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STRUCTURE OF PHENAZINOMYCIN, A NOVEL ANTITUMOR ANTIBIOTIC

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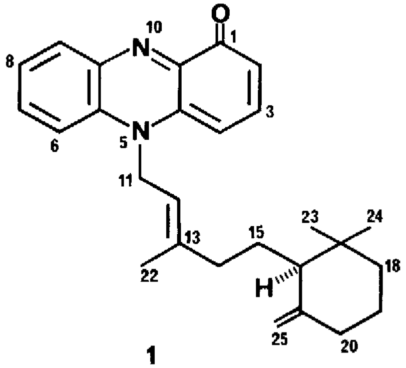
Summary: Structure and absolute configuration of a novel antitumor antibiotic phenazinomycin was determined on the basis of the spectral data and chemical degradation. Phenazinomycin is the first phenazinone alkaloid which possesses sesquiterpene moiety in its structure.

In the course of our screening program for new antitumor antibiotics, a novel antibiotic phenazinomycin (1) was isolated from the mycelial extracts of Streptomyces WK-2057[1)]. This compound showed stronger cytotoxic activity against adriamycin-resistant P388 leukemia cells (IC_{50} 3.1 μg/ml) rather than against normal P388 leukemia cells (IC_{50} 25 μg/ml) and showed antitumor activity against S180 *in vivo* (ILS 140%, 22.2 mg/kg/day x 9, ip). This paper deals with the structure elucidation of phenazinomycin (1).



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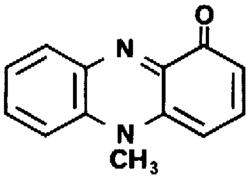
Molecular formula of phenazinomycin2) was established to be $C_{27}H_{32}N_2O$ by the combination of high resolution mass spectral data and ^1H and ^{13}C NMR spectra. In the UV spectrum of phenazinomycin, a characteristic absorption maximum at 745 nm was observed and which is quite similar to that of pyocyanine (2, 5-methyl-1-phenazinone)4). Pyocyanine (2) was prepared4) and the physico-chemical properties of 2 were compared with those of phenazinomycin. The ^1H NMR spectrum of 2 exhibited two sets of aromatic proton signals and the chemical shifts and coupling patterns of both sets [(δ 6.50, 6.58 and 8.05) and (δ 7.71, 7.87, 8.05 and 8.30)] were identical with those of phenazinomycin (Table 1). As a consequence, it was estimated that a 1-phenazinone moiety was involved in the structure of phenazinomycin. The existence of a carbonyl signal at δ 178.8 and eleven other signals in the ^{13}C NMR spectrum of phenazinomycin (Table 1) also verify the hypothesis proposed. The structure of the remaining portion attached to N_5 position consists of fifteen carbons was deduced by the NMR spectral studies of phenazinomycin. In view of the observation of an nOe effect between C_{11}-methylene and C_4-H and C_6-H, the position (N_5) of this unit was confirmed (Fig. 1).



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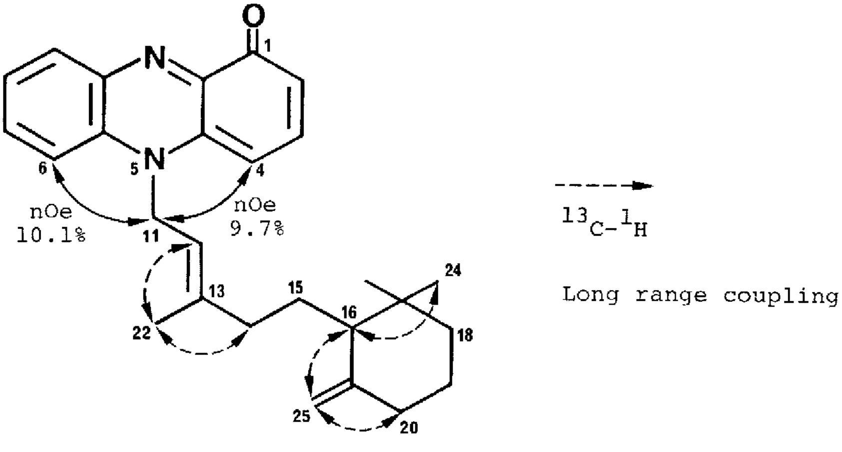
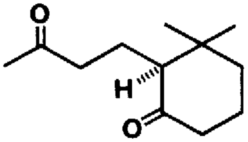


Fig. 1. ^{13}C-^1H Long range coupling pattern and nOe in phenazinomycin (1).

Table 1. NMR spectral data of phenazinomycin (1) and pyocyanine (2).

	1		**2**
No.	δ_C	δ_H	δ_C
1	178.8 s	-	178.2 s
2	115.8 d	6.59 (1H, d, J = 8.4 Hz)	115.5 d
3	146.9 d	7.90 (1H, dd, J = 8.4, 8.4 Hz)	146.5 d
4	94.5 d	6.39 (1H, d, J = 8.4 Hz)	94.4 d
4a	135.7 s	-	136.1 s
5a	133.9 s	-	133.7 s
6	116.5 d	7.88 (1H, d, J = 7.8 Hz)	116.5 d
7	137.5 d	8.02 (1H, dd, J = 7.8, 7.8 Hz)	137.4 d
8	127.3 d	7.70 (1H, dd, J = 7.8, 7.8 Hz)	127.2 d
9	134.2 d	8.33 (1H, d, J = 7.8 Hz)	134.2 d
9a	138.3 s	-	138.0 s
10a	147.6 s	-	147.6 s
5-CH_3			36.1 q
11	48.3 t	5.32 (2H, d, J = 5.2 Hz)	-
12	117.6 d	5.14 (1H, t, J = 5.2 Hz)	-
13	144.1 s	-	-
14	38.8 t	1.98 (2H, m)	-
15	25.2 t	1.57 (2H, m)	-
16	54.4 d	1.65 (1H, m)	-
17	35.8 s	-	-
18	37.6 t	1.28 (2H, m)	-
19	24.8 t	1.48 (2H, m)	-
20	33.6 t	1.90 (2H, m)	-
21	150.4 s	-	-
22	17.1 q	2.00 (3H, s)	-
23	26.5 q	0.78 (3H, s)	-
24	28.9 q	0.84 (3H, s)	-
25	109.7 t	4.39 (1H, s)	-
		4.66 (1H, s)	-

Phenazinomycin was degradated by ozonolysis[5] to afford 3 ($C_{12}H_{20}O_2$, M^+ 196) which had been previously obtained by oxidation of trixagol with $KMnO_4$/acetone[7]. This fact also supports the partial structure of phenazinomycin as described above. The absolute configuration of the asymmetric carbon of 3 was determined to be S by comparing the CD spectrum of this compound with those of the previously reported products[5,7] and the absolute configuration of C_{16} of phenazinomycin (1) was concluded to be R.



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From all of the observations described above, the structure of phenazinomycin was concluded to be 1. This is the first report of a compound which possesses both of phenazinone and sesquiterpene moieties in a structure.

Acknowledgements This work was supported, in part, by Grants-in-Aid from the ministry of Health and Welfare and the Ministry of Education, Science and Culture, and by funds from the Japan Keirin Association.

References and Notes

1) S. Ōmura, S. Eda, S. Funayama, Y. Takahashi and K. Komiyama: *J. Antibiotics*, in press.
2) Phenazinomycin was obtained as dark blue powder: mp. 113-118°C; $[\alpha]_D^{25}$ -49° (c=0.45, CH_3OH); Anal. C 81.26, H 8.12, N 6.29, Calcd. for $C_{27}H_{32}N_2O$, C 80.96, H 8.05, N 6.99; UV λ_{max} (CH_3OH) 240 (24500), 321 (27200), 745 nm (6600); IR ν_{max} (KBr) 2938, 1633, 1501, 1467, 1446, 1243, 1159, 887, 755, 723 cm^{-1}; EI-MS *m/z* 400 (M^+), 275, 263, 247, 223, 196, 109, 95, 81, 69, 55; HR-MS found 400.251, Calcd. for $C_{27}H_{32}N_2O$ 400.252; 500 MHz ^1H NMR (CD_3OD) and 125.76 MHz ^{13}C NMR (CD_3OD) see Table 1.
3) A.R. Surry: *Organic Synthesis*, **3**, 753-756 (1955).
4) Pyocyanine was obtained as dark blue powder: mp. 129-131°C; UV λ_{max} (CH_3OH) 239 (21600), 319 (24300), 746 nm (5800); IR ν_{max} (KBr) 1626, 1603, 1560, 1492, 1460, 1258, 1169 cm^{-1}; EI-MS *m/z* 210 (M^+), 196, 181, 168, 140, 102, 91, 84, 77, 63, 51; 300 MHz ^1H NMR (CD_3OD) δ 4.01 (3H, s, N_5-CH_3), 6.50 (1H, d, *J* = 8.4 Hz, C_4-H), 6.58 (1H, d, *J* = 8.4 Hz, C_2-H), 7.71 (1H, dd, *J* = 8.2, 8.2 Hz, C_8-H), 7.87 (1H, d, *J* = 8.2 Hz, C_6-H), 8.05 (1H, dd, *J* = 8.4, 8.4 Hz, C_3-H), 8.05 (1H, dd, *J* = 8.2, 8.2 Hz, C_7-H), 8.30 (1H, d, *J* = 8.2 Hz, C_9-H); 75.47 MHz ^{13}C NMR (CD_3OD) see Table 1.
5) H. Wu, H. Nakamura, J. Kobayashi, M. Kobayashi, Y. Ohizumi and Y. Hirata: *Bull. Chem. Soc. Jpn.*, **59**, 2495-2504 (1986).
6) Phenazinomycin (**1**, 40 mg, 0.1 mmol) was dissolved in methanol and was saturated with ozone at -78°C for 30 min. After excess ozone was removed by N_2 stream and to this solution was added triphenylphosphin (52 mg, 0.2 mmol). The solution was stirred successively at 0°C for 30 min and at r.t. over night. The solution was chromatographed over silica gel (4 g) with hexane-ethyl acetate (10:1) as an eluent to yield **3** as a colorless syrup: Yield 5.0 mg; $[\alpha]_D^{25}$ +5.5° (c = 0.25, $CHCl_3$); CD $[\theta]_{292}$ +8232; IR ν_{max} ($CHCl_3$) 2962, 1710, 1368, 1162 cm^{-1}; EI-MS *m/z* 196 (M^+), 181, 163, 139, 123, 111, 95, 83, 69, 55; 300 MHz ^1H NMR (CD_3OD) δ 0.78 (3H, s), 1.09 (3H, s), 2.12 (3H, s), 1.5-2.6 (11H, m); 75.47 MHz ^{13}C NMR (CD_3OD) δ 18.1 (t), 21.8 (q), 23.1 (t), 29.6 (q), 30.0 (q), 39.4 (s), 39.8 (t), 41.5 (t), 42.6 (t), 60.0 (d), 209.0 (s), 213.4 (s).
7) J.D.P. Teresa, E. Caballero, C. Caballero, M. Medarde, A.F. Barrero and M. Grande: *Tetrahedron Lett.*, **19**, 3491-3494 (1978).

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