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The Structure of Diphenazithionin, a Novel Antioxidant from *Streptomyces griseus* ISP 5236

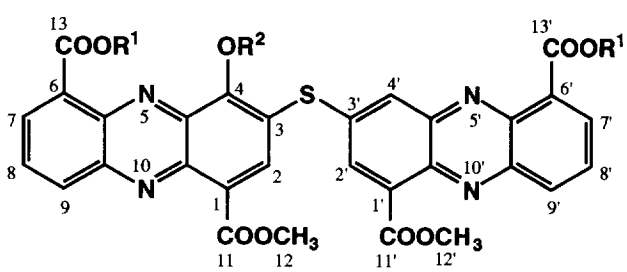
Yoshiko Hosoya, Hayamitsu Adachi, Hikaru Nakamura, Yoshio Nishimura, Hiroshi Naganawa, Yoshiro Okami* and Tomio Takeuchi

Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

Abstract: A novel member of the phenazine group of metabolites, diphenazithionin, has been isolated as an inhibitor of lipid peroxidation from *Streptomyces griseus* ISP 5236. It possesses a unique structure composing of two different phenazinecarboxylic acids connected by a sulfur linkage.

Free radicals are involved in causation of several significant human diseases including inflammation, ischemia repulfusion injury, rheumatism, *etc.*[1] Moreover, lipid peroxidation possibly plays an important role in inducing carcinogenesis or aging of cells.[2] Vitamins such as vitamin C or E are well-known agents to inhibit oxidation, but other potent and stable antioxidizing drugs can still be expected from nature. Actinomycetes have provided many kinds of potent antioxidants with low molecular weight.[3] We have investigated physiological responses and inducible secondary metabolites of organisms under oxidizing conditions, and designed a new screening methodology[4] using oxidizing agents added to the fermentation medium for actinomycetes. As a result of the screening, we isolated diphenazithionin (**1**), an inhibitor of lipid peroxidation which has a novel structure with the phenazine skeleton, from a culture filtrate of *Streptomyces griseus* ISP 5236. Here we report the structure elucidation by an X-ray crystallographic experiment.

Figure 1

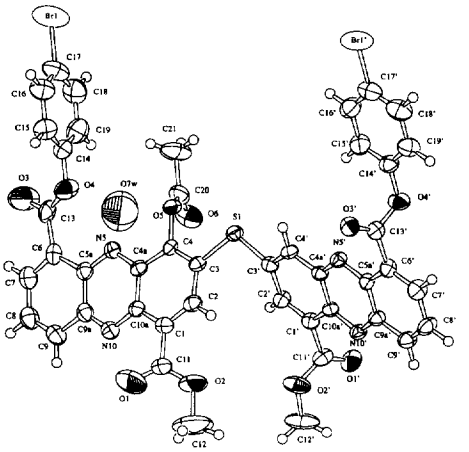


1: R^1=R^2=H (Diphenazithionin)

2: R^1=*p*-BrC_6H_4, R^2=$COCH_3$

Compound **1** [dark green powder, HR-FAB MS $(M+H)^+$ *m/z* 611.0878, $C_{30}H_{19}N_4O_9S$ (Calcd. 611.0873)] has two methyl esters and two carboxylic acid groups (δ_H 4.22 and 4.24, δ_C 168.5, 169.0, 170.2 and 170.6)[5]. Only 15 protons, including the two methyl groups, were also observed in the ^1H NMR spectrum[5]. The UV spectrum (λmax: 260, 305, 375 and 442) and the characteristic low-field chemical shifts of ^1H NMR spectrum showed a typical feature of the phenazine skeleton similar to phencomycin[6], a carboxylated phenazine compound. These facts suggested that the major skeletal structure was composed of two phenazine components.

Figure 2. X-Ray crystal structure of 2.



In order to prepare a single crystal for the X-ray crystallography experiment, we examined acetylation of the hydroxy group (CH_3COCl, NaOH, $[(CH_3(CH_2)_3]_4N(HSO_4)$, CH_3CN)[7] and esterification of two carboxyl groups with 4-bromophenol (*p*-BrC_6H_4OH, Et_3N, BOP, CH_2Cl_2)[8]. The resulting ester **2** crystallized as brown needle crystals [mp 218-219°C (decomp)] in the triclinic space group $P\bar{1}$ with a=12.756(2), b=16.027(2), c=10.501(3) A°, and α=95.95(2), β=102.92(2), γ=75.88(1)°, V=2026.4(7) A°^{3}, Z=2. All reflections were collected at 21°C, λ(CuKα)-1.54178 A°, ω-2θ scan mode to 2θmax=120.2°. Of the 6350 reflections collected, 6026 reflections were unique. The structure was solved by direct methods (SHELX86), and the non-hydrogen atoms were anisotropically refined, including the hydrogen atoms in the calculated position. The final cycle of full-matrix least-squares refinement was based on 4446 observed reflections (I>2.00 σ(I)) and converged with R=0.077. Crystallographic parameters have been deposited with the Cambridge Crystallographic Data Center. An ORTEP drawing of **2** is shown in Fig. 2.

As shown in Fig. 2, the present study clarified the structure of diphenazithionin as 4-hydroxy-3,3'-thio-di(1-carbomethoxyphenazine-6-carboxylic acid).

The phenazine compounds have often been isolated from streptomycetes as antibiotics[9] and also as antioxidants, but we have reported in this paper the first discovery of diphenazithionin, a dimeric phenazinecarboxylic acid linked by a sulfur atom, showing strong antioxidant activity.

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5. ^{1}H-NMR (TFA-d, 500 MHz) δ 9.52 (s, 2-H) 9.30 (d, *J*=7.2 Hz, 7'-H), 8.47 (dd, *J*=7.2, 6.1 Hz, 8'-H), 8.96 (d, *J*=7.2 Hz, 9'-H), 8.83 (brs, *J*=0.8 Hz, 2'-H), 8.36 (brs, J=0.8 Hz, 4'-H), 9.24 (d, *J*=6.1 Hz, 7-H), 8.79 (dd, *J*=7.2, 6.1 Hz, 8-H), 9.21 (d, *J*=7.2 Hz, 9-H), 4.22 (3H, s, 12-H), 4.24 (3H, s, 12'-H); ^{13}C-NMR (TFA-d, 125 MHz) δ 56.0 (12'), 56.4 (12), 114.3 (3), 120.7, 121.4 (4'), 128.5 (9), 130.4, 130.8 (6), 134.6 (9a), 134.9 (4), 134.9 (8'), 135.6, 135.7, 137.6 (9'), 138.8, 139.1 (2'), 141.2 (7), 142.3 (10'a), 143.0 (8), 143.2 (6'), 145.7 (7'), 153.4 (2), 154.8, 165.2 (1), 168.5 (11'), 169.0 (11), 170.2 (13'), 170.6 (13).
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