Decomposition of *N*-(2-Chloroethyl)-*N*-nitrosoureas in Aqueous Media

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A reinvestigation of the aqueous decomposition of *N*-(2-chloroethyl)-*N*-nitrosoureas has shown that their mode of decomposition is dependent upon whether or not the solution is buffered at or near physiological pH. In distilled water, the 2-chloroethyl compounds decompose with the loss of 1 mol, or slightly less, of chloride ion per mole of nitrosourea and the formation of acetaldehyde and 3–4% of 2-chloroethanol. In buffer, the yield of 2-chloroethanol increases to 0.3–0.6 mol per mole of nitrosourea, the yield of chloride ion decreases to 0.5 mol per mole of nitrosourea, and the yield of acetaldehyde decreases to 0.1–0.4 mol per mole of nitrosourea. Evidence for the formation of the vinyl cation, a possible precursor of acetaldehyde, in these reactions is presented. In contrast to the results obtained with the *N*-(2-chloroethyl)-*N*-nitrosoureas, the decomposition of *N,N'*-bis(2-fluoroethyl)-*N*-nitrosourea in distilled water gave almost 1 mol of 2-fluoroethanol per mole of nitrosourea and only 0.04 mol of acetaldehyde per mole of nitrosourea.

Some time ago the decomposition of *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), the first nitrosourea found to have useful clinical activity,[1] in water was studied in these laboratories and found to be anomolous in that major products were 2-chloroethanamine hydrochloride and acetaldehyde rather than the expected *N,N'*-bis(2-chloroethyl)urea and 2-chloroethanol, which was detected only in small amounts by ether extraction of the spent reaction solution followed by gas chromatographic examination of the ether extract.[2] The course of the reaction was followed by nitrogen evolution (GC) and by titration of the acid released[3] and of the chloride ion formed. All these methods indicated an apparent first-order reaction, and the calculated specific rate constants were in good agreement. Acid (1 mol) and 1 mol of chloride ion per mole of BCNU were released, and the nonvolatile residue recovered was identified as 2-chloroethanamine hydrochloride (~1 mol/mol of BCNU). Thus, it is clear that in distilled water almost all of the BCNU decomposed in the abnormal manner.

The decomposition of BCNU in phosphate buffer was also followed by GC analysis of the nitrogen and carbon dioxide evolved. The principal product in the nonvolatile residue from this decomposition was identified as 2-(2-chloroethylamino)-2-oxazoline hydrochloride. About 0.2 mol of acetaldehyde per mole of BCNU was detected, but under the same conditions the recovery of a measured amount of acetaldehyde from phosphate buffer was only 57%. Ether extraction of the spent reaction solution and identification of 2-chloroethanol were not undertaken in this case. However, the course of the reaction was followed by chloride ion titration, which showed that 1.5 mol of chloride ion per mole of BCNU was formed and the reaction rate as measured by chloride ion release was not first order, although by nitrogen evolution it was. These facts appeared to be explained by the oxazoline formation referred to above.[2]

Since the recently reported results of Kramer et al.[4] on the alkylation of DNA by BCNU can only be reasonably explained by assuming reaction of DNA with either 2-chloroethanol or the 2-chloroethyl cation (more likely), we reinvestigated the decomposition of BCNU in carbon dioxide free distilled water and in phosphate buffer at pH 7.0 using a different technique. Aliquots were removed from the reaction mixtures at intervals and injected directly into the gas chromatograph through an air-cooled port. Using this technique, we have confirmed our finding that the principal volatile product of the decomposition of BCNU in water is acetaldehyde with a small amount of 2-chloroethanol (~4%) also being detected (see Table I). The nonvolatile residue is 2-chloroethanamine hydrochloride containing traces of *N,N'*-bis(2-chloroethyl)urea and the oxazoline (MS). In phosphate buffer, however, about 0.4 mol of 2-chloroethanol and 0.2 mol of acetaldehyde per mole of BCNU were formed. That this difference was not specifically dependent upon the phosphate ion, which is known to catalyze the decomposition of the nitrosoureas,[2,5] was shown by following the decomposition in acetate (pH 7.0) and Tris (pH 7.4) buffers, a procedure that gave results similar to those in phosphate buffer.

Because of the complications involved in the determination of chloride ion release from BCNU (since chloride ion can and apparently, in buffer, does come from both sides of the molecule), we turned our attention to the unambiguous case of *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), which has been found by Reed to produce both acetaldehyde and 2-chloroethanol in phosphate buffer.[6] Decomposition in distilled water–dioxane gave only a trace of 2-chloroethanol along with nitrogen, carbon dioxide, cyclohexanamine hydrochloride, and 0.75 mol of acetadehyde per mole of CCNU (the addition of dioxane was necessary to solubilize CCNU at this concentration). But again in phosphate buffer–dioxane the reaction is quantitatively different. About 0.1 mol of 2-chloroethanol and 0.3 mol of acetaldehyde per mole of CCNU are formed. Chloride ion titration, however, showed that 0.77 mol of chloride ion per mole of CCNU is formed *even in phosphate buffer*, indicating that abormal decomposition is the major mode of decomposition in this case also. The contrast between these results and those obtained with BCNU in pure phosphate buffer raised the question of the effect of dioxane on the reaction and caused us to repeat the experiment at a greater dilution in order to effect solution of CCNU in pure buffer. The concentration used is the theoretical maximum, momentary drug level[7] that could be obtained in a mouse given an LD_{10} of CCNU. Under these simulated physiological conditions, the decomposition was similar to that observed for BCNU in buffer. Roughly half the CCNU decomposed to 2-chloroethanol and half to chloride ion (and acetaldehyde). Since May, Boose, and Reed[8] have recently identified *N*-(2-chloroethyl)-*N'*-(*cis*-4-hydroxycyclohexyl)-*N*-nitrosourea as the major oxidation product of CCNU by liver microsomes, this compound was allowed to decompose in buffer at physiological concentration and gave similar results.

The decomposition of three other *N*-(2-chloroethyl)-*N*-nitrosoureas—chlorozotocin, of interest because of its low bone marrow toxicity and water solubility,[9] and two buffer-soluble derivatives of MeCCNU, *cis*- and *trans*-4-[3-(2-chloroethyl)-3-nitrosoureido]cyclohexaneacetic acid (NSC-204816 and NSC-175377), which are highly active in animal solid tumor systems[10]—has also been studied by the

Table I. Aqueous Decomposition of Selected *N*-(2-Chloroethyl)-*N*-nitrosoureas[a]

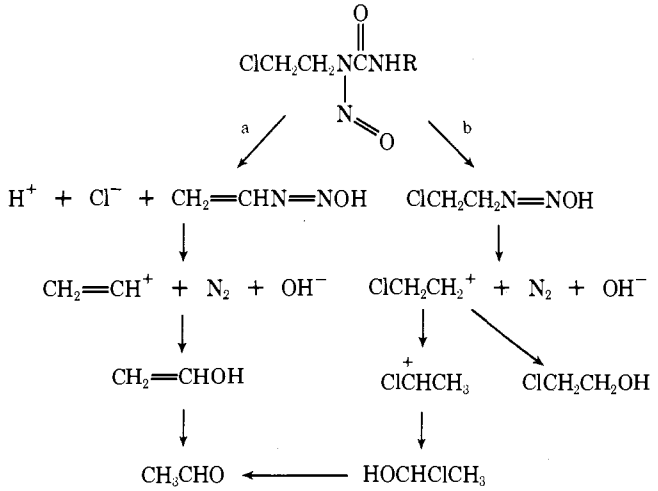
Compd	Concn, m*M*	Solvent[b,c]	Mol/mol of nitrosourea (time, hr)		
			Acetaldehyde	2-Chloroethanol	Chloride ion
BCNU	19.3	A	0.40 (21)	0.043 (21)	1.06 (24)
	19.6	B	0.21 (6)	0.44 (6)	0.48 (11)
	19.0	C	0.23 (3)	0.62 (24)	
	19.8	D	0.11 (24)	0.62 (24)	
CCNU	10.5	E	0.75 (23)	0.007 (23)	0.71 (23)
	11.9	F	0.48 (21)	0.10 (21)	0.77 (24)
	0.32	A	0.83 (10.5)	<0.03 (10.5)	0.98 (30)
	0.33	G	0.39 (7.5)	0.52 (24)	0.50 (48)
cis-4-HO-CCNU	0.35	A	0.36 (20)	0.025 (20)	0.85 (20)
	0.31	G	0.17 (20)	0.55 (20)	0.47 (20)
Chlorozotocin	11.2	A	0.32 (24)	0.041 (24)	
	5.8	B	0.1 (3.2)	0.49 (3.2)	
NSC-204816	5.8	B	0.3 (3.2)	0.35 (30)	
NSC-175377	6.8	B	0.3 (3.2)	0.32 (30)	
BFNU	0.97	A	0.044 (20)	0.95[d] (20)	

[a] Acetaldehyde, 2-chloroethanol, and 2-fluoroethanol were determined by GC; other minor products detected but not identified are probably chloroethene and 1,2-dichloroethane (cf. ref 11). Chloride ion was determined by titration. [b] A, boiled distilled H_2O; B, phosphate buffer, pH 7.0; C, acetate buffer, pH 7.0; D, Tris buffer, pH 7.4; E, 1:2 (v/v) dioxane–boiled distilled H_2O; F, 1:2 (v/v) dioxane–phosphate buffer, pH 7.0; G, phosphate buffer, pH 7.4. [c] At 37°. [d] 2-Fluoroethanol.

techniques described above, and the results, similar to those obtained with BCNU and CCNU, are given in Table I.

In our previous publication, loss of chloride ion via an oxazoline intermediate was proposed to explain the formation of acetaldehyde, since no acetaldehyde was detected in the diazotization of 2-chloroethanamine,[2] which should give rise to the 2-chloroethyl cation. Recently, Colvin et al.[11] have shown that this diazotization does produce acetaldehyde, and using a different procedure we have confirmed their observations and have also found that acetaldehyde is rapidly consumed when mixed with the gases evolved from the diazotization reaction (carried out in a closed system), which probably explains our previous failure to detect this product.[2] In view of these results, it is possible to explain the formation of acetaldehyde either by loss of HCl to give the vinyl cation (a) or by rearrangement of the 2-chloroethyl cation before reaction with water (b) (Scheme I). However, it should be recognized that, because

Scheme I



of the results reported herein, whatever similarity exists between the product ratios in the reaction of BCNU in phosphate buffer and the diazotization of 2-chloroethanamine in strong mineral acid[11] must be fortuitous. Furthermore, the decomposition of 5-[3-(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide in distilled water, an excellent precursor of the 2-chloroethyl cation under mild conditions, gave a 70% yield of 2-chloroethanol,[12] in contrast to the results obtained with BCNU in distilled water (Table I). Verification of the vinyl cation was found in the identification (MS and GC) of bromoethene as a volatile product of the decomposition of BCNU in saturated sodium bromide solution. Unexplained is the shift in the ratio of acetaldehyde (or chloride ion) to 2-chloroethanol by buffering the media, but the failure of *N,N'*-bis(2-fluoroethyl)-*N*-nitrosourea (BFNU) to produce significant amounts of acetaldehyde even in distilled water (see Table I) can probably be attributed to the greater stability of the carbon–fluorine bond.

In summary, this study of seven *N*-(2-chloroethyl)-*N*-nitrosoureas has shown that the products of the decomposition of these compounds in aqueous media is quantitatively dependent upon pH and that in buffered solutions approximately equal amounts of 2-chloroethanol and chloride ion are formed. Acetaldehyde, the major product resulting from loss of chloride ion, is difficult to quantitate but was found in yields approaching 0.5 mol per mole of nitrosourea.

Experimental Section

GC analyses were performed on a Hewlett-Packard 5750 research chromatograph equipped with a flame ionization detector. Mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer. Preparations of *cis*-4-[3-(2-chloroethyl)-3-nitrosoureido]cyclohexaneacetic acid, its trans isomer, and *N*-(2-chloroethyl)-*N'*-(*cis*-4-hydroxycyclohexyl)-*N*-nitrosourea will be described elsewhere. Buffers were prepared as previously noted.[2]

Aqueous Decomposition of BCNU, CCNU, and Related Nitrosoureas (Table I). The decompositions were carried out at 37 or 50° (water bath) in a 10-ml test tube that was reduced at one end to accommodate a small silicone rubber septum. A measured volume of solvent (CO_2-free distilled water or pH 7 buffer) was added to a weighed amount of the nitrosourea (usually 1–4 mg/ml) sufficient to produce 0.01–0.02 *M* solutions, and the reaction vessel was stoppered and shaken to effect solution, which was complete in less than 1 min. BCNU and chlorozotocin were soluble in both water and buffer, the *cis*- and *trans*-cyclohexaneacetic acid derivatives were soluble in buffer, but CCNU was first dissolved in 1,4-dioxane and diluted with water or buffer (1:2 v/v). At intervals, 3-μl aliquots were withdrawn by syringe for GC analysis of acetal-

dehyde and 2-chloroethanol; in some experiments, larger aliquots (up to 1 ml) were removed for chloride ion titration. No correction was made for the acetaldehyde present in the head space, but most experiments were conducted with little head space. For decompositions of CCNU, BFNU, and *cis*-4-hydroxy-CCNU at "physiological concentration" (~70 μg/ml),[7] the reaction vessel was large enough to contain 100 ml of solution with little head space. Aliquots (5 μl) were taken for GC analysis; a 50-ml aliquot of the spent solution was taken for chloride ion titration.

Determination of Acetaldehyde and 2-Chloroethanol by Gas Chromatography. The glass column (1.85 m × 6.4 mm o.d.) packed with 10% Carbowax 20M on 60–80 mesh Gas Chrom Q support was of a design that permitted on-column injection of samples. The injection port and that portion of the column near the inlet septum were maintained at 30° by an auxiliary stream of cool air continuously flushing the external port area. Aliquots of the reaction mixtures were injected directly on-column at the cold port. Volatiles were swept into the hot (90°) zone by the 50 ml/min helium flow, nonvolatile reaction products and unchanged reactant remaining in the cool area of the column. At intervals, usually at the end of an 8-hr day, the column was removed and the first few inches of packing and the accumulated, nonvolatile materials were removed and replaced with unused packing. The accumulation of unchanged nitrosourea in the cool zone presented no observable interference with an effective analysis of the volatiles from subsequently injected samples.

The major chromatographic peaks observed by this procedure and also observed on a Chromosorb 101 column in some cases were identified by their retention times as acetaldehyde and 2-chloroethanol, whose identity was subsequently confirmed by MS in separate experiments. Minor components were observed in some reactions but were not identified and quantitated. Quantitation of the major peaks was achieved by comparison with standard solutions of acetaldehyde and 2-chloroethanol, whose respective retention times were 0.4 and 8.1 min.

Chloride Ion Titration. Aliquots, 1 ml or less from 0.01 to 0.02 *M* runs, were added to cold water (100 ml) and titrated with standard 0.015 *N* mercuric nitrate solution.[13] The 50-ml aliquots from 70 μg/ml runs in phosphate buffer were acidified with 5% HNO_3 to pH 2.4–2.6 before titration.

Decomposition of BCNU and CCNU in pH 7.0 Phosphate Buffer. Identification of 2-Chloroethanol and Nonvolatile Products. A mixture of BCNU (500 mg) and pH 7.0 phosphate buffer (100 ml) was stirred at 50° for 1 hr. The reaction solution was divided into 50-ml portions A and B. A was saturated with NaCl and extracted with Et_2O (8 × 50 ml). Evaporation of the dried (Na_2SO_4) extract under reduced pressure left a yellow oil: GC major peak corresponding to $Cl(CH_2)_2OH$ (retention time 8.1 min); mass spectrum *m/e* 80 [M^+, $Cl(CH_2)_2OH$]. B was concentrated in vacuo and the residue examined by TLC and MS. For TLC (silica gel, H_2O, ninhydrin and heat), the residue was extracted with hot EtOH and the ethanolic solution spotted on plates. The three components separated by TLC and identified by comparison with authentic samples were *N,N'*-bis(2-chloroethyl)urea, 2-(2-chloroethylamino)-2-oxazoline hydrochloride, and 2-chloroethanamine hydrochloride. These compounds were also detected by MS.

A solution prepared by dissolving CCNU (500 mg) in EtOH (50 ml) and diluting to 100 ml with pH 7.0 phosphate buffer was stirred at 50° for 2 hr. The reaction solution was distilled in vacuo at 30° and then up to 120° to remove volatiles, which were collected in a cold (Dry Ice–acetone) trap and examined for the presence of 1,2-ethanediol by TLC [silica gel, 9:1 v/v $CHCl_3$–MeOH, charring after $(NH_4)_2SO_4$–H_2SO_4 spray]. No 1,2-ethanediol was detected in the collected distillate. The distillation residue was extracted with hot EtOH and the extract examined by TLC (silica gel, 99:1 v/v $CHCl_3$–MeOH, ninhydrin and heat) and MS. The three spots were identified as *N,N'*-dicyclohexylurea, cyclohexanamine hydrochloride, and unchanged CCNU by TLC comparison with authentic samples. These compounds were also detected by MS.

Decomposition of BCNU in Water. Chloride Ion by Potentiometric Titration. A solution of BCNU (160 mg) in CO_2-free water (40 ml) was kept at 50° for 22 hr. A 20-ml aliquot was titrated potentiometrically with 0.05 *N* NaOH solution; the result expressed as moles of Cl^- or $Cl(CH_2)_2NH_2 \cdot HCl$/mol of BCNU was 1.07. A 20-ml aliquot was evaporated in vacuo and the residue examined by MS (70 eV): *m/e* 79 [M^+, $Cl(CH_2)_2NH_2$ from M · HCl], 184 [M^+, $[Cl(CH_2)_2NH]_2CO$], 148 [M^+, 2-(2-chloroethylamino)-2-oxazoline from M · HCl].

Decomposition of 0.02 *M* Solution of BCNU in pH 7.0 Phosphate Buffer. Identity of Nonvolatile Products. A solution of BCNU (2.1 mg, 0.10 mmol) in pH 7.0 phosphate buffer (5.0 ml) was stirred at 37° for 19 hr and then evaporated under reduced pressure. The residue was examined by MS (70 eV): *m/e* 184 [M^+, $[Cl(CH_2)_2NH]_2CO$], 148 [M^+, 2-(2-chloroethylamino)-2-oxazoline from M · HCl], 79 [M^+, $Cl(CH_2)_2NH_2$ from M · HCl].

Diazotization of 2-Chloroethanamine. Identity of Products. The procedure used was based on that reported for the deamination of methanamine.[14] H_2SO_4 (2 *N*, 1.85 ml) was added during 1 hr to a cold (5–10°), gently stirred solution of 2-chloroethanamine hydrochloride (290 mg) and $NaNO_2$ (520 mg) in H_2O (5.5 ml) covered by Et_2O (15 ml, for entrapment of volatile organic products). The two-phase reaction mixture was kept cold (ice–water bath) before GC analysis of each layer on a Chromosorb 101 column. 2-Chloroethanol (major) was found in the aqueous layer; 1,2-dichloroethane (major), acetaldehyde (minor), and chloroethene (minor) were found in the Et_2O layer. The products were identified by GC comparisons with authentic samples. In a deamination conducted with a more concentrated aqueous solution at room temperature in a 10-ml vessel sealed with a septum and with no addition of Et_2O (pressure being relieved at intervals through the septum), GC analysis revealed rapid diminution of the acetaldehyde content of a known vapor sample when mixed with samples of the vapor above the reaction mixture.

Decomposition of BCNU in Saturated NaBr Solution. A solution of BCNU (11 mg) in a saturated solution (9.0 ml) of NaBr in H_2O was allowed to decompose at 37° in the closed 10-ml reaction vessel described above. The 1-ml head space was immediately evacuated by syringe in order to reduce the air content of the vapor above the reaction mixture. Aliquots of the vapor (<0.05 ml) were taken for MS analysis after 1.5, 3.0, and 6.0 hr. The mass spectrum (70 eV) observed after 3.0 hr was typical: *m/e* (rel intensity) [142 (6.8), 144, (8.9), 146 (1.9); M^+, $BrCH_2CH_2Cl$], [107 (3.8), 109 (1.9); $BrCH_2CH_2^+$], [106 (6.1), 108 (5.5); M^+, $CH_2{=}CHBr$], [63 (100), 65 (32.8); $ClCH_2CH_2^+$], [62 (8.0), 64 (7.4); $CH_2{=}CHCl$], [49 (6.4), 51 (2.2); $ClCH_2^+$], [27 (86.2), $CH_2{=}CH^+$], [26 (30.2), C_2H_2]. These selected peaks were compared with the spectra of authentic $BrCH_2CH_2Cl$ [*m/e* (rel intensity) [142 (7.1), 144 (9.2), 146 (2.1)], [107 (3.3), 109 (1.8)], [106 (1.5), 108 (1.2)], [63 (100), 65 (33.3)], [62 (9.5), 64 (4.8)], [49 (6.8), 51 (1.8)], [27 (99.1)], [26 (31.5)]] and $CH_2{=}CHBr$ [*m/e* (rel intensity) [106 (45.0), 108 (42.2)], [80 (2.8), 82 (2.2); HBr], [79 (5.5), 81 (5.6); Br^+], [27 (100)], [26 (18.3)]]. The 106 and 108 peaks in the spectrum of $BrCH_2CH_2Cl$ are fragments of the M^+ peaks at 142, 144, and 146 and are significantly less intense than the corresponding peaks in the spectrum of the 3-hr vapor sample; both $BrCH_2CH_2Cl$ and $CH_2{=}CHBr$ are, therefore, present in the 3-hr vapor sample.

The identity of bromoethene as a reaction product was confirmed by GC analysis of a 6-hr vapor sample, separation being effected on a Chromosorb 101 column. Bromoethene and 1-bromo-2-chloroethane were identified by comparison of retention times with those of authentic samples.

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2-Halo Derivatives of 3'-Acetamido-3'-deoxyadenosine

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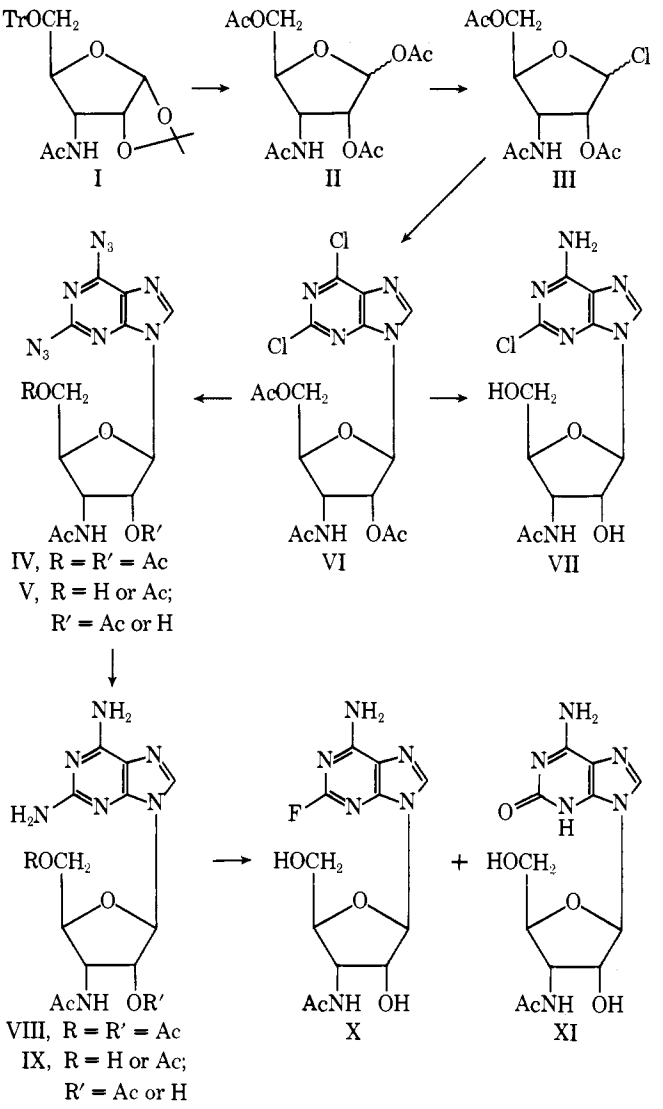
9-(3-Acetamido-2,5-di-*O*-acetyl-3-deoxy-β-D-ribofuranosyl)-2,6-dichloropurine (VI), prepared by the mercuric cyanide catalyzed reaction of 3-acetamido-2,5-di-*O*-acetyl-3-deoxy-D-ribofuranosyl chloride (III) with 2,6-dichloropurine, was converted by standard reactions to 3'-acetamido-2-chloro-3'-deoxyadenosine (VII) and 3'-acetamido-3'-deoxy-2-fluoroadenosine (X). The 2-chloroadenine nucleoside VII was not cytotoxic, but the 2-fluoroadenine nucleoside X was moderately so, and its cytotoxicity to a subline of H.Ep.-2 cells resistant to 2-fluoroadenine indicates that its activity is due to the intact nucleoside.

3'-Acetamido-3'-deoxyadenosine, found in the culture filtrates of *Helminthosporium* together with 3'-amino-3'-deoxyadenosine, does not inhibit the growth of Ehrlich ascites tumor cells as the latter compound does.[1] It has been proposed[1] that this lack of activity might be due to (1) rapid deamination, (2) cleavage of the glycosyl bond, (3) inhibition of phosphorylation of the nucleoside by the *N*-acetyl group, and (4) a combination of these factors.

We have prepared the 2-chloro (VII) and the 2-fluoro (X) derivatives of 3'-acetamido-3'-deoxyadenosine to study the effects of these halogens on the biologic activity of this compound. Acetolysis of 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-5-trityl-α-D-ribofuranose (I)[2] gave 3-acetamido-3-deoxy-1,2,5-tri-*O*-acetyl-β-D-ribofuranose (β-II)[3] in 22% yield. Later, using the acetolysis conditions of Anisuzzaman and Whistler,[3] the yield was raised to 51%. Fusion of β-II with 2,6-dichloropurine gave a mixture of four nucleosides, the α- and β-ribonucleosides and the α- and β-arabinonucleosides,[4] the latter pair resulting from epimerization at C_2 of β-II under the conditions of the fusion reaction.[5]

In an effort to improve the yield of 9-(3-acetamido-2,5-di-*O*-acetyl-3-deoxy-β-D-ribofuranosyl)-2,6-dichloropurine (VI) by eliminating the arabinonucleosides formed in the fusion reaction, the tetraacetate was treated with ether–HCl to convert it to the chloro sugar III, which was allowed to react with 2,6-dichloropurine in nitromethane in the presence of mercuric cyanide[6] and in benzene with molecular sieves[7] (Linde AW-500). Although both these reactions gave a high yield of the β anomer VI contaminated with only a trace of other nucleosides, the nitromethane–mercury cyanide procedure was somewhat superior. Assignment of the β configuration is based on the trans rule[8] and on the coupling constant (2 Hz) of the anomeric proton.[9] Treatment of VI with methanolic ammonia gave 3'-acetamido-2-chloro-3'-deoxyadenosine (VII). Reaction of VI with sodium azide resulted in some O-deacetylation as well as displacement of the chlorines of the purine giving a mixture of IV and V which was reduced sluggishly with Pd/C[10] to give 3'-acetamido-3'-deoxy-2',5'-di-*O*-acetyl-2-aminoadenosine (VIII) containing O-deacetylated nucleosides IX. This mixture (VIII and IX) when subjected to the modified Schiemann reaction[10] gave primarily 3'-acetamido-3'-deoxy-2-fluoroadenosine (X) and the isoguanine nucleoside XI, cleavage of the remaining *O*-acetyl groups occurring in the acidic medium (Scheme I).

Scheme I



Cytotoxicity. 3'-Acetamido-2-chloro-3'-deoxyadenosine (VII) was without effect on H.Ep.-2 cells in culture at the highest level tested (20 μmol/l.), but 3'-acetamido-3'-