Synthesis of 2-Deamino- and 2-Deamino-2-nitroactinomycin D

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A single step synthesis of 2-deaminoactinomycin D (**3a**) and 2-deamino-2-nitroactinomycin D (**2a**) arising from actinomycin D (**1a**, AMD) is reported. Structural confirmation was made by nmr, ir and chemical conversion to known materials.

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The naturally occurring antibiotic actinomycin D (AMD, **1a**), whose potent activity in the treatment of Wilm's tumor [1] and gestational choriocarcinoma [2] is well-known in the clinic, has been used extensively as a molecular probe in studies related to RNA metabolism due to its specific inhibition of RNA synthesis [3-5]. The mechanism of action of AMD derives from its ability to intercalate with double-stranded DNA, and the overall result of this interaction is the inhibition of DNA-dependent RNA synthesis [6-8]. Work from our laboratories has shown that the actinomycin molecule can be altered at the N^2-site without adversely affecting its DNA-binding and antitumor properties [9-11,16,17] and that a free amino group at C-2 is not crucial for the antitumor activity of AMD. In some cases N^2-amine modified analogs acted even as superior antitumor agents especially when they were activated *in vivo* [12-15]. A similar trend has been observed in certain N^2-spin-labelled analogs of AMD which are prone to internal bioactivation to free radical species. These analogs have shown marked activity against P388 tumor in mice in spite of the loss of their binding affinity for DNA [12,13].

In addition to the antibiotic's action of binding to DNA and inhibiting biochemical reactions involving DNA, it is known to cause chromosomal damage and appears to require active cell processes for this to occur [14,15]. In light of the similarity of quinone containing antibiotics (anthracyclines, mitomycin, streptonigrin) and the quinonimine structure of AMD, Bachur and his coworkers proposed a mechanism involving enzymatic reduction of AMD to a free radical intermediate with subsequent transfer of the electron to oxygen to yield superoxide. These free radicals may be the critical activated form of the antibiotic to cause intracellular DNA-damage [14,15].

In our continuing efforts to synthesize improved antitumor actinomycin D derivatives capable of bioactivation to free radical species, we examined the possibility of introducing a nitro group into actinomycin D at the 2-position to enhance its radical forming ability in the presence of red-ox enzymes in cells, and also in the proliferating tumor cells. A possible route to achieve this was suggested by the work of Jones and Robins [18] who were able to convert a series of 8-aminopurines by the action of nitrous acid *via* diazotization to 8-nitropurines. In this manner a nitro group was introduced into the 2-position of the imidazole moiety in the purine ring system. This approach was then

FIGURE 1

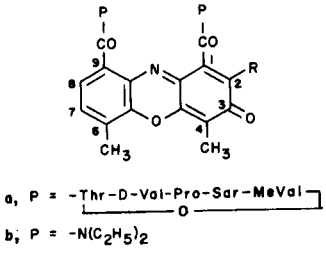
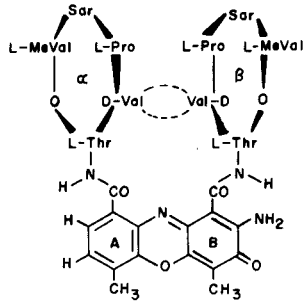
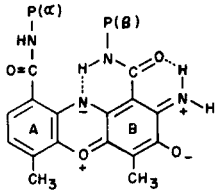
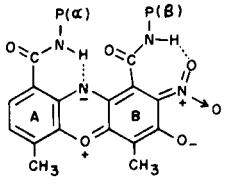


FIGURE 2





1a



2a

followed by the synthesis of yet another antibiotic azomycin by Beaman and coworkers [19], who succeeded in making the naturally occurring 2-nitroimidazole in appreciable yields from 2-aminoimidazole after diazotization in the presence of cupric sulfate.

Table I

Comparison of NMR Chemical Shifts (ppm) of Protons in Actinomycin D (**1a**), 2-Deamino-2-nitroactinomycin D (**2a**) and 2-Deaminoactinomycin D (**3a**)

Proton	**1a** [a] ppm (J)	**2a** ppm (J)	**3a** ppm (J)
NHC=O (Val)	8.09 (5.7)	9.58 (7.2)	8.67 (7.2)
	7.94 (6.0)	9.48 (7.2)	8.57 (7.2)
NHC=O (Thr)	7.82 (6.2)	8.51 (7.2)	7.78 (7.2)
	7.20 (6.8)	6.92 (7.2)	6.66 (7.2)
$ArNH_2$	7.3-7.4 (br)	—	—
ArC_8H	7.64 (7.8)	7.53 (7.8)	7.32 (7.8)
ArC_7H	7.37 (7.8)	8.08 (7.8)	7.41 (7.8)
ArC_2H	—	—	7.01
α-CH (MeVal)	6.03 (7.5)	6.27 (7.5)	6.49 (7.5)
	5.98 (7.5)	6.01 (7.5)	6.32 (7.5)
β-CH (Thr)	5.21 (2.5)	5.60 (2.5)	5.88 (2.5)
	5.15 (6.0)	5.16 (6.0)	5.35 (6.0)
α-CH (Thr)	4.62 (6.5)	4.86 (6.0)	4.73 (6.0)
	4.51 (2.5)	4.75 (2.5)	4.61 (2.5)
$6\text{-}CH_3$	2.56	2.43	2.38
$4\text{-}CH_3$	2.24	2.17	2.03

[a] These data are from Arison and Hoogstein [26] and compare well with our data.

Initially, we attempted this reaction with chromophore model compound **1b** (R = NH_2) in which the pentapeptide lactone amide moieties at positions 1 and 9 in AMD (**1a**) were replaced by diethylamino groups [20]. Reaction of the diazonium salt with nitrous acid in the presence of cupric sulfate by a modification of the procedure of Beaman and coworkers [19] indeed afforded the desired 2-nitro chromophore **2b** (R = NO_2) and also the 2-H chromophore **3b** (R = H) by elimination of diazo group as products [20]. Encouraged by these findings we examined this reaction with AMD (**1a**) under identical conditions. The resulting 2-deamino-2-nitroactinomycin D (**2a**) and 2-deaminoactinomycin D (**3a**) were isolated by preparative layer chromatography and were characterized by ir and nmr. The identity of **2** was also established by ir, nmr and tlc behavior, confirming a nitro group has been introduced at the desired 2-position of the phenoxazinone moiety. Furthermore, the reduction of the 2-nitro group in the compound (**2a**) in the presence of platinum oxide and hydrogen in 95% ethanol at 40-45 atmosphere pressure (16 hours) generated the starting compound (**1a**) (R = NH_2) (*via* the intermediate 3,10-dihydro derivative) in quantitative yield. Catalytic hydrogenation of the 2-deamino compound **3a**, on the other hand, regenerated the material (**3a**) after aerial oxidation of the intermediately reduced product [10].

Proton nmr chemical shifts of **1a-3a** are summarized in Table 1 and are consistent with the proposed structures. Assignments were based on the spectral characteristics of AMD and on those of authentic model analogs **2b** and **3b** reported recently by us [20]. We observed that the same coupling constants between C_7-H and C_8-H are present in nmr of AMD (**1a**) that are observed for both compounds **2a** (R = NO_2) and **3a** (R = H). Furthermore, the chemical shift data are in accord with the observation that C_7-H and C_8-H are shifted upfield in the 2-deamino compound **3a** compared to the 2-nitro compound **2a** and AMD (**1a**) due to an overall increase in the electron density of the A ring in **3a**. A singlet at 7.01, which is absent in the spectra of the 2-nitro derivative **2a** and AMD, is assigned to a resonance of the C_2-H of the chromophore in **3a**. These assignments are consistent with the unambiguous assignments of the C_2-H, C_7-H and C_8-H protons of model systems (**1b-3b**) made recently by pmr, cmr and gated non-decoupling with NOE cmr experiments [20] and with the values assigned by Mosher for **3a** [21].

In general, the spectra indicate that the conformations of the two peptide lactone rings in both **2a** and **3a** differ from each other and also from AMD. There is a significant downfield shift of the valine-NH signals in these analogs relative to AMD. This implies that the peptide conformations have been altered, and it is likely the hydrogen bonds between the D-valine-NH of one ring and the D-valine-carbonyl of the other ring as found in **1a** [22], are perhaps disrupted in **2a** and **3a**. The large downfield shift of the β-threonine-NH proton resonance in the case of the 2-deamino-2-nitro derivative **2a** compared to **1a** and **3a** is consistent with the proposed alternate resonance structure in Figure 2. The charge distribution predicted by this structure would favor hydrogen bond formation between the ring nitrogen (N_{10}) and α-threonine-NH, as well as between the 2-nitroxyl oxygen and the β-threonine-NH. These interactions would be expected to result in a downfield shift for the threonine-NH protons with a larger shift for those in the ring adjacent to $-NO_2$ on the chromophore. The nmr data in Table 1 support these interpretations.

In the infrared, a band at 1585 cm^{-1} which is ascribed to planarity of the chromophore [23], is absent both in the spectra of **2a** and **3a**, implying the loss of planarity in the chromophore.

It is worth mentioning that in the crystals of **2a** two molecules of acetone and three molecules of water are associated with each molecule of **2a** (Experimental). Previous workers [24] have observed that the *gem*-diol groups of hexafluoro acetone sesquihydrate in aqueous medium disrupt the interannular hydrogen bonds [22] between the peptide lactones in AMD (Figure 2). We have found that chloral hydrate can also disrupt these bonds in a non-polar benzene solvent [11]. If acetone sesquihydrate similarly disrupts these bonds in chloroform solution then the ob-

served changes in the peptide conformations may, in part, be due to the *gem*-diol interactions.

Preliminary esr and oxygen consumption experiments show that **2a** is superior to both **3a** and AMD (**1a**) in radical forming activities. Also, the previously reported model analogs **1b-3b** are inferior in these activities to the corresponding peptide substituted analogs **1a-3a** [25]. Detailed reports on these results and implications in their activities in normal and tumor cells will be reported shortly.

EXPERIMENTAL

The ir spectra were taken with a Perkin Elmer Model 457A grating spectrophotometer in potassium bromide pellets, uv spectra were measured 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, W. 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 PF 254 (E. Merck, Darmstadt, Germany). The compounds were detected by visual examination under uv light (254 nm). Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Evaporation of solvents was done under diminished pressure at less than 40° using a rotary evaporator.

2-Deamino-2-nitroactinomycin D (**2a**) and 2-Deaminoactinomycin D (**3a**).

A solution of the actinomycin D (**1a**) (161.25 mg, 0.125 mmole) was dissolved in a mixture containing 10 ml of water, 0.05 ml of concentrated sulfuric acid and 0.25 ml of fluoboric acid (48% solution in water); the solution was then cooled to −20° in an ice-salt bath and a solution of sodium nitrite (87 mg, 1.25 mmoles) in 5 ml of water was added dropwise to the phenoxazinonium sulfate solution. The mixture was stirred at −10° for 1 hour and then added to a solution of cupric sulfate (0.625 g, 2.5 mmoles) in 25 ml of water at 4°. An additional 87 mg (1.25 mmoles) of sodium nitrite was added to this mixture and the mixture stirred at 4° for 24 hours. 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 red residue. This residue was fractionated by preparative layer chromatography using 1:8 acetone-chloroform to give two major fractions; that with orange band R_f 0.47 was assigned structure **3a** and a purple with R_f 0.27 (R_f of **1a** 0.5) was proved to have structure **2a**. These major fractions were repurified by preparative layer chromatography until they were homogeneous and the bands were extracted with 1:1 acetone-chloroform.

Compound **2a** was obtained as a dark red solid (41 mg, 25%). In the infrared the nitro bands appeared at 1385 and 1515 cm^{-1}. A band at 1585 cm^{-1} (ascribed to planarity of the chromophore) [23] which is present in AMD (**1a**) was absent, uv (methanol): λ max nm (ε), 319 (20,000), 371 (8500), 474-486 (br) (2900); (0.1*M* phosphate, *p*H 7.0): 320 (19,300), 372 (7900), 492-501 (br) (2600).

Anal. Calcd. for $C_{62}H_{84}N_{12}O_{18}\cdot 2CH_3COCH_3\cdot 3H_2O$: C, 56.35; H, 6.63; N, 11.60. Found: C, 56.15; H, 6.85; N, 11.54.

Compound **3a** was obtained as an orange red solid (13 mg, 8%). In the infrared bands for nitro were absent. The band at 1585 cm^{-1} found in AMD (**1a**) was also absent; uv (methanol): λ max nm (ε), 303-306 (8333); (0.1*M* phosphate, *p*H 7.0); 315-318 (7600).

Anal. Calcd. for $C_{62}H_{85}N_{11}O_{16}\cdot 3CH_3CO_2C_2H_5\cdot 1H_2O$: C, 58.38; H, 7.30; N, 10.12. Found: C, 58.43; H, 7.53; N, 9.70.

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