Synthesis and CMR Analysis of Phenoxazinone Derivatives Related to the Actinomycin D Chromophore

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Synthesis of 2-deamino- and 2-deamino-2-nitro-chromophores of actinomycin was accomplished by reaction of nitrous acid in fluoroboric acid *via* diazotization of the 2-amino group in the chromophore. Structural assignments were made by cmr and pmr.

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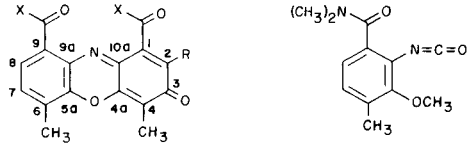
Among a variety of clinically useful antitumor agents actinomycin D (AMD, **1b**) is one of the few possessing curative effects against two different kinds of tumors [1,2], namely Wilms' tumor [3] and gestational choriocarcinoma [4]. Unfortunately, its spectrum of antitumor activity in man is relatively narrow and its administration difficult due to its high toxicity. The development of modified actinomycins with a broader range of activity is thus highly desirable. The mechanism of action of AMD results from its ability to intercalate into double stranded DNA, and the overall effect is the inhibition of DNA dependent RNA synthesis [5]. Although intercalation is generally believed to account for the antineoplastic acitivity of the drugs [5], recent evidence suggests that cell death may result from damage to DNA caused by bioreductive activation of the antibiotic to a free radical state [6] by microsomal and nuclear flavoproteins. In order to increase the therapeutic effectiveness while simultaneously decreasing the high toxicity associated with AMD treatment a large number of AMD derivatives have been evaluated for their biological activities [7-9]. Previous studies emphasized that the intact peptide lactones and the 2-amino, 3-quinoid oxo, and 4- and 6-methyl groups of the phenoxazinone ring are necessary for activity [5]. Our work clearly demonstrated that the molecule of AMD can be altered at the C-7 and N^2-sites without damage to its DNA-binding and antitumor properties [10]. We observed that the presence of a free amino group at C-2 is not essential for the antitumor activities and that some analogs with poor afinity for DNA can even act as superior agents if they can be activated *in vivo* [8,11]. Sinha *et al.* [12] have reported that certain spin-labelled N^2 analogs of AMD which can be internally activated to free radical species are more effective against P-388 tumor in mice and this activity was demonstrated in spite of the loss of their DNA intercalative binding property. Bachur suggested that antitumor activity of AMD may derive partly from the quinoneimino chromophore which like other quinones may act as a substrate for red-ox enzyme mediated transformation to quinoneimino radicals leading to chromosomal damages in cells [6,13]. Sinha further demonstrated that a spin-labelled AMD analog is a better substrate for certain red-ox enzymes, and shows improved activity *in vivo* [14].

As part of our continuing effort to synthesize improved antitumor actinomycin D derivatives we planned to introduce 2-nitro function into actinomycin D. We believed that the nitro group could enhance its radical forming capability both in epr and in presence of red-ox enzymes in cells, especially proliferating tumor cells. A possible route to this compound was suggested by the work of Jones and Robins [15] who, by the action of nitrous acid were able to convert a series of 8-aminopurines, *via* diazotization, to 8-nitropurines. Thus a nitro group was introduced into the 2-position of the imidazole moiety in the purine ring system. This work was then extended to the synthesis of the antibiotic azomycin by Beaman *et al.* [16] who were successful in converting 2-aminoimidazole into the naturally occurring 2-nitroimidazole in improved yields in the presence of cupric sulfate. Our initial attempts were directed at actinomycin chromophore model compound **1a** (R = NH_2) in which the pentapeptide lactone amide moieties at positions 1 and 9 in AMD (**1b**) were replaced by diethylamino groups. Diazotization of the model compound **1a** with nitrous acid-fluoroboric acid by a modification of the procedure of Beaman *et al.* [16], followed by the reaction of the diazonium salt with nitrous acid in the presence of



1-3

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

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

c, X = -OH

	R
1	$-NH_2$
2	$-NO_2$
3	$-H$

Table I

Proton NMR Spectral Data of Phenoxazinone Derivatives [a]

Compound	7-H	8-H	2-NH_2	2-H	4-CH_3	6-CH_3	-$N(CH_2$-$CH_3)_2$	-$N(CH_2$-$CH_3)_2$
1a	7.28 (d)	7.14 (d)	5.44 (brs)	—	2.22 (s)	2.50 (s)	2.93-3.77 (m)	0.89-1.38 (m)
2a	7.49 (d)	7.07 (d)	—	—	2.19 (s)	2.49 (s)	2.84-3.84 (m)	0.75-1.33 (m)
3a	7.38 (d)	7.11 (d)	—	6.79 (s)	2.14 (s)	2.47 (s)	2.93-3.69 (m)	0.89-1.38 (m)

[a] 90 MHz spectrum of deuteriochloroform solution, chemical shifts in parts per million (δ) to low field from internal tetramethylsilane.

cupric sulfate afforded 2-nitro chromophore **2a** (R = NO_2) and the 2-H chromophore **3a** (R = H) by deamination of the chromophore **1a**, along with other minor products.

Catalytic reduction of the model compound **2a** in the presence of platinum oxide in 95% ethanol at 40-45 atmosphere pressure produced the starting compound (**1a**) quantitatively (*via* an aminophenol intermediate) [17] indicating a nitro group is introduced at the desired 2-position. A similar reduction of **3a** led to the recovery of the starting material only. Proton (pmr) and C-nmr (cmr) spectra of the analogs **1a-3a** are summarized in Tables 1 and 2.

Carbon magnetic resonance (cmr) chemical shift assignments for compounds **1a-3a** are based upon selective proton decoupling and gated non-decoupling (with NOE) experiments and comparison with the model actinocin (**1c**) whose cmr spectra has been reported. Selective irradiation of the downfield aromatic proton in **1a** and **3a** (7.28 and 7.38 respectively) resulted in collapse of the downfield doublets (δ 130.7 and 134.2) in the cmr spectra to broad absorption peaks with discernable long range coupling. The upfield doublets (δ 122.1 and 122.2, respectively) in the cmr spectra of **1a** and **3a** collapsed to very narrow doublets during selective irradiation of the downfield aromatic proton as a result of the small chemical shift differences between the C-7 and C-8 protons. Gated non-decoupling with NOE revealed simple upfield doublets at δ 122.1, 123.0, and 122.2 for **1a-3a** respectively, while the downfield resonances at δ 130.7, 136.1 and 134.2 appeared as a doublet of quartets indicating long range coupling to the C-6 methyl substituent. These results clearly establish the C-7 and C-8 cmr chemical shift assignments. They also establish the C-7 and C-8 proton chemical shift assignments in the pmr spectra confirming the downfield absorption of the C-7 proton relative to the C-8 proton in **1a** and **3a**. This is a reversal of previous assignments [17]. Similarly, the C-7 carbon resonance is downfield from the C-8 absorption in contrast to the assignments reported for actinocin (**1c**) [18]. The trend in cmr chemical shift assignments for C-6 to C-9 correlate very well with calculated values (C-6 to C-9, δ 125.1, 126.3, 120.5, 131.1 respectively) using substituent tables and part structure **4** as a model [19].

Gated non-decoupling with NOE also revealed several absorptions in the spectra of **1a-3a** (**1a**: δ 112.5, 144.9, 140.8; **2a**: δ 115.8, 145.2, 142.2; **3a**: δ 115.6, 145.1, 142.1) which appeared to exhibit long range coupling to methyl substituents expected for carbon atoms 4, 4a and 5a. Similarly, the absorptions at δ 129.6, and 128.9 in the spectra of **1a** to **3a** appeared as doublets and were assigned to carbon atom 9. The absorptions at δ 137.0, 136.3 in the spectra of **2a** and **3a** also appeared as doublets and were assigned to carbon atom 9a. The remaining assignments were made using actinocin (**1c**) as a model [18].

The C-1 and C-2 absorptions of **1a** to **3a** do not reflect a simple variation suggesting the influence of both electronic and steric effects. Additivity rules have previously been shown not to be applicable for ortho substituted

Table II

^{13}C-NMR Chemical Shifts of Phenoxazinone Derivatives [a]

C-atom	**1a**	**2a**	**3a**
1	107.3	132.3	146.5
2	145.3	134.5	130.6
3	179.4	174.5	184.9
4	112.5	115.8	115.6
4a	144.9	145.2	145.1
5a	140.8	142.2	142.1
6	125.8	126.4	126.0
7	130.7	136.1	134.2
8	122.1	123.0	122.2
9	129.6	129.6	128.9
9a	134.6	137.0	136.3
10a	142.8	144.7	141.4
C4-Me	7.6	7.8	7.4
C6-Me	14.8	14.6	14.7
C1-C0	165.6	159.5	164.6
C9-CO	168.0	166.4	167.0
-$N(CH_2CH_3)_2$	38.8	38.5	38.8
	39.2	39.1	38.9
	43.1	43.3	43.1
	43.4	43.3	43.1
-$N(CH_2CH_3)_2$	12.9	12.0	12.7
	13.1	12.9	12.9
	14.2	13.2	13.9
	14.8	13.9	13.9

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

nitrobenzenes and *N,N*-dimethylanilines suggesting that electronic interactions are less important in these conjugated but sterically crowded systems [20].

We have successfully applied these nuclear magnetic resonance assignments to corresponding actinomycin D analogs. These will be reported shortly.

Conclusions.

Using a novel single step reaction we have replaced the 2-amino group in a model derivative of actinomycin D to 2-nitro and 2-H functions. These new derivatives have already demonstrated better radical forming abilities *via* chemi-reduction both by spectroscopy and epr [21]. We believe that our studies on analogs of actinomycin D will result in agents with novel pharmacochemical properties and improved antitumor activity.

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 90 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 (4:1) 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 PF254 (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-Amino-1-9-*bis*-(*N,N*-diethylcarbamoyl)-4,6-dimethyl-3*H*-phenoxazin-3-one (**1a**) with Nitrous Acid.

A solution of the phenoxazinone (**1a**) (219 mg, 0.5 mmole) was dissolved in a mixture containing 20 ml of water, 0.2 ml of concentrated sulfuric acid and 1 ml of fluoroboric acid (48% solution in water). The solution was then cooled to −20° in an ice-salt bath and a solution of sodium nitrite (345 mg, 5 mmoles) in 5 ml of water was added dropwise to the cooled phenoxazinonium sulfate solution. The mixture was stirred at −10° for 1 hour, then added to a solution of cupric sulfate (2.5 g, 10 mmoles) in 50 ml of water. An additional 345 mg (5 mmoles) of sodium nitrite was added to this mixture and the mixture was stirred at room temperature overnight. The *p*H of the mixture was then adjusted to approximately 2.0 with dilute nitric acid. The mixture was repeatedly extracted with ethyl acetate (6 × 25 ml), dried over sodium sulfate and the solvent removed under reduced pressure to leave a crude dark brown residue. The residue from two batches was deposited on top of a silica gel column (125 g) and fractionated by elution with 1:8 acetone:chloroform (1200 ml) to give two major fractions, purple which was proved to have structure **2a** and orange which has structure **3a**, followed by 1:1 acetone:chloroform (600 ml) to give a third fluorescent fraction which is an unknown at the present time. The tlc of purple band indicated it to be contaminated with an unknown minor component (R_f 0.84) and the orange component and the orange fraction was contaminated with small amounts of the purple component. These major fractions were repurified by preparative layer chromatography to homogeneity using 1:8 acetone:chloroform and bands were extracted with 1:1 acetone:chloroform.

Compound **2a** was obtained as a red solid (114 mg, 24%), R_f 0.81 (R_f of **1a** 0.5); uv: λ max nm (ε × 10^{-3}) 515 (5.80), 385 (10.85), inf 260 (16.47) and 235 (22.46). In the infrared bands for nitro appeared at 1385 and 1505 cm^{-1}.

Anal. Calcd. for $C_{24}H_{28}N_4O_6 \cdot 2CH_3COCH_3$: C, 61.64; H, 6.85; N, 9.59. Found: C, 61.84; H, 6.66; N, 9.41.

Compound **3a** was obtained as a orange red solid (52 mg, 12%), R_f 0.72; uv: λ max nm (ε × 10^{-3}) 495 (8.88), 375 (13.87), 267 (20.47) and 242 (22.99). In the infrared bands for nitro or amino were absent.

Anal. Calcd. for $C_{24}H_{29}N_3O_4 \cdot H_2O$: C, 65.30; H, 7.03; N, 9.52. Found: C, 64.79; H, 6.94; N, 9.16.

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REFERENCES AND NOTES

[1] E. Frei, III, *Cancer Chemother. Rep.*, **58**, 49 (1974).

[2] P. A. Friedman and A. Cerami in "Cancer Medicine", J. F. Holland and E. Frei, III, eds, Lea and Febiger, Philadelphia, PA, 1973, pp 835-839.

[3] S. Farber, *J. Am. Med. Assoc.*, **198**, 828 (1966).

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

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

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

[7] H. Brockmann and H. Lackner, *Naturwissenschaften*, **57**, 384 (1964).

[8] S. K. Sengupta, M. S. Madhavarao, C. Kelly and J. Blondin, *J. Med. Chem.*, **26**, 1631 (1983).

[9] S. K. Sengupta, J. E. Anderson and C. Kelly, *J. Med. Chem.*, **25**, 1214 (1982).

[10] S. K. Sengupta, J. E. Anderson and Y. Kogan, *J. Med. Chem.*, **24**, 1052 (1981).

[11] S. K. Sengupta, D. H. Trites, M. S. Madhavarao and W. R. Beltz, *J. Med. Chem.*, **22**, 797 (1979).

[12] B. K. Sinha, M. G. Cox, C. F. Chignell and R. L. Cysyk, *J. Med. Chem.*, **22**, 1051 (1979).

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

[14] B. K. Sinha and M. G. Cox, *Mol. Pharmacol.*, **17**, 432 (1980).

[15] J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **82**, 3773 (1980).

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

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

[18] U. Hollstein, E. Breitmaier and G. Jung, *J. Am. Chem. Soc.*, **96**, 8036 (1974).

[19a] G. C. Levy and G. L. Nelson in "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972, Chapter 4, pp 80-81; [b] Incremental values for *N,N*-dimethylbenzamide were obtained from "The Sadtler Guide to Carbon-13 NMR Spectra", W. W. Simons, ed, Sadtler Research Laboratories, Philadelphia, 1983, pp 605-607.

[20] P. C. Lauterbur, *J. Chem. Phys.*, **38**, 1415 (1963).

[21] S. K. Sengupta *et al.* unpublished results.