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Mycoediketopiperazine, a novel fungal metabolite from a *Papularia* sp.

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Abstract—A novel metabolite, mycoediketopiperazine, was isolated from a fungi culture (M-204036). Its structure and absolute stereochemistry was established by spectroscopic studies, including single-crystal X-ray diffraction analysis.

In the process of searching for useful natural products, it is generally agreed that a diverse and less exploited repertoire of microbes is essential to obtain a variety of novel metabolites.[1] Many novel secondary metabolites have been isolated from ordinary surroundings such as: plant,[2] terrestrial microorganisms, and marine organism etc.[3–5] Furthermore some secondary metabolites of novel structure and high bioactivity may also be isolated from extreme environment[6] such as high temperature, high pressure, concentrated acid or bases etc. But less attention is focused on fungi isolated from polluted environment, which might provide a good alternative to search for useful natural products. Here we have uncovered a novel metabolite by the name of mycoediketopiperazine (compound **1**, Fig. 1) from fungal fermentation designated as A4-Z-1, which was isolated from polluted environment. According to the vegetal characteristics of the fungus, A4-Z-1 was identified as a *Papularia* fungus.[7,8] This communication focuses on the isolation and structure elucidation of the new compound mycoediketopiperazine.

The fungus for production was isolated from a highly contaminated river in southern China. In the present experiment, the fungus strain was cultured on potato dextrose agar (PDA) medium with gentamicin for 7 days at 25 °C. After thalli were separated by filtration, it was extracted thoroughly with ethyl acetate. The extract was concentrated in vacuo and subjected to silica-gel flashes column chromatography.

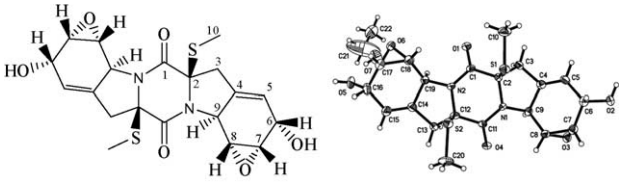

Figure 1. Molecular structure of mycoediketopiperazine and ORTEP diagram (40% probability ellipsoids) showing crystallographic atom numbering and solid-state conformation.

Mycoediketopiperazine was obtained as colorless sheet crystals from methanol. Its molecular formula $C_{20}H_{22}N_2O_6S_2$ was established by ESIMS $[M+Na]^+$ $m/z = 473.0$ and HREIMS $[M-SCH_3]^+$ $m/z = 403.0959$ ($\Delta 0.4$ mmu from calculated value). Its IR spectrum contained absorption bands at 1663 (NHCO), 3409 (OH), 814 (alkene), 1311 (SCH_3), 1257, 837 (epoxide) cm^{-1}. Table 1 lists the ^1H and ^{13}C NMR spectroscopic data and the assignment according to DEPT and COSY study. The 10 resonances of the ^{13}C NMR spectrum were assigned to groups of one primary methyl, one methene, four sp^3-hybridized methines, one

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Table 1. ^{1}H (500 Hz) and ^{13}C (125 Hz) NMR data of mycoediketopiperazine in DMSO

Assignment	δ C[a]	δ H (multiplicity, $J_{H/H}$ Hz)		^{1}H–^{1}H COSY
1	165.8 (q)			
2	71.2 (q)			
3	39.5 (s)	2.81 (Ha, d)	14	3b
		3.01 (Hb, d)	14	3a
4	133.6 (q)			
5	124.2 (t)	5.80 (1H, d)	6	6
6	61.8 (t)	4.45 (1H, dd)	6, 3, 8	5, 7, OH
7	53.1 (t)	3.38 (1H, m)	3, 3	6, 8
8	57.5 (t)	3.62 (1H, d)	3	7
9	55.5 (t)	4.23 (1H, s)		
10	13.9 (q)	2.08 (3H, d)		
OH		5.01 (1H, d)	8	6

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

trisubstituted double bond, one quaternary carbon, and one carbonyl, respectively. The ^{1}H NMR display nine signals in total, including one methyl group, one olefinic proton, three carbinols, one methene proton (split up into two signals), one methine proton. The NMR data indicated the existence of ten carbons, including one methyl, one methene, five methines, and three quaternary carbons. However, the molecular formula required 20 carbons; it means that the molecular structure is symmetry. And the further ESI-MS/MS fragment at *m/z* 403 $[M-SCH_3]^+$ implied that there was methylsulfanyl group.

The NMR data also implied that the molecule is symmetrical. On the other hand, the NMR data assigned eight oxygen-bonded carbons, including two carbonyl and six carbinol groups. However, the molecular formula contains six oxygens. From these results, the presence of two epoxide bridges between two carbinols was deduced. An epoxide between C-7 and C-8 was supported by the ^{1}H–^{1}H correlations of H-8 to H-7 (Table 1). Further analysis of proton spectra, including a H–H COSY study, indicated that H-6 (δ 4.43) correlates to the carbinol protons H-7 (δ 3.38). There is also a coupling between H-6 and olefinic protons H-5 (δ 5.80). The above observation and the ^{1}H–^{13}C correlation (Table 1), revealed that this system is as a six-membered ring with one double bond and an epoxide.

Based on $^{1}H/^{1}H$-coupling constants, NOE (NOESY), and X-ray diffraction studies, the absolute stereochemistry structure of the compound **1** was assigned. First of all, due to the fact that there was strong H-6/H-7 and H-7/H-8 COSY and the coupling constants $J_{H6/H7}$ and $J_{H7/H8}$ were less than 3 Hz, H-6, H-7, and H-8 were determined to occupy the same face of the molecular plane. Furthermore, the coupling constant $J_{H5/H6}$ was about 6 Hz, which was correspondent to a dihedral angle of H-5/H-6 at the range of 40°. No coupling between H-8 and H-9 indicated that H-8 and H-9 had a relative orientation of 180°. The structure and absolute stereochemistry (Fig. 1), as depicted, were firmly established by IR, ^{1}H NMR, ^{13}C NMR, MS, and X-ray crystallographic analysis.

Optical rotation was measured with a Perkin–Elmer 341 automatic polarimeter $[\alpha]_D^{20}$ −45.2 (*c* 1.0, CH_3OH:DMSO = 1:1). Melting point was determined on a Yanaco MP-500 micro melting point apparatus and was uncorrected (Mp = 182–184 °C). Compound **1** exhibited low potent cytotoxic activity in the KB cell line (IC_{50} 120 μg/mL) by the MTT assay protocol which was adapted from that described by Mosmann.[9]

Crystallographic data: $C_{20}H_{22}N_2O_6S_2{\cdot}C_2H_5OH$; $M = 450.53$, Tetragonal, space group $P212121$, $a = 9.469(3)$ Å, $b = 9.469(3)$ Å, $c = 23.915(6)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 2144.1(8)$ Å^3, $Z = 4$, Dc = 1.538 g cm^{-3}, λ (Mo Kα) = 0.7013 Å, $F(000) = 1048$, $T = 293$ K, colorless sheet, size $0.32 \times 0.30 \times 0.05$ mm, 12,979 independent measured reflections, refinement based on F^2 to give R_1 $[F^2 > 2\sigma(F^2)] = 0.0612$, $w_2 = 0.1523$ for 4957 observed reflections, and 298 parameters. Computations were carried out using the SHELXTL-97 program package. CCDC reference number is 204904. Copies of this information may be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44 1223 336 033; email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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References and notes

1. Sponga, F.; Cavaletti, L.; Lazzarini, A.; Borghi, A.; Ciciliato, I. *Biotechnology* **1999**, *70*, 65–69.
2. Ramachandra, R. S.; Ravishankar, G. A. *Biotechnol. Adv.* **2002**, *20*, 101–153.
3. Cai, P.; McPhail, A. T.; Krainer, E.; Katz, B.; Pearce, C.; Boros, C. *Tetrahedron Lett.* **1999**, *40*, 1479–1482.
4. Takahashi, C.; Minoura, K.; Yamada, T.; Numata, A.; Kushida, K. *Tetrahedron* **1995**, *51*, 3483–3498.
5. Burja, A. M.; Abou-Mansour, E.; Banaigs, B.; Payri, C.; Burgess, J. G.; Wright, P. C. *Microbiol. Methods* **2002**, *48*, 207–219.
6. Tepšic, K.; Gunde-Cimerman, N.; Frisvad, J. C. *FEMS Microbiol. Lett.* **1997**, *157*, 9–12.
7. Barnett, H. L.; Hunter, B. B. *Illustrated Genera of Imperfect Fungi*, 3rd ed.; China Scientific, 1977 (Chinese version) 144–145.
8. Hawksworth, D. L.; Sutton, B. C.; Ainsworth, G. C. *Dictionary of the Fungi*, 7th ed.; Commonwealth Mycological Institute: Kew, Surrey, 1983, 1–500.
9. Mosmann, F. *Immunol. Methods* **1983**, *65*, 55–63.