ductive polymers. We are currently developing a mechanistic model for this enhanced order. We are also exploring the extent to which other types of polymers can be ordered via the template method. Template synthesis may prove to be a general procedure for obtaining highly ordered nanoscopic polymeric fibrils.

Acknowledgment. This work was supported by the Office of Naval Research and the Air Force Office of Scientific Research.

Structure of Pyrrolosine: A Novel Inhibitor of RNA Synthesis, from the Actinomycete *Streptomyces albus*

Susumu Ikegami,* Haruhiko Isomura, and Noboru Tsuchimori

Faculty of Applied Biological Sciences
Hiroshima University, Kagamiyama
Higashihiroshima-shi, Hiroshima 724, Japan

Yasuko T. Osano, Tetsuro Hayase, Tomoko Yugami, Haruyuki Ohkishi, and Takao Matsuzaki

Research Center, Mitsubishi-Kasei Corp.
Kamoshida-cho, Midori-ku, Yokohama-shi
Kanagawa 227, Japan

Received August 24, 1990

During the last 20 years, our knowledge of the biosynthesis of RNA has expanded greatly, due largely to the discovery and use of various inhibitors of RNA synthesis. Several inhibitors of RNA synthesis have been used as anticancer, antiviral, and antibacterial agents.[1] However, there are very few, if any, specific inhibitors of RNA synthesis without undesirable side effects on cellular functions. We have searched for microbial products capable of arresting the development of starfish embryos specifically at the early blastula stage, when RNA synthesis becomes active, without interfering with cell division at the morula stage or with blastula formation, which are independent of RNA synthesis.[2] We encountered a culture broth conditioned by the actinomycete *Streptomyces albus* A282, which showed marked activity in the starfish assay and which led us to isolate the active component. Now we report the structure elucidation of the active constituent, pyrrolosine.

The culture broth filtrate (17 L) was passed through a column of Diaion HP-20.[3] The absorbed material was eluted with MeOH. The eluate was subjected to low-pressure column chromatography on silanized silica gel (H_2O/5% MeOH), then alumina (70% MeOH), and finally Robar RP-8[4] (10% MeOH), to give pyrrolosine (**1**) (246 mg) as colorless plates, $[\theta]_{240}$ +2300° (*c* 0.0014, MeOH). Pyrrolosine (10 μg/mL) inhibited RNA synthesis of starfish (*Asterina pectinifera*) embryos at blastulation and halted embryonic development just after completion of blastulation. Furthermore, pyrrolosine inhibited cell growth of transformed human fibroblast KMST-6 cells and mouse mammary carcinoma FM3A cells at IC_{50} 13 and 27 ng/mL, respectively. The molecular formula of $C_{11}H_{13}N_3O_5$ was established by an MH^+ ion peak at *m/z* 268 in the FAB mass spectrum and by elemental analysis. Pyrrolosine showed UV absorption maxima [$\lambda_{max}^{H_2O}$ (pH 7.0) 220 (ε 10000), 242 (4400), 272 nm (4200); (pH 1.5) 225 (5800), 268 (7500 sh), 276 (7800), 285 nm (5200 sh)]. ^{13}C NMR spectra[5] revealed 11 carbon signals, five of which are assignable to a ribosyl unit. Detailed 270- and 400-MHz NMR analyses[6] including H–H COSY[7] and C–H COSY experiments[8] (^1J and long range) suggested strongly that the structure of pyrrolosine is 7-(β-ribofuranosyl)-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidine. This *C*-nucleoside analogue of inosine was reported as having been synthesized and was designated 9-deazainosine by Lim et al.[9] However, HPLC analysis (Radialpak NVC18,[10] 0.8 × 10 cm; 20% MeOH) revealed that the retention volume of 9-deazainosine[11] was 2.9 mL whereas that of pyrrolosine was 3.8 mL. Furthermore, the UV spectrum[12] of 9-deazainosine was different from that of pyrrolosine. Therefore, the structure of pyrrolosine was deduced by single-crystal X-ray diffraction. Repeated crystallization from a mixture of MeOH and ethyl acetate afforded colorless hexagonal plates (methanol monosolvate; mp 105–110 °C) belonging to the orthorhombic space group $P2_12_12_1$ with a = 9.994 (1) Å, b = 19.441 (1) Å, c = 7.026 (1) Å, and Z = 4. Intensities were measured in the ω–1.33θ scan mode on an Enraf-Nonius CAD4 diffractometer, using graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). Correction was made for Lorentz and polarization factors but not for absorption. Of 1655 independent reflections measured in a range of $2° < 2\theta < 149°$, 31 reflections with $|F_o| < 1.0\sigma(|F_o|)$ were considered unobserved.

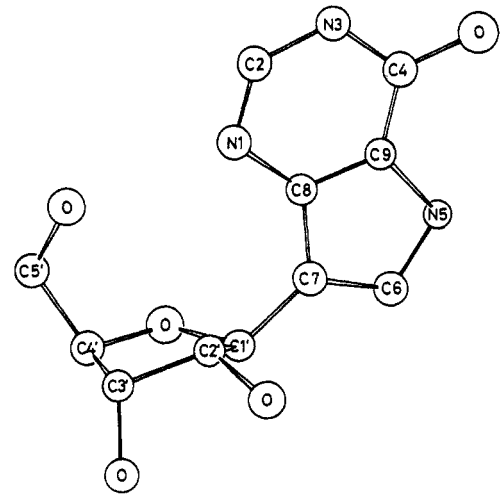


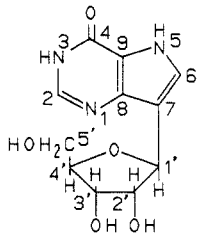
Figure 1. Computer-generated perspective drawing of pyrrolosine. Hydrogens are omitted for clarity, and no absolute configuration is implied.

The structure was determined by direct methods coupled with the MULTAN 11/82 program.[13] Its parameters were refined by the full-matrix least-squares method using anisotropic temperature factors for non-hydrogen atoms. The final R value for 1624 reflections used in the refinement process was 0.065. A computer-generated perspective drawing of the final X-ray model of pyrrolosine (**1**) is given in Figure 1. The X-ray experiment did not define the absolute configuration, so the enantiomer shown is arbitrary. The structure of pyrrolosine is the same as that proposed for synthetic 9-deazainosine.

Synthetic "9-deazainosine" was shown to be toxic in vitro to protozoan cells but not to mammalian cells.[14] Starfish embryos developed normally in the presence of the reputed "9-deazainosine"

(1) Sung, S. C. *Life Sci* **1974**, *15*, 359–369.
(2) Isomura, H.; Itoh, N.; Ikegami, S. *Biochim. Biophys. Acta* **1989**, *1007*, 343–349.
(3) Mitsubishi-Kasei Corp., Tokyo, Japan.
(4) E. Merk, Darmstadt, West Germany.
(5) ^{13}C NMR (D_2O, 67.8 MHz): δ 62.9 (t, C-5′), 72.7 (d, C-3′), 75.6 (d, C-2′), 76.4 (d, C-1′), 86.2 (d, C-4′), 119.3 (s), 135.3 (s), 147.2 (s), 149.4 (d, C-6), 150.6 (s, C-4), 153.1 (d, C-2).
(6) ^1H NMR (D_2O, 270 MHz): δ 3.84 (2 H, br AB q, J = 14.0 Hz, H-5′), 4.22 (1 H, br m, H-4′), 4.32 (1 H, br d, J = 7.2 Hz, H-3′), 4.52 (1 H, dd, J = 7.4 and 7.2 Hz, H-2′), 5.04 (1 H, d, J = 7.4 Hz, H-1′), 8.02 (1 H, s, H-6), 8.15 (1 H, s, H-2).
(7) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542–561.
(8) Morris, G. A.; Hall, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 4703–4714.
(9) Lim, M.; Klein, R. S.; Fox, J. J. *Tetrahedron Lett.* **1980**, *21*, 1013–1016.
(10) Waters Assoc., Milford, MA.
(11) Generous gift of Dr. Robert S. Klein, Memmorial Sloan-Kettering Cancer Center Walker Laboratory, Rye, NY.
(12) $\lambda_{max}^{H_2O}$ (pH 7.0) 230 (ε 16600), 260 nm (5900).
(13) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M. *MULTAN 11/82, a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data*; University of York: York, England, 1982.
(14) LaFon, S. W.; Nelson, D. J.; Berens, R. L.; Marr, J. J. *J. Biol. Chem.* **1985**, *260*, 9660–9665.

0002-7863/90/1512-9668$02.50/0



1

(200 μg/mL). Synthetic "9-deazainosine" was active in vivo against several pathogenic hemoflagellate species including one affecting AIDS patients.[15] The remarkable specificity in the pharmacological action of the reported "9-deazainosine"[9] requires prompt reinvestigation of its chemical structure.

Acknowledgment. This work was supported by grants from the Fisheries Agency, Japan, and by a Grant-in-Aid for Scientific Research (No. 2151037) from the Ministry of Education, Science and Culture, Japan.

Supplementary Material Available: Tables of final atomic coordinates, thermal parameters, bond distances, and bond angles for **1** (4 pages); table of observed and calculated structure factors for **1** (7 pages). Ordering information is given on any current masthead page.

(15) Marr, J. J.; Berens, R. L.; Cohn, N. K.; Nelson, D. J.; Klein, R. S. *Antimicrob. Agents Chemother.* **1984**, *25*, 292-295.

Site-Specific Atom Transfer from DNA to a Bound Ligand Defines the Geometry of a DNA–Calicheamicin γ_1^I Complex

James J. De Voss and Craig A. Townsend*

Department of Chemistry, The Johns Hopkins University Baltimore, Maryland 21218

Wei-Dong Ding, George O. Morton, and George A. Ellestad*

American Cyanamid Company, Medical Research Division Lederle Laboratories, Pearl River, New York 10965

Nada Zein

American Cyanamid Company, Agricultural Division Princeton, New Jersey 08540

Alethea B. Tabor and Stuart L. Schreiber*

Department of Chemistry, Harvard University 12 Oxford Street, Cambridge, Massachusetts 02138

Received July 18, 1990

Among the emerging class of diynene antitumor antibiotics, calicheamicin γ_1^I (CLM) shows the greatest sequence selectivity in its cleavages of double-stranded DNA.[1] We describe in this paper atom-transfer studies that both establish the identity of one of the two principal DNA-bound hydrogens abstracted by the proposed thiol-activated form of the drug (**1** in Scheme I)[5] and quantify the efficiency of the overall process. As the transfer is specific to only one of the acetylenes in CLM, it is possible to define the major orientation of the drug in the minor groove at a particular cleavage site. These preliminary findings illustrate the utility of the site-specific atom-transfer method to derive detailed structural information, and they point to further experimental steps to be taken toward a deeper understanding of the underlying issues of molecular recognition and mechanism posed by the reactions of CLM with DNA.

Scheme I

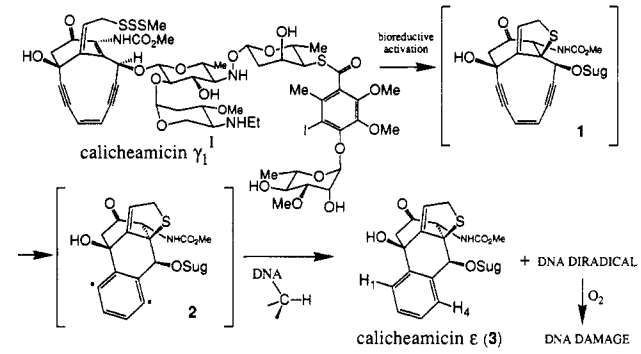
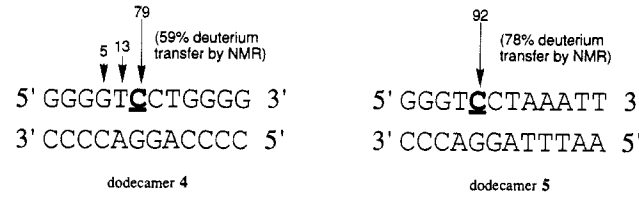


Chart I



Atom-transfer experiments are precedented between tritiated thymidine residues in λ DNA and the neocarzinostatin chromophore (NCS-chrom)[6] and for reaction of both the NCS-chrom[7] and CLM[8] in deuteriated media in the presence and absence of calf thymus DNA. However, in none of these experiments was it possible to correlate the precise location of the abstracted hydrogens in the reduced drug to specific loci on the DNA strand from which they were transferred. While for NCS-chrom the identities of the DNA hydrogens removed in major and minor reaction processes are well studied, for CLM it has only been possible to infer these sites on the basis of electrophoretic mobilities of cleavage fragments.[1,9] To secure experimentally the suspected transfer of a deoxyribose 5'-hydrogen from DNA to CLM and to establish unambiguously the orientation of the calicheamicin γ_1^I aglycon in the minor groove at a specific cleavage site, two site specifically labeled duplex oligonucleotides **4** and **5** (Chart I) were synthesized and purified by HPLC.[10] Each dodecamer contained an internal 5'-TCCT/AGGA in which the 5'-cytidine (C) carbon bore two deuteriums (C^2H_2).[11] Reaction, therefore, of CLM with these oligonucleotides in the presence of a thiol would be anticipated to result in transfer of a single deuterium to the

(1) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198-1201. Compare the sequence selectivities of the neocarzinostatin chromophore,[2] esperamicin A,[3] and dynemicin A.[4]

(2) Goldberg, I. H. In *Mechanisms of DNA Damage and Repair*; Simic, M. G., Grossman, L., Upton, A. C., Eds.; Plenum: New York, 1987; pp 231-244.

(3) Long, B. H.; Golik, S.; Forenza, S.; Ward, B.; Rehfuss, R.; Dabrowiak, J. C.; Catino, J. J.; Musial, S. T.; Brookshire, K. W.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2-6. Sugiura, Y.; Uesawa, Y.; Takahashi, Y.; Kuwahara, J.; Golik, J.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7672-7676.

(4) Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3831-3835.

(5) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464-3466. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-3468. De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554-4556.

(6) Kappen, L. S.; Goldberg, I. H. *Nucleic Acids Res.* **1985**, *13*, 1637-1648.

(7) Chin, D.-H.; Zeng, C.-h; Costello, C. E.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 8106-8114.

(8) Zein, N.; McGahren, W. J.; Morton, G. O.; Ashcroft, J.; Ellestad, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 6888-6890.

(9) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697-699.

(10) The syntheses of [5'-$^2H_2$]DMT-d(cytidine)(Bz)cyanoethyl phosphoramidite will be described elsewhere. The synthesis and purification of dodecamers **4** and **5** were carried out by conventional methods and will be reported in a full paper.

(11) Earlier experiments to investigate the sequence selectivity of DNA cleavage by CLM had shown TCCT sites to be particularly favored for cutting.[1]

0002-7863/90/1512-9669$02.50/0