SULFUR-CONTAINING DIOXOPIPERAZINE DERIVATIVES FROM EMERICELLA HETEROTHALLICA[1]

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Abstract ——— Emethacins A (**1**) and B (**2**), sulfur-containing dioxopiperazines, were isolated from Emericella heterothallica ATCC 16824, along with three dioxopiperazine derivatives (**3-5**). Emethacin B was identical with (3R,6R)-3,6-dibenzyl-3,6-bis(methylthio)-2,5-dioxopiperazine (**2**) which had been already isolated from Aspergillus terreus. The structure of the new compound, emethacin A (**1**), was confirmed by spectroscopic and chemical correlation as (3R,6Z)-3-benzyl-6-benzylidene-3-methylthio-2,5-dioxopiperazine. Compound **3** was (3S,6Z)-3-benzyl-6- benzylidene-2,5-dioxopiperazine, which was the enantiomer of the compound **7** isolated from Streptomyces noursei.

Recently the antifungal epidithiodioxopiperazines, emestrin[2] and dithiosilvatin,[3] were isolated from Emericella striata (Rai, Tewari & Mukerji) Malloch & Cain and Aspergillus silvaticus Fennell & Raper, respectively. In the course of screening for dioxopiperazine derivatives from Emericella spp., a novel pyrazinone derivative designated emeheterone (**6**)[4] was isolated from Emericella heterothallica (Kwon, Fennell & Raper) Malloch & Cain (mating type a), strain ATCC 16824. We are reporting in this paper the isolation of two sulfur-containing dioxopiperazines designated emethacins A (**1**) and B (**2**) from the same extract, along with two dioxopiperazines (**3** and **4**). Another dioxopiperazine

derivative (**5**) was isolated from the mycelial chloroform extract of *E. heterothallica*.

Dioxopiperzines **4** and **5** were found to be identical with (3*S*,6*S*)-3,6-dibenzyl-2,5-dioxopiperazine (L-phenylalanine anhydride)[5] and (3*Z*,6*Z*)-3,6-dibenzylidene-2,5-dioxopiperazine,[6] respectively, which were isolated from *Streptomyces noursei* Brown & *al.* in Hazen & Brown.[7] Compound **3** was identical with (3*R*,6*Z*)-3-benzyl-6-benzylidene-2,5-dioxopiperazine (**7**)[8] which had been isolated by Shin *et al.* from *St. noursei*[7] except the optical rotation, the sign of which was opposite in each other, even though the value of the optical rotation of compound **3** was low, which meaned the partial racemization. Therefore, the structure of **3** was confirmed as (3*S*,6*Z*)-3-benzyl-6-benzylidene-2,5-dioxopiperazine, which was the enantiomer of **7** isolated from *St. noursei*. (3*S*,6*S*)-Dibenzyl-2,5-dioxopiperazine (**4**) and (3*Z*,6*Z*)-dibenzylidene-2,5-dioxopiperazine (**5**) were also isolated from *Penicillium nigricans* Bainier & Thom[9] and *St. thioluteus*,[10] respectively.

One of the sulfur-containing dioxopiperazine, emethacin B (**2**), was identical in all respect, including the optical rotation, with the (3*R*,6*R*)-3,6-dibenzyl-3,6-bis(methylthio)-2,5-dioxopiperazine isolated from *Aspergillus terreus* Thom as biosynthetic precursor of bisdethiobis(methylthio)acetylaranotin (**8**).[11]

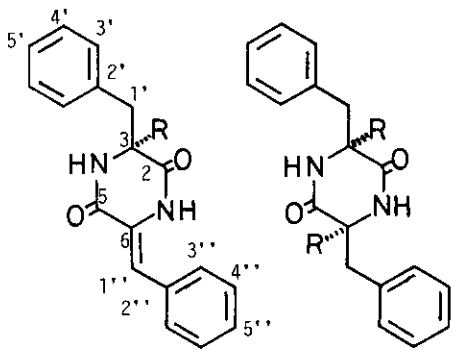
The other sulfur-containing dioxopiperazine, emethacin A (**1**), which was positive (dark brown) against palladium chloride reagent[12] as same as **2**, was shown to have the molecular formula as $C_{18}H_{15}N_2O_2S$ from the high resolution ms. The strong fragment ion at *m/z* 291 [$(M\text{-}SMe)^+$] and the ^1H nmr signal at δ 2.26 (3H) suggested the presence of one methylthio group in **1**. The ^1H nmr signals at δ 7.21-7.45 (10H), 3.18 (1H), and 3.57 (1H) suggested the presence of a phenyl group and a benzyl group, the latter was supported also by the fragment ion at *m/z* 91 [$(C_6H_5CH_2)^+$]. The other proton signals at δ 6.26 (1H) and 7.85 (1H), and 6.84 (1H) were assigned as two amide protons and a vinylic proton adjacent to the phenyl group, respectively. From the above results, the structure of emethacin A was assumed as **1**. The ^{13}C nmr signals (Table) also supported this structure. In order to confirm this structure including the stereochemistry, emethacin B (**2**) was refluxed with sodium carbonate to give (methylthio)dioxopiperazine derivative, which was identified with naturally occurring emethacin A (**1**) in all the respect, especially optical rotaions, though the value of the synthetic one was low. Therefore the absolute configuration of emethacin A was confirmed as of *R*-configuration. The streochemistry of the double bond

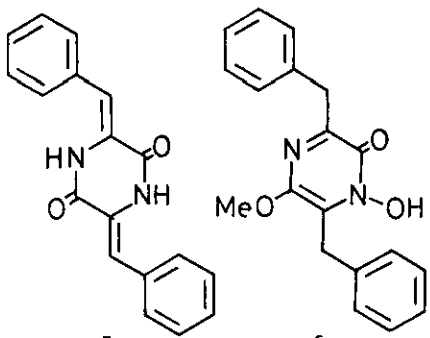
in **1** was confirmed as Z-configuration from the comparison of the ^{1}H nmr chemical shift of the vinylic proton.[6] From the above results, the structure of emethacin A (**1**) was confirmed as (3S,6Z)-3-benzyl-6-benzylidene-3-methylthio-2,5-dioxopiperazine.

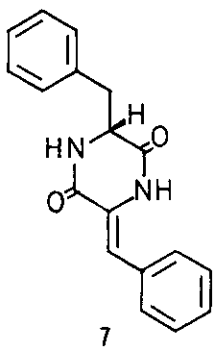
Emethacin A (**1**) is the first example of the naturally occurring mono(methylthio)-dioxopiperazine. It is interesting that mono- and bis(methylthio)dioxopiperazines, emethacins A (**1**) and B (**2**), respectively, was isolated along with the

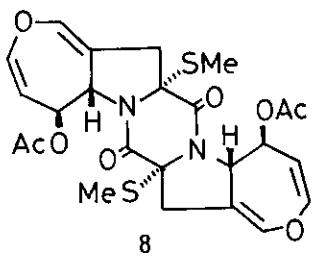
Table. ^{13}C Nmr chemical shifts of emethacin A (**1**) and the related compounds in $(CD_3)_2SO$

Carbon	1	2	5
C-2	163.57	165.55	157.29
C-3	67.16	65.15	126,02
C-5	160.11	165.55	157.29
C-6	125.63	65.15	126.02
C-1'	42.07	43.08	114.55
C-2'	135.84	134.87	132.73
C-3'	130.67	130.07	128.74
C-4'	127.89	127.96	128.31
C-5'	126.87	126.78	127.72
C-1''	114.38	43.08	114.55
C-2''	132.84	134.87	132.73
C-3''	129.15	130.07	128.74
C-4''	128.54	127.96	128.31
C-5''	127.99	126.78	127.72
3-SMe	12.80	13.68	
6-SMe		13.68	









other dioxopiperazines (**3** - **5**) and pyrazinone derivative (**6**)[4] from the same fungus, E. heterothallica.

EXPERIMENTAL

Melting points were uncorrected. The following instruments were used: optical rotation; JASCO DIP-181 spectrometer: mass spectra; JEOL JMS-D 300 spectrometer: uv spectra; Hitachi 124 spectrophotometer: ir spectra; JASCO IR-810 spectrophotometer: ^{1}H nmr spectra; JEOL JNM-GX 270 spectrometer: ^{13}C nmr spectra; JEOL JNM-GX 400 spectrometer: 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 Dioxopiperazine Derivatives

Emericella heterothallica, strain ATCC 16824, was cultivated at 27°C for 21 days in Czapek-Dox medium containing 0.1 % yeast extract. The culture filtrate (30 l) was extracted with dichloromethane at pH 2, and the organic layer was dried (Na_2SO_4). The evaporated residue (9.1 g) was chromatographed on silica gel with chloroform followed by lplc using cyclohexane-chloroform (2:1) to give emethacin A (**1**) (20 mg) and then using cyclohexane-chloroform (1:1) to obtain emethacin B (**2**) (300 mg). 3-Benzyl-6-benzylidene-2,5-dioxopiperazine (**3**) (20 mg) and 3,6-dibenzyl-2,5-dioxopiperazine (**4**) (50 mg) were obtained as the eluate of chloroform-methanol (100:1) and (30:1), respectively, from the above same chromatography. The dried mycelia (250 g) were pulverized and extracted with chloroform, and the chloroform solution was dried (Na_2SO_4) and concentrated under reduced pressure. The residue (16 g) was chromatographed on silica gel with chloroform followed by the chromatography on silica gel with benzene-acetone (100:1) to give ergosterol (1.2 g) and with benzene-acetone (50:1) to give 3,6-dibenzylidene-2,5-dioxopiperazine (**5**) (30 mg).

Emethacin A (1)

Compound **1** was obtained as colorless plates, mp 293-295°C, $[\alpha]_{350}^{25}$ -389° (c=0.009 in chloroform), ir ν_{max}^{KBr} cm^{-1}: 3200, 3060 (NH), 1670 (CON), uv λ_{max}^{MeOH} nm (log ε): 226 sh (4.13), 298 (4.16), elecron impact ms m/z: 338.1091 (M^+, 338.1089 for $C_{19}H_{18}N_2O_2S$, 4%), 291.1135 [291.1134 for $C_{18}H_{15}N_2O_2$, $(M-SMe)^+$, 100%], 91 [$(C_6H_5CH_2)^+$, 52%], ^{1}H nmr ($CDCl_3$) δ: 2.26 (3H, s, SMe), 3.18 (1H, d, J=14.0 Hz, CH_2), 3.57 (1H, d, J=14.0 Hz, CH_2), 6.26 (1H, br s, NH), 6.84 (1H, s, PhCH=C), 7.21-7.45 (10H, m, ArH), 7.85 (1H, br s, NH).

Emethacin B (2)

Compound **2** was obtained as colorless needles, mp 298-300°C, $[\alpha]_{546}^{25}$ -168° (c=0.05 in chloroform), ir ν_{max}^{KBr} cm^{-1}: 3180, 3070 (NH), 1670 (CON), electron impact ms m/z: 386 (M^+, 2%), 339 [$(M-SMe)^+$, 48%], 290 (100%), 118 (70%), 91 [$(C_6H_5CH_2)^+$, 68%], ^1H nmr ($CDCl_3$) δ: 2.26 (6H, s, SMe×2), 2.86 (2H, d, J=13.4 Hz), 3.29 (2H, d, J=13.4 Hz), 6.89-7.09 (10H, m, ArH), 8.81 (2H, NH×2). This compound was identified with (3R,6R)-3,6-dibenzyl-3,6-bis(methylthio)-2,5-dioxopiperazine (**2**) isolated from Aspergillus terreus[11] by the comparison of ir, uv, and ^1H nmr spectra, and optical rotation.

Other Dioxopiperazines (3 - 5)

(3S,6Z)-3-Benzyl-6-benzylidene-2,5-dioxopiperazine (**3**) was obtained as colorless plates, mp 281-283°C, $[\alpha]_D^{25}$ +192° (c=0.05 in pyridine), ir ν_{max}^{KBr} cm^{-1}: 3200, 3060 (NH), 1675, 1630 (CON), uv λ_{max}^{MeOH} nm (log ε): 295 (4.15), electron impact ms m/z: 292 (M^+, 83%), 201 [$(M-C_6H_5CH_2)^+$, 100%], 173 (52%), 91 [$(C_6H_5CH_2)^+$, 94%], ^1H nmr [$(CD_3)_2SO$] δ: 2.97 (1H, dd, J=13.4, 4.9 Hz, CH_2CH), 3.16 (1H, dd, J=13.4, 4.0 Hz, CH_2CH), 4.34 (1H, dd, J=4.9, 4.0 Hz, CH_2CH), 7.15 (1H, s, CH=C), 7.12-7.36 (10H, m, ArH), 8.44 (1H, br s, NH), 9.68 (1H, br s, NH). This compound was identified with (3R,6Z)-3-benzyl-6-benzylidene-2,5-dioxopiperazine (**7**) from St. noursei[7] in the comparison of their uv, ir, and ^1H nmr spectra, but the optical rotation was opposite with each other.[8]

(3S,6S)-3,6-Dibenzyl-2,5-dioxopiperazine (**4**) was obtained as crystalline powder, mp >300°C, $[\alpha]_D^{25}$ -118° (c=0.05 in pyridine), ir ν_{max}^{KBr} cm^{-1}: 3200, 3070 (NH), 1670,1600 (CON), elecron impact ms m/z: 294 (M^+, 51%), 203 [$(M-C_6H_5CH_2)^+$, 32%], 175 (30%), 91 [$(C_6H_5CH_2)^+$, 100%], ^1H nmr [$(CD_3)_2SO$] δ: 2.23 (2H, dd, J=13.7, 6.4 Hz), 2.57 (2H, dd, J=13.7, 4.6 Hz), 3.97 (2H, dd, J=6.4, 4.6 Hz), 7.04 (4H, br d, J=8.6 Hz, ArH), 7.21-7.32 (6H, m, ArH), 7.92 (2H, br s, NH×2). This compound was identified with compound **4** isolated from St. noursei[7] by the comparison of their ir and ^1H nmr spectra, and optical rotation.[5]

(3Z,6Z)-3,6-Dibenzylidene-3,5-dioxopiperazine (**5**) was obtained as pale yellow needles, mp >300°C, ir ν_{max}^{KBr} cm^{-1}: 3220, 3100 (NH), 1680,1625 (CON), uv λ_{max}^{MeOH} nm (log ε): 233 sh (3.67), 334 (3.95), electron impact ms m/z: 290 (M^+, 100%), 118 (53%), ^1H nmr ($CHCl_3$) δ: 7.04 (2H, s, CH=C×2), 7.36-7.51 (10H, m, ArH), 8.17 (2H, br s, NH×2). This compound was identified with compound **5** isolated from St. noursei[7] by the comparison of their uv, ir, and ^1H nmr spectra.[6]

Treatment of Emethacin B (2) with Sodium Carbonate

Emethacin B (**2**) (200 mg) was dissolved in acetone (2 ml), and 5% sodium carbonate (4 ml) was added to the solution. The mixture was refluxed for 30 min and acetone was evaporated under reduced pressure. The reaction mixture was extracted with ethyl acetate, and the organic layer was dried (Na_2SO_4). The evaporated residue was purified by lplc using cyclohexane-chloroform (2:1) to give (methylthio)dioxopiperazine derivative (**1**) (24 mg). This compound was identical with naturally occurring emethacin A by the comparison of the ir and ord spectra, and by the mixed fusion.

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