emethallicin F (2) has the same carbon skeleton as emethallicins A (3), B (4), and D (6). These compounds suggested that the biogenetic relationship of emethallicins in Emericella heterothallica, i.e.: The benzene rings of phenylalanine anhydride or its derivative, which had been isolated from the extract of the culture filtrate,[7] was oxidized and esterified with phenylacetic acid to give the compound having two dihydrobenzene rings, emethallicin E (1). Then emethallicin F (2) was derived by oxygenation of one of the dihydrobenzene ring of 1 followed by the oxygenation of one of the phenylacetate moiety to give emethallicin A (3). The insertion of the sulfur atoms gave the trisulfide, emethallicin D (6), and then the tetrasulfide, emethallicin B (4), which was further oxygenated to give emethallicin C (5). Emethallicins A (3) and B (4), and emethallicin D monoacetate (7) have a potent inhibitory activity upon compound 48/80-induced histamine release from mast cells,[1,2] and emethallicins E (1) and F (2) also have fairly strong inhibitory activities. The IC_{50} values for inhibition of histamine release of 1 and 2 were determined as 1.0×10^{-7} and 2.0×10^{-7} M, respectively.

EXPERIMENTAL

Melting points were uncorrected. The following instruments were used: optical rotation; JASCO DIP-370 spectropolarimeter: mass spectra; JEOL JMX-HS-110 spectrometer: uv spectra; Hitachi U-3210 spectrophotometer: ir spectra; JASCO IR-810 spectrophotometer: ^{1}H nmr and ^{13}C nmr spectra; JEOL JNM-GX-400 spectro-

meter: cd spectra; JASCO J-600 spectropolarimeter: low pressure liquid chromatography (lplc); Chemco Low-Prep 81-M-2 in a glass column (200×10 mm) packed with silica gel CQ-3 (30-50 μm; Wako). Abbreviations: s=singlet, d=doublet, m=multiplet, br=broad, sh=shoulder.

Isolation of Emethallicins E (1) and F (2) from Emericella heterothallica

E. heterothallica, strain ATCC 16847, was cultivated at 27°C for 14 days with 30 Roux flasks containing 250 ml of Czapek-Dox medium supplemented with 0.1 % yeast extract in each flask. The dried mycelium (53 g) was pulverized and extracted with chloroform at room temperature. The evaporated residue (4.2 g) was chromatographed on silica gel with benzene-acetone (100:1). The eluate was rechromatographed on silica gel to give the fraction eluated with n-hexane-ethyl acetate (4:1) and then the fraction eluated with n-hexane-ethyl acetate (3:1) after the removal of ergosterol (280 mg) with n-hexane-ethyl acetate (5:1). The former fraction was further purified by lplc using n-hexane-benzene (1:1) to give emethallicin E (1) (50 mg) and emethallicin F (2) (30 mg) was obtained from the later fraction by lplc using n-hexane-benzene (1:1).

Emethallicin E (1)

Compound **1** was obtained as white amorphous powder, $[\alpha]_D^{20}$ -104° (c=0.30 in chloroform). Fast-atom bombardment ms m/z: 625 $[(M+1)^+]$. Anal. Calcd for $C_{34}H_{28}N_2O_6S_2$: C, 65.36; H, 4.52; N, 4.48; S, 10.27. Found: C, 65.33; H, 4.48; N, 4.70, S, 10.16. Uv λ_{max}^{MeOH} nm (log ε): 270 (4.11). Ir ν_{max}^{KBr} cm^{-1}: 1740 (COO), 1700 (CON). ^{1}H Nmr ($CDCl_3$) δ: 2.87 (2H, d, J=17.7 Hz, 7α-H, 14α-H), 3.72 (2H, dd, J=17.7, 1.8 Hz, 7β-H, 14β-H), 3.74 (4H, br s, 2'-H_2, 2"-H_2), 5.02 (2H, ddd, J=13.4, 1.8, 1.8 Hz, 4a-H, 11a-H), 5.50 (2H, br d, J=9.8 Hz, 2-H, 9-H), 5.90-6.05 (4H, m, 1-H, 3-H, 8-H, 10-H), 6.05 (2H, br d, J=13.4 Hz, 4-H, 11-H), 7.25-7.35 (10H, m, Ar-H). Cd (c=1.6×10^{-5} in methanol) [θ] (nm): -100800 (217), -93500 sh (237), +63200 (273), -3700 (328).

Emethallicin F (2)

Compound **2** was obtained as pale yellow amorphous powder, $[\alpha]_D^{20}$ -221° (c=1.17 in chloroform). Fast-atom bombardment ms m/z: 641 $[(M+1)^+]$. Anal. calcd for $C_{34}H_{28}N_2O_7S_2$: C, 63.74; H, 4.41; N, 4.37; S, 10.01. Found: C, 63.91; H, 4.33; N, 4.65; S, 10.22. Uv λ_{max}^{MeOH} nm (log ε): 264 sh (3.77). Ir ν_{max}^{KBr} cm^{-1}: 1740 (COO), 1700 (CON). ^{1}H Nmr ($CDCl_3$) δ: 2.81 (1H, d, J=17.7 Hz, 8α-H), 2.97 (1H, d, J=18.3 Hz, 15α-H), 3.56 (1H, d, J=15.9 Hz, 2'-H), 3.63 (1H, d, J=15.9 Hz, 2'-H), 3.74 (2H, br s, 2"-H_2), 3.78 (1H, br d, J=17.7 Hz, 8β-H), 4.03 (1H, ddd, J=18.3, 1.8,

1.8 Hz, 15β-H), 4.55 (1H, dd, J=8.5, 1.8 Hz, 4-H), 5.02 (1H, br d, J=13.4 Hz, 12a-H), 5.13 (1H, ddd, J=8.5, 2.4, 1.8 Hz, 5a-H), 5.49 (1H, br d, J=9.2 Hz, 10-H), 5.71 (1H, ddd, J=8.5, 1.8, 1.8 Hz, 5-H), 5.90-6.05 (2H, m, 9-H, 11-H), 6.03 (1H, br d, J=13.4 Hz, 12-H), 6.29 (1H, dd, J=8.5, 1.8 Hz, 3-H), 6.62 (1H, dd, J=2.4, 1.8 Hz, 1-H), 7.25-7.35 (10H, m, Ar-H). Cd (c=1.88×10^{-5} in methanol [θ] (nm): -78500 (224), -70300 sh (234), +21000 (272), -2400 (340).

Hydrolysis of Emethallicin A (3) Followed by Phenylacetylation

1N-Sodium hydroxide (2 ml) was added to a solution of emethallicin A (3) (100 mg) in acetone (2 ml) and the reaction mixture was stirred at room temperature for 30 min. The mixture was poured into water and extracted with ethyl acetate, and the solvent was evaporated. The extract was dissolved in pyridine (2 ml), and phenylacetic anhydride (500 mg) and 4-dimethylaminopyridine (30 mg) were added to the solution. The mixture was kept to stand at room temperature overnight. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The residue obtained by evaporation of the extract was purified by lplc with hexane-ethyl acetate (5:1) to afford compound **2** (15 mg). This compound was identical with naturally occurring emethallicin F (2) on the basis of the comparison of the ^1H nmr and ir spectra and the optacal rotation.

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