2,3,4,4a,5,6-Hexahydro-1*H*,8*H*-pyrido[1′,2′:3,4]pyrimidino-[2,1-*b*]quinazolin-8-one·HCl (8).—To a suspension of anthranilic acid (13.7 g) in dimethylacetamide (100 ml) **4a** (20 g) was added, and the mixt was heated to 150–160° for 3 hr. After cooling the solvent was evapd *in vacuo*, and the residue was dissolved in $CHCl_3$. The soln was extd with 2 *N* NaOH and with H_2O. The org phase was dried (Na_2SO_4) and evapd, and the residue was chromatogd on silica gel (Merck AG, 0.05–0.2 mm). The product was eluted with $CHCl_3$. After evapn the residue was dissolved in EtOH (80 ml), and the soln was satd with anhyd HCl. On addn of Et_2O (300 ml) the hydrochloride crystd, it was removed by filtration (5.9 g, 20%) and dried *in vacuo* (0.1 mm) at 60°, mp 267–270°. *Anal.* ($C_{15}H_{18}N_3OCl$) N, O, Cl.

Analogously **1,2,3,4,13,13a-hexahydro-7-methyl-11*H*-pyrido-[1′,2′:3,4]imidazo[2,1-*b*]quinazolin-11-one** (**7e**) was prepd in 10% yield by the above procedure from **3a** and 3-methylanthranilic acid, mp 129–130°. *Anal.* ($C_{15}H_{17}N_3O$) C, H, N.

1,2,3,4,13,13a-Hexahydro-8-methoxy-11*H*-pyrido[1′,2′:3,4]-imidazo[2,1-*b*]quinazolin-11-one (**7f**).—A mixt of 4-methoxyanthranilic acid (6.0 g) and **3a** (6.0 g) in 25 ml of DMAC was heated for 4 hr at 150–160°. The solvent was evapd and the remaining solid dissolved in CH_2Cl_2. The soln was washed with 2 *N* NaOH (50 ml) twice with water (100 ml), dried (Na_2SO_4), and evapd *in vacuo*. The residue crystd from Et_2O, 4.0 g (42%), mp 154–157°. *Anal.* ($C_{16}H_{17}N_3O_2$) C, H, N, O.

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Notes

Nucleic Acids. 12. Synthesis of the L Enantiomer of 1-β-Arabinofuranosylcytosine and of $O^2,O^{2'}$-Anhydro-1-β-D-arabinofuranosylcytosine

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1-β-D-Arabinofuranosylcytosine[1a,b] (cytarabine, *ara*-cytidine, *ara*-C, cytosine arabinoside, Cytosar), has proven efficacious in the treatment of acute leukemias and lymphomas[2a–f] and is an inhibitor of DNA synthesis,[3a–e] DNA viruses,[4a,b] and rodent tumors,[5a–j] and inhibits growth of various mammalian cell lines.[3a–e]

A derivative of *ara*-C, 5′-(1-adamantoyl)-*ara*-C, has been shown to possess superior therapeutic properties (compared to *ara*-C) in the treatment of L1210 leukemic mice[6] and to possess greater immunosuppressive activity in this species[7a,b] and in the rat.[7b,8] Recent reports have described the synthesis of a series of 5′ esters of *ara*-C and the superiority of some of these derivatives as antileukemic and immunosuppressant drugs.[9a,b]

Sanchez and Orgel have recently described a convenient synthesis of *ara*-C utilizing 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (I) as the key intermediate.[10] In this synthesis, cyanamide is treated with D(−)-arabinose to yield the sugar–oxazoline derivative (I), which is then condensed with cyanoacetylene to give the $O^2,O^{2'}$-anhydro derivative (II) of *ara*-C. II, without isolation, is hydrolyzed to *ara*-C. Utilizing this synthetic route, but substituting 2-amino-β-L-arabinofurano[1′2′:4,5]-2-oxazoline for the D isomer, we have prepared the L enantiomer of *ara*-C and have tested it for biological activity. We have further devised a method for the isolation of the $O^2,O^{2'}$-anhydro derivative II of D-*ara*-C, a compd that may prove to be an intermediate for the preparation of a number of derivatives of *ara*-C itself.

The preparation of $O^2,O^{2'}$-anhydro-1-β-D-arabinofuranosylcytosine was first reported by Walwick, *et al.*,[1b] who obtained this product in the form of its hydrochloride by the action of prostatic phosphatase on the 3′,5′-diphosphate of the anhydro derivative. This diphosphate had been obtained by phosphorylation of cytidine with polyphosphoric acid. Nagyvary[11] prepared the 3′-phosphate ester of the $O^2,O^{2'}$-anhydronucleoside *via* a polytrimethyl silylated derivative of cytidine 2′,3′-cyclic phosphate. The 3′-phosphate can be dephosphorylated enzymatically. Doerr and Fox[12] had prepared this anhydro nucleoside from 2′-deoxy-2′-chlorocytidine. None of these methods offers a convenient process for the preparation of the anhydro compd. We have now prepared $O^2,O^{2'}$-anhydro-1-β-D-arabinofuranosylcytosine directly from the aminooxazoline. For this purpose, the aminooxazoline I was converted to its hydrochloride and this was condensed with cyanoacetylene to give directly II·HCl.

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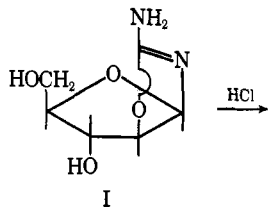

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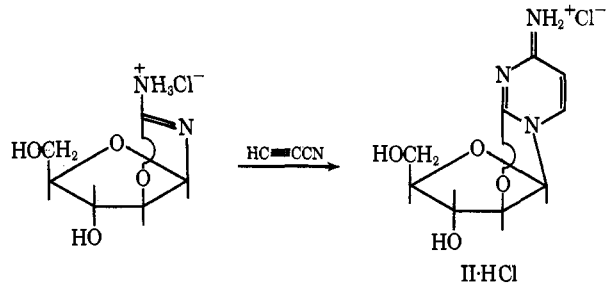

II·HCl

II·HCl crystallized from the reaction mixture in pure form. The conversion of the aminooxazoline I to its hydrochloride greatly reduces its nucleophilicity, of course, and heating for several hr is required to obtain condensation with cyanoacetylene and subsequent cyclization to the HCl salt of II. On the other hand, the free base of the aminooxazoline I reacts exothermically with cyanoacetylene, and base-catalyzed conversion to *ara*-C *via* the intermediate anhydro compd II is complete within a few min.[10]

Since completion of this work, Sanchez, *et al.*, have reported the preparation of $O^2,O^{2'}$-anhydroarabinofuranosylcytosine by a process that involves condensation of cyanoacetylene with the aminooxazoline I followed by isolation of the anhydro derivative as its acetate salt.[13]

The L analog of *ara*-C was inactive (at doses of up to 300 mg/kg per day ip daily for 5 days) when tested *in vivo* in the mouse as an antileukemic agent against L1210. The method employed in studying the effect of agents on survival of L1210 leukemic mice was similar to that described by the Cancer Chemotherapy National Service Center.[14] The L analog proved to be inactive as an immunosuppressant at 200 mg/kg ip and po when tested in the mouse hemagglutinin test as described by Gray, *et al.*[15] It neither potentiated nor inhibited the activity of *ara*-C in this test. It was inactive as an antiviral agent in the *in vitro* cell culture test described by Renis, *et al.*[16]

These results parallel those with other L-nucleosides. Yamaoka, *et al.*, have prepared the 1-α-L-arabinofuranosyl derivatives of thymine, cytosine, and uracil.[17] Preliminary screening studies of the L-nucleosides showed no significant activity against L1210 mouse leukemia or Burkett's tumor cells in tissue culture. The cytosine nucleoside was not deaminated by human liver or mouse kidney homogenates, nor did it inhibit the deamination of 1-β-arabinofuranosylcytosine in those systems.[17] These workers also prepared the α-L-xylo and α-L-lyxo derivatives of thymine, uracil, and cytosine, and obtained identical results in biological studies with those obtained with the 1-α-arabinofuranosyl derivatives.[17] Smrž and Farkaš have reported that 1-α-L-lyxofuranosylthymine is not cleaved by nucleoside phosphorylase from *Escherichia coli*.[18] The $O^2,O^{2'}$-anhydro derivative of *ara*-C was active against herpes virus *in vitro*, but was inactive against several RNA viruses. This compd showed no activity in the hemagglutinin test in the mouse when administered ip at 200 mg/kg, but showed some inhibition when given orally on days 1–5 at 200 mg/kg.

When it was administered as a single 200 mg/kg ip dose to L1210 leukemic mice one day after tumor inoculation, a 46% increase in life span (ILS) was obtained. Oral administration (single 500 mg/kg dose) yielded a 39% ILS. The corresponding results with *ara*-C under the same conditions (doses, routes, schedules) were 20 and 28% ILS, respectively.

Experimental Section[19]

2-Amino-β-L-arabinofurano[1′,2′:4,5]-2-oxazoline.—L-(+)-Arabinose (45 g, 0.3 mole) and 25.2 g (0.6 mole) of cyanamide were stirred in a mixt of 15 ml of 6 *M* NH_4OH and 75 ml of MeOH for 5 hr at room temp. The mixt was stored for 72 hr in the cold with stirring, then cooled in an ice–salt bath for several hr. The solid was collected, washed with cold MeOH and then Et_2O, and air-dried. The yield was 25.3 g (47%); mp 179–180° dec; $[\alpha]_D^{25}$ −21° (*c* 1, H_2O). *Anal.* ($C_6H_{10}N_2O_4$) C, H, N. The nmr and ir spectra were superimposable on those for the D compd.[10]

1-β-L-Arabinofuranosylcytosine·HCl (L-*ara*-C·HCl) was prepd as described for D-*ara*-C·HCl,[10] substituting 2-amino-β-L-arabinofurano[1′,2′:4,5]-2-oxazoline for the D isomer. The product was recrystd from DMF–EtOAc: yield, 52%; mp 198–200° dec. A sample was recrystd from MeOH for analysis; mp 200° dec; $[\alpha]_D^{25}$ − 130° (*c* 1, H_2O) (+129° for the D form[1a]). *Anal.* ($C_9H_{13}N_3O_5$·HCl) C, H, N, Cl.

$O^2,O^{2'}$-Anhydro-1-β-D-arabinofuranosylcytosine·HCl.—2-Amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (52.2 g, 0.3 mole) was suspended in 300 ml of MeOH and 27.0 ml of concd HCl was added. The mixt was stirred to effect soln, and the solvent was evapd *in vacuo*. The hydrochloride of the oxazoline was obtained as a glassy residue, which was further dried *in vacuo*. This material was dissolved in 300 ml of DMF, and about 17 g (10% excess) of cyanoacetylene[10] was added. The soln was heated at 95° for 2 hr to effect cyclization. During this time the product sepd as a white cryst solid. The mixt was cooled, the product was collected, washed with DMF and then Et_2O, and dried: yield, 41.5 g (53%); mp 260° dec (lit., 248–250° dec;[1b] 260° dec[12]); $[\alpha]_D^{25}$ −79° (*c* 1, DMSO). *Anal.* ($C_9H_{11}N_3O_4$·HCl) C, H, N, Cl.

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4(5)-(2-Amino-1-hydroxyalkyl)imidazoles[1a]

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