A NOVEL CLASS OF DIKETOPIPERAZINES

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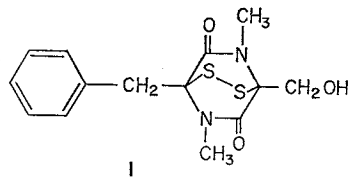
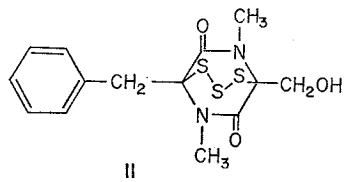
(Received for publication May 26, 1973)

In the course of our screening for antibiotics, NRRL 3888, an unidentified* fungus, was observed to produce three new substances related to the *epi*dithiadiketopiperazines such as gliotoxin[1], sporidesmin[2], and aranotin[3,4].

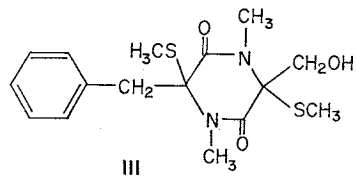
The following structures were determined for these metabolites: 2-benzyl-1, 4-dimethyl-5-hydroxymethyl-2, 5-*epi*-dithia-3, 6-diketopiperazine (**I**), 2-benzyl-1, 4-dimethyl-5-hydroxymethyl-2, 5-*epi*trithia-3, 6-diketopiperazine (**II**), and the bisdethiadi(methylthio) analogue of **I** (**III**).



I



II

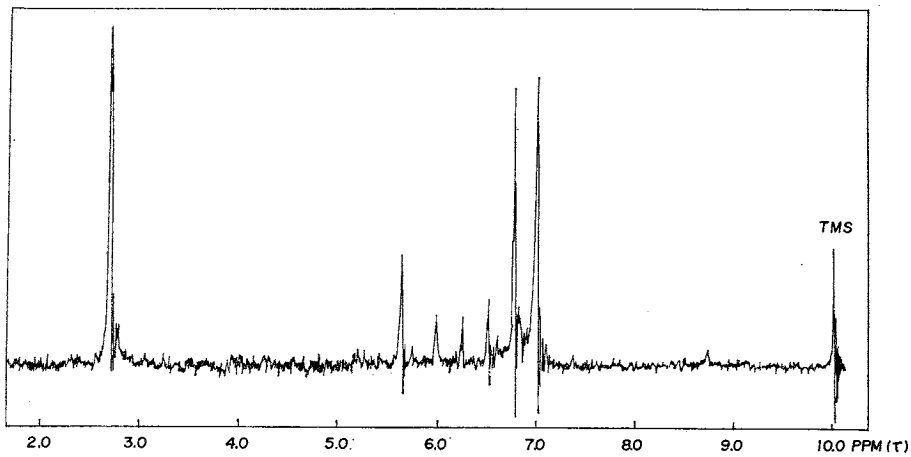


III

These structures are of particular interest because they represent the benzyldiketopiperazine suggested by NEUSS, *et al.*, 1968[5] as a late intermediate in the biogenesis of gliotoxin type and aranotin type metabolites. The possibility of such an intermediate was later corroborated by the finding that phenylalanine and not *m*-tyrosine is a precursor of both gliotoxin[6] and the aranotins.[7] The discovery of these three metabolites, notwithstanding the obvious limitations of such a statement, lends some support to the proposed biosynthetic scheme and at least shows that synthesis of the dithiadiketopiperazine moiety can preceed oxidation of the phenyl group and formation of the pyrrolidine ring.

The structures of these metabolites were deduced from proton magnetic resonance (pmr) and mass spectral data and from the chemical correlation between these metabolities and appropriately selected reference compounds. In the pmr spectrum of **I** (Fig. 1) the signal at τ 2.70 represents five aromatic protons. Singlet resonances at τ 6.80 and 7.04 arise from the 4-N and 1-N-CH_3 groups. Resonances arising from

Fig. 1. The proton magnetic resonance spectrum of compound **I** in $CDCl_3$ at 60 MHz.



* In spite of considerable effort generously afforded by the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, NRRL 3888 remained sterile, therefore making it impossible to identify the fungus.

Table 1. Antifungal spectra

Test organism	MIC (mcg/ml)		
	Gliotoxin	Compound **I**	Compound **III**
Candida albicans 10231	12.5	6.2	12.5
Epidermaphyton floccosum Wise	3.1	0.78	3.1
Microsporum canis VB	3.1	1.56	6.2
Microsporum gypseum 1236	25	6.2	25
Trichophyton mentagrophytes 9533	3.1	0.78	6.2
Microsporum audouinii 10216	6.2	1.56	12.5
Trichophyton tonsurans 10217	3.1	1.56	6.2
Trichophyton rubrum Robinson	6.2	6.2	12.5

the two methylene groups and observed as a singlet at τ 5.65 for the $-CH_2OH$ group and an AB quartet with doublets at τ 5.90 and 6.39 (J_{AB} =16 Hz), attributable to the benzylic-CH_2, are similar to those reported for dehydrogliotoxin[8,9]. The additional singlet observed in this region at τ 6.52 is caused by residual solvent methanol. The OH resonance was not observed. The singlets produced by the N-CH_3 protons shifted to 6.9 and 6.93 ppm in the pmr spectrum of **II**, while **III** produced additional signals at 7.7 (s, S-CH_3) and 7.85 (s, S-CH_3).

The mass spectra of substances **I**, **II**, and **III** exhibited relatively simple fragmentation. I produced a weak molecular ion *m/e* 324 ($C_{14}H_{16}N_2O_3S_2$) and a strong fragment ion at *m/e* 260 (m-S_2)$^+$, while **II** produced weak ions at *m/e* 356, 324, and 292 which were interpreted to represent a stepwise desulfurization of a homologue of **I**. The bisdethiadi (methylthio) analogue, **III**, produced a weak molecular ion, *m/e* 354 ($C_{16}H_{22}N_2O_3S_2$), and strong fragment ions at *m/e* 307 ($C_{15}H_{19}N_2O_3S$) and *m/e* 260 ($C_{14}H_{16}N_2O_3$). The mass spectra of **I**, **II**, and **III** each exhibited fragment ions at *m/e* 242 ($C_{14}H_{14}N_2O_2$), 231 ($C_{13}H_{15}N_2O_2$), and 91 (trophylium ion).

Versus a series of dermatophytes, as shown in Table 1, compounds **I** and **II** are relatively potent antifungal antibiotics whose spectra and potency are very similar to that of gliotoxin. The toxicity of these compounds, however, is also similar to that of gliotoxin [LD_{50} (mouse, i.p.): **I**, 35~50 mg/kg; **II**, 24~40 mg/kg]. Also like gliotoxin, **I** and **II** exhibit antibacterial activity primarily against Gram-positive organisms. Compound **III**, like the bisdethiadi (methylthio) analogues reported in the literature,[5] is not active against fungi and bacteria and is relatively non-toxic to mice [LD_{50} (i.p.): >200 mg/kg].

Acknowledgement

The authors wish to thank Dr. R. EGAN for pmr spectra, Dr. M. LEVENBERG for mass spectra, Ms. M. JACKSON and Mr. C. S. WADLEY for *in vitro* data, and particularly Mr. W. ANDRES for many helpful suggestions.

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