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PRELIMINARY COMMUNICATIONS

PROPOSED STRUCTURES OF THE PYRROLO(1,4)BENZODIAZEPINE ANTIBIOTIC-DEOXYRIBONUCLEIC ACID ADDUCTS

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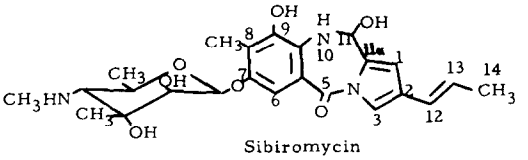
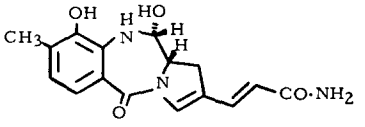
Anthramycin, tomaymycin, sibiromycin and the neothramycins A and B are potent antitumor agents produced by various actinomycetes (1). These drugs have been shown to form covalent adducts with DNA (2-5) resulting in inhibition of nucleic acid synthesis (6,7), and, at least in the case of anthramycin, induction of unscheduled DNA synthesis (8), recombinational events in yeast (9) and sister chromatid exchange in skin fibroblasts (10). Since the reactions of these compounds with DNA appear to be unique (1,11,12) and lead to unusual biological effects relative to other DNA reactive drugs, such as lack of bone marrow depression (13) and very high potency (1), we have attempted to determine the precise mechanism for interaction of these drugs with DNA. We have recently published a proposed structure for the anthramycin-DNA adduct (14) and this communication extends this proposal to accommodate all other known members of this group of antitumor compounds. This proposal is based upon a comparison of the effects of binding of these drugs on the structure of DNA and on structure-activity relationships of derivatives of the neothramycins A and B (5).

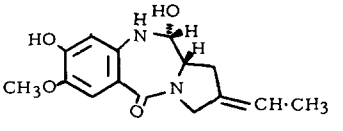
Examination of the structures of the pyrrolo(1,4)benzodiazepine antibiotics (Fig. 1) reveals that each of the antibiotics has certain structural features. In addition to the pyrrolo(1,4)benzodiazepine nucleus, all possess a 10,11-carbinolamine or, in the case of the neothramycins, a 10,11-enamine which is hydrated in aqueous solution to give the carbinolamine. This is the DNA reactive group on these antibiotics (1,2,5,12) which is



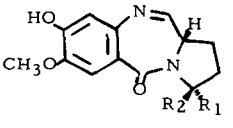
Sibiromycin



Anthramycin



Tomaymycin



R_1 = OH, R_2 = H Neothramycin A
R_1 = H , R_2 = OH Neothramycin B

Fig. 1. Structures of the pyrrolo(1,4)benzodiazepine antibiotics.

believed to form a covalent adduct through the 2-amino group of guanine (14), producing a labile covalent aminal linkage which may be stabilized by secondary hydrogen bonding interactions. The adducts are stable only as long as the helical structure of DNA is maintained, since treatment with acid (2,11), heat (5,15) or DNA digestion enzymes (15) leads to loss of the intact drug from DNA. Based upon information on the probable covalent binding sites on DNA and anthramycin together with the known crystal conformation of anthramycin (16), we have suggested that anthramycin is bound within the minor groove of DNA (see Fig. 2A), such that the right-handed twist of the molecule follows the minor groove (14). From this model certain predictions can be made, including that drug binding should not distort DNA. Using S_1 nuclease and BND-cellulose chromatography as probes for detection of distortion or local denaturation of DNA, we have shown that anthramycin does not distort DNA in any detectable manner (14).

In order to extend these findings to the other pyrrolo(1,4)benzodiazepine antibiotic-DNA adducts, we have prepared Corey, Pauling and Koltun (CPK) molecular space filling models of the tomaymycin, sibiromycin and neothramycin A and B adducts with DNA (Fig. 2B-E). In each case the proposed adducts are analogous to that suggested for the anthramycin-DNA adduct (14), i.e. attached through C-11 of the drug to N-2 of guanine by an aminal linkage. According to our models (Fig. 2A-E), each of the drugs fits snugly in the narrow groove without

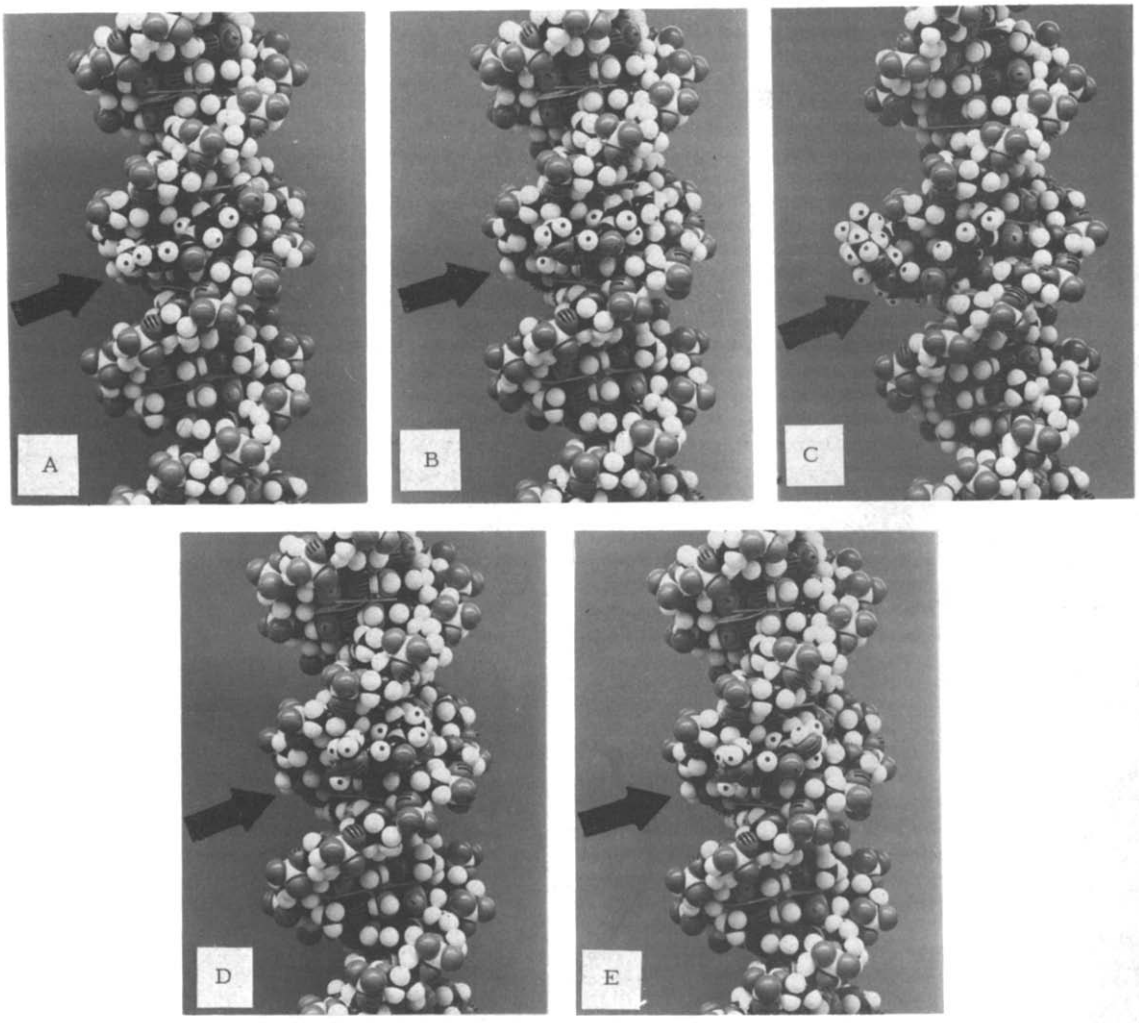


Fig. 2. CPK space filling models of the (A) anthramycin, (B) tomaymycin, (C) sibiromycin, (D) neothramycin A and (E) neothramycin B-DNA adducts.

distortion of the helix. The bulky amino sugar of sibiromycin is the only part of any of the antibiotics which extends outside the groove of DNA (Fig. 2C).

To probe the drug modified-DNA for distortion, S_1 nuclease digestions and benzoylated napthalated DEAE (BND)-cellulose chromatography were carried out as previously described (14). In neither case did any of the antibiotics produce distortion of DNA that could be detected by these methods. Typical experimental results using sibiromycin modified-DNA in conjunction with S_1 nuclease are shown in Fig. 3. In fact, in the case of each drug, the susceptibility of DNA to S_1 nuclease was decreased by drug binding. However, this was not due to inhibition of S_1 nuclease activity towards unmodified DNA, since when untreated DNA was added to S_1 nuclease digestions of the drug-DNA adducts, normal kinetics of nucleotide release was found (14).

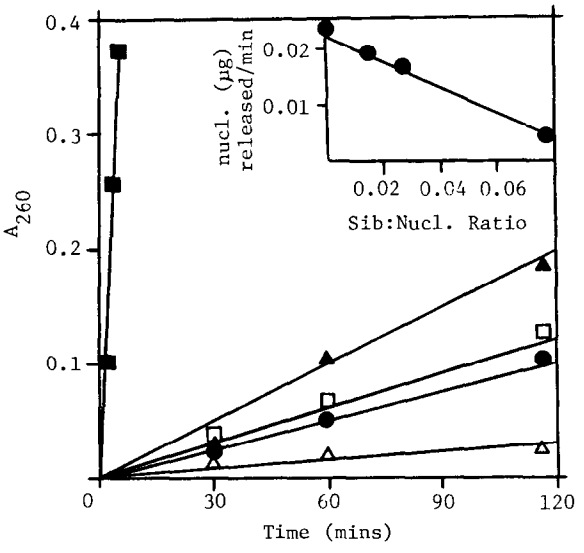


Fig. 3. Time course hydrolysis of calf thymus DNA during digestion with S_1 nuclease in the presence of increasing amounts of bound sibiromycin. Assays were carried out exactly as described by Hurley and Petrusek (14) as modified from Vogt (17). The anthramycin:nucleotide ratio was 0 (▲), 0.015 (□), 0.031 (●), 0.058 (△), and heat denatured DNA (■). Inset, dependence of the nucleotides (μg) released per min against sibiromycin:nucleotide ratios.

Hydrogen bonding stabilization of each of the covalent adducts is predicted through the N-10 proton and the phenolic proton at C-9 of anthramycin and sibiromycin. With the exception of sibiromycin the N-10 proton of each of the antibiotics should be hydrogen bonded to the 2-keto group of thymine or cytosine in the adjacent base pair on the same strand to which the drug is covalently bound. The replacement of the common sp^3 carbon atom at C-11 in anthramycin, tomaymycin and the neothramycins by an sp^2 carbon atom in sibiromycin should affect the conformation of the 7-membered ring such that the N-10 proton hydrogen bonds to the 2-keto group in cytosine in the same base pair to which the drug is covalently bound. The phenolic proton at C-9 of anthramycin should be hydrogen bonded to the 2-keto group in cytosine in the same base pair to which the drug is covalently bound, whereas the equivalent proton in sibiromycin should be hydrogen bonded to the 2-keto group of thymine or cytosine in the adjacent base pair on the same strand to which the drug is covalently bound. In sibiromycin the amino sugar can further stabilize the adduct by interaction with the deoxyribose phosphate backbone of DNA.

A much stronger case for the proposed models of these drug-DNA adducts could be made if subtle changes in the structures of these drugs would drastically affect binding to DNA in predictable ways based upon the CPK models shown in Fig. 2. The isomeric neothramycins

A and B and their 3-methoxy and butoxy derivatives are available and have been tested by Maruyama et al. (5), using fluorescence enhancement measurements, for their ability to bind to calf thymus DNA. Individual members of the three isomeric pairs show surprisingly different DNA binding abilities such that while neothramycin A binds more strongly to DNA than do neothramycin B and its 3-methoxy and 3-butoxy derivatives, the 3-methoxy and 3-butoxy derivatives of neothramycin A do not bind to DNA. Reassuringly this is precisely what would be predicted from the CPK models of the neothramycin A and B adducts, since only in the case of neothramycin A is hydrogen bonding between the 3-hydroxy proton and O1' of the deoxyribose phosphate backbone of DNA possible. This explains the stronger binding of neothramycin A relative to neothramycin B and its derivatives. Furthermore, steric hindrance due to methylation or butylation of neothramycin A at position 3 preverts only these derivatives of this isomer of neothramycin from binding to DNA. These fluorescence measurements obtained independently by the Japanese group supply strong confirmatory evidence that our proposed structures for these drug-DNA adducts are correct. The inability of the 9-methoxy derivatives of anthramycin (18,19) and sibiromycin (20) and the 11-acetyl derivative of anthramycin (12) to bind to DNA is also predicted from the CPK models since steric hindrance prevents their binding to DNA.

In summary, these results show the generality of our proposed model of the anthramycin-DNA adduct (14) to the other known members of this series of antitumor compounds. The fact that the experiments reported in this communication and the published data from other laboratories (5,12,18,19) fit precisely and without exception into our proposed model provides further compelling evidence that the proposed CPK models are correct.

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REFERENCES

1. L. Hurley, J. Antibiot., Tokyo 30, 349 (1977).
2. L. H. Hurley, C. Gairola and M. Zmijewski, Biochim. biophys. Acta 475, 521 (1977).
3. Y. Nishioka, T. Beppu, M. Kohsaka and K. Arima, J. Antibiot., Tokyo 25, 660 (1972).
4. G. G. Gause and Y. V. Dudnik, Studia Biophys. 31/32, 395 (1972).
5. I. N. Maruyama, N. Tanaka, S. Kondo and H. Umezawa, J. Antibiot., Tokyo 32, 928 (1979).
6. K. W. Kohn, in Antibiotics III. Mechanism of Action of Antimicrobial and Antitumor Agents (Eds. J. W. Corcoran and F. E. Hahn), pp. 3-11. Springer, New York (1975).
7. I. N. Maruyama, H. Suzuki and N. Tanaka, J. Antibiot., Tokyo 31, 761 (1978).
8. L. Hurley, C. Chandler, T. Garner and S. Zimmer, J. biol. Chem. 254, 605 (1979).
9. M. A. Hannan, L. H. Hurley and C. Gairola, Cancer Res. 38, 2795 (1978).
10. S. Ved Brat, R. S. Verma and H. Dosik, Mutation Res. 63, 325 (1979).
11. K. W. Kohn, D. Glaubiger and C. L. Spears, Biochim. biophys. Acta 361, 288 (1974).
12. J. W. Lown and A. V. Joshua, Biochem. Pharmac. 28, 2017 (1979).
13. S. Korman and M. D. Tendler, J. new Drugs 5, 275 (1965).
14. L. H. Hurley and R. Petrusek, Nature, Lond. 282, 529 (1979).
15. L. Hurley, C. Allen, J. Feola and W. Lubawy, Cancer Res. 39, 3134 (1979).
16. A. Mostad, C. Romming and B. Storm, Acta chem. scand. B32, 639 (1978).
17. V. Vogt, Eur. J. Biochem. 33, 192 (1973).
18. V. Stefanovic, Biochem. Pharmac. 17, 315 (1968).
19. S. B. Horowitz, S. C. Chang, A. D. Grollman and A. B. Borkovec, Science 174, 159 (1971).
20. L. I. Kozmyan, Y. V. Dudnik and N. G. Shepelevteva, Antibiotiki 23, 602 (1978).