Soc., Perkin Trans. 1, 2674 (1972).
(10) R. A. Earl and L. B. Townsend, J. Heterocycl. Chem., 9, 1141 (1972).
(11) (a) Y. F. Shealy, J. A. Montgomery, and W. Russel Laster, Jr., Biochem. Pharmacol., 11, 674 (1962); (b) Y. F. Shealy, C. A. Krauth, and J. A. Montgomery, J. Org. Chem., 27, 2150 (1962).
(12) C. W. Noell and C. C. Cheng, J. Med. Chem., 12, 545 (1969).

Synthesis and Antitumor Properties of 7-Deoxy-7-[(*cis*- and *trans*-3-aminocyclohexane)thio]carminomycinone

John M. Essery,* Terrence W. Doyle, and Richard H. Schreiber

Research Division, Bristol Laboratories, Syracuse, New York 13201. *Received May 21, 1979*

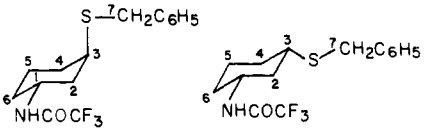
The synthesis of analogues of carminomycin in which the daunosamine group has been replaced by (*cis*- and *trans*-3-aminocyclohexane)thio moieties is described. The new compounds were found to exhibit none of the antitumor or antibiotic activity associated with carminomycin.

The recent disclosure by Tong et al.[1] of the preparation of 7-[(aminoalkyl)thio] derivatives of daunomycinone prompts us to report the synthesis of similar derivatives in the carminomycin series. We sought to replace the daunosaminyl residue of carminomycin (1) with the (3-aminocyclohexane)thio moiety with the expectation that the C_1–S bond would be considerably more stable in vivo than is the glycosidic linkage in 1. We also hoped that the C_7–S bond might prove more resistant to in vivo reduction than the C_7–O bond in 1. In view of the close steric relationship of the analogue to the parent drug, it was expected that a stable complex with DNA could be formed by the analogue.[2]

Chemistry. The desired 3-aminocyclohexanethiols were prepared in the following manner. Base-catalyzed addition of benzyl mercaptan to cyclohexenone (**5**) proceded smoothly to give **6**, which was converted directly to the oxime acetate **7** without further purification. Reduction of **7** with diborane gave the desired *S*-benzyl-3-aminocyclohexanethiol (**8**) as a mixture of cis and trans isomers in which the cis isomer slightly predominated. The overall yield of **8** from **5** was 87%. The isomer ratios were determined by measuring the peak height of the ^{1}H NMR signals for the S-CH_2Ph group for each isomer (at δ 3.69 for the trans isomer **8a** and δ 3.74 for the cis isomer **8b** in $CDCl_3$). Since we anticipated coupling of the thiols to carminomycinone (**2**) as their *N*-trifluoroacetamides, the mixture of amines **8** was converted to the amides **9** via reaction with trifluoroacetic anhydride. While both the amine mixture and the amide mixture were not resolvable by TLC (silica gel using a variety of developing solvents), base-line resolution of the amide isomer mixture was achieved on μ-Porasil high-performance LC columns using methylene chloride–hexane (9:1) as the eluting solvent [k' (isomer 1) = 1.08; k' (isomer 2) = 1.31]. The analytical separation was scaled to a Prep LC/500 scale (5-g loads). The samples were recycled seven times, with the front and back of the main band being shaved on each pass. Excellent material recovery was experienced, and each isomer was obtained enriched to 95% purity. Analysis of the ^{1}H and ^{13}C NMR spectra of the isomers permitted assignment of the trans stereochemistry to the minor isomer 1 and cis stereochemistry to isomer 2.

The ^{1}H NMR spectrum of **9b** exhibited features compatible with a conformationally rigid cyclohexane ring, while the spectrum of **9a** was consistent with an equilibrating set of conformers as evidenced by the methylene envelope between δ 1.3 and 2.0 integrating for 8 protons. In **9b**, the peak width at half-height for the C*H*-S signal was ~18 Hz, which is consistent for an axial proton. The peak widths at half-height for the C*H*-N and C*H*-S signals in the ^{1}H NMR spectrum of **9a** were 18 and 14 Hz respectively, suggesting that the conformer of **9a** in which C–S bond is axial is the major contributor to the time-averaged spectrum of **9a**. These assignments were confirmed by an analysis of the ^{13}C NMR spectra of **9a** and **9b**. The ^{13}C NMR assignments were made on the basis of model compounds from the literature and theoretical considerations.[3] The observed line positions, together with their assignments, are listed in Table I. It has been observed that sterically crowded atoms in a cyclohexane ring, such as the 1, 3, and 5 carbons in cyclohexanes carrying an axial substituent, are found at higher field than similar carbon atoms not subject to steric compression effects. Thus, **9a** having carbon-1, -3, and -5 at highest field was assigned to the trans isomer in agreement with the ^{1}H NMR assignment. While C_1 and C_5 exhibit normal compression shifts of ~4.4 ppm on going from **9b** to **9a**, C_3 shows only a minimal compression shift of 1.4 ppm. This is further support of the suggestion from the ^{1}H NMR spectrum that **9a**-A is the major conformer of **9a** in solution.

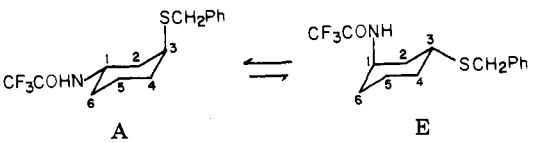
Table I. ^{13}C NMR Listings for **9a** and **9b**[a]



9a **9b**

carbon	9a	9b
1	45.9	50.4
2	40.8	43.5
3	31.1	32.5
4	35.3	35.9
5	20.4	24.7
6	35.3	34.5
7	40.2	41.1

[a] In parts per million downfield from Me_4Si. Recorded in $CDCl_3$.

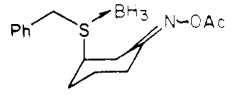


A E

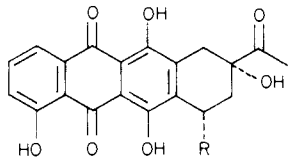
The ratio of cis to trans isomers of **8** produced on diborane reduction of the oxime deserves some comment. While a variety of methods for the reduction of oximes to

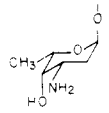
0022-2623/79/1822-1425$01.00/0

amines have been reported, we chose to use diborane in order to maximize the yield of trans isomer. The reduction of oxime acetates in conformationally rigid molecules has been reported to proceed in a highly stereoselective manner to give the product of hydride approach from the sterically least hindered side.[4] In the sugar series, the reaction has been studied extensively by Lemieux et al.,[5] with selectivities for the production of the equatorial amine as high as 95% being reported. It was our expectation that complexation of the borane with the sulfide linkage might lead to deliverance of the hydride from the same side as the sulfide linkage, thus leading to greater yields of trans isomer than might otherwise be expected. Presumably, such an intermediate might be represented by structure 11. The stability and use of borane–sulfide complexes for reductions has been reviewed by Lane.[6]

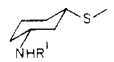


11



1, R = 

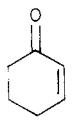
2, R = OH

3a, R^1 = $COCF_3$; R = 

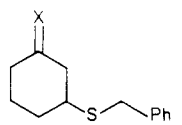
b, R^1 = H; R =

4a, R^1 = $COCF_3$; R = 

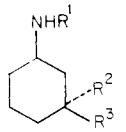
b, R^1 = H; R =



5



6, X = O
7, X = NOAc



8a, R^1 = H = R^3; R^2 = SCH_2Ph
b, R^1 = H = R^2; R^3 = SCH_2Ph
9a, R^1 = $COCF_3$; R^2 = SCH_2Ph; R^3 = H
b, R^1 = $COCF_3$; R^3 = SCH_2Ph; R^2 = H
10a, R^1 = $COCF_3$; R^2 = SH; R^3 = H
b, R^1 = $COCF_3$; R^2 = H; R^3 = SH
12a, R^1 = R^3 = H; R^2 = SH
b, R^1 = R^2 = H; R^3 = SH

In several runs under a variety of conditions (order of addition of reagents and reaction temperature), the yield of trans isomer ranged from 45 to 48%. This yield is considerably in excess of that expected based purely on steric considerations alone.

Initially, the N-protected thiols **10a** and **10b** (obtained by sodium–liquid ammonia reductive cleavage of the *S*-benzyl group) were allowed to react with carminomycinone (**2**) in trifluoroacetic acid solution to provide the new sulfides **4a** and **3a**. However, it was found that hydrolysis of the trifluoroacetamide function in the coupled products was incomplete when using conditions (0.1 N sodium hydroxide–tetrahydrofuran at 0 °C) normally employed when *N*-(trifluoroacetyl)daunosamine is coupled to similar aglycones.[7] Neither carrying out the reaction at room temperature nor substitution of methanol for the tetrahydrofuran resulted in complete hydrolysis. It is possible that this low reactivity may be due to the lack of an adjacent hydroxyl group which can assist in the hydrolysis of *N*-(trifluoroacetyl)daunosamine. It was therefore decided to remove the amide before coupling to the aglycone **2**. Each of the amides **9a** and **9b** was subjected to potassium hydroxide hydrolysis, and the oily products **8a** and **8b** were characterized as their hydrochlorides. Reduction with sodium in liquid ammonia afforded the aminothiols **12a** and **12b**, which were not isolated but coupled directly with **2** in trifluoroacetic acid solution. In this manner, the cis isomer **12b** provided the sulfide **3b** in which the C_7 proton absorbed at 4.58 ppm [in contrast to the value of 5.30 ppm due to this proton in the NMR spectrum of carminomycin (**1**)]. Furthermore, this signal appeared as a multiplet with $J_{7,8a} + J_{7,8b} = 8$ Hz indicating a pseudoequatorial configuration for this proton;[8] i.e., the new substituent at C_7 had the same stereochemistry as in the natural product **1**. From the *trans*-aminothiol **12a** was obtained the sulfide **4b** in which the NMR data again indicated the pseudoaxial configuration of the sulfur substituent. In this case, a second component was isolated which from the complexity of the NMR spectrum and the considerably broader multiplet due to the C_7 proton was assumed to be a mixture of **4b** and its C_7 epimer.

Biological Results. The antitumor antibiotic carminomycin (**1**) has been shown to induce phage production from lysogenic *M. lysodiekticus*,[9] and Price et al.[10] have discussed the in vitro screening of antitumor antibiotics by measuring the induction of lysogenic *E. coli* W1709 (λ). Using their procedure, we found no induction at concentrations up to 50 μg/mL for the new compounds **3b** and **4b**, whereas carminomycin (**1**) was active (T/C 6.2–6.9) at 3.1 μg/mL. That this test was predictive of the in vivo antitumor activity was shown by the lack of activity of **3b** when tested against L-1210 leukemic mice by the method of Geran et al.[11] The drug was administered as a single dose ip and failed to give a significant effect (i.e., T/C < 125) at dosages from 0.25 to 32 mg/kg, whereas carminomycin (**1**) tested at the same time gave T/C = 157 at 0.64 mg/kg. When tested for antibiotic activity against *B. subtilis* ATCC 6633 via a plate assay, carminomycin (**1**) gave a zone of inhibition of 19 mm at 3.1 μg/mL, whereas **3b** and **4b** had values of 10.3 and 10.5 mm at 50 μg/mL. The new sulfide analogues were thus without the antitumor and antibacterial properties of the natural product carminomycin.

Experimental Section

All melting points are uncorrected. NMR spectra were recorded on a Varian HA-100 or XL-100 spectrometer using $CDCl_3$ as solvent, unless otherwise specified, and tetramethylsilane as internal standard. IR spectra were recorded on a Beckman IR-4240 and UV–visible spectra on a Beckman Acta III spectrophotometer. Carminomycin and carminomycinone were obtained from the figaroic acid complex produced by *Streptosporangium sp.* strain C-31, 751 ATCC 31129.[12,13]

3-(*N*-Trifluoroacetamido)cyclohexanethiol (9). To a mixture of cyclohexenone (9.6 g, 0.1 mol) and benzyl mercaptan (12.4 g, 0.1 mol) was added 10 mL of pyridine followed by 5 drops of triethylamine. An exothermic reaction ensued. The NMR

spectrum of an aliquot of the mixture indicated complete reaction in 1 h, as shown by the complete absence of the olefinic signals of cyclohexenone. After 1 h, 50 mL of MeOH and 7.0 g (0.101 mol) of hydroxylamine hydrochloride were added. The solution was stored at 23 °C for 18 h, following which the solvent was removed at reduced pressure. The residue was taken up in 100 mL of CH_2Cl_2 and 10 mL of pyridine was added. To this mixture, a solution of 10 mL of acetyl chloride in 50 mL of CH_2Cl_2 was added over 10 min. The solution was stirred for 1 h and partitioned between CH_2Cl_2 and water. The organic phase was washed with 10% HCl and then with saturated $NaHCO_3$. The solution was dried over Na_2SO_4, filtered, and evaporated to yield 28.3 g of an oil, the NMR spectrum of which was compatible with the oxime acetate **7**: ^1H NMR δ 7.26 (s, 5 H, Ph), 3.75 (s, 2 H, SCH_2), 2.12 (s, 3 H, $COCH_3$), 3.5–1.0 (m, 9 H).

The oxime acetate **7** was taken up in 300 mL of dry THF and cooled to 0 °C. A solution of borane in THF (0.97 M BH_3, 300 mL) was added over 30 min. The solution was stirred at 0–5 °C for 1 h and then heated at reflux for an additional 1.5 h. The solution was then cooled and stored for 18 h at ambient temperature (~23 °C). To the cooled solution (0 °C) was added 200 mL of 20% NaOH slowly. The solution was brought to reflux and boiled for 4 h. The phases were separated and the aqueous phase was extracted with 3 × 200 mL portions of Et_2O. The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was taken up in Et_2O, and HCl gas was added to precipitate the amine **8** as its salt: yield 20.45 g (80%). From the filtrate an additional 1.6 g of amine (7%) was recovered.

To a suspension of 20.45 g of the hydrochloride of **8** in 200 mL of CH_2Cl_2 was added 14.2 mL of triethylamine, followed by 25 mL of trifluoroacetic anhydride. After 1 h, the solvent was removed at reduced pressure and the residue partitioned between Et_2O and water. The organic phase was dried, treated with 1 g of activated carbon, filtered, and evaporated to yield 19.2 g of a mixture of isomers of **9**. The solids were analyzed by high-performance LC on μ-Porasil columns using 10% hexane in CH_2Cl_2 as the developing solvent. The ratio of isomers was 47:53, as estimated from the peak heighth of the isomers.

The isomers were separated by preparative high-performance LC using a Waters Prep LC/500 apparatus. Two silica gel PrepPak cartridges were used which had been equilibrated with CH_2Cl_2–Skellysolve B (9:1). Thus, 3.75 g of the amide mixture **9** described above was dissolved in 17 mL of CH_2Cl_2. The solution was injected on the head of the first column. The flow rate was set at 500 mL/min. The first peak (solvent front) was collected and the second recycled once, before any material was removed. On the second pass, both the front and back of the main peak were collected, with the center being recycled. A total of seven recycles were made with the fractions being analyzed by analytical high-performance LC and combined accordingly.

In subsequent runs, the amount of material loaded was increased to 5.0 g each. Fractions containing >90% of one isomer were combined with all intermediate fractions being rechromatographed. From 18.7 g of crude **9** were obtained 7.45 g of **9a** and 7.50 g of **9b** of ~95% purity. Each sample was recrystallized from benzene. **9a**, isomer 1: mp 102–103 °C; ^1H NMR δ 7.26 (s, 5 H, Ph), 6.06 (br s, 1 H, NH), 4.23 (m, 1 H, C_3 H, peak width at half-height = 18 Hz), 3.71 (s, 2 H, CH_2Ph) 2.86 (m, 1 H, C_1 H, peak width at half-height = 14 Hz), 1.3–2.0 (m, 8 H). Anal. ($C_{15}H_{18}F_3NOS$) C, H, N. **9b**, isomer 2: mp 116–117 °C; ^1H NMR δ 7.25 (m, 5 H, Ph), 6.45 (br s, 1 H, NH), 3.72 (2 H, s, CH_2Ph), 3.75 (br m, 1 H, C_3H, obscured by CH_2Ph signal), 2.56 (m, 1 H, C_1H), 2.24 (dm, 1 H), 1.85 (m, 3 H), 1.25 (m, 4 H). Anal. ($C_{15}H_{18}F_3NOS$) C, H.

***cis*-3-Amino-*S*-benzylcyclohexanethiol (8b).** A solution containing 0.2 g of the *cis*-trifluoroacetamide **9b** and 0.2 g of KOH in 15 mL of 50% aqueous EtOH was heated under reflux for 1 h. The solution was cooled, diluted with H_2O, and extracted with CH_2Cl_2. The extracts were washed with H_2O and dried over Na_2SO_4, and the solvent was evaporated to give 130 mg of oil. This was dissolved in dry Et_2O and treated with a solution of dry HCl in Et_2O. The precipitated solid was recrystallized from aqueous acetone to provide the hydrochloride of **8b** as colorless needles: mp 171–172 °C; ^1H NMR (acetone-d_6–D_2O) δ 7.27 (m, 5 H, Ph), 3.78 (s, 2 H, SCH_2), 4.20 (m, 1 H, C_3H, peak width C_3 H, at half-height = 22 Hz), 2.28–2.78 (m, 2 H, includes C_1 H), 1.70–2.20 (m, partially obscured by solvent), 1.00–1.65 (m, 4 H). Anal. ($C_{13}H_{19}NS$·HCl·0.5H_2O) C, H, N.

***trans*-3-Amino-*S*-benzylcyclohexanethiol (8a).** Similarly, the *trans*-trifluoroacetamide **9a** provided **8a** as its hydrochloride: mp 133–134 °C; ^1H NMR (acetone-d_6–D_2O) δ 7.36 (m, 5 H, Ph), 3.82 (s, 2 H, SCH_2), 3.50–3.80 (m, 1 H, C_3 H, peak width at half-height = 22 Hz), 3.26 (m, 1 H, C_1 H, peak width at half-height = 10 Hz), 1.60–2.20 (m, partially obscured by solvent). Anal. ($C_{13}H_{19}NS$·HCl·0.5H_2O) C, H, N, S.

7-Deoxy-7-[(*cis*-3-aminocyclohexane)thio]carminomycinone (3b). A portion of **8b**·HCl was extracted into CH_2Cl_2 from an aqueous solution at pH 9.0 to provide 71 mg (0.32 mmol) of **8b**. This was dissolved in 10 mL of liquid NH_3 containing 1 mL of THF, and to the stirred solution was added, in portions, sodium until a blue color persisted. Following the addition of NH_4Cl, NH_3 was evaporated under a N_2 atmosphere. The solid residue was dissolved in trifluoroacetic acid (4 mL), and insoluble salts were removed by filtration. Carminomycinone (**2**; 77 mg, 0.2 mmol) was added to the filtrate (assumed to contain 0.32 mmol of **12b**) and the solution was stored for 17 h at 40 °C. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2, which was then extracted with four portions of aqueous HCl at pH 3.5. The combined extracts were adjusted to pH 8.0 with dilute NaOH, and the product was extracted into CH_2Cl_2. These extracts were washed with brine and dried over Na_2SO_4, and the solvent was evaporated to provide a solid which was triturated with Et_2O to give 19 mg of **3b** as a red solid: mp 205–207 °C; IR (KBr) 1710, 1600 cm^{-1}; ^1H NMR δ 7.90–7.60 (m, 2 H, C_1 H, C_2 H), 7.29 (dd, 1 H, C_3 H), 4.58 (m, 1 H, C_7 H, $J_{7,8a} + J_{7,8b}$ = 8 Hz), 3.35–2.60 (m, 4 H, includes C_{10} H, $C_{1'}$ H, $C_{3'}$ H), 2.50–2.25 (m, 5 H, includes $COCH_3$ and C_8 H), 2.05–1.70 and 1.45–1.00 (m, ring CH_2 protons). Anal. ($C_{26}H_{27}NO_7S$·H_2O) C, H, N.

7-Deoxy-7-[(*trans*-3-aminocyclohexane)thio]carminomycinone (4b). In a similar manner, a sample of **8a**·HCl was converted to 140 mg (0.63 mmol) of **8a**, which was reduced and treated as before to provide a solution of **12a** (assumed 0.63 mmol) in 10 mL of trifluoroacetic acid. To this solution was added 222 mg (0.58 mmol) of carminomycinone (**2**), and the solution was stored at 40 °C for 19 h. A small amount of orange solid was removed by filtration, and the solvent was removed from the filtrate under reduced pressure. The residue was shaken with CH_2Cl_2 and saturated aqueous $NaHCO_3$, and the organic phase was separated and exhaustively extracted with aqueous HCl at pH 3.0. The combined aqueous extracts were basified to pH 8.5 (with NaOH), and the product was extracted into $CHCl_3$ to afford 48 mg of purple solid which was purified by high-performance LC on a Partisil 20 column using CH_2Cl_2–MeOH–concentrated NH_4OH (90:10:1) as the mobile phase. The desired product (**3a**) was obtained as a red solid: yield 6.5 mg; mp 175–177 °C; IR ($CHCl_3$) 1715, 1600 cm^{-1}; ^1H NMR δ 7.96–7.62 (m, 2 H, C_1 H, C_2 H), 7.30 (dd partially obscured by solvent, C_3 H), 4.56 (m, 1 H, C_7 H, $J_{7,8a} + J_{7,8b}$ = 8 Hz), 3.58 (br, 1 H, $C_{3'}$ H), 3.40–2.90 (m, 3 H, includes C_{10} H and $C_{1'}$ H), 2.44–2.30 (m, 5 H, includes $COCH_3$ and C_8 H), 2.04–1.50 (m, 8 H); UV-vis λ_{max} (MeOH) 234 nm (ϵ 20 300), 253 (16 700), 494 (9160), 529 (8050). Anal. ($C_{26}H_{27}NO_7S$·H_2O) C, H, N.

Acknowledgment. The authors thank Drs. W. T. Bradner and J. A. Bush of these laboratories for supplying the in vivo and in vitro antitumor data.

References and Notes

(1) G. L. Tong, T. H. Smith, D. W. Henry, and E. M. Acton, "Abstracts of Papers", 175th National Meeting of the American Chemical Society, Anaheim, Calif., Mar 13–17, 1978, American Chemical Society, Washington, D.C., MEDI 48.

(2) A. DiMarco and F. Arcamone, *Arzneim.-Forsch.*, **25**, 368 (1975).

(3) (a) The ^{13}C NMR spectra of both *cis*- and *trans*-1,3-dimethylcyclohexane have been assigned and were used as a departure point for calculation of the spectra to be expected for **8a** and **8b**: D. K. Dalling and D. M. Grant, *J. Am. Chem. Soc.*, **89**, 6612 (1967); A. S. Perlin and H. J. Koch, *Can. J. Chem.*, **48**, 2639 (1970). (b) G. C. Levy and G. L. Nelson,

"Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, London, Sydney, and Toronto, 1972.
(4) A. Hassner and P. Catsoulacos, *J. Chem. Soc., Chem. Commun.*, 590 (1967).
(5) R. U. Lemieux, K. James, T. L. Nagabhushan, and Y. Ito, *Can. J. Chem.*, **51**, 33 (1973).
(6) C. F. Lane, *Chem. Rev.*, **76**, 773 (1976).
(7) E. M. Acton, A. N. Fujiwara, and D. W. Henry, *J. Med. Chem.*, 17, 659 (1974).
(8) P. F. Wiley, R. B. Kelly, E. L. Caron, V. H. Wiley, J. H. Johnson, F. A. MacKellar, and S. A. Mizsak, *J. Am. Chem. Soc.*, **99**, 542 (1977).
(9) Y. V. Dudnik, L. N. Ostanina, L. I. Kozmyan, and G. F. Gauze, *Antibiotiki*, **19**, 514 (1974).
(10) K. E. Price, R. E. Buck, and J. Lein, *Appl. Microbiol.*, **12**, 428 (1964).
(11) R. I. Geran, N. N. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 9 (1972).
(12) W. T. Bradner, J. A. Bush, and D. E. Nettleton Jr., U.S. Patent 4112071 (1978).
(13) M. C. Wani, H. L. Taylor, M. E. Wall, A. T. McPhail, and K. D. Onan, *J. Am. Chem. Soc.*, **97**, 5955 (1975).

Nucleoside Conjugates as Potential Antitumor Agents. 2. Synthesis and Biological Activity of 1-(β-D-Arabinofuranosyl)cytosine Conjugates of Prednisolone and Prednisone[1]

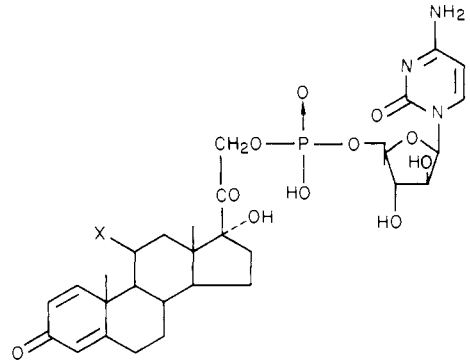
Chung Il Hong,* Alexander Nechaev, and Charles R. West

Department of Neurosurgery, Roswell Park Memorial Institute, Buffalo, New York 14263. *Received December 27, 1978*

Two of the new anticancer drugs recently synthesized in our laboratory from conjugation of *ara*-C[2] and several corticosteroids linked through a phosphodiester bond include prednisolone- (I) and prednisone-p-*ara*-C (II). They were demonstrated to be enzymatically hydrolyzed to the corresponding steroid and *ara*-CMP and the latter was further shown to be hydrolyzed to *ara*-C by phosphodiesterase I, snake venom, 5'-nucleotidase, and acid phosphatase. However, the conjugates were shown to be resistant to hydrolysis by alkaline phosphatase. The activity of conjugates I and II against L1210 lymphoid leukemia in female mice ($C_3D_2F_1/J$) was significantly greater than that of *ara*-C alone or in combination with the steroid. In fact, when the optimum dosage of 75 (μmol/kg)/day × 5 was used, the administration of *ara*-C alone was followed by an increased life span (ILS) of 45%. This result is similar to that previously reported.[3] With the same equimolar doses of mixtures of *ara*-C and either prednisolone or prednisone, the ILS values were 40 and 44%, respectively. However, when the conjugates were used, the ILS values were 89 and 100%, respectively. These findings seem promising and have provided the bases for continued study of these new compounds.

Because 1-(β-D-arabinofuranosyl)cytosine (*ara*-C) has a specific mode of action at the S phase of the cell cycle[4] and is rapidly deactivated by cytidine deaminase,[5] it requires a very complex and precise dosage schedule to be efficacious. As a result of its rapid degradation, the plasma half-life in patients is generally too short for effective and convenient administration.[6] In an attempt to overcome some of these difficulties, others[3,7-10] have synthesized lipophilic derivatives. For example, *ara*-C 5'-phosphate L-1,2-dipalmitin has recently been developed and reported to show strong therapeutic potential.[10] Furthermore, studies[11] on the use of steroids as covalent carriers of cytotoxic groups, including nucleoside bases,[12] have been presented. The present study was designed to (1) synthesize conjugates of *ara*-C and steroids and (2) to determine the extent to which the favorable response of *ara*-C might be enhanced by the chemical linkage of this compound to steroids. A description of the synthesis and biological activity of conjugates of *ara*-C and two steroids, prednisolone- (I) and prednisone-p-*ara*-C (II), is provided in the present report.

Chemistry. Three chemical reactions were used sequentially to prepare *ara*-C conjugates of prednisolone and of prednisone. The first was the direct phosphorylation of *ara*-C with $POCl_3$ and $(EtO)_3PO$ by a modified method of Yoshigawa et al.[13] and Hong et al.[14] By this method, the yield for *ara*-CMP was 80%. In the next step, the latter compound was acetylated with Ac_2O in the presence of pyridine. The Ac_3-*ara*-CMP prepared demonstrated one spot on TLC, and the mobilities in various solvents were identical with that of the compound prepared by phosphorylation of Ac_3-*ara*-C.[15] The observed UV maxima for the pyridine-free compound were 243 and 290 nm and were near that for Ac_3-*ara*-C (245 and 295 nm). Finally, Ac_3-*ara*-CMP was condensed with 2 molar equiv of either prednisolone or prednisone. This was analogous to the preparation of prednisolone 21-phosphate by condensation of prednisolone with 2-cyanoethyl phosphate in the presence of DCC and pyridine.[16] After these procedures were completed and the protective groups removed, conjugates I and II were separated on a DE-52 column using a HOAc gradient (0–1.5 N). The conjugates were actually eluted out in 1–1.5 N HOAc fractions and the yield for each was 30%. Attempts at condensation of prednisolone 21-phosphate or prednisone 21-phosphate with Ac_3-*ara*-C in the presence of DCC and pyridine at room temperature and at reflux were not successful.



I, X = OH
II, X = =O

Confirmation of the molecular structures of the conjugates was provided by elemental analysis; UV, IR, and NMR spectra; and especially by chemical and enzymatic hydrolyses of the phosphodiester bonds. Hydrolyses of

0022-2623/79/1822-1428$01.00/0