Experimental Section

Melting points were obtained on a Thomas-Hoover Unimelt using open capillary tubes and are uncorrected. Evaporations were performed at diminished pressure on a rotary evaporator. Petroleum ether refers to that fraction boiling from 30–60°. Analyses indicated only by the symbols of the elements were within ±0.3% of the theoretical values. The ir spectra were obtained on a Perkin-Elmer Model 137 recording spectrophotometer and the uv spectra by A. Kalowsky using a Cary Model 11 spectrophotometer. A Varian Associates A-60A instrument was used by Dr. A. W. Douglas and staff for recording nmr spectra. The authors are grateful to Mr. R. N. Boos and associates for microanalyses and to Mr. T. E. Lanza for helpful advice.

DL-*N*-Acetyl-3-chloroalanine Methyl Ester (DL-II). The method of Rothstein[9] was modified so as to provide a higher yield. A suspension of 257 g (1.48 mol) of DL-3-chloroalanine methyl ester hydrochloride (DL-V, Cyclo Chemical) in 1.8 l. of anhydrous benzene was mixed with 234 g (3.0 mol) of acetyl chloride and heated under reflux for 2.5 hr. A small amount of solid was removed by filtration and into the stirred filtrate was poured 4.5 l. of petroleum ether over a 30-min period. Overnight cooling gave a solid which was collected, washed with 500 ml of petroleum ether, and dissolved in 6.5 l. of ether. The solution was evaporated to a volume of 400 ml and diluted with 1.5 l. of petroleum ether. Filtration, followed by washing with petroleum ether, gave 224 g (88%) of pure product, mp 77.5–79.5° (lit.[9] mp 79–80°).

DL-2-Amino-3-(hydroxyamino)propionic Acid (DL-III). Under anhydrous conditions 77.9 g (0.43 mol) of DL-II was dissolved in 710 ml of absolute ethanol and to the solution was added over 1.2 hr a solution of sodium *anti*-benzaldoximate prepared by adding 105 g (0.87 mol) of *anti*-benzaldoxime[7] to a solution of 19.9 g (0.87 g-atom) of sodium metal in 710 ml of absolute ethanol. The reaction mixture was neutralized with 30 ml of saturated ethanolic HCl, cooled to 4°, filtered, and evaporated to give 187 g of an oil. This was heated under reflux with 850 ml of concentrated HCl for 30 min. Most of the HCl gas was next removed by evaporation in a bath at 45°, causing a solid to separate which was removed by filtration. The filtrate was washed with three 850-ml portions of ether and concentrated to dryness. The residue was evaporated with three small portions (*ca.* 50 ml) of H_2O and finally taken up in 180 ml of H_2O. This solution was washed with six 400-ml portions of ether, separated, and cooled to 3°, and the pH was adjusted to 6.5 by the addition of concentrated aqueous ammonia. The solution was treated with Darco KB, filtered, and cooled to 0°, and to it was added 2.23 l. of absolute ethanol with stirring over 1.2 hr. The solid that separated was collected and washed with ethanol and ether to yield 34.4 g (66%) of the product, mp 180–181° dec (lit.[4] mp 163–165° dec). The compound reduced Fehling's solution and its ir spectrum (Nujol) was superimposable upon that of an authentic sample.‡

DL-2-Amino-3-(hydroxynitrosamino)propionic Acid (DL-I). A solution of 91.1 g (0.76 mol) of DL-III in 765 ml of 1 *N* HCl was treated (17 min) at 0° with 52.3 g (0.76 mol) of $NaNO_2$. A solid began to separate after about half of the nitrite had been added. Addition of 1.1 l. of cold absolute ethanol completed the precipitation of the product, which was filtered and washed with ethanol and ether to give 97.5 g (86%) of slightly yellow powder (air dried), mp 189° dec. Purification was performed by dissolving 75.0 g (0.504 equiv) in 475 ml of 1 *N* NaOH (0.475 equiv) to give a faintly turbid solution (pH 6.2). This was treated with Darco KB, filtered, and cooled in an ice bath, and the pH was adjusted to 5.4 by the dropwise addition of 28 ml of acetic acid. The precipitate was collected and washed successively with 100-ml portions of H_2O, ethanol, and ether. Drying over KOH pellets at diminished pressure afforded 60.3 g (79%) of ash-free, white powder, mp 193° dec (lit.[4] mp 185°). Uv, ir, and nmr spectra of the product were in accord with expectation.

DL-*N*-(2-Acetamido-2-carboxyethyl)-α-phenylnitrone Ethyl Ester (DL-IV). A solution of 0.01 mol of sodium *anti*-benzaldoximate in 40 ml of absolute ethanol, prepared as prescribed above, was combined with 1.8 g (0.01 mol) of L-*N*-acetyl-3-chloroalanine methyl ester (L-II), $[\alpha]^{25}D$ −16.4° (*c* 0.93, H_2O), prepared as described in the literature,[10] and heated at 65–70° for 45 min. Concentration to dryness gave 3.0 g of an oil which solidified when treated with 15 ml of ether. The optically inactive solid (1.0 g) was dissolved in absolute ethanol (3 ml), evaporated to dryness, and triturated with ether to yield 200 mg (7%) of product: mp 128–130°; $\lambda_{max}^{ethanol}$ [nm ($\epsilon \times 10^{-3}$)] 296 (20.0), 229 (7.3), and 224 (8.3); ir and nmr spectra were as expected. *Anal.* ($C_{14}H_{18}N_2O_4$) C, H, N.

DL-*N*-Carbethoxy-3-chloroalanine Methyl Ester (DL-VI). Under anhydrous conditions 5.2 g (0.03 mol) of DL-V was mixed with 7.5 g (0.09 mol) of $NaHCO_3$ and 6.5 g (0.06 mol) of ethyl chloroformate. The mixture was stirred for 4.5 hr at room temperature and filtered and the filtrate was concentrated to give 5.3 g (84%) of product, mp 50–56°. The analytical sample was crystallized from petroleum ether–ether: mp 64–66°; ir and nmr spectra were as expected; tlc, single spot, R_f 0.9, silica gel G, ethyl acetate, visualized with I_2 vapor. *Anal.* ($C_7H_{12}ClNO_4$) C, H, Cl, N.

L-*N*-Carbethoxy-3-chloroalanine Methyl Ester (L-VI). This stereoisomer was prepared in the manner described above for the DL form, starting with L-V (Cyclo Chemical). The product was obtained as crystals from petroleum ether–ether in 75% yield; mp 57–59°; $[\alpha]^{25}D$ −29° (*c* 2, dimethylformamide). Attempts to react L-VI with sodium *anti*-benzaldoximate under various conditions resulted in complete loss of optical activity in the reaction products, even under mild conditions.

‡We are grateful to Dr. G. C. Lancini of Lepetit S.p.A. for providing an authentic sample of this compound.

References

(1) Y. K. S. Murthy, J. E. Thiemann, C. Coronelli, and P. Sensi, *Nature (London)*, 211, 1198 (1966).
(2) C. Coronelli, C. R. Pasqualucci, G. Tamoni, and G. G. Gallo, *Farmaco, Ed. Sci.*, 21, 269 (1966).
(3) D. Fumarola, *Pharmacology*, 3, 215 (1970).
(4) G. C. Lancini, E. Lazzari, and A. Diena, *Farmaco, Ed. Sci.*, 24, 169 (1969).
(5) E. Buehler and G. B. Brown, *J. Org. Chem.*, 32, 265 (1967).
(6) E. Bellasio, F. Parravicini, A. Vigevani, and E. Testa, *Gazz. Chim. Ital.*, 98, 1014 (1968).
(7) E. F. Schoenewaldt, R. B. Kinnel, and P. Davis, *J. Org. Chem.*, 33, 4270 (1968).
(8) B. Liberek and Z. Palacz, *Rocz. Chem.*, 45, 1173 (1971).
(9) E. Rothstein, *J. Chem. Soc.*, 1968 (1949).
(10) L. Benoiton, *Can. J. Chem.*, 46, 1549 (1968).

The N^6-(Dimethylamino)methylene Derivative of 9-β-D-Arabinofuranosyladenine as an Antiviral Agent

Stephen Hanessian*

Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan 48106. *Received July 13, 1972*

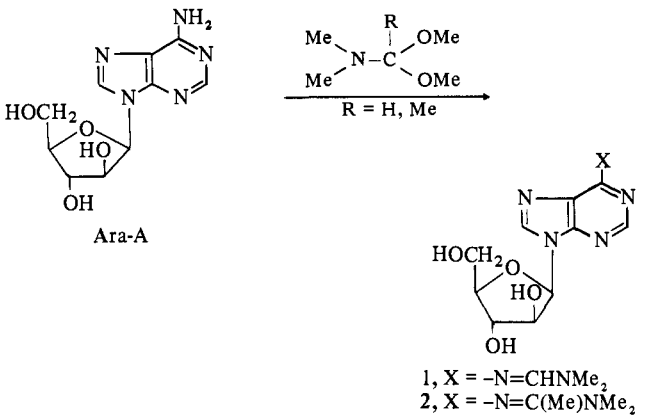
Recent reports[1,2] from these laboratories presented information on the antiviral compound, 9-β-D-arabinofuranosyladenine (Ara-A), which shows significant therapeutic activity against herpes simplex and vaccinia viruses in cell cultures and experimental animals. In addition, Ara-A exhibits an inhibitory effect on a variety of other DNA viruses in cell culture but little if any on RNA viruses.[1,3]

The antiviral activity of nucleosides may be limited or altered due to enzymatic or chemical transformation *in vivo*. The distribution and metabolic fate of such compound may thus be affected by such processes. In this regard it is noteworthy that unlike its 2 epimer adenosine, Ara-A is relatively insoluble in water (≃1 mg/ml). It seemed desirable to prepare derivatives of Ara-A that would combine the properties of greater solubility in water and the regeneration of the parent nucleoside in a sustained fashion.

We wish to report in this note on the preparation of a water-soluble derivative of Ara-A and to comment on its antiviral activity and related properties. The new derivative is the N^6-(dimethylamino)methylene analog **1** (Scheme I) which was prepared in high yield by the reaction of Ara-A with *N,N*-dimethylformamide dimethyl acetal[4] in DMF.

*Address correspondence to the Department of Chemistry, University of Montreal, Montreal, Quebec, Canada.

Scheme 1



The corresponding N^6-(dimethylamino)ethylidene derivative **2** was also prepared in what appears to be a general reaction of the N^6-amino group in Ara-A with such amide acetals.[5]

As reported by Žemlička,[6] adenosine and *N,N*-dimethylformamide dimethyl acetal afford N^6-(dimethylamino)methylene[2′,3′-*O*-(dimethylamino)methylene]adenosine, in which the *O*-acetal linkage was found to be extremely labile. The corresponding 2,3-*O*-(dimethylamino)ethylidene and α-(dimethylamino)benzylidene acetals in methyl β-D-ribofuranoside have been shown to be versatile O-protecting groups for vicinal *cis*-diols.[7] In the case of Ara-A, the reaction with amide acetals is confined to the N^6-amino group due to the unfavorable disposition of the hydroxyl groups in the carbohydrate moiety.

The crystalline N^6-(dimethylamino)methylene derivative **1**, of Ara-A, was approximately 40 times more soluble in water than the parent compound. In aqueous solutions, **1** was slowly hydrolyzed into Ara-A, but this could be prolonged in buffered solutions.

Biological Data. Table I lists the results of testing Ara-A and its derivatives **1** and **2** against herpes simplex and vaccinia viruses in H.Ep no. 2 cell cultures by the plaque reduction technique.[8] Compound **1** and Ara-A were not cytotoxic at levels of 31.2 μg/ml. Compound **2** was noncytotoxic but inactive at the same level; at 100 μg/ml, it was slightly cytotoxic but the level of activity was comparatively low (55 and 48% plaque reduction against herpes simplex and vaccinia). Acute toxicity studies in mice indicate that compound **1**, like the parent Ara-A, is relatively nontoxic. It compared favorably with Ara-A in its behavior against experimental herpes simplex infection in mice as shown in Table II. Although Ara-A and compound **1** are equally nontoxic, they are not necessarily of equal chemotherapeutic activity when given in the doses indicated in Table II. In a separate test involving Ara-A and compound **2**, no survivors were noted for the synthetic analog. This is attributed to its ineffectiveness rather than its toxicity. Whereas Ara-A has a pronounced and preferential inhibitory effect on DNA synthesis in cell cultures, compound **1** was inert, which possibly implies that under the conditions of the test, a conversion of **1** into Ara-A could not be effected (Table III). Although compound **1** could conceivably possess antiviral properties *per se*, it is also possible that prior hydrolysis to Ara-A is responsible for its behavior. Thus, because of the ready water solubility of **1**, and its conversion into the parent nucleoside, it can be used in solution to supply the relatively insoluble Ara-A in a sustained fashion.

Table I. *In Vitro* Antiviral Activity and Mouse Toxocity Data

Compd	Acute ip mouse toxicity, mg/kg	Antiviral activity[a] Herpes simplex μg/ml	Herpes simplex % PR[b]	Vaccinia μg/ml	Vaccinia % PR[b]
Ara-A	$LD_0 \geq 4000$ $LD_{50} = 4460 \pm 90$	31.2	91	31.2	92
1	$LD_{50} > 3200$	31.2	95	31.2	87
2	$LD_{50} > 200 < 250$	100.0	55	100.0	48

[a]Measured by the plaque reduction technique[8] with H.Ep no. 2 cells making up the monolayer. [b]Plaque reduction.

Table II. Herpes Simplex Chemotherapy *in Vivo*[a]

Compd	mg/kg × 4[b]	Survivors[c] total	M.D.D.[d]
Ara-A	400	4/10 (9/20)	15 (7.9)
	200	3/10 (7/20)	14 (7.6)
1	400	3/10 (2/10)	12 (4.1)
	200	4/10 (3/10)	13 (6.7)
Control	0	0/10	(6.2)

[a]Herpes simplex virus experimental intracerebral infection in 16–18-g Carworth mice. [b]Ip route. [c]Treatment was started 24 hr previrus infection and continued BID (four times). Values between parentheses are for treatment started 24 hr postvirus infection. [d]Mean day of death.

Table III. Inhibition of DNA, RNA, and Protein Synthesis[a]

Compd	Molar concn for 50% inhibition of synthesis DNA	RNA	Protein
Ara-A	3×10^{-5}	NI[b]	NI
1	NI at 1×10^{-3}	NI	NI

[a]Inhibition of synthesis in H.Ep no. 2 cells was measured by incubation of labeled thymidine, uridine, and leucine for 1 hr, separation of the nucleoprotein fractions, and determining the specific count. [b]Determined by the amount of isotope taken up by H.Ep no. 2 cells in the presence and absence of drugs.

Experimental Section

Melting points are uncorrected. Ultraviolet spectra were recorded on a Cary-14 recording spectrophotometer. Thin-layer chromatography was performed with plates coated with silica gel GF_{254} and the spots were detected with a sulfuric acid spray and by visualization under a uv lamp.

N^6-(Dimethylamino)methylene(9-β-D-arabinofuranosyl)adenine (**1**). To a stirred suspension of Ara-A (1.5 g) in 30 ml of anhydrous DMF was added 15 ml of DMF dimethyl acetal. After stirring for 1–2 hr the solution became homogeneous and after stirring a total of 18 hr, the solution was diluted with an excess of Et_2O. The crystalline precipitate was filtered, washed with Et_2O, and dried to give the product **1** (1.7 g, 90%) which was chromatographically homogenous (tlc, $CHCl_3$–MeOH, 10:3). Recrystallization from a mixture of MeOH, CH_2Cl_2, and Et_2O gave an analytical sample: mp 214–215° dec; λ max 310, 232 mμ (EtOH). *Anal.* ($C_{13}H_{18}N_6O_4$) C, H, N.

The product **1** is soluble in water to the extent of 40 mg/ml. It is soluble in aqueous neutral buffers and in solvents such as DMF, DMA, DMSO, various alcohols, etc. Aqueous or buffered solutions of **1** (40 mg/ml) began to deposit Ara-A after standing at room temperature during 3–4 days. The rate of hydrolysis was slowest in the region of pH 8.5. The crystalline product was stable when stored in tight bottles at low temperature and hydrolysis to Ara-A was minimal after several months.

N^6-(Dimethylamino)ethylidene(9-β-D-arabinofuranosyl)adenine (**2**). To a stirred suspension of Ara-A (3.5 g) in 30 ml of DMAC was added 30 ml of DMAC dimethyl acetal and the mixture was stirred in the dark for 16 hr. The pale yellow homogeneous solution was diluted with a large volume of Et_2O and the resulting precipitate was filtered and dried. The solid was dissolved in CH_2Cl_2 containing a small amount of MeOH, and Et_2O was added to precipitate the product as an amorphous colorless solid that showed essentially one spot on chromatograms ($CHCl_3$–MeOH, 10:3): yield 4 g (91%); λ max 304 mμ (EtOH). The solubility of the product was at least 200 mg/ml in water or aqueous buffers. Such solutions began to deposit Ara-A after 7–10 days.

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References

(1) F. A. Miller, G. J. Dixon, J. Ehrlich, B. J. Sloan, and I. W. McLean, Jr., *Antimicrob. Ag. Chemother.*, 136 (1968).
(2) B. J. Sloan, F. A. Miller, I. W. McLean, Jr., and H. E. Machamer, *ibid.*, 161 (1968).
(3) F. M. Schabel, *Chemotherapy*, 13, 321 (1968).
(4) H. Meerwein, P. Borner, O. Fuchs, J. J. Sasse, H. Schrodt, and J. Spille, *Chem. Ber.*, 89, 2060 (1956); H. Meerwein, W. Florian, G. Schon, and G. Stopp, *Justus Liebigs Ann. Chem.*, **641**, 1 (1961).
(5) S. Hanessian, German Patent 1,811,267; *Chem. Abstr.*, 71, 124858w (1969).
(6) J. Žemlička, *Collect. Czech. Chem. Commun.*, **28**, 1060 (1963).
(7) S. Hanessian and E. Moralioglu, *Can. J. Chem.*, **50**, 233 (1972); *Tetrahedron Lett.*, 813 (1971).
(8) R. Dulbecco, *Proc. Nat. Acad. Sci. U. S.*, 38, 747 (1952); G. S. Hsiung and J. L. Melnick, *Virology*, **1**, 533 (1955).

Synthesis of Potential Anticancer Agents. Preparation of Some 1-Deazapurines and Pyrimidines†

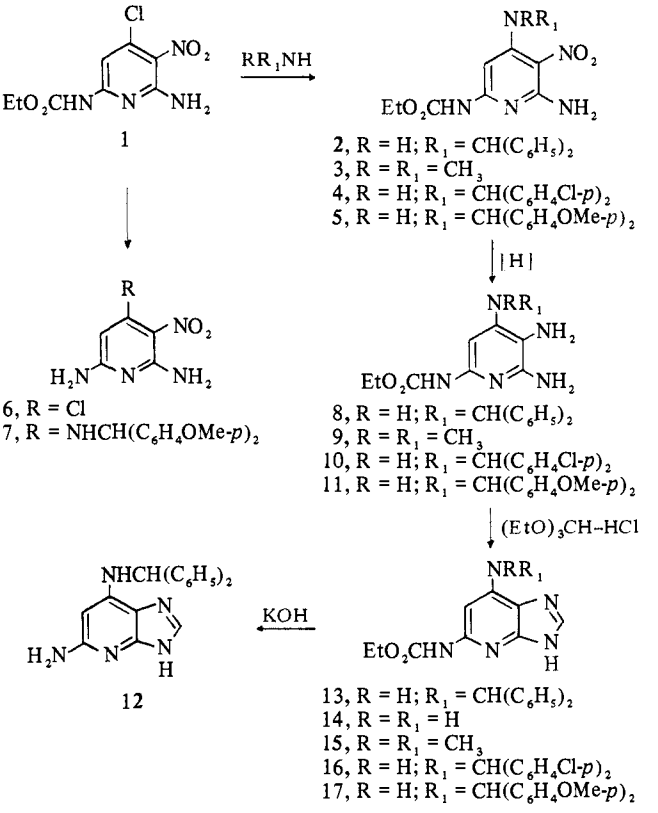
Carroll Temple, Jr.,* Buford H. Smith, Robert D. Elliott, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205. *Received August 14, 1972*

During an investigation of the preparation of 1-deazapurines (imidazo[4,5-*b*]pyridine) from pyridine precursors, several intermediates were found to possess activity in the leukemia L1210 test system. We report the preparation of these compounds and the search for activity in some pyrimidine and purine analogs.

Reaction of **1** with diphenylmethylamine gave **2**.[1] Hydrogenation of **2** in the presence of Raney Ni gave the 5,6-diaminopyridine **8**, which was cyclized with the triethyl orthoformate-concentrated HCl reagent[2] to give **13**. That cyclization of **8** occurred between the primary amino groups rather than between the diphenylmethylamino and the 5-amino groups was established by the pmr spectrum of the product, which showed spin-spin coupling between the NH and CH of the $(C_6H_5)_2CHNH$ moiety [δ_{TMS} 9.04 d, 6.02 d (J = 8.0 Hz)]. After the addition of D_2O the NH doublet collapsed and the CH doublet appeared as a singlet. Analogous ring closures have been observed in the pyrimidine series.[3] Removal of the diphenylmethyl group of **13** with HBr–HOAc gave **14**, and hydrolysis of the urethane group of **13** with KOH–EtOH gave **12**. Reaction of **1** with dimethylamine and bis(*p*-chlorophenyl)methylamine, respectively, gave **3** and **4**, which were converted to **15** and **16** *via* **9** and **10**. Treatment of **1** with bis(*p*-methoxyphenyl)methylamine gave a mixture of **5**, **6**, and **7**, which was separated to give each pure compound. Hydrogenation of **5** gave **11**, but the condensation of the latter with triethyl orthoformate to give **17** resulted in a mixture from which no pure compounds were isolated. Apparently the increased nucleophilicity of the NH of the 4-[bis(*p*-methoxyphenyl)methylamino] group directed some cyclization to this nitrogen atom. The deamino derivatives **20** and **21** of **13** and **8** were prepared by similar procedures from the known pyridine intermediate **18**[4] *via* **19** (Scheme I).

Scheme I



Reaction of **22**[5] with diphenylmethylamine and its bis(*p*-chlorophenyl) and bis(*p*-methoxyphenyl) derivatives, respectively, gave **23**, **24**, and **25**. Reduction of the nitro group of **23** with Raney Ni and hydrogen gave **29**. The hydrogenation of **24** and **25** was attempted, but the isolation of a pure sample of the corresponding 5-aminopyrimidines was unsuccessful. The product from **25** was identified as 2,4,5,6-tetraaminopyrimidine, resulting from reductive removal of the bis(*p*-methoxyphenyl)methyl group (Scheme II).

Amination of **26** with diphenylmethylamine and its bis(*p*-chlorophenyl) derivative, respectively, gave **27** and **28**. The properties of the new compounds are summarized in Table I and typical procedures are given in the Experimental Section.

Compounds were tested against L1210 leukemia cells implanted intraperitoneally in mice[6] on single dose and chronic schedules; the test results indicated that single-dose treatment was superior in all cases. The diaminopyridine **8** showed a 22% ILS at 200 mg/kg on the single-dose schedule but no activity on the chronic schedule.‡ In the latter test toxicity was observed at 400 mg/kg/day, qd 1–15. The corresponding bis(*p*-chlorophenyl) and bis(*p*-methoxyphenyl) derivatives **10** and **11**, the deaminopyridine **21**, and the pyridine analog **29** appeared to be inactive. The most active compound, **13**, gave a 59% ILS at a dose of 40 mg/kg on the single-dose schedule (toxic 200 mg/kg) and a 24% ILS at a dose of 20 mg/kg/day on the chronic schedule (toxic 45 mg/kg/day, qd 1–9).§ The corresponding dimethylamino and

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‡Against Walker carcinosarcoma 256 (im) in the rat at 200 mg/kg (qd 3–6); tumor weight of treated animals was 9% of controls.

§For comparison, 6-mercaptopurine at 15 mg/kg, qd 1–15, gave a 53% ILS.[7]