Novel Epitetrathiodioxopiperazines, Emethallicins B and C, as Potent Inhibitors of Compound 48/80-induced Histamine Release, from *Emericella heterothallica*

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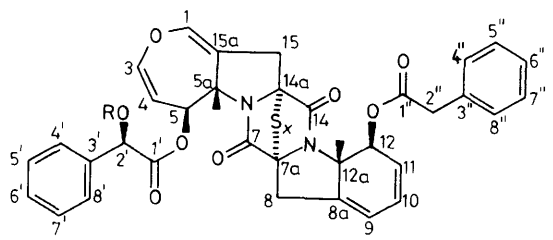
The structure elucidation of emethallicins B (**1**) and C (**2**), novel epitetrathiodioxopiperazines, possessing strong inhibitory activity of the histamine release from mast cells, isolated from the mycelial extract of *Emericella heterothallica* (mating type a), is reported, based on their ^1H and ^{13}C n.m.r. spectra and a chemical correlation of emethallicin A (**10**) with apoaranotin (**11**); they are diesters of two C_6–C_2 acids having the same basic skeletons as apoaranotin (**11**) and aranotin (**13**), respectively.

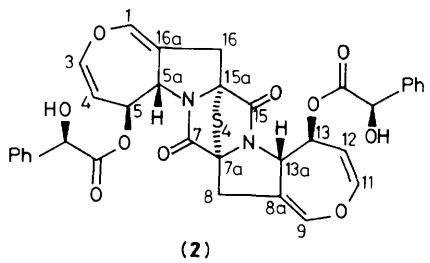
In a previous paper,[1] we reported the isolation of the pyrazinone derivative, emehetetone (**3**), from the culture filtrate of *Emericella heterothallica* (Kwon, Fennell & Raper) Malloch & Cain, strain ATCC 16824 (mating type a). Recently, sulphur-containing dioxopiperazines designated as emethacins A (**4**) and B (**5**) were isolated from the same extract, along with two dioxopiperazines, (**6**) and (**7**).[2] During our search for sulphur-containing compounds related to (**3**)—(**7**), two novel compounds designated as emethallicins B (**1**), needles [from ethyl acetate–methanol (1:1, v/v)], m.p. 180—182 °C, $[\alpha]_D$ −268 ° (*c* 0.30, chloroform), and C (**2**), pale yellow needles (from acetone), m.p. 193—195 °C, $[\alpha]_D$ −312 ° (*c* 0.20, chloroform), were isolated from the mycelial chloroform extract of the above fungus along with another dioxopiperazine (**8**).

Positive fast-atom bombardment mass spectrometry [*m/z* 721 $(M+1)^+$ for (**1**) and 753 $(M+1)^+$ for (**2**)] and elemental analyses of (**1**) and (**2**) confirmed their molecular formulae as $C_{34}H_{28}N_2O_8S_4$ and $C_{34}H_{28}N_2O_{10}S_4$, respectively. A positive colouration with silver nitrate (dark brown)[3] of (**1**) and (**2**) suggested the presence of a polythio bond. Emethallicin B (**1**) had $\lambda_{max.}$ (MeOH) 259sh (log ε 3.89), 265sh (3.86), and 284sh nm (3.69); $\nu_{max.}$ (KBr) 3450 (OH), 1735, 1720 (CO_2), and 1680 (CON) cm^{-1}; and $\delta_H(CD_3SOCD_3)$ 3.075 (1H, d, *J* 15.9 Hz, 8α-H), 3.101 (1H, d, *J* 16.2 Hz, 15α-H), 3.451 (1H, br. d, *J* 15.9 Hz, 8β-H), 3.470 (1H, br. d, *J* 16.2 Hz, 15β-H), 3.713 (1H, d, *J* 16.1 Hz, 2″-H), 3.764 (1H, d, *J* 16.1 Hz, 2″-H), 4.241 (1H, dd, *J* 8.2 and 1.8 Hz, 4-H), 5.035 (1H, ddd, *J* 8.4, 2.1, and 1.8 Hz, 5-H), 5.083 (1H, br. d, *J* 8.4 Hz, 5a-H), 5.182 (1H, s, 2′-H), 5.261 (1H, br. d, *J* 13.7 Hz, 12a-H), 5.609 (1H, br. d, *J* 8.7 Hz, 10-H), 5.641 (1H, br. d, *J* 13.7 Hz, 12-H), 6.031 (2H, m, 9-H and 11-H), 6.337 (1H, dd, *J* 8.2 and 2.1 Hz, 3-H), 6.779 (1H, br. s, 1-H), 7.24—7.37 (6H, m, ArH), 7.395 (2H, br. d, *J* 7.0 Hz, 4″-H and 8″-H), and 7.533 (2H, br. d, *J* 7.1 Hz, 4′-H and 8′-H), whereas emethallicin C (**2**) had $\lambda_{max.}$ (MeOH) 230sh (log ε 4.59) and 290sh nm (3.35); $\nu_{max.}$ (KBr) 3480 (OH), 1730 (CO_2), and 1680 (CON) cm^{-1}; and δ_H (CD_3COCD_3) 3.212 (2H, d, *J* 16.4 Hz, 8α-H and 16α-H), 3.566 (2H, ddd, *J* 16.4, 2.5, and 2.0 Hz, 8β-H and 16β-H), 4.409 (2H, dd, *J* 8.2 and 1.7 Hz, 4-H and 12-H), 4.873 (2H, d, *J* 5.6 Hz, 2′-OH and 2″-OH), 5.202 (2H, ddd, *J* 8.5, 2.0, and 2.0 Hz, 5a-H and 13a-H), 5.256 (2H, ddd, *J* 8.5, 2.2, and 1.7 Hz, 5-H and 13-H), 5.362 (2H, d, *J* 5.6 Hz, 2′-H and 2″-H), 6.332 (2H, dd, *J* 8.2 and 2.2 Hz, 3-H and 11-H), 6.780 (2H, dd, *J* 2.5 and 2.0 Hz, 1-H and 9-H), 7.325 (2H, br. t, *J* 7.3 Hz, 6′-H and 6″-H), 7.391 (4H, br. t, *J* 7.3 Hz, 5′-H, 7′-H, 5″-H, and 7″-H), and 7.667 (4H, br. d, *J* 7.3 Hz, 4′-H, 8′-H, 4″-H, and 8″-H).

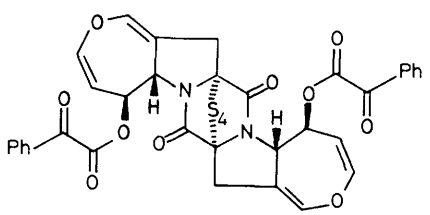
Acetylation of (**1**) gave a monoacetate (**9**), amorphous powder, $[\alpha]_D$ −363 ° (*c* 0.98, chloroform). The ^1H and ^{13}C n.m.r. (Table 1) spectra, the homonuclear ^1H–^1H shift correlation (^1H–^1H COSY) spectrum, and the heteronuclear ^1H–^{13}C shift correlation (^1H–^{13}C COSY) spectrum indicated



(**1**) : R = H, *x* = 4
(**9**) : R = Ac, *x* = 4
(**10**) : R = H, *x* = 2



(**2**)



(**12**)

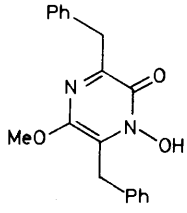
the structure of emethallicin B (**1**). Compound (**1**) was converted to the dithio derivative, emethallicin A (**10**),† by desulphurization with triphenylphosphine. Alkaline hydrolysis of (**10**) followed by acetylation gave compound (**11**),[4] m.p. 201—204 °C, which had the same properties, including optical rotation, as aranotin originally isolated from *Arachniotus aureus* (Eidam) Schroeter as an antiviral agent.[5] The absolute configuration of the epipolythiodioxopiperazine moiety in (**1**) was also determined by the comparison of its c.d. curve with those of related compounds.[6]

† Emethallicin A (**10**) is also a metabolite of this fungus, but we could isolate this compound only as a monoacetate because it was a minor component.

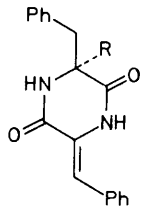
Table 1. ^{13}C N.m.r. chemical shifts of emethallicins B (**1**) and C (**2**) in CD_3SOCD_3.

Carbon	(1)	Carbon	(2)
1	137.97 (Dm)	1	138.01 (Dm)
3	140.24 (Dm)	3	140.33 (Dm)
4	104.66 (Ddd)	4	104.71 (Dm)
5	70.71 (Dbrdd)	5	70.78 (Dm)
5a	60.22 (Dm)	5a	60.36 (Dm)
7	165.29 (Sd)	7	165.38 (Sdd)
7a	75.97 (Sdd)	7a	76.22 (Sdd)
8	40.05 (Tdd)	8	40.41 (Tdd)
8a	133.50 (Sm)	8a	109.58 (Sm)
9	119.79 (Dm)	9	138.01 (Dm)
10	125.38 (Dm)	11	140.33 (Dm)
11	127.68 (Ddd)	12	104.71 (Dm)
12	74.26 (Dbrdd)	13	70.78 (Dm)
12a	63.74 (Dm)	13a	60.36 (Dm)
14	166.16 (Sdd)	15	165.38 (Sdd)
14a	80.04 (Sdd)	15a	76.22 (Sdd)
15	40.49 (Tdd)	16	40.41 (Tdd)
15a	109.50 (Sm)	16a	109.58 (Sm)
1′	171.55 (Sdd)	1′	171.66 (Sdd)
2′	72.44 (Dbrs)	2′	72.51 (Dt)
3′	139.19 (Sm)	3′	139.24 (St)
4′(8′)	126.73 (Dm)	4′(8′)	126.81 (Dm)
5′(7′)	128.09 (Dd)[a]	5′(7′)	128.18 (Dd)
6′	128.18 (Dm)	6′	127.80 (Dt)
1″	170.33 (Std)	1″	171.66 (Sdd)
2″	40.37 (Tt)	2″	72.51 (Dt)
3″	134.18 (Sm)	3″	139.24 (St)
4″(8″)	129.47 (Dm)	4″(8″)	126.81 (Dm)
5″(7″)	128.14 (Dd)[a]	5″(7″)	128.18 (Dd)
6″	126.65 (Dt)	6″	127.80 (Dt)

[a] Assignments may be reversed.

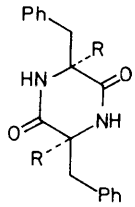


(3)



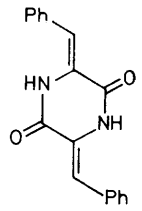
(4) R = SMe

(6) R = H

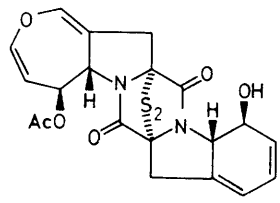


(5) R = SMe

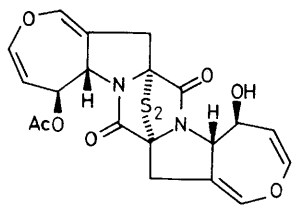
(7) R = H



(8)



(11)



(13)

The positions of the esters of phenylacetic and mandelic acids were determined from the homonuclear ^1H–^1H nuclear Overhauser enhancement (n.O.e.) correlation (NOESY) spectrum of (**1**). Alkaline hydrolysis of (**1**) followed by methylation with diazomethane gave (*R*)-(−)-methyl mandelate.[7,8] Consequently, the structure of emethallicin B (**1**) was confirmed including the absolute stereochemistry.

Oxidation of emethallicin C (**2**) with manganese dioxide gave the dioxo derivative (**12**), needles (from benzene), m.p. 198—200 °C. Compound (**2**) showed approximately half the number of ^1H and ^{13}C n.m.r. (Table 1) signals which were shown by (**1**), and therefore compound (**2**) should have a symmetrical structure. ^1H–^1H and ^1H–^{13}C COSY spectra suggested that the structure of emethallicin C is as shown in (**2**), *i.e.*, the structure of (**2**) had the same basic skeleton as aranotin (**13**).[9] The relative structure was determined by analysis of the NOESY spectrum. The c.d. curve of (**2**), which was superimposable on that of (**1**), confirmed the absolute configuration of the basic skeleton.[6] Alkaline hydrolysis of (**2**) followed by methylation with diazomethane gave (*R*)-(−)-methyl mandelate.[7,8]

Emethallicins B (**1**) and C (**2**) have the same basic skeletons, including the absolute stereochemistry, as apoaranotin (**11**)[4] and aranotin (**13**),[9] respectively, originally isolated from *Arachniotus aureus*. Although phenylacetic acid and its derivatives have been isolated from several fungi, this is the first time that mandelic acid has been isolated, as a free acid or its ester, from fungi. Compounds (**1**) and (**2**) are rare examples of C_6–C_2 acids in ester form.

Emethallins B (**1**) and C (**2**) show potent inhibitory activity of compound 48/80-induced histamine release from mast cells. The IC_{50} values of these compounds were determined as 8.0×10^{-8} M for (**1**) and 1.0×10^{-6} M for (**2**).‡

Received, 31st January 1989; Com. 9/00482C

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‡ Details of this inhibitory activity will be published elsewhere.