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THE SYNTHESIS AND ANTITUMOR ACTIVITY OF ARABINOSYL-5-AZACYTOSINE

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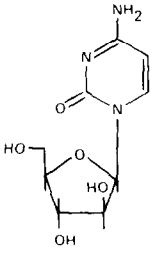
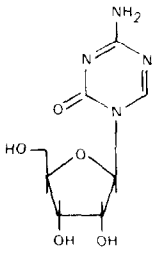
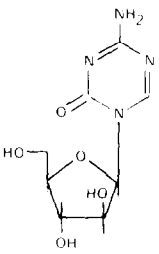
The antitumor and antiviral activity (1) of 1-β-D-arabinofuranosylcytosine (ara-C) prompted us to initiate a synthetic effort to prepare the 5-aza bioisostere of ara-C. The arabinosyl 5-aza derivative was additionally attractive as a synthetic goal because of our interest (2,3) in nucleosides related to 5-azacytidine (5-AC) which is used clinically in the treatment of leukemia (4). In terms of chemical structure 1-β-D-arabinofuranosyl-5-azacytosine (ara-AC) contains elements of both ara-C and 5-AC. The



ara-C



5-AC



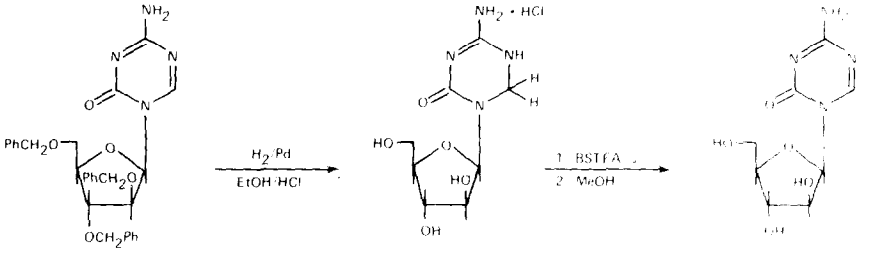
ara-AC

inherent synthetic difficulty in the preparation of ara-AC lies in the instability of the triazine ring with respect to reduction and nucleophilic reactions. Because 5,6-dihydro-5-azacytidine is much more stable to nucleophilic attack than 5-AC (2), a reasonable strategy for the synthesis of ara-AC would indicate utilization of the dihydro derivative of ara-AC as a synthetic intermediate. In a final synthetic step, dehydrogenation to give ara-AC might be accomplished via the novel thermal elimination reaction of a per-trimethylsilylated derivative analogous to the conversion of dihydro-5-azacytidine to 5-AC (5).

Materials and Methods. In an earlier, unsuccessful attempt to synthesize ara-AC, the preparation of 1-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-5-azacytosine (I) was described (6). This intermediate seemed an appropriate starting material for the present synthesis which is shown in Scheme 1. Hydrogenation of I in ethanolic hydrogen chloride led to the reduction of the 5,6-double bond in the triazine ring as well as effecting the

hydrogenolysis of the benzyl protecting groups to provide 5,6-dihydro-1-β-D-arabinofuranosyl-5-azacytosine hydrochloride (II) in 79% yield: mp 166-169° dec.; $[\alpha]^{24}_{D}$ -26° (c 1.0, H_2O); NMR (Me_2SO-d_6) δ 5.77 (doublet, J = 5Hz, 1H, C_1,H), 4.70 (broad singlet, 2H, C_6H).

Scheme I



I II III

Treatment of II at room temperature with bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) in acetonitrile solution readily gave the pentakis-trimethylsilyl derivative of II as shown by combined gas chromatography-mass spectrometry (GC-MS) analysis*. After 53 hours reflux of the silylation solution, a complete conversion to III as the tetrakis-trimethylsilyl derivative was evident by GC-MS. Evaporation of the reaction solution gave a syrup which was boiled with methanol to remove the trimethylsilylgroups by solvolysis. Arabinosyl-5-azacytosine (III) crystallized from solution in 82% yield: mp 223-225° dec., $[\alpha]^{24}_{D}$ 122° (c 1.0, H_2O). The NMR spectrum of III in Me_2SO-d_6 solution showed the C-6 aromatic proton of the triazine nucleus at δ 8.22 as a sharp singlet and the C_1, anomeric proton at δ 6.03 as a doublet (J = 4Hz). In comparison, the corresponding singlet due to the aromatic C-6 proton of 5-AC in the same solvent appeared at δ 8.60. In the spectrum of the reduced nucleoside (II), the low-field aromatic singlet, characteristic of III and 5-AC, was replaced by a singlet at δ 4.70 attributable to the pair of C-6 methylene protons.

The UV spectrum of III in water solution showed a maximum at 243 nm (ε 6800); an hour later the maximum shifted to 242 nm and the extinction coefficient increased to 7200. The respective values recorded for 5-AC at the same time intervals in the same solvent were 241 nm (ε 6,800) and 241 nm (ε 7,700) (8). The UV behavior of 5-AC has been explained (7,8) by a facile hydrolysis reaction which gives rise to a hydrolysis product that has a larger extinction coefficient at approximately the same wavelength. Since the arabinosyl derivative (III) has very similar UV characteristics, it can be assumed that III is also

*The GC-MS system and operating conditions are described in reference 3 along with other instrumentation and procedures relevant to this report.

unstable with respect to hydrolysis producing a similar hydrolysis product, with enhanced absorptivity, at approximately the same rate as 5-AC.

The antitumor activity of ara-AC (III) was determined in parallel experiments with 5-AC using the murine leukemia L1210 system according to protocols (9) set forth by the National Cancer Institute. Dose-response assays were conducted for each compound using six CDF_1 mice to evaluate each dose level on a day 1, 5 and 9 treatment schedule. In order to minimize drug loss to hydrolytic decomposition, ara-AC and 5-AC were administered within 15 min. after dissolution in physiological saline. The results of three identical experiments are recorded in Table 1. Increase in life span of test animals beyond the survival time of untreated control animals expressed as a percentage (%ILS) was used to evaluate antitumor activity. Activity is defined here as a %ILS $\geq$ 25%.

Results and Discussion. Although the optimal dose for ara-AC apparently has not yet been achieved, the data presented in Table 1 indicate that ara-AC has antitumor activity in the L1210 test system which is comparable to that produced by 5-AC. As additional supplies of ara-AC become available, the effect of different administration schedules will be investigated, in addition to the determination of the optimal dose. In order to reveal possible differences in the antitumor effect of ara-AC and 5-AC, further parallel studies will be conducted using a subline of L1210 resistant to 5-AC and tumor systems

Table 1. Comparison of ara-AC and 5-AC Against L1210 Leukemia[a]

ara-AC				5-AC			
Dose (mg/kg)	% ILS control number[b]			Dose (mg/kg)	% ILS control number[b]		
	8937	8946	8947		8937	8946	8947
200		100	90	40	45	35	61
100	75	82	64	20	83	129	78
50	63	48	73	10	75	88	135
25	29	49	67	5	49	56	40
12.5	32	32	51	2.5	17	47	28
	28	23	21	1.25	18	32	36
			19				
			5				

[a]) cells intraperitoneally (i.p.) on Day 0. Test compounds d i.p. on Days 1, 5 and 9. [b] Untreated animals in control mean survival times of 8.7, 8.5 and 8.8 days, respectively.

refractory to 5-AC. Since the higher dose levels of ara-AC relative to 5-AC necessary to produce an equivalent response might in part be due to rapid in vivo deamination of the former (which calls to mind the metabolic fate of ara-C), drug combination experiments with the deaminase inhibitor, tetrahydrouridine, might indicate the possibility of lower effective ara-AC dosages.

The high-yield dehydrogenation reaction of reduced ara-AC (II) through a per-trimethylsilylated derivative was central to the successful synthesis of ara-AC which enabled the biological evaluation of this elusive nucleoside. Other labile triazine nucleosides, difficult to prepare by conventional routes, might also be prepared in a similar way via the dihydro intermediate.

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