On the Formation of "Symmetrical" Phenoxazinone Derivative Related to the Actinomycin D Chromophore

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In studies related to the synthesis of 2-deamino chromophores of actinomycin by reaction of nitrous acid in fluoroboric acid *via* diazotization of the 2-amino group in the chromophore, the unknown fluorescent product is identified as the symmetrical phenoxazinone by cmr and pmr.

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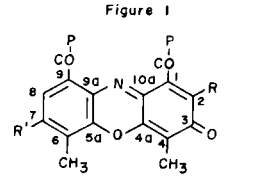
Actinomycin D (AMD, **1b**) is an antibiotic known for its antineoplastic action [1,2]; it is very effective in the treatment of Wilms' tumor in children [3] and gestational choriocarcinoma in adults [4]. The antitumor activity of AMD is believed to result, in part, from its intercalation with double-stranded DNA in the cell. From x-ray analysis [5], nmr and other spectral studies [6,7] it is now established that AMD binds to duplex DNA by intercalation of its chromophore between guanosine-cytosine sequences, with the peptide portion lying in the narrow groove of the DNA helix. This drug-DNA interaction appears to be responsible for AMD's selective inhibition of DNA dependent RNA synthesis [8]. However, recent evidence suggests that DNA breaks in cells can be mediated *via* free-radicals which derive from AMD phenoxazinone ring system [9,10]. Thus, in addition to the antibiotic's ability to bind to and inhibit biochemical reactions involving DNA, it may also cause chromosomal damage in cells by generating reactive reduced oxygen species [11,12].

Figure 1



a, P = $-N(C_2H_5)_2$

b, P = -Thr-D-Val-Pro-Sar-MeVal-O

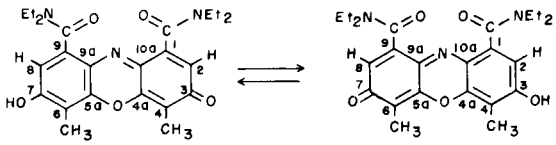
	R	R'
1	$-NH_2$	-H
2	$-NO_2$	-H
3	-H	-H
4	-H	-OH
5	-H	$-OCH_3$

In our continuing search for actinomycins possessing improved antitumor activity, low toxicity and/or broader range of activity, we recently introduced a nitro group at the 2-position of the phenoxazinone moiety in actinomycin D (**1b**) [13] and also in a model derivative of actinomycin (**1a**) (Figure 1) in which the pentapeptide lactone amide moieties at positions 1 and 9 of AMD (**1b**) are replaced by diethylamino groups [14]. Diazotization of the model compound **1a** with nitrous acid-fluoroboric acid by a modification of the procedure of Beamen *et al.* [15] followed by the reaction of the diazonium salt with nitrous acid in the presence of cupric sulfate was found to produce the 2-nitro chromophore **2a** (R = NO_2) and the 2-deamino chromophore **3a** (R = H) along with an unidentified fluorescent fraction which was eluted with 1:1 acetone:chloroform [14]. In this communication we have identified this unknown fluorescent product as the symmetrical phenoxazinone derivative **4a**, evidenced by proton and carbon magnetic resonance properties and microanalytical data.

Phenoxazinone **4a** was obtained as a dark red solid in 10% yield, R_f 0.036 (silica gel, 1:8 acetone:chloroform); uv:

Table I

^{13}C-NMR Chemical Shifts of Symmetrical Phenoxazinone Derivative **4a** [a]

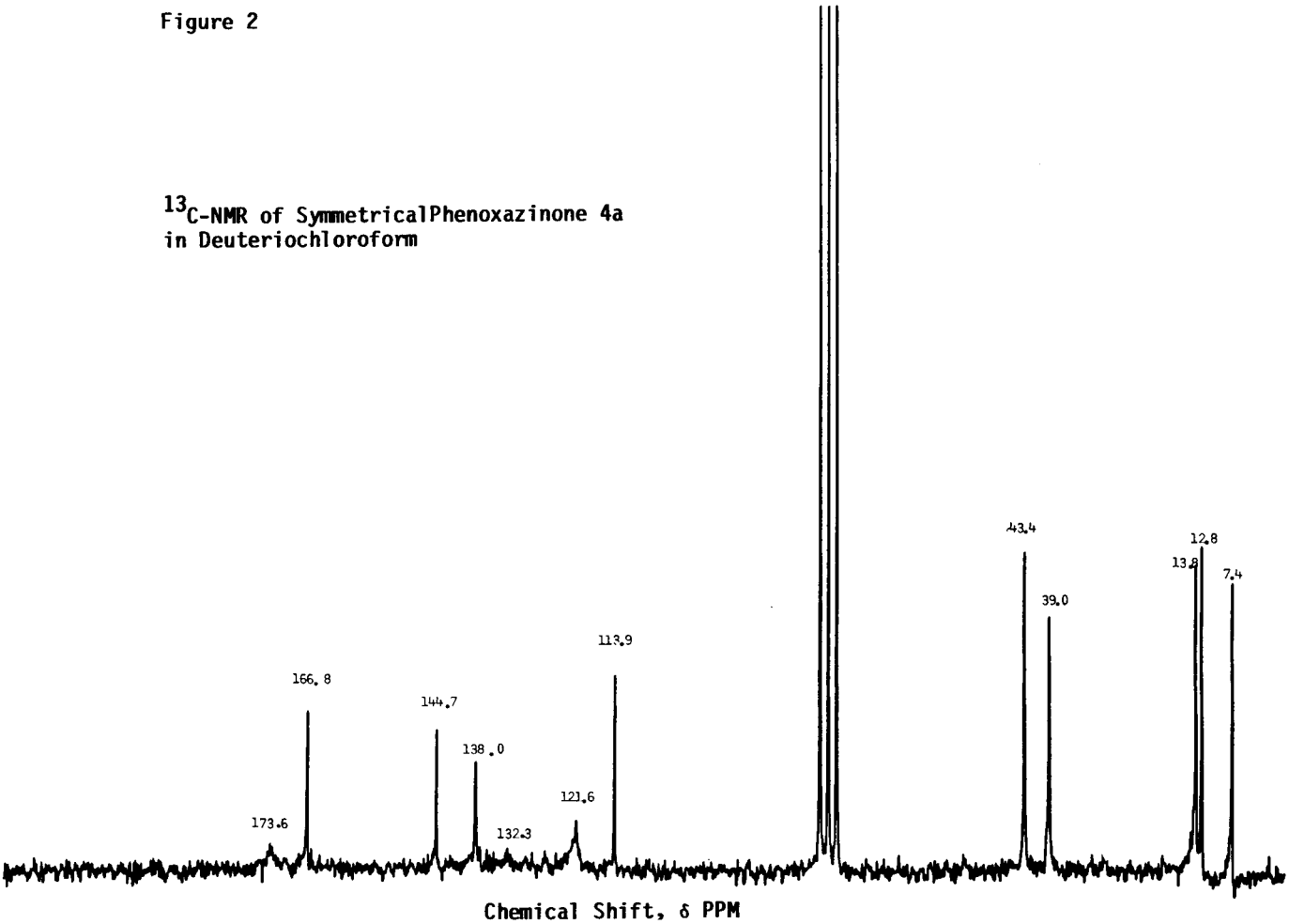


C-atom	
1,9	138.0
2,8	121.6 (broad)
3,7	173.6 (broad)
4,6	113.9
4a,5a	144.7
9a,10a	132.3 (broad)
C4,C6-Me	7.4
C1,C9-CO	166.8
$-N(CH_2CH_3)_2$	39.0
	43.4
$-N(CH_2CH_3)_2$	12.8
	13.8

[a] Chemical shifts are in parts per million downfield from tetramethylsilane and are referenced with respect to that internal marker in deuteriochloroform.

Figure 2

^{13}C-NMR of Symmetrical Phenoxazinone 4a in Deuteriochloroform



λ max nm (ε × 10^{-3}) 485 (8.24), inf 427 (7.24), inf 263 (12.33), inf 245 (20.11), 227 (27.81). Fluorescence spectrum in 95% ethanol: excit. (λ max nm) 310, 370, and 485, emission (φ max nm) 615. In the infrared prominent O-H stretch appeared at 3425 cm^{-1} indicating the presence of a free hydroxyl group. *Anal.* Calcd. for $C_{24}H_{29}N_3O_5 \cdot H_2O$: C, 63.02; H, 6.78; N, 9.19. Found: C, 62.76; H, 6.69; N, 9.07. Its nmr is consistent with **4a** being a symmetrical molecule and in an equilibrium mixture of its tautomeric forms, *i.e.* a 3-OH, and a 7-OH derivative (Table I); [δ 6.72 (s, C 2 or 8, 2H), 5.74 (brs, O*H*), 3.78-2.93 (m, N-(C*H*$_2$-CH$_3$)$_2$, 8H), 2.01 (s, C 4,6-(CH$_3$)$_2$, 6H), 1.38-0.84 (m, N-(CH$_2$-C*H*$_3$)$_2$, 12H)]. From the nmr chemical shift (δ) values it is evident that the two ring methyls and also the two ring hydrogens at C-2 and C-8, respectively, are equivalent due to a rapid tautomerization between the quinoid and the benzenoid rings. That this is a symmetrical molecule was further confirmed by its cmr whose chemical shift (δ) values are summarized in Table I.

The cmr spectrum of **4a** displays twelve absorptions (Figure 2) and in combination with the combustion analysis establishes the symmetrical nature of the molecular structure on the cmr time scale. INEPT[1] pulse sequences and SFORD experiments revealed methine (δ 121.6), methylene (δ 39.0 and 43.4), and methyl (δ 7.4, 12.8, and 13.8) absorptions. The carbon absorptions of the ethyl groups on the amide nitrogen atoms were readily apparent while the absorptions at δ 7.4, 113.9, 144.7, and 166.8 were assigned by comparison with phenoxazinone derivatives **1a-3a** for which cmr chemical shift assignments had been established [14]. The absorptions at δ 121.6, 132.3, and 173.6 are broad and of low intensity consistent with an equilibration of tuatomers on the time scale of the cmr experiment. The absorption at δ 138.0 is roughly the average of the C-1 (δ 146.5) and C-9 (δ 128.9) absorptions in **3a** and accordingly was assigned to C-1 and C-9 in **4a**. The absorptions at δ 121.6, 173.6, and 132.3 show an increased shielding (approximately 10 ppm) relative to the C-2 (δ 130.6), C-3 (δ 184.9), and C-10a (δ 141.4) absorptions in **3a** as expected from a consideration of resonance structures and were assigned to C-2 and C-8, C-3 and C-7, and C-9a and C-10a, respectively, in structure **4a**.

To further prove its structure, the hydroxy function in the symmetrical phenoxazinone **4a** was converted into its

methyl ether derivative **5a** by alkylation with methyl iodide in dry acetone at ambient temperature in the presence of anhydrous potassium carbonate [16]. The methoxy derivative was obtained in nearly quantitative yield and showed a single spot on tlc. Its identity was established by nmr (see Experimental).

EXPERIMENTAL

The ir spectra were taken with a Perkin Elmer Model 457A Grating spectrophotometer in potassium bromide pellets, uv spectra were measured for solutions in ethanol with a Gilford Model 250 spectrophotometer, and nmr spectra were determined on a JEOL-FX 90Q spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Analytical tlc's were done on 5 × 20 cm precoated glass plates with a 0.25 mm layer of silica gel-25 (Macherey-Nagel, West Germany) with chloroform:-acetone (8:1) as the developing agent and preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2 mm layer of silica gel PF-254 (E. Merck, Darmstadt, Germany). The compounds were detected by visual examination under uv light (254 nm). Microanalyses were determined by Galbraith Laboratories, Knoxville, Tennessee.

Reaction of 2-Deamino-1,9-bis-(*N,N*-diethylcarbamoyl)-4,6-dimethyl-7-hydroxy-3*H*-phenoxazin-3-one (**4a**) with Methyl Iodide.

A solution of the 2-deaminophenoxazinone (**4a**) (9.14 mg, 0.02 mmole) in dry acetone (10 ml) was allowed to react with 200 μl of methyl iodide in the presence of anhydrous potassium carbonate (10 mg) in a nitrogen atmosphere at room temperature for 16 hours. The orange reaction mixture was filtered to remove inorganic salts and the filtrate was concentrated to dryness. The residue was purified by preparative layer chromatography to homogeneity using 1:16 acetone:chloroform and the orange colored band was extracted with 1:4 acetone:chloroform.

Compound **5a** was obtained as a red solid (8 mg, 87%); R_f 0.27; uv: λ max nm (ϵ 10^{-3}) 475 (3.96), 438 (3.96), inf 253 (19.09), 227 (41.74). In the infrared the bands for an O-H stretch were absent; nmr: (δ) 6.77 (s, C-8(2), 1H), 6.71 (s, C-2(8), 1H), 3.96 (s, C-3(7)-OCH_3, 3H), 3.73-2.93 (m, N-(CH_2-CH_3)$_2$, 8H), 2.29 (s, C-6(4)-CH_3, 3H), 2.13 (s, C-4(6)-CH_3, 3H), 1.51-0.78 (m, N-(CH_2-CH_3)$_2$, 12H).

Anal. Calcd. for $C_{25}H_{31}N_3O_5 \cdot 2CH_3COCH_3 \cdot H_2O$: C, 63.37; H, 7.66; N, 7.16. Found: C, 63.07; H, 7.21; N, 6.99.

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REFERENCES AND NOTES

[1] U. Hollstein, *Chem. Rev.*, **74**, 625 (1974).

[2] H. Brockmann, *et al.*, *Cancer Chemother. Rep.*, **58**, 1 (1974).

[3] S. Farber, *J. Am. Med. Assoc.*, **198**, 820 (1966).

[4] J. L. Lewis, Jr., *Cancer*, **30**, 1517 (1972).

[5] H. M. Sobell and S. C. Jain, *J. Mol. Biol.*, **68**, 21 (1972).

[6] H. Lackner, *Angew Chem., Int. Ed. Engl.*, **14**, 375 (1975).

[7] W. Muller and D. M. Crothers, *J. Mol. Biol.*, **35**, 251 (1968).

[8] J. Meienhofer and E. Atherton, in "Structure Activity Relationships Among the Semisynthetic Antibiotics", D. Perlman, ed, Academic Press, New York, 1977, pp 427-529.

[9] N. R. Bachur, M. V. Gee and S. L. Gordon, *Proc. Am. Assoc. Cancer Res.*, **19**, 75 (1978).

[10] N. R. Bachur, S. L. Gordon, M. V. Gee and H. Kon, *Proc. Natl. Acad. Sci., U.S.A.*, **76**, 954 (1979).

[11] W. Ostertag and W. Kersten, *Exp. Cell Res.*, **39**, 296 (1965).

[12] H. Nakazawa, Feng-te E. Chou, P. A. Andrews and N. R. Bachur, *J. Org. Chem.*, **46**, 1493 (1981).

[13] R. K. Sehgal, R. K. Dieter and S. K. Sengupta, *J. Heterocyclic Chem.*, **22**, 665 (1985).

[14] R. K. Sehgal, R. K. Dieter and S. K. Sengupta, *J. Heterocyclic Chem.*, **21**, 1661 (1984).

[15] A. G. Beaman, W. Tautz, T. Gabriel and R. Duschinsky, *J. Am. Chem. Soc.*, **87**, 389 (1965).

[16] S. K. Sengupta, S. K. Tinter, H. Lazarus, B. L. Brown and E. J. Modest, *J. Med. Chem.*, **18**, 1175 (1975).