Notes

Synthesis of 1-β-L-Arabinofuranosylcytosine, the Enantiomer of Cytosine Arabinoside[1]

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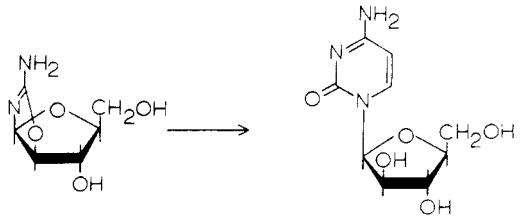
Wu and Chargaff,[2] in a study of the enantiomers of natural nucleosides, have determined that L-uridine is an acceptor for phosphate transfer from carrot phosphotransferase as well as nucleoside phosphotransferase from human prostate. In a similar study using L-adenosine, Shimizu and coworkers[3] have determined that L-adenosine was 50% deaminated in the time that the D enantiomer was completely converted to D-inosine. Snake venom 5′-nucleotidase did not accept L-adenylic acid as a substrate. It is apparent from these examples that enzymes differ widely in their ability to accept L nucleosides as substrates. It was therefore of interest to prepare the enantiomer of the anticancer agent, cytosine arabinoside, since the D enantiomer is known to undergo a rapid enzymatic deamination to form an inactive metabolite, 1-β-D-arabinofuranosyluracil.[4]

An extension of the procedure of Sanchez and Orgel[5] has provided a novel approach to the synthesis of the β-L-nucleoside. Treatment of L-arabinose with cyanamide in MeOH containing concd NH_4OH gave 2-amino-β-L-arabinofurano[1′,2′-4,5]-2-oxazoline (**1**) in good yield. Ring closure of **1** with cyanoacetylene furnished a cyclonucleoside intermediate, which was hydrolyzed, by NH_4OH without isolation, to 1-β-L-arabinofuranosyl cytosine hydrochloride (**2**).

SCHEME I



1 **2**

Anticancer Evaluation.—Results received to date indicate that **2** possesses no significant activity[6] against lymphoid leukemia L1210 or the Ridgway osteogenic sarcoma.

(1) Synonyms for cytosine arabinoside are: Ara-C, cytarabine, and 1-β-D-arabinofuranosylcytosine.

(2) A. F. Wu and E. Chargaff, *Proc. Nat. Acad. Sci. U. S.*, **63**, 1222 (1969).

(3) B. Shimizu, M. Asai, H. Hieda, M. Miyaki, and H. Okazaki, *Chem. Pharm. Bull.*, **13**, 616 (1965).

(4) R. P. Panzica, R. K. Robins, and L. B. Townsend, *J. Med. Chem.*, **14**, 259 (1971), and ref cited therein.

(5) R. A. Sanchez and L. E. Orgel, *J. Mol. Biol.*, **47**, 531 (1970); this procedure was employed recently by A. Holy, *Tetrahedron Lett.*, 189 (1971).

(6) The authors with to thank Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, N.C.I., for the lymphoid leukemia L-1210 evaluation and Dr. C. Chester Stock of the Sloan-Kettering Institute for Cancer Research for the Ridgway osteogenic sarcoma evaluation.

Enzymatic Investigation.—1-β-L-Arabinofuranosylcytosine·HCl did not function as a substrate in an *Escherichia coli* cytidine aminohydrolase system.[7]

Experimental Section[8]

2-Amino-β-L-arabinofurano[1′,2′-4,5]-2-oxazoline (1).—To a suspension of L-arabinose (30.0 g, 0.2 mole) in MeOH (100 ml) was added cyanamide (16.8 g, 0.4 mole) and concd NH_4OH (10 ml). The stoppered flask has stirred at ambient temp for 24 hr and cooled to 5°, and the solid was filtered and washed with cold *i*-PrOH (24.3 g, 70%). The anal. sample was recrystd from aq MeOH: mp 175° (dec with bubbling), $[\alpha]^{25}D$ + 16.1° (*c* 1.0, H_2O); no uv spectrum above 220 nm. *Anal.* ($C_6H_{10}N_2O_4$) C, H, N.

1-β-L-Arabinofuranosylcytosine (2).—A suspension of **1** (6.96 g, 0.04 mole) in dimethylacetamide (20 ml) was cooled in an ice bath and cyanoacetylene[9] (2.5 ml, 0.04 mole) was added by syringe through a serum cap to the partially evacuated flask. The reaction mixt was allowed to warm to room temp and after 40 min was poured into 1 *N* NH_4OH (100 ml). The soln was heat at 70° for 15 min and the dark mixt was then evapd to dryness *in vacuo*. Two 25-ml portions of MeOH were successively added and evapd *in vacuo*. Addition of 3% dry HCl in MeOH and vol reduction caused crystn. Filtration furnished a crude solid which was recrystd with charcoal from MeOH–EtOAc (7.26 g, 68%). The anal. sample was recrystd from aq *i*-PrOH: mp 197° dec, $[\alpha]^{25}D$ −127.7° (*c* 1.0, H_2O); $\lambda_{max}^{pH\ 1}$ 280 nm (ε 14,000), $\lambda_{max}^{pH\ 7}$ 272 nm (10,700). *Anal.* ($C_9H_{13}N_3O_5 \cdot HCl$) C, H, N.

(7) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960); the authors wish to thank Mr. Randy J. Bauer for the gift of purified *E. coli* cytidine aminohydrolase.

(8) Satisfactory analytical data (C, H, N within ±0.4% of theoretical values) were obtained from MHW Laboratories, Garden City, Mich. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncor. The uv spectra were recorded on a Cary 15 spectrophotometer and optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital readout polarimeter.

(9) C. H. Moureu and J. C. Bongrand, *C.R. Acad. Sci.*, **151**, 946 (1910).

Preliminary Studies on the Antitumor Activity of Some Phosphatidyl Nitrogen Mustard Derivatives

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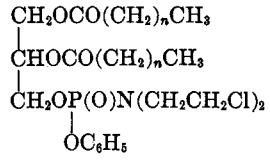
As part of our studies on exploring the use of phospholipid moieties for the potential dual function of transport and latentiation of the bis(2-chloroethyl)-amino group, we synthesized a number of phosphatidyl nitrogen mustard intermediates (**3a–3h**, Table I) following the general procedure of Batrakov, *et al.*,[1] who have synthesized **3c** but did not report on its antitumor activity.[1] Derivatives **3a–3g**, as well as nonlipid synthetic precursors **1** and **2** were tested against leukemia L-1210 in mice[2] and found to afford

(1) S. G. Batrakov, Y. G. Molotovskii, V. V. Dorogov, and L. D. Bergel'son, *Zh. Obshch. Khim.*, **37**, 426 (1967); *Chem. Abstr.*, **67**, 99595 (1969).

(2) Screening data were furnished by Vitro Laboratories, Silver Spring, Md., under contract to the Cancer Chemotherapy National Service Center; **8h** was not available in sufficient quantity for screening.

TABLE I

1,2-Diglycerides 3-[Hydrogen bis(2-chloroethyl)phosphoramidate phenyl ester]



Compd	n^a	Empirical formula	Yield, %	Mp, °C	$n^{20}D$	R_f^b	Analyses[c]
3a	16	$C_{49}H_{88}Cl_2NO_7P$	58	29–32		0.33	C, H, N
3b		$C_{49}H_{84}Cl_2NO_7P$	30		1.4807	0.30	C, H, N
3c	14	$C_{45}N_{80}Cl_2NO_7P$	53	37–39		0.33	C, H, N
3d	12	$C_{41}H_{72}Cl_2NO_7P$	60	39–40		0.30	C, H, N
3e	10	$C_{37}H_{64}Cl_2NO_7P$	59		1.4802	0.30	C, H
3f	8	$C_{33}H_{56}Cl_2NO_7P$	59		1.4803	0.27	C, H, N
3g	7	$C_{31}H_{52}Cl_2NO_7P$	54		1.4838	0.27	C, H, N
3h	6	$C_{29}H_{48}Cl_2NO_7P$	25		1.4800	0.26	C, H, N

[a] 3b = 1,2-disubstituted unsatd olein [hydrogen bis(2-chloroethyl)phosphoramidate Ph ester]. [b] Silica,[5] hexane–Et_2O (60:40). [c] See ref 5.

no significant prolongation of survival time. These results strongly indicate that lack of activity is this tumor of the "cytotoxic" N mustard moiety of these analogs is not related to significant differences in lipid solubilities or other physicochemical properties, and broadly confirm literature reports[3,4] that antineoplastic activity in phosphoramidate and phosphoroesteramidic mustards is greatly diminished or abolished by substituents such as Ph.

Experimental Section[5]

N,N-Bis(2-chloroethyl)phosphoramidic acid (2,2-dimethyl-1,3-dioxolanyl-4-yl)methyl phenyl ester (**1**) and *N,N*-bis(2-chloroethyl)phosphoramidic acid 2,3-dihydroxypropyl phenyl ester (**2**) were prepd by the method of Batrakov, *et al.*;[1] upon prepn **2** was used directly for acylation reactions. Deriv **3b** was prepd according to the procedure described for the synthesis of **3a–3h** except that acylation was carried out at 70°.[1] Spectral data (ir, pmr) were nearly identical for each **3** homolog and are reported only for **3c**. Some physical properties of **3a–3h** are included in Table I.

1,2-Diglycerides 3-[Hydrogen bis(2-chloroethyl)phosphoramidate, phenyl ester] (3a–3h).—To 1.48 g (0.004 mole) of **2** in 8 ml of pyridine (0°) was added 3.3 g (0.012 mole) of palmitoyl chloride, and the mixt was kept stirring at room temp for 48 hr. The reaction mixt then was poured into ice H_2O and the solid material was extd with three 25-ml portions of Et_2O. The exts were combined and washed successively with H_2O, ice-cold 2% H_2SO_4, and again with H_2O. The soln was dried ($MgSO_4$), filtered, and concd under reduced pressure to give 4 g of a crude yellow oil, which was placed on an Al_2O_3 (100 g) column (60 × 2 cm) and was eluted with 600 ml of $CHCl_3$. Concn of the eluate gave 2.6 g of a yellow viscous liq which showed a major (R_f 0.33) and a minor (R_f 0.6) spot on tlc (silica gel, hexane–Et_2O, 60:40). The viscous material then was chromatogd on a silica gel (50 g) column (60 × 2 cm) with hexane–Et_2O (60:40) as eluent (450 ml). Fractions which showed one spot (R_f 0.33) were combined and concd under reduced pressure to obtain 1.8 g (53%) of a colorless oil. The oil was dissolved in 15 ml of petr ether (bp 30–80°) and kept at −10° for 8 hr, during which time an amorphous, white solid sepd. Recrystn using Norite A (boiling, 5 min) gave **3c**: mp 37–39° (lit.[1] 35.5–37°); R_f 0.33, silica gel, hexane–ether (60:40); ir (Nujol) 1748 (C=O), 1262 (P=O), 1200 (POC_{arom}) 1090 (PN), 1060 (POC_{aliph}), 762 cm^{-1} (CCl); 100-MHz pmr ($CDCl_3$) τ 2.64–2.85 (m, 5), 4.73 (t, 1), 5.52–5.98 (m, 4) 6.33–6.88 (m, 8), 7.72 (t, 4), 8.75 (s, 52) 9.14 (t, 6).

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(3) R. P. Bratzel, R. B. Ross, T. H. Goodridge, W. T. Huntress, M. T. Flather, and D. E. Johnson, *Cancer Chemother. Rep.*, **26**, 281 (1963).

(4) O. M. Friedman, *ibid.*, **51**, 347 (1967).

(5) Fatty acyl chlorides were obtained from Eastman Kodak Co., Rochester, N. Y. 14650, and Sigma Chemical Co., St. Louis, Mo. 63118, and were used without further purification. Silicar tlc 7GF (Mallinckrodt) was used for tlc analyses; column chromatog purifications were made with aluminum oxide, neutral, and silica gel (E. Merck AG). The ir spectra (neat, Nujol) were detd with Beckman IR-8 and Perkin-Elmer 521 spectrophotometers, and pmr spectra with Varian A-60 and HA-100 spectrometers using $CDCl_3$ (Me_4Si) as solvent. Melting points were detd with a Thomas-Hoover capillary melting point apparatus and are uncor. Refractive indices were detd with a Bausch and Lomb Abbe-3L refractometer. Where analyses (Table I) are indicated only by the symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theor values. Analyses were performed by Micro-Analysis, Inc., Marshallton, Wilmington, Del.

New Thio Derivatives of Carcinogenic Arylamines. 5. Ring-Substituted Methylthio-4-acetamidostilbenes[1a]

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In earlier papers in this series we described the synthesis of some new thiofluorenes[1b–d] related to the metabolism of the carcinogen 2-acetamidofluorene, and of a similar new thio derivative of 4-acetamidodiphenyl.[1e]

The carcinogen *N*-hydroxy-4-acetamidostilbene [*N*-(OH)-4-AAS] yielded a more complex metabolic pic-

(1) (a) Supported in part by a grant (CA-01744) from the National Cancer Institute, National Institutes of Health, and in part by Research Career Development Award 5-K3-CA-14,991 (T.L.F.); (b) T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *J. Med. Chem.*, **10**, 936 (1967); (c) M. J. Namkung and T. L. Fletcher, *ibid.*, **11**, 1235 (1968); (d) H.-L. Pan, M. J. Namkung, and T. L. Fletcher, *ibid.*, **11**, 1236 (1968); (e) T. L. Fletcher, C.-A. Cole, H.-L. Pan, and M. J. Namkung, *ibid.*, **13**, 784 (1970).