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NOTES

New Dioxopiperazine Metabolites from a *Fusarium* Species Separated from a Marine Alga

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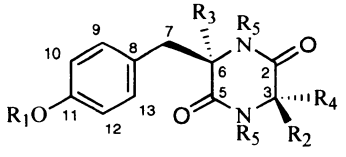
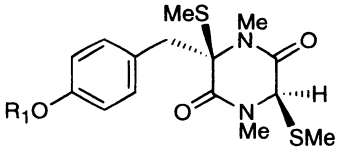
In our continuing search for antitumor metabolites from microorganisms isolated from marine organisms,[1] we have isolated a strain of *Fusarium chlamydosporum* OUPS-N124 as producing microorganisms of cytotoxic materials from the marine alga *Carpopeltis affinis*. Investigation for metabolites of this fungal strain has led to the isolation of two new sulfur-containing dioxopiperazine derivatives (**1** and **2**), designated fusaperazines A and B, respectively, in addition to four known compounds (**3**~**6**). We report herein the isolation and stereostructures of **1** and **2** (Fig. 1), and the absolute configuration of the known compounds **3** and **4**, previously undetermined.

As shown in Experimental, the fungal strain was cultured and the AcOEt extract of the culture filtrate was purified by a combination of some column chromatography and recrysatallization to afford **1**~**6**. The known compounds **3**~**6** were identified by comparison of spectral data with published values[2~5] as Sch 54794, Sch 54796, 3-[(4-hydroxyphenyl)-methyl]-1,4-dimethyl-3,6-bis(methylthio)-2,5-piperazinedione and *cis*-bis(methylthio)silvatin, respectively.

Fusaperazine A (**1**) had the molecular formula $C_{13}H_{16}N_2O_3S_2$ established by HREIMS as shown in Table 1. Its IR spectrum exhibited absorption bands for a hydroxy and/or an amino group, an amide and an aromatic ring. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 2) by DEPT and ^1H-^{13}C COSY experiments revealed the presence of two methylthio groups, one sp^3-hybridized methylene, one sp^3-methine and one quaternary sp^3-carbon each bearing a sulfur atom, two secondary amides, a hydroxy group, and one 1,4-disubstituted benzene. The lowfield chemical shift for one quaternary carbon of the aromatic ring suggested that one substituent on benzene is a hydroxy group. The connection of these functional groups was demonstrasted on the basis of HMBC correlations summarized in Fig. 2, and the planar structure of **1** was elucidated. The relative stereochemistry of **1** was elucidated by comparison of the ^1H chemical shifts of H-3 and 6-SMe in **1** with those of the known compounds **3** and **4** (Table 2) which were measured in DMSO-d_6 instead of $CDCl_3$ containing CD_3OD reported previously.[3] As observed in **3**,

Fig. 1. Structures of dioxopiperazines.





1 R_1 = H, R_2 = H, R_3 = R_4 = SMe, R_5 = H
2* R_1 = isoprenyl, R_2 = SMe, R_3 = OMe, R_4 = R_5 = H
3 R_1 = isoprenyl, R_2 = H, R_3 = R_4 = S Me, R_5 = H
4 R_1 = isoprenyl, R_2 = R_3 = SMe, R_4 = R_5 = H
7 R_1 = isoprenyl, R_2 = H, R_3 = R_4 = SMe, R_5 = M e
8 R_1 = isoprenyl, R_2 = R_3 = SMe, R_4 = H, R_5 = Me

* relative chemistry

5 : R_1 = H
6 : R_1 = isoprenyl

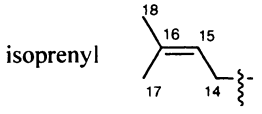


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Table 1. Physico-chemical properties of fusaperazine A (**1**) and B (**2**).

	1	**2**
Appearance	Colorless needles	Colorless needles
$[\alpha]_D^{16}$	−110.8° (*c* 0.20, DMSO)	−75.2° (*c* 0.08, dioxane)
HREIMS		
Found:	312.0597 (M^+)	364.1461 (M^+)
Calcd:	312.0601 (for $C_{13}H_{16}O_3N_2S_2$)	364.1456 (for $C_{18}H_{24}O_4N_2S_1$)
Molecular formula	$C_{13}H_{16}O_3N_2S_2$	$C_{18}H_{24}O_4N_2S_1$
UV λ_{max} (EtOH) nm (log ε)	226 (4.25), 278 (3.83), 285 (3.81)	227 (3.62), 277 (3.16), 283 (3.14)
IR ν_{max} (KBr) cm^{-1}	3396, 1662, 1617, 1517	3438, 1718, 1710, 1651
CD λ_{max} (EtOH) nm ($\Delta\varepsilon$)	297 (0), 270 (−1.42), 242(0), 232 (+2.74), 226 (0), 213 (−15.1) (*c* 4.80 x 10^{-5} M in EtOH)	260 (0), 240 (−0.50), 234.5(0), 225 (+3.31) (*c* 4.58 x 10^{-5} M in EtOH)
TLC Rf[a]	0.07	0.2
Solubility		
soluble	DMSO, MeOH	DMSO, dioxane, MeOH
insoluble	*n* -hexane	*n* -hexane

[a] Silica gel (5% MeOH in CH_2Cl_2).

Table 2. NMR spectral data of **1**~**4** in DMSO-d_6.[a]

	1			**2**			**3**			**4**		
Position	δ_H[b]	*J* /Hz	δ_C	δ_H	*J* /Hz	δ_C	δ_H	*J* /Hz	δ_C	δ_H	*J* /Hz	δ_C
1	8.99 s			8.90 s			9.01 s			9.06 s		
2			165.03 (q)[c]			165.28 (q)			165.20 (q)			164.50 (q)
3	4.52 d	2.9	57.85 (t)	5.03 d	2.0	57.44 (t)	4.56 d	3.4	58.04 (t)	5.06 d	1.5	57.39 (t)
4	8.77 d	2.9		8.84 d	2.0		8.78 d	3.4		8.58 d	1.5	
5			164.55 (q)			163.69 (q)			164.59 (q)			163.91 (q)
6			65.41 (q)			87.75 (q)			65.33 (q)			67.75 (q)
7 A	2.83 d	13.1	42.15 (s)	2.75 d	13.1	42.23 (s)	2.87 d	13.3	42.09 (s)	2.81 d	13.1	41.32 (s)
B	3.38 d	13.1		3.28 d	13.1		3.43 d	13.3		3.40 d	13.1	
8			125.13 (q)			126.27 (q)			126.93 (q)			126.99 (q)
9	7.05 d	8.1	131.53 (t)	7.10 d	8.8	131.89 (t)	7.17 d	8.7	131.68 (t)	7.16 d	8.6	131.97 (t)
10	6.61 d	8.1	114.62 (t)	6.81 d	8.8	114.32 (t)	6.79 d	8.7	113.95 (t)	6.80 d	8.6	114.20 (t)
11			156.15 (q)			157.64 (q)			157.41 (q)			157.65 (q)
12	6.61 d	8.1	114.62 (t)	6.81 d	8.8	114.32 (t)	6.79 d	8.7	113.95 (t)	6.80 d	8.6	114.20 (t)
13	7.05 d	8.1	131.53 (t)	7.10 d	8.8	131.89 (t)	7.17 d	8.7	131.68 (t)	7.16 d	8.6	131.97 (t)
14				4.48 d	6.6	64.22 (s)	4.46 d	6.7	64.10 (s)	4.45 d	6.7	64.21 (s)
15				5.37 br t	6.6	120.14 (t)	5.40 br t	6.7	120.04 (t)	5.37 br t	6.7	120.10 (t)
16						136.90 (q)			136.96 (q)			136.91 (q)
17				1.68 s		18.05 (p)	1.69 s		18.01 (p)	1.67 s		18.05 (p)
18				1.72 s		25.47 (p)	1.73 s		25.43 (p)	1.71 s		25.46 (p)
3-SMe	2.23 s		14.98 (p)	1.23 s		10.34 (p)	2.23 s		15.17 (p)	1.12 s		9.09 (p)
6-SMe	2.26 s		13.24 (p)				2.26 s		13.41 (p)	2.14 s		12.72 (p)
6-OMe				3.10 s		50.22 (p)						
11-OH	9.27 s											

[a] Measured at 300 and 75.4 MHz for ^1H and ^{13}C, respectively (Varian XL-300).

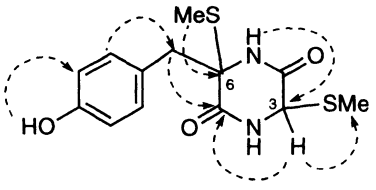
[b] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* /Hz).

[c] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

the H-3 signal of **1** in DMSO-d_6 appeared shifted upfield (δ 4.52) by virtue of a shielding effect of the benzene ring, while the 6-SMe signal showed an usual chemical shift (δ 2.23) as observed with 3-SMe of **1** and **3**, and 6-SMe of **3**. This evidence suggested the *cis* arrangement of two methylthio groups in **1**. Compound **1** exhibited the CD spectrum, comparable to that of **3**, of which the absolute configuration was determined as described below. From this finding, the absolute configuration of **1** was deduced as 3*S*, 6*S*.

Fusaperazine B (**2**) was assigned the molecular formula $C_{18}H_{24}N_2O_4S$ deduced from HREIMS. The general features of its ^1H and ^{13}C NMR spectra (Table 2) closely resembled those of **1** except that the signals for the hydroxy group and one of the methylthio groups in **1** were replaced by those of isoprenyloxy and methoxy groups in **2**. The chemical shift of the carbon signal for C-6 (δ 87.78) implied that the methoxy group was linked at C-6. The *trans* arrangement of **2** was deduced from the upfield chemical shift (δ 1.23) of the 3-SMe proton due to a shielding effect of the aromatic ring. Thus, the relative stereostructure of **2** was elucidated. The dioxopiperazine such as fusaperazine B (**2**) having a methoxy group at the α-carbon of an amino acid residue appear to be unusual, but it has been already reported that some of other dioxopiperazines having OH/OMe groups were produced by several genera of fungi and actinomyces such as *Penicillium*,[6,7] *Phoma*[8] and *Streptomyces*.[9] It was confirmed by a modeling experiment that any methylthio groups of the dioxopiperazines **1** and **3**~**6** isolated in this experiment could be never exchanged by a methoxy group under the conditions of the isolation procedure, implying that fusaperazine B (**2**) is a true metabolite and not an artifact.

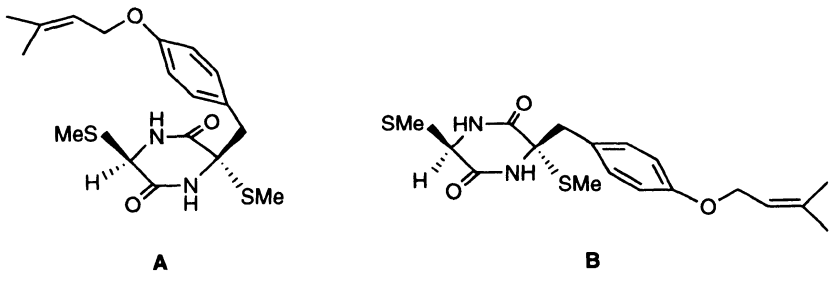
Fig. 2. Selected HMBC correrations in fusaperazine (**1**).



Among the known compounds **3**~**6**, the absolute configuration of **3** and **4** has been remained undecided. In order to determine their absolute configuration, the following reactions were carried out. Compound **3** was treated with MeI to give *N*-methyl derivatives **7** and **8**. Contrary to our expectation, derivative **7** had the opposite sign of specific optical rotation to that of the natural product **6** with the 3*R*, 6*R*-configuration, and exhibited the symmetrical CD curve with that of **6**, implying that **7** is the enantiomer of **6**. This evidence led to the absolute configuration (3*S*, 6*S*) for **3**. Epimerization of **3** by treatment with NaH followed by HCl afforded **4** together with recovered **3**, implying that the absolute styereochemistry of **4** is 3*R*, 6*S*. Compound **5** exhibited almost the same CD curve as **6**, supporting the 3*R*, 6*R*-configuration of **5** reported previously.[3]

It has been deduced from NOE experiments by CHU and co-workers[2] that compound **4** exist preferentially in a B type of conformer in $CDCl_3$ containing a small amount of CD_3OD (Fig. 3). This conformation was also confirmed by our NOESY experiments, in which NOE correlations from 6-SMe to H-10 and H-3, and from 3-SMe to H-3 were observed. In this solvent the 6-SMe proton signal of **4** appeared shifted upfield (δ 1.48) by virtue of a shielding effect of the benzene ring.[2] On the other hand, the 3-SMe proton signal of **4** was found shifted upfield (δ 1.12) when measured in DMSO-d_6, suggesting that **4** exist in an A type of conformer in the solvent. The A type of conformation of **4** in DMSO-d_6 was supported by NOE correlations from 3-

Fig. 3. Comformers for **4**.



SMe to H-9, H-10, H-14, H-15 and H-3, and from 6-SMe to H-3 observed in the NOESY of **4**. Incidentally, assignments for the methylthio groups of **4** were confirmed by HMBC correlations (3-SMe/C-3 and 6-SMe/C-6).

Among compounds **1**~**6**, **1**, **3** and **6** exhibited week cytotoxic activities (ED_{50} 22.8, 21.5 and 7.7 μg/ml, respectively) against P388 lymphocytic leukemia cells, whereas others were inactive (ED_{50} >100 μg/ml).

Biogenetically, it is very interesting that the same fungal strain produced two kinds of derivatives with an opposite chirality (**1** and **5**, or **3** and **6**). The similar result has been observed in the metabolites (penostatins A and B) from a strain of *Penicillium* sp., reported previouly by our research group.[10]

Experimental

Culturing and Isolation of Metabolites

According to the method reported previously,[11] a strain of *F. chlamydosporum* OUPS-N124 was initially isolated from the marine alga *C. affinis*, collected in the coast of Toyooka city (Hyogo Prefecture) in July, 1993. The fungal strain was grown in a liquid medium (259 liters) containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5 for four weeks at 27°C. The AcOEt extract (37.3 g) of the culture filtrate was passed through Sephadex LH-20, using CH_2Cl_2-MeOH (1:1) as the eluent. The second fraction (16.8 g) was chromatographed on silica gel with a gradient of MeOH-CH_2Cl_2 as the eluent to afford the CH_2Cl_2 eluate (2.17 g, Fr. 1), 2 fractions of the MeOH-CH_2Cl_2 (1:99) eluates (70 and 121 mg, Fr. 2 and Fr. 3, respectively), and the MeOH-CH_2Cl_2 (2:98) (70 mg, Fr. 4) and (5:95) (76 mg, Fr. 5) eluates. Fr. 1~5 yielded **3** (389.0 mg), **5** (14.0 mg), **4** (15.2 mg), **2** (2.8 mg) and **1** (28.0 mg), respectively, after purification by recrystallization. The mother liquor of recrystallization for Fr. 1 was chromatographed on silica gel with a gradient of *n*-hexane-CH_2Cl_2 as the eluent and the final separation of the *n*-hexane-CH_2Cl_2 (2:98) eluate by HPLC [ODS, MeOH-H_2O (4:1)] gave **6** (58.1 mg).

Sch 54794 (3)

Colorless needles (CH_2Cl_2), mp 200~203°C; $[\alpha]_D^{28}$ −70.4° (*c* 0.43, DMSO); EIMS *m/z* 380 (M^+); CD [*c* 4.95×10^{-5} M, EtOH] ($\Delta\varepsilon$) 291 (0), 267.3 (−1.33), 239.3 (0), 232.4 (+0.55), 224.7 (0), 214 (−16.0) nm. ^1H and ^{13}C NMR data in $CDCl_3$ containing CD_3OD and the other spectral data were identical with published values.[2]

Sch 54796 (4)

Colorless needles (CH_2Cl_2), mp 209~211°C; $[\alpha]_D^{16}$ −18.1° (*c* 0.23, DMSO); EIMS *m/z* 380 (M^+); CD [*c* 3.50×10^{-5} M, EtOH] ($\Delta\varepsilon$) 290 (+0.86), 282.5 (0), 242 (−3.46), 231 (−2.38), 215 (−7.36) nm. ^1H and ^{13}C NMR data in $CDCl_3$ containing CD_3OD and the other spectral data were identical with published values.[2]

3-[(4-Hydroxyphenyl)methyl]-1,4-dimethyl-3,6-bis(methylthio)-2,5-piperazinedione (5)

Colorless needles (CH_2Cl_2), mp 67~69°C; $[\alpha]_D^{16}$ −38.1° (*c* 0.13, $CHCl_3$); EIMS *m/z* 340 (M^+); CD [*c* 5.44×10^{-5} M, EtOH] ($\Delta\varepsilon$) 294 (0), 245 (+2.98), 229 (0), 211 (+6.27). The other spectral data were identical with published values.[3]

cis-Bis(methylthio)silvatin (6)

Colorless oil; $[\alpha]_D^{16}$ −43.9° (*c* 0.41, $CHCl_3$); EIMS *m/z* 408 (M^+); CD [*c* 5.96×10^{-5} M, EtOH] ($\Delta\varepsilon$) 300 (0), 237 (+2.67), 230 (+1.91) 216 (+7.62) nm. The other spectral data were identical with published values.[4]

N-Methylation of 3

A solution of **3** (38 mg, 0.1 mmol) in THF (4 ml) was added gradually to a suspension of NaH (13.2 mg, 60%, 3.3 eq.) in THF (1 ml) at 0°C. After stirring for 30 minutes, MeI (0.1 ml) was added and stirred further for 30 minutes. The reaction mixture was quenched with water, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography [EtOAc-hexane (1:1)] followed by HPLC [ODS, MeOH-H_2O (4:1)] to afford **7** (5.1 mg) and **8** (3.2 mg). **7**: Colorless oil, $[\alpha]_D^{28}$ +39.8° (*c* 0.50, $CHCl_3$); EIMS *m/z* 408 (M^+); CD [*c* 2.27×10^{-5} M, EtOH] ($\Delta\varepsilon$) 300 (0), 237 (−2.67), 230 (−2.00), 216 (−7.34) nm. The spectral data except for specific optical rotation and CD spectrum were identical with those of **6**. **8**: Colorless needles ($CHCl_2$), mp 130~132°C, $[\alpha]_D^{28}$ −25.2° (*c* 0.14, $CHCl_3$); EIMS *m/z* 408 (M^+). The spectral data except for specific optical rotation were identical with those of *trans*-bis(methylthio)silvatin.[4]

Epimerization of 3

The procedure described for *N*-methylation of **3** was carried out for **3** (38 mg, 0.1 mmol) using 10% HCl (0.1 ml) instead of MeI to afford **3** (2.7 mg) and **4** (1.7 mg).

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References

1) AMAGATA, T.; M. DOI, T. OHTA, K. MINOURA & A. NUMATA: Absolute stereostructures of novel cytotoxic metabolites, gymnastatins A~E, from a *Gymnascella* species separated from a *Halichodria* sponge. J. Chem. Soc. Perkin Trans. 1: 3585~3599, 1998 and references cited therein.
2) CHU, M.; R. MIERZWA, I. TRUUMEES, F. GENTILE, M. PETEL, V. GULLO, T.-M. CHAN & M. S. PUAR: Two novel diketopiperazines isolated from the fungus *Tolypocladium* sp. Tetrahedron Lett. 34: 7537~7540, 1993
3) HANSON, J. R. & M. A. O'LEARY: New piperazinedione metabolites of *Gliocladium deliquescens*. J. Chem. Soc., Perkin Trans. 1: 218~220, 1981
4) KAWAHARA, N.; K. NOZAWA, S. NAKAJAMA & K. KAWAI: Studies on fungal products. Part 13. Isolation and structures of dithiosilvatin and silvatione, novel dioxopiperazine derivatives from *Aspergillus silvaticus*. J. Chem. Soc., Perkin Trans. 1: 2099~2101, 1987
5) WANG, G.-Y.-S.; L. M. ABRELL, A. AVELAR, B. M. BORGESON & P. CREWS: New hirsutane based sesquiterpenes from salt water cultures of a marine sponge-derived fungus and the terrestrial fungus *Coriolus consors*. Tetrahedron 54: 7335~7342, 1998
6) NOZAWA, K.; S.-I. UDAGAWA, S. NAKAJIMA & K.-I. KAWAI: A dioxopiperazine derivative from *Penicillium megasporum*. Phytochemistry 28: 929~931, 1989
7) AYER, W. A.; I. VAN ALTENA & L. M. BROWNE: Three piperazinediones and a drimane diterpenoid from *Penicillium brevi-compactum*. Phytochemistry 29: 1661~1665, 1990
8) PEDRAS, M. S. C. & C. J. BIESENTHAL: Isolation, structure determination, and phytotoxicity of unusual dioxopiperazines from the phytopathogenic fungus *Phoma lingam*. Phytochemistry 58: 905~909, 2001
9) KING, R. R.; C. H. LAWRENCE & L. A. CALHOUN: Chemistry of phytotoxins associated with *Streptomyces scabies*, the casual organism of potato common scab. J. Agric. Food Chem. 40: 834~837, 1992
10) TAKAHASHI, C.; K. MINOURA, T. OKA, T. OHTA, S. HAGISITA & A. NUMATA: Absolute stereostructures of novel cytotoxic metabolites, penostatins A~E, from a *Penicillium* species separated from an *Enteromorpha* alga. Tetrahedron 55: 14353~14368, 1999
11) TAKAHASHI, C.; A. NUMATA, Y. ITO, E. MATSUMURA, H. ARAKI, H. IWAKI & K. KUSHIDA: Leptosins, antitumour metabolites of a fungus isolated from a marine alga. J. Chem. Soc. Perkin Trans. 1: 1859~1864, 1994