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Condensation of a tricyclic phenoxazin-2-amino-3-one (a model for the actinomycin D ring system) with acetaldehyde or benzaldehyde or with pyruvic acid as an acetaldehyde precursor gave tetracyclic 5*H*-oxazolo[4,5-*b*]phenoxazines. The pyruvic acid reaction proceeds efficiently in one step. The latter compounds were oxidized in a novel reaction by 2,3-dichloro-5,6-dicyanobenzoquinone exclusively at position 8 in the benzenoid ring. Uv, ir, nmr, and mass spectrometric data are reported.

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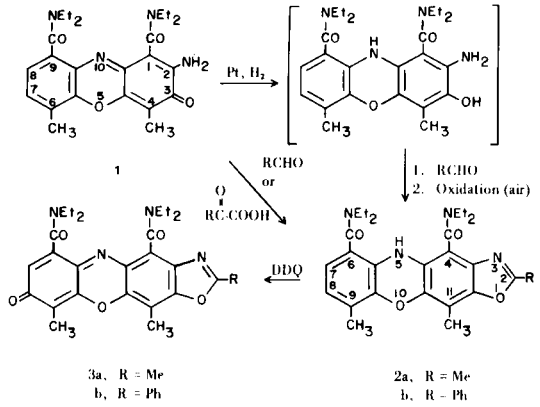
We have been interested in the design of analogs and derivatives of actinomycin D (AMD) as selective, fluorescent chromosomal DNA-binding agents (1) and as pharmacologically modified compounds that might exhibit improved properties as experimental cancer chemotherapeutic agents (1,2). Meienhofer and Atherton (3) have published an excellent review of the chemistry, biological activity, binding specificity, pharmacology and clinical antitumor activity of the actinomycins.

The 2-amino and 3-oxo functions, in addition to the peptide lactones, have been implicated as necessary for optimal interaction of the AMD molecule with DNA, and DNA-interaction has been assumed to be the probable basis for the biological activity of the antibiotic (3). For this reason, we began our program of modification of the AMD chromophore by introducing substitution at positions 7 and 8, in order to avoid interference with the DNA-binding functionality of the molecule. We have published (1,2) on the synthesis and biological activity of three 7-substituted AMD derivatives, which were synthesized *via* new methods first tested successfully with a chromophore model system (**1**), wherein the cyclic pentapeptide lactone amide moieties at positions 1 and 9 of the phenoxazinone ring system have been replaced by diethylcarboxamide groups.

It is of interest that recent work by Moore *et al.*, (4), as well as our own (1,2), indicates that, despite reduced antibacterial and DNA-binding properties, certain analogs and derivatives of AMD can show pronounced antitumor activity in experimental mouse tumor systems.

As part of our program, we have designed AMD derivatives in which the 2-amino and 3-oxo functions are substituted by the addition of a fourth ring as a protective group [an oxazinone (2,5) or an oxazole (6)] with the intent of removing the additional ring at a later stage, either by chemical or metabolic means. The purpose of this note is to describe preliminary work on oxazolo analogs of the AMD chromophore model ring system.

In order to establish the chemical route to these oxazoles, we chose as a model the diethylamide, **1** (2) for our initial studies. This compound, which possesses the same 2-aminophenoxazin-3-one chromophoric ring system as that of AMD, also exhibits the same ultraviolet absorption properties and quite similar nmr proton shifts (7). When



1 is dissolved in methanol and reduced catalytically with platinum oxide and hydrogen, the red-yellow color of the solution is discharged due to formation of an intermediate aminophenol, which is highly susceptible to aerial oxidation and reverts to the original chromophore **1**. In the absence of air, the aminophenol can add a mole of aldehyde to form an oxazoline, which upon exposure to air is spontaneously oxidized to an oxazole. Thus with acetaldehyde, compound **2a**, and with benzaldehyde, compound **2b**, are formed. Unfortunately we found these room temperature reactions to be relatively inconsistent and the yields unreliable.

Reproducible results and greater yields are obtained at elevated temperatures when a high-boiling aldehyde, such as benzaldehyde, or a α-keto acid (as a precursor of acetaldehyde) is employed. With pyruvic acid, acetaldehyde is generated *in situ*, so that condensation proceeds in high yield at 60-70°. Benzaldehyde, however, requires a higher temperature (180°) but gives consistently better yields of **2b**. The 2-phenyloxazole, **2b**, shows ultraviolet absorption characteristic of a conjugated chromophore.

The 2-methyloxazole **2a** did not show the long wavelength visible absorption maximum expected for a benzoxazole (6). The ultraviolet absorption maxima at 253 and 351 nm suggested that **2a** could be an oxazoline derivative, *i.e.*, a 2,3-dihydro derivative of **2a** (Table I). However, both low and high resolution mass spectra revealed a molecular weight consistent with **2a** only, and no mass peak for the presumed 2,3-dihydro derivative of **2a** could be

Table I

UV Absorption Spectra

λ max chloroform nm ($\epsilon \times 10^{-3}$)

2a 351 (8.99) and 253 (33.60)

3a 495 (11.09), 382 (13.27), 295 inf (6.83), 276 (23.41), and 263 (22.90)

2b 387 (16.03), 288 (13.17), and 253 (34.04)

3b 504 (15.27), 379 (15.53), 322 (22.37), 302 (20.53), 282 (21.59), and 253 (18.00)

Table II

Nmr Spectral Data
τ, deuteriochloroform

Compound	8-H	7-H	9-Me	11-Me	5-NH	2-Me
2a	3.50	3.28	7.78	7.76	3.05	7.45
3a	-	3.25	7.85	7.65	-	7.42
2b	3.50	3.28	7.82	7.60	2.95	-
3b	-	3.25	7.83	7.33	-	-

found. In addition, the nmr spectrum of **2a** showed the expected chemical shift for the oxazole ring 2-methyl protons at 7.42 τ (Table II); oxazoline ring 2-methyl protons would give higher τ values and doublets (6). The chemical shifts of the 9- and 11-methyls and the 7 and 8 aromatic protons of the phenoxazine ring are in agreement with the assigned oxazolo phenoxazine ring system for both **2a** and **2b** (Table II).

Formation of the oxazole derivative increases the electrophilicity of the original aromatic ring, so that 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) easily oxidizes the ring system of **2a** and **2b** to the 8-oxo compounds. The phenyloxazole **2b** gives better yields of **3b**. We have reported a similar oxidation for an oxazinone-substituted phenoxazine ring (2). The same 8-oxo compound, **3b**, can be obtained with potassium ferricyanide or bromine in lower yield (7).

The proton-chemical shifts in chloroform (Table II) are consistent with the structures of **3a** and **3b**. Table I shows the ultraviolet absorption characteristics.

EXPERIMENTAL

Melting points were measured in Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are uncorrected. Ir spectra were taken with a Perkin-Elmer Model 137B double-beam spectrophotometer, and nmr spectra were determined on a Varian A-60 spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Tlc's were done on Eastman silica gel chromagram sheets, with chloroform-acetone (4:1) as the developing solvent. Microanalyses were determined by Galbraith Laboratories, Knoxville, Tenn. Mass spectra were recorded using AEI MS-9 and CEC 21-103 low resolution mass spectrometers and a CEC 21-110B high resolution mass spectrometer; the sample was introduced at a probe temp. of 175°.

4,6-Bis(*N*,*N*-diethylcarbamoyl)-2,9,11-trimethyl-5*H*-oxazolo[4,5-*b*]phenoxazine (**2a**).

(*A*). A solution of **1** (220 mg., 0.5 mmole) in methanol (40 ml.) was hydrogenated over platinum oxide (60 mg.) for 1 hour. The intial red-yellow color of **1** was discharged, and the solution was carefully filtered with the aid of nitrogen pressure into a second flask, which was also kept under nitrogen. The solution was concentrated under reduced pressure to 1 ml. and flushed with nitrogen; 10 ml. of acetaldehyde was added, and the reaction was then allowed to proceed for 19 hours at room temperature. The color of the solution turned reddish-brown. Evaporation of volatile solvent gave a brown oil, which solidified on treatment with benzene and petroleum ether at a temperature of 4°. Recrystallization from ligroin (b.p. 60-90°) gave 158 mg. (68%) of **2a**, a brown-red solid, m.p. 184-186°. Tlc showed a single spot at R_f = 0.61 (R_f of **1** = 0.5).

Anal. Calcd. for $C_{26}H_{32}N_4O_4$: C, 67.22; H, 6.94; N, 12.06. Found: C, 66.76; H, 7.14; N, 11.94.

This is the best of several reactions, some of which were unsuccessful.

(*B*). A solution of **1** (110 mg., 0.25 mmole) in methanol (15 ml.) containing pyruvic acid (1 ml.) was refluxed for 6 hours. The reaction mixture was cooled, the solvent was evaporated, and the residue was extracted with chloroform (20 ml.). The chloroform extract was washed with water (3 x 10 ml.) to free it from pyruvic acid and then dried (sodium sulfate) and evaporated. The brown residue crystallized from ligroin (b.p. 60-90°) gave a reddish-brown solid (**2a**), 83 mg. (71.5%), m.p. 184-186°, which was identical with an authentic sample obtained *via* method *A* (tlc, ir, uv, nmr).

Mass Spectrometric Analysis: Sample **2a**, temperature of probe, 175°, Composition; $C_{26}H_{32}N_4O_4$ Calcd: 464.242234. Observed: 464.240782.

Method *B* is decidely preferable to method *A*.

4,6-Bis(*N*,*N*-diethylcarbamoyl)-9,11-dimethyl-2-phenyl-5*H*-oxazolo[4,5-*b*]-phenoxazine (**2b**).

(*A*). When a solution of **1** (220 mg., 0.5 mmole) was hydrogenated (platinum oxide, 60 mg.) and then allowed to react with benzaldehyde (2 ml.), as described earlier for **2a** (experiment *A*), a brown oil was obtained. Trituration with benzene-ligroin (b.p. 60-90°) and crystallization from ligroin yielded a red-brown solid **2b**, 160 mg. (63%), m.p. 218-219°; R_f 0.77.

Anal. Calcd. for $C_{31}H_{34}N_4O_4$: C, 70.69; H, 6.50; N, 10.63. Found: C, 70.34; H, 6.57; N, 10.59.

Yields of this reaction can be variable.

(*B*). A suspension of **1** (440 mg., 1 mmole) in benzaldehyde (5 ml.) was stirred at a bath temperature of 180° under nitrogen. After 5 hours, tlc (silica gel, chloroform:acetone, 4:1) showed no spot for **1** (R_f 0.50), but only a faster-moving spot of **2b** (R_f 0.78). The reaction mixture was then cooled to room temperature and evaporated under vacuum, and the residue was crystallized from a 1:1 mixture of benzene and heptane (3.5 ml.) yielding a red-brown solid **2b** (427 mg., 81%), m.p. 218-219°, identical with the solid obtained in *A* (ir, nmr, tlc, and mixed m.p. 218-219°).

4,6-Bis(*N*,*N*-diethylcarbamoyl)-8*H*-8-oxo-2,9,11-trimethyloxazolo-[4,5-*b*]phenoxazine (**3a**).

A solution of **2a** (150 mg.) in *p*-dioxane (10 ml.) and DDQ (300 mg.) was refluxed for 30 minutes. The dioxane was then evaporated and dichloromethane (10 ml.) was added to the residue. Refrigeration gave a red solid (2,3-dichloro-5,6-dicyanohydroquinone) which was collected, and the filtrate was chromatographed over neutral alumina (Woelm) in a chloroform-acetone mixture (4:1). The faster-moving fraction, containing one major orange

spot (R_f 0.46), was rechromatographed over silica gel, using the same solvent system. The fractions having only one fast-moving red spot (R_f 0.46) were evaporated and recrystallized from carbon tetrachloride and heptane (1:1). Compound **3a** was obtained as a red solid (60 mg., 40%) m.p. 271-272° dec.

Anal. Calcd. for $C_{26}H_{30}N_4O_5$: C, 65.25; H, 6.31; N, 11.70. Found: C, 65.26; H, 6.57; N, 11.65.

4,6-Bis(*N,N*-diethylcarbamoyl)-8*H*-8-oxo-9,11-dimethyl-2-phenyloxazolo[4,5-*b*]phenoxazine (**3b**).

In a run similar to that described above, **2b** (100 mg., 0.191 mmole) and DDQ (100 mg., 0.44 mmole) were stirred under nitrogen in a solution of dry methanol (10 ml.) at ambient temperature for 30 mintues. Evaporation of solvent and chromatography of the residue, as described for **3a**, gave after crystallization from benzene, a red solid (**3b**, 73 mg. 72%), m.p. 287-289°, R_f 0.76.

Anal. Calcd. for $C_{31}H_{32}N_4O_5$: C, 68.87; H, 5.97; N, 10.37. Found: C, 68.71; H, 6.31; N, 10.39.

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