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MOLECULAR MECHANISM OF BINDING OF PYRROLO(1,4)BENZODIAZEPINE ANTITUMOUR AGENTS TO DEOXYRIBONUCLEIC ACID—

ANTHRAMYCIN AND TOMAYMYCIN*

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Abstract—The synthesis of 3,3-dimethyl-4-oxo-3,4-dihydroquinoline (**16**), 3,3-dimethyl-4-oxo-2-methoxy-1,2,3,4-tetrahydroquinoline (**17**), and of 11*a*-*S*-pyrrolo(1,4)benzodiazepine (**21**) as models to study the mechanism of action of the pyrrolo(1,4)benzodiazepine antitumour antibiotics is described. Both **16** and **21** readily add nucleophiles to the imine bond but only **21**, like the parent antibiotics, readily produces covalent attachment to DNA. The extent of binding of the pyrrolo(1,4)benzodiazepine antibiotics to DNA, measured by suppression of ethidium fluorescence, is proportional to the antibiotic concentration and is partly reversed by a heat-denaturation–renaturation cycle. The extent of binding of the pyrrolo(1,4)benzodiazepines to DNA is also promoted by lower pH (range 4.7 to 9) and higher temperatures (range 0–51°), and the DNA–antibiotic complex is stable to dialysis. There is no evidence that these antibiotics intercalate into DNA, assayed by calf thymus topoisomerase, but they are more reactive toward relaxed PM2-DNA than to supercoiled DNA. Examination of DNA binding of the antitumour antibiotics and their analogues to DNAs of different base composition and separately in conjunction with sequence specific binding agents showed little base preference for the binding. Reaction of the pyrrolo(1,4)benzodiazepines with DNA produces neither depurination, assayed with endonuclease VI, nor strand scission. A free or potential carbinolamine or imine function at the 10, 11 positions in a benzo(1,4)diazepine nucleus is an absolute requirement for DNA binding or for reaction with nucleophiles. These results with the native antibiotics and their analogues, in particular the *N*-acetyl compound **7** favor a molecular mechanism of action by acid-promoted addition of biological nucleophiles to the 10, 11 conjugated imine closely analogous to that proposed for the antitumour agent maytansine.

Anthramycin and tomaymycin belong to the pyrrolo(1,4)benzodiazepine class of antitumor agents. They have been isolated from *Streptomyces refuineus* var. *thermotolerans* [1, 3] and *Streptomyces achromogenes* var. *tomaymyceticus* [4, 5] respectively. Anthramycin was shown to have antibiotic, antitumor, antiprotozoal and chemosterilant activity against houseflies. Tomaymycin has been shown to have antitumor, antiviral and antibiotic activities. Other known members of this class of antibiotics are sibiromycin [6–8] and neothramycins A and B [9, 10]. Another antibiotic, dextrochrisin of unknown structure has also been included in this group [11]. The total synthesis of anthramycin [12] and neothramycins [11] has been reported. The structures of these antibiotics are shown in Fig. 1.

A structural feature common to all these antibiotics is the pyrrolo(1,4)benzodiazepine nucleus. They all contain a carbinolamine function at position 10, 11 ("imine" in the case of neothramycins), a phenolic hydroxy group and an unsaturated side chain on the pyrrole ring. The pyrrole ring can have various degrees of unsaturation. Thus, although these antibiotics lack any of the structural features responsible for tight binding to DNA, they react specifically with it to form nearly irreversible complexes. Several attempts have been made to establish the molecular mechanism of this binding between DNA and anthramycin [13–22], tomaymycin [21–23] and sibiromycin [24–28], and also to correlate the biological activity of anthramycin and several analogues and derivatives with their structures [29, 30]. In spite of these studies, the exact nature of this binding and the functional group requirements of these antibiotics for reaction with DNA remained speculative. We decided to examine the reactions of anthramycin, tomaymycin and other structurally related compounds with different DNAs by the ethidium fluorescence assay [31, 32] with a view to elucidating the molecular mechanism of binding of pyrrolo(1,4)benzodiazepine antibiotics with DNA and to establish the functional group requirements of these compounds for reaction with DNA. Recently, these techniques have been used to examine the interaction of certain antitumor antibiotics with nucleic acids [33–37].

* Studies related to antitumour antibiotics, Part XVI.

† Anthramycin and tomaymycin are most commonly supplied as the 11-methyl ethers, in which form they are more stable. The terms anthramycin and tomaymycin will be used in this paper to designate the methyl ethers.

MATERIALS AND METHODS

Anthramycin† was kindly supplied by Dr. Harry B. Wood of the National Cancer Institute, Bethesda, MD.

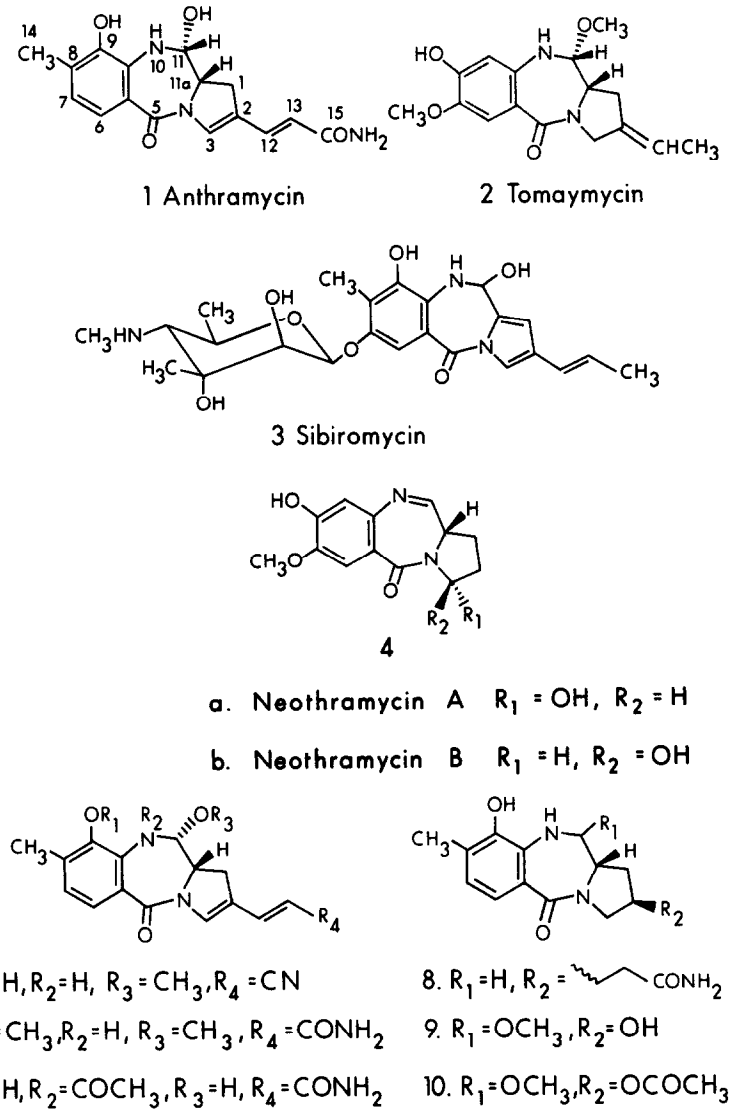

Fig. 1. Structures of the pyrrolo(1,4)benzodiazepine antitumor antibiotics, derivatives and analogues.

Anthramycin and several analogues were also kindly supplied by Hoffmann-La Roche Inc., Nutley, NJ. Tomaymycin* was a gift from Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan. Other compounds were prepared as described below. Solutions of the compounds were prepared in dimethylsulfoxide and stored at 0°. Ethidium bromide was purchased from Sigma Chemicals, St. Louis, MO. The λ, PM2-CCC and calf thymus DNAs were obtained as described previously [32]. *Escherichia coli* and *Clostridium perfringens* DNAs were purchased from Sigma. The calf thymus topoisomerase was prepared according to the method of Herrick and Alberts [38]. Endonuclease VI was purified according to the method of Verly and Rassart [39] from *E. coli* BATCC 11303.

Melting points were determined on a Fischer–Johns apparatus and are uncorrected. The i.r. spectra were recorded on a Nicolet 7199 F.T. spectrophotometer, and only the principal sharply defined peaks are reported. The n.m.r. spectra were recorded on Varian A-60 and A-100 analytical spectrometers. The spectra were measured on approximately 10–15% (w/v) solutions in appropriate deuterated solvents with tetramethylsilane as standard. Line positions are reported in parts per million from the reference. Absorption spectra were recorded with a Beckman model DB spectrophotometer. Mass spectra were determined on an Associated Electrical Industries MS-9 double focusing high resolution mass spectrometer. The ionization energy in general was 70 eV. Peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15,000. Kieselgel DF-5 (Camag, Switzerland) and Eastman Kodak precoated sheets were used for thin-layer chromatography. Preparative layer chromatography was performed with 20 × 20 cm, 1 mm thick plates coated with silica gel (E.M. Reagents, Germany). Microanalyses were carried out by Mrs. D. Mahlow of this department.

2-(o-Nitrobenzoyl)isobutyraldehyde (**11**). This compound was prepared by a procedure similar to that reported by Inukai and Yoshizawa [40]. To a stirred solution of 11.13 g (0.06 mole) *o*-nitrobenzoyl chlo-

* See footnote † on p. 2017.