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Studies on Biologically Active Nucleosides and Nucleotides. IV.[1] Synthesis of 1-β-D-Arabinofuranosyl-2-aralkylamino-1,4-dihydro-4-iminopyrimidine Hydrochlorides

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Some 1-β-D-arabinofuranosyl-2-aralkylamino-1,4-dihydro-4-iminopyrimidine hydrochlorides (2a—c) were prepared by the reaction of 2,2′-anhydro-1-(β-D-arabinofuranosyl)-cytosine hydrochloride (**1**) with the corresponding aralkylamines (1 equiv.) in methanol. Treatment of **1** with an excess of the amines gave 1-β-D-arabinofuranosyl-2-aralkylamino-1,4-dihydro-4-aralkyliminopyrimidine hydrochlorides (**3a,b**). These compounds were inactive against *leukemia L-1210* and *Ehrlich carcinoma* in mice.

Keywords——derivatives of araC; anhydro-araC; depot form; aminolysis; antitumor activity; antiviral activity

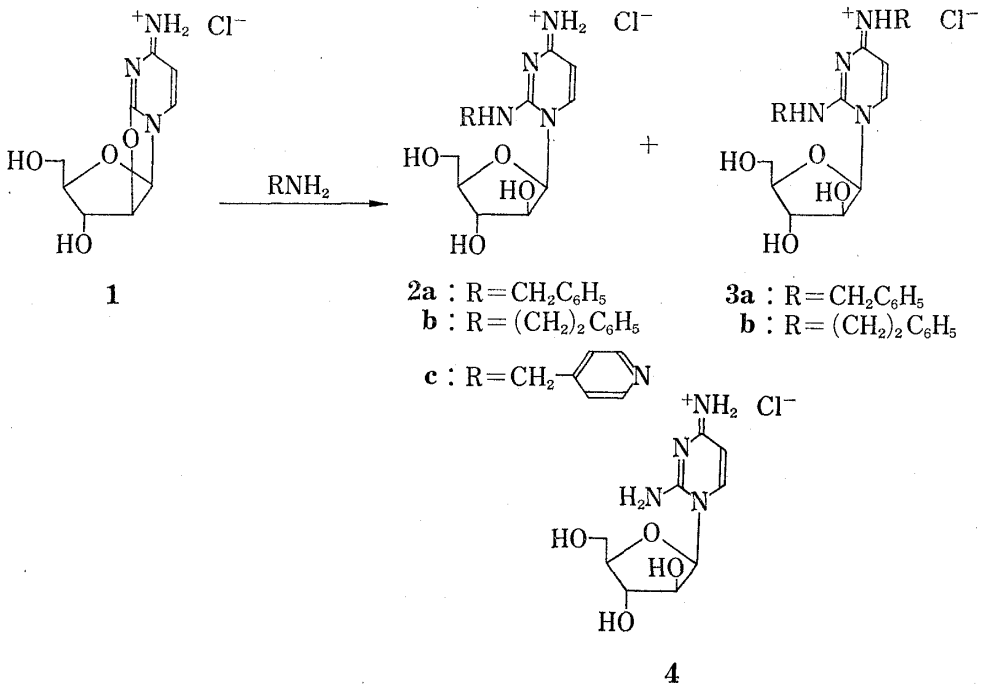
1-β-D-Arabinofuranosylcytosine (araC) has been proved effective in the treatment of acute leukemias and lymphomas.[3] In cell culture, araC inhibits the multiplication of DNA-virus.[4] AraC, however, undergoes rapid enzymatic deamination *in vivo* to the biologically inactive 1-(β-D-arabinofuranosyl)uracil.[5] Numerous derivatives[6] of araC have been prepared in an attempt to develop more clinically useful drugs. Some of them have been found to exhibit superior therapeutic properties to the parent compound. For example, 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (**1**) is a highly effective antitumor agent[7] with toxicity somewhat less than that of araC.[8] This compound is resistant to deamination[9] and the enhanced activity is probably related to a slow, nonenzymatic hydrolysis to give sustained levels of araC.[10]

Doerr and Fox have described the synthesis and chemical properties of 1-β-D-arabinofuranosyl-2-amino-1,4-dihydro-4-iminopyrimidine hydrochloride (**4**).[11] This compound is unstable in water, being gradually converted to **1** and araC. On the basis of the properties, Fox has assumed that **4** may act as a depot form of araC *in vivo*.[12] In fact, **4** was found to be active against leukemic mice,[12] though details of the activity have not been reported yet. These findings prompted us to prepare the C_2-aralkylamino analogs of **4** in order to find novel derivatives of araC with more potent antitumor activity.

The reaction of **1** with 1 equiv. of benzylamine was carried out in methanol at room temperature for 5 days. By direct crystallization of the crude product two compounds were

1) Part III: K. Kondo, T. Adachi, and I. Inoue, *J. Org. Chem.*, **42**, 3967 (1977).
2) Location: *16-89, Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan.*
3) B.D. Clarkson, *Cancer*, **30**, 1572 (1972).
4) L.A. Feldman and F. Rapp, *Proc. Soc. Exptl. Biol. Med.*, **122**, 243 (1966).
5) W.A. Creasey, R.J. Papac, M.E. Markiuo, P. Calabresi, and A.D. Welch, *Biochem. Pharmacol.*, **15**, 1417 (1966).
6) E.K. Hamamura, M. Prystasz, J.P.H. Verheyden, J.G. Moffatt, K. Yamaguchi, N. Uchida, K. Sato, A. Nomura, O. Shiratori, S. Takase, and K. Katagiri, *J. Med. Chem.*, **19**, 654 (1976).
7) A. Hoshi, F. Kanzawa, and K. Kuretani, *Gann*, **63**, 353 (1972).
8) H. Hirayama. K. Sugihara, T. Sugihara, K. Wakigawa, M. Iwamura, H. Ohkuma, and J. Hikita, *Pharmacometrics*, **8**, 353 (1974).
9) A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi, and Y. Arai, *Gann*, **62**, 145 (1971).
10) M.C. Wang, R.A. Sharma, and A. Bloch, *Cancer Res.*, **33**, 1265 (1973).
11) I.L. Doerr and J.J. Fox, *J. Org. Chem.*, **32**, 1462 (1967).
12) J.J. Fox, *Pure. Appl. Chem.*, **18**, 223 (1969).

isolated in crystalline form. The major product, isolated in 63% yield, was proved to be the desired 1-β-D-arabinofuranosyl-2-benzylamino-1,4-dihydro-4-iminopyrimidine hydrochloride (**2a**).[13] The structure of **2a** was confirmed by its proton magnetic resonance (^{1}H NMR)



1

2a : R=$CH_2C_6H_5$
b : R=$(CH_2)_2C_6H_5$
c : R=CH_2-(pyridin-4-yl)

3a : R=$CH_2C_6H_5$
b : R=$(CH_2)_2C_6H_5$

4

Chart 1

spectrum and elemental analysis. The spectrum of **2a** showed the presence of a $C_{2'}$ hydroxy group, and the signal for the $C_{2'}$H was located roughly 1.0 ppm upfield relative to that of **1**. This strongly suggests that S_N2 displacement of **1** by benzylamine took place on the C_2 of the pyrimidine ring, as expected. The minor product was obtained in 6% yield, and its ^{1}H NMR spectrum and elemental analysis showed this compound to be 1-β-D-arabinofuranosyl-2-benzylamino-1,4-dihydro-4-benzyliminopyrimidine hydrochloride (**3a**).[13] When the above reaction was carried out with an excess of benzylamine at reflux temperature the yield of **3a** increased to 27%. The formation of 2,4-bisaralkylamino derivatives is not unexpected, since cytidine is known to react with primary amines to give the corresponding N^4-substituted derivatives.[14] Very recently, it has been reported by Cook *et al.*[15] that the reaction of 2,2′-anhydro-1-(β-D-arabinofuranosyl)-5-fluorocytosine with a simple alkylamine afforded the corresponding 2,4-bisalkylamino derivatives. Similar reactions of **1** with 1 equiv. of phenethylamine and with 4-aminomethylpyridine afforded 1-β-D-arabinofuranosyl-2-(phenethylamino)-1,4-dihydro-4-iminopyrimidine hydrochloride (**2b**) and 1-β-D-arabinofuranosyl-2-(pyridine-4-methylamino)-1,4-dihydro-4-iminopyridine hydrochloride (**2c**) in yields of 49 and 25%, respectively. Treatment of **1** with an excess of phenethylamine gave 1-β-D-arabinofuranosyl-2-(phenethylamino)-1,4-dihydro-4-(phenethylimino)pyrimidine hydrochloride (**3b**) in 31% yield.

Compounds **2a**—**c** are stable in crystalline form. However, chromatographic and ultraviolet examination indicated that these compounds decomposed gradually in aqueous solution at room temperature and resulted in complete hydrolysis to araC after 1 month.

13) The true tautomeric form of the neutral species of **2** and **3** is unknown. In this paper these compounds have been tentatively named as a *para*-quinoid rather than the tautomeric *ortho*-quinoid structure. See also ref. 11.
14) T. Kanai, M. Ichino, A. Hoshi, F. Kanzawa, and K. Kuretani, *J. Med. Chem.*, **17**, 1076 (1974).
15) A.F. Cook, M.J. Holman, and P.W. Trown, Abstr. Papers, *Am. Chem. Soc., Joint Conf.* **1977**, 18.

Antiviral activity against *vaccinia* virus and *Newcastle disease* virus (NDV) of compounds **2a**—**c** and **3a**,**b** was examined in monolayer cultures of primary chick embryo cells (CEC). Under the test conditions compounds **2a**—**c** showed significant antiviral activity at wide range of concentrations. Compounds **2a** and **2b** inhibited completely cytopathic effect of *vaccinia* virus at concentrations up to 3.12 μg/ml. Compound **2c** showed relatively weak activity, producing an inhibition at concentrations up to 12.5 μg/ml. In comparative experiments, araC was effective at concentrations up to 0.78 μg/ml. Chromatographic and ultraviolet examination indicated that under the conditions of the antiviral assay hydrolysis of the 2-benzylamino and 2-phenethylamino derivatives (**2a**, **2b**) to araC is rapid and roughly 80% of **2a** and **2b** were converted to araC. 2-(Pyridine-4-methylamino) derivative (**2c**) is somewhat less labile and roughly 30% of **2c** was hydrolyzed to araC. Accordingly, the inhibition of the preparation of *vaccinia* virus shown in the present experiment probably due to araC formed rather than **2a**—**c**. The decreased antiviral activity of **2c**, which is hydrolyzed to araC to the less extent than **2a** and **2b**, supports this assumption.

As anticipated, all compounds prepared in this study were inactive against NDV.

All compounds reported herein were also tested for antitumor activities against *L*-1210 *leukemia* and *Ehrlich carcinoma* in mice. However, none of the compounds have shown the activities at 200 mg/kg/day (5 days) *i.p.*

Experimental

Melting points were uncorrected. ^{1}H NMR spectra were obtained at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in Me_2SO-d_6. Spectra are recorded in parts per million downfield of an internal standard of tetramethylsilane.

Ultraviolet (UV) spectra were measured on a Hitachi EPS-3T spectrometer. Paper electrophoresis (PE) was carried out on Toyo No. 51A paper using acetate buffer (pH 3.7—3.8).[9] Spots were detected by UV examination.

Reactions of 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrochloride (1) with Benzylamine——A. Using a 1 : 1 Ratio: To a solution of **1** (2.0 g, 7.65 mmol) in methanol (200 ml) was added benzylamine (0.82 g, 7.65 mmol). The solution was allowed to stand at room temperature for 5 days and then the solvent was evaporated *in vacuo*. The residue was washed with ether and crystallized from water (15 ml), giving 0.2 g (6%) of 1-β-D-arabinofuranosyl-2-benzylamino-1,4-dihydro-4-benzyliminopyrimidine hydrochloride (**3a**): mp 181—182° (dec.); $\lambda_{max}^{H_2O}$ (pH=1) nm (ε): 227 (sh, 21700), 247 (sh, 19200). NMR δ 3.6—4.2 (4H, m, C_3' H, C_4' H, and C_5' H_2), 4.3—4.8 (5H, m, C_2' H, and 2×$ArCH_2$), 5.2—6.1 (3H, br., 3×OH), 6.22 (1H, d, J=5 Hz, C_1' H), 6.34 (1H, d, J=8 Hz, C_5H), 7.0—7.6 (10H, m, ArH), 8.17 (1H, d, J=8 Hz, C_6H), 9.5—9.9 (1H, br.s, NH). *Anal.* Calcd. for $C_{23}H_{26}N_4O_4 \cdot HCl \cdot 0.5H_2O$: C, 59.03; H, 5.82; N, 11.97; Cl, 7.58. Found: C, 59.35; H, 5.89; N, 11.93; Cl, 7.58.

The mother liquors from the crystallization of **3a** were evaporated and the residue was crystallized from ethanol to give 1.9 g (63%) of 1-β-D-arabinofuranosyl-2-benzylamino-1,4-dihydro-4-iminopyrimidine hydrochloride (**2a**) as a hemiethanolate with mp 131—132°. Recrystallization from water gave **2a** as the hemihydrate with mp 135—137° (dec.): $\lambda_{max}^{H_2O}$ (pH=1) nm (ε): 224 (22500), 270 (sh, 5300). NMR δ 3.6—4.2 (4H, m, C_3' H, C_4' H, and C_5' H_2), 4.3—4.8 (3H, m, C_2' H and $ArCH_2$), 5.41 (1H, t, C_5' OH), 5.69 (1H, d, C_2' OH), 5.95 (1H, d, C_3' OH), 6.22 (1H, d, J=5.5 Hz, C_1' H), 6.27 (1H, d, J=7.5 Hz, C_5H), 7.1—7.6 (5H, m, ArH), 8.0—8.4 (2H, m, C_6H and NH), 8.4—8.7 (1H, br.s, NH), 8.8—9.2 (1H, br.s, NH). *Anal.* Calcd. for $C_{16}H_{20}N_4O_4 \cdot HCl \cdot 0.5H_2O$: C, 50.86; H, 5.87; N, 14.83; Cl, 9.58. Found: C, 50.84; H, 5.98; N, 14.62; Cl, 9.42.

B. Using a 1 : 6 Ratio. A solution of **1** (0.5 g, 1.9 mmol) and benzylamine (1.2 g, 11.5 mmol) in methanol (50 ml) was heated under reflux for 7 hr. The solvent was evaporated *in vacuo* and the residue was washed with ether. Crystallization of the residue from water followed by recrystallization from the same solvent gave 0.24 g (27%) of **3a** with mp 181—182° (dec.), identical with that above.

1-β-D-Arabinofuranosyl-2-(phenethylamino)-1,4-dihydro-4-iminopyrimidine Hydrochloride (2b)——was prepared as described for **2a**. The product was recrystallized from 30% ethanol: yield, 49%; mp 104—107° (dec.); $\lambda_{max}^{H_2O}$ (pH=1) nm (ε): 220 (23600), 275 (sh, 6100). *Anal.* Calcd. for $C_{17}H_{22}N_4O_4 \cdot HCl \cdot 2/3H_2O$: C, 51.71; H, 6.21; N, 14.19; Cl, 8.98. Found: C, 51.78; H, 6.40; N, 14.59; Cl, 9.21.

1-β-D-Arabinofuranosyl-2-(phenethylamino)-1,4-dihydro-4-(phenethylimino)pyrimidine Hydrochloride (3b)——A solution of **1** (1.0 g, 3.8 mmol) and phenethylamine (2.8 g, 22.9 mmol) in methanol (100 ml) was heated at 40—45° for 4 days. The reaction mixture was worked up as in B above. Recrystallization from ethanol gave 0.58 g (31%) of **3b** with mp 187—188° (dec.); $\lambda_{max}^{H_2O}$ (pH=1) nm (ε): 225 (sh, 20900) 250 (sh, 16600). *Anal.* Calcd. for $C_{25}H_{30}N_4O_4 \cdot HCl$: C, 61.66; H, 6.42; N, 11.50; Cl, 7.28. Found: C, 61.55; H, 6.50; N, 11.79; Cl, 7.50.

1-β-D-Arabinofuranosyl-2-(pyridine-4-methylamino)-1,4-dihydro-4-iminopyrimidine Hydrochloride (2a) ——To a solution of **1** (3.0 g, 11.5 mmol) in methanol (300 ml) was added 4-aminomethylpyridine (1.2 g, 11.5 mmol). The solution was allowed to stand at room temperature for 6 days. The solvent was evaporated *in vacuo* and the residue was washed with ether. Examination of the product by PE (2000 V, 1.5 hr) showed that it consisted of a mixture of **2c** and **1** in a ratio of 1: 1. The resulting residue was extracted with hot ethanol (200 ml) and the extract was filtered. The filtrate was evaporated to dryness *in vacuo* and recrystallization of the residue from ethanol gave 1.2 g (25%) of **2c** with mp 133—135° (dec.): $\lambda_{max}^{H_2O}$ (pH=2) nm (ε): 218 (23 800) 240 (sh, 18200), 273 (sh, 9000). *Anal.* Calcd. for $C_{15}H_{19}N_5O_4 \cdot HCl \cdot 2/3C_2H_5OH$: C, 47.90; H, 6.15; N, 17.10; Cl, 8.66. Found: C, 47.94; H, 5.85; N, 17.23; Cl, 8.74.

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