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

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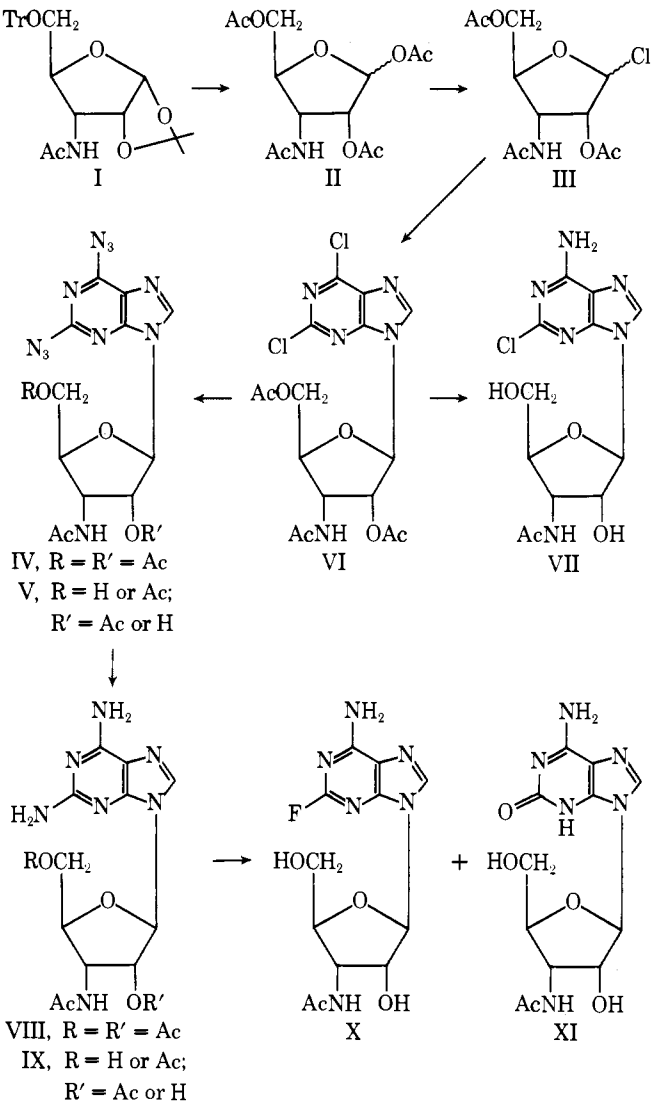
9-(3-Acetamido-2,5-di-*O*-acetyl-3-deoxy-β-D-ribofuranosyl)-2,6-dichloropurine (VI), prepared by the mercuric cyanide catalyzed reaction of 3-acetamido-2,5-di-*O*-acetyl-3-deoxy-D-ribofuranosyl chloride (III) with 2,6-dichloropurine, was converted by standard reactions to 3'-acetamido-2-chloro-3'-deoxyadenosine (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'-

Table I. Cytotoxicity to H.Ep.-2 Cells in Culture

Compound	H.Ep.-2/0 ED_{50}(H.Ep.-2), μmol/l.[a]	Deg of resistance[b] H.Ep.-2/FA	H.Ep.-2/FA/FAR
2-Fluoroadenine	0.03	>2000	>2000
2-Fluoroadenosine	0.02	20	>2000
2-Chloroadenine	10	>20	
2-Chloroadenosine	7	>10	>10
X	20	1	>3
VII	>60	<1	

[a] The concentration required to inhibit the growth of cells, as measured by clone counts, to 50% of controls. See ref 10. [b] Ratio to the ED_{50} of the compound for the wild H.Ep.-2 line to its ED_{50} for the resistant subline (/FA = resistant to 2-fluoroadenine; /FA/FAR = resistant to 2-fluoroadenine and 2-fluoroadenosine).

deoxy-2-fluoroadenosine (X) inhibited the growth of these cells to 50% of controls at 20 μmol/l. (see Table I). The cytotoxicity of X clearly indicates that deamination may be a limiting factor in the biologic activity of the parent nucleoside, since the introduction of fluorine at C-2 of adenosine and its analogs is known to produce nucleosides resistant to the action of adenosine deaminase.[11] Furthermore, the subline of H.Ep.-2 cells resistant to 2-fluoroadenine[12] is still sensitive to X, whereas the subline resistant to both 2-fluoroadenine and 2-fluoroadenosine[13] is resistant to X, indicating that X is not cleaved to any extent to 2-fluoroadenine but must be phosphorylated to some extent by the adenosine kinase of H.Ep.-2 cells. However, since cytotoxicity appears to correlate well with ease of phosphorylation,[14] it is probably not a good substrate. The inactivity of 3′-acetamido-3′-deoxyadenosine[1] may result from very limited production of the nucleotide as a consequence of its relative K_m values for the deaminase and the kinase.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. The uv absorption spectra were determined in aqueous media with a Cary Model 17 spectrophotometer. The ^1H NMR spectra were obtained with a Varian XL-100 spectrometer in the solvents indicated with tetramethylsilane as an internal reference. Chemical shifts quoted in the case of multiplets are measured from the approximate center. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Mass spectral data (MS) were taken from low-resolution spectra determined with a Hitachi Perkin-Elmer RMU-7 double-focusing instrument (70 eV); M = molecular ion.

3-Acetamido-3-deoxy-1,2,5-tri-*O*-acetyl-D-ribofuranose (II). To a solution of I (47.2 g, 0.1 mol) in 500 ml of glacial acetic acid and 57.5 ml of acetic anhydride was added 35 ml of concentrated H_2SO_4 keeping the temperature below 20°. After addition was complete, the mixture was allowed to stand at room temperature for 20 hr before it was poured into 1 l. of ice water. Triphenylcarbinol was removed by filtration and the water solution extracted with $CHCl_3$ (1.5 l.). The $CHCl_3$ extract was washed with bicarbonate solution and then with water and dried over $MgSO_4$. Evaporation gave a light yellow oil that solidified on standing: yield 7.05 g (22%).

In a later run using acetolysis conditions of Anisuzzaman and Whistler,[3] a 51% yield of a purer material was obtained. This sample melted at 99–101° and its ^1H NMR spectrum agreed well with the literature.[3]

3-Acetamido-3-deoxy-2,5-di-*O*-acetyl-D-ribofuranosyl Chloride (III). A solution of β-II (475 mg) in ether saturated with HCl and containing 0.5 ml of acetyl chloride was allowed to stand 4 days at 4°. The solution was evaporated to dryness and the residue dissolved in toluene. This solution was evaporated and the process repeated three times giving a light-colored oil: ^1H NMR ($CDCl_3$) δ 2.08, 2.11, and 2.2 (3 s, Me of Ac), 4.4 (m, H_4 and H_5), 5.15 (m, H_3), 5.34 (d, $J_{1,2}$ = 2.5 Hz, H_2), 6.02 (s, H_1 of β anomer), 6.5 (d, $J_{1,2}$ = ~3 Hz, H_1 of α anomer), 9.9 ppm (broad singlet, NH). A small doublet at 6.1 indicated the presence of another sugar. This oil was used in the coupling reaction without further purification.

9-(3-Acetamido-3-deoxy-2,5-di-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (VI). A. A mixture of III (from 475 mg of II), 2,6-dichloropurine (284 mg), and molecular sieve (3 g, AW-500) in benzene (30 ml) was refluxed with stirring for 1 hr. An additional 3 g of sieve was added and the solution was refluxed for 1 more hr. The sieve was removed by filtration and extracted with $CHCl_3$. Evaporation of the benzene solution and $CHCl_3$ extracts gave 511 mg of β-VI as a yellow foam.

B. A mixture of III (from 3.72 g of II), 2,6-dichloropurine (2.2 g), and $Hg(CN)_2$ (3.36 g) in 135 ml of nitromethane was refluxed for 3 hr before it was evaporated to dryness. A $CHCl_3$ solution of the residue was filtered and washed with water. The water was back extracted with $CHCl_3$ and the combined $CHCl_3$ solutions were dried over $MgSO_4$ before they were evaporated: yield of yellow foam, 4.55 g (87%); ^1H NMR ($CDCl_3$) δ 2.08, 2.10, and 2.22 (3 s, Me of Ac), 4.4 (m, $2H_{5'}$ and $H_{4'}$), 5.22 (q, $H_{3'}$), 5.6 (q, $H_{2'}$), 6.15 (d, $J_{1'2'}$ = ~2 Hz, $H_{1'}$), 6.3 (d, NH), 8.35 ppm (s, H_8). This material, which showed only trace impurities on TLC and which was identical with the material prepared as described in A, was used in the next steps without further purification.

3′-Acetamido-3′-deoxy-2-chloroadenosine (VII). A solution of VI (947 mg, 2.1 mmol) in 100 ml of alcoholic ammonia (saturated at 0°) was allowed to stand at room temperature for 4 days before it was evaporated to dryness. After extraction with petroleum ether (3 × 50 ml), the residue was recrystallized from methanol (charcoal) to give a white solid, which was dried for 6 hr at 100° over P_2O_5: yield 304 mg; mp >255° dec; $[\alpha]^{25}D$ 15.9 ± 1.1° (*c* 0.66, aqueous MeOH); λ_{max} in nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl 265 (12.4); λ_{max} in nm ($\epsilon \times 10^{-3}$) pH 7 buffer and 0.1 *N* NaOH 265 (13.4); ^1H NMR (DMSO-d_6) δ 1.92 (s, CH_3), 3.65 (m, $2H_{5'}$), 4.03 (m, $H_{4'}$), 4.45 (m, $H_{2'}$ and $H_{3'}$), 5.09 (t, $O_{5'}H$), 5.93 (d, $J_{1'2'}$ = 2 Hz, $H_{1'}$), 5.95 (d, $O_{2'}H$), 7.83 (br s, NH_2), 8.0 (d, NH), 8.45 ppm (s, H_8). *Anal.* ($C_{12}H_{15}ClN_6O_4$) C, H, N.

The residue from the crystallization was purified by column chromatography on silica gel using $CHCl_3$–MeOH (17:3) as eluent followed by recrystallization from methanol: yield 143 mg; total yield 447 mg (62%).

3′-Acetamido-3′-deoxy-2-aminoadenosine and Its Mono- and Diacetates (VIII and IX). A solution of VI (3.5 g, 8 mmol) and sodium azide (1.04 g, 16 mmol) in 80 ml of 90% ethanol was refluxed for 1 hr, chilled, filtered to remove NaCl, and diluted with 100 ml of ethanol. The diazides IV and V were reduced at atmospheric pressure with 5% Pd/C catalyst (0.65 g). The hydrogen atmosphere was changed at 0.5, 1, 2.5, and 5 hr. Since reduction was still not complete, the mixture was hydrogenated for an additional 21 hr with fresh catalyst and finally in a Parr shaker for 6 hr at 40 psi. Chromatography and mass spectrometry indicated that a mixture of nucleosides was obtained: *m/e* 407 (M of VIII)$^+$, 365 (M of 3′-acetamido-3-deoxy-2-aminoadenosine monoacetate)$^+$, 323 (M of 3′-acetamido-3-deoxy-2-aminoadenosine)$^+$, 253 (sugar)$^+$, 216 (sugar − Ac + H)$^+$, 179 (base + CHO)$^+$, 175 (sugar − 2Ac + 2H)$^+$, 150 (base + H)$^+$; yield 2.94 g. This material was used in the next step without further purification.

3′-Acetamido-3′-deoxy-2-fluoroadenosine (X). To a solution of VIII and IX (300 mg) in 6 ml of 48% fluoboric acid chilled to −20° was added $NaNO_2$ (138 mg in 1 ml of H_2O) dropwise over a period of 15 min. After the mixture was stirred for 20 min, 10 ml of $CHCl_3$ was added, and the mixture was neutralized with 50% NaOH at −30°. The insoluble solid that formed was removed by filtration, triturated with alcohol–ether, and dried: yield of 3′-acet-

amido-3′-deoxyisoguanosine (TLC) 60 mg. Evaporation of the water layer from the reaction mixture gave a solid residue which was triturated with methanol (2 × 25 ml). Evaporation of the methanol gave a solid which was chromatographed on a silica gel column using $CHCl_3$–MeOH (9:1) as eluent: yield of white solid 81 mg (17%); mp 248–250° dec; λ_{max} in nm ($\epsilon \times 10^{-3}$) 0.1 *N* HCl 262 (20.1), 268 sh; λ_{max} in nm ($\epsilon \times 10^{-3}$) pH 7 buffer and 0.1 *N* NaOH 262 (22.2), 268 sh; ^1H NMR (DMSO-d_6) δ 1.95 (s, Me of Ac), 3.65 (m, $H_{5'}$), 4.0 (m, $H_{4'}$), 4.45 (m, $H_{3'}$ and $H_{2'}$), 5.05 (t, OH at $C_{5'}$), 5.9 (d, $J_{1'2'}$ = 2.3 Hz, $H_{1'}$), 5.95 (d, OH at $C_{2'}$), 7.8 (br s, NH_2), 7.9 (d, NH), 8.4 ppm (s, H_8). *Anal.* ($C_{12}H_{15}FN_6O_4 \cdot 0.25\ H_2O$) C, H, N.

Acknowledgments. This investigation was supported by Contract NO1-CM-43762 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. The authors wish to thank Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for the analytical and spectral data reported and Mrs. Martha Thorpe for her help in the interpretation of the NMR spectra. They also wish to thank Dr. L. L. Bennett, Jr., and Miss Jean Carpenter for the cytotoxicity data reported.

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2-Aryloxymethyl-2,3,5,6-tetrahydro-1,4-oxazines, a New Class of Antidepressants

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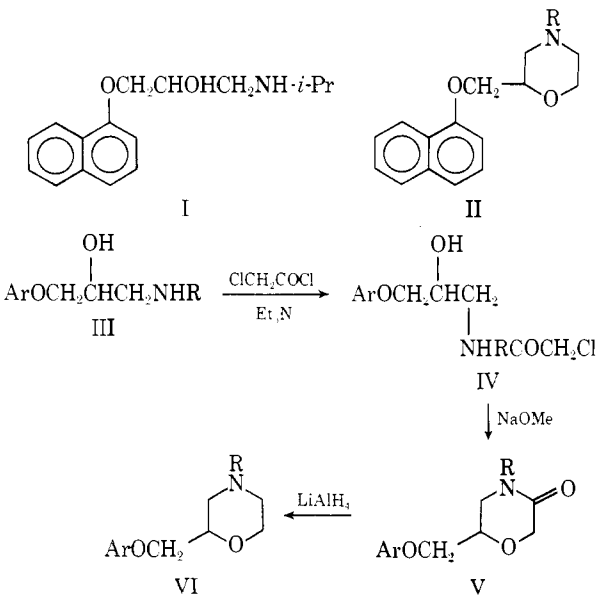
Some 2-aryloxymethyl-2,3,5,6-tetrahydro-1,4-oxazines have been shown to possess marked antidepressant activity. The 1,4-oxazines were synthesized by lithium aluminum hydride reduction of the readily available 6-aryloxymethyl-2,3,5,6-tetrahydro-1,4-oxazin-3-ones. High antidepressant activity was associated with ortho substitution of the 2-phenoxymethyl group and with 1,4-oxazines devoid of 4-substituents.

Effects on the central nervous system have been reported to be produced by the β-receptor blocking drug propranolol (I).[1,2] During a research program investigating the central nervous system properties of chemical structures related to the aryloxypropanolamines III, a number of 2-(naphthoxymethyl)-2,3,5,6-tetrahydro-1,4-oxazines II were synthesized and shown to possess potent antidepressant properties. This observation led us to synthesize a wide variety of 2-aryloxymethyl-2,3,5,6-tetrahydro-1,4-oxazines and evaluate more fully their biological activity.[3]

Syntheses. The tetrahydrooxazines (VI) were conveniently synthesized from the propanolamines III.[5-7] Treatment of III with chloroacetyl chloride under basic conditions gave the amides IV, which were readily cyclized utilizing sodium methoxide to produce the lactams V. The 1,4-oxazin-3-ones when crystalline were isolated and the compounds characterized are listed in Table II. The yields of the 1,4-oxazinones based on the propanolamines were in the 40–60% range. Lithium aluminum hydride reduction of these lactams gave the required tetrahydrooxazines in 30–60% yield. The chloroacetylation stage also produced some O-acylated product, but the ester was readily removed by acid treatment. The secondary amines VI (R = H) could also be prepared in this way starting with the primary amines III (R = H), but better yields (20% overall based on propanolamine) were obtained by preparing the N-benzylated compound VI (R = $PhCH_2$–) and then removing the benzyl group by hydrogenolysis. The N-benzylated propanolamines III (R = $PhCH_2$–) were conveniently prepared from benzylamine and 3-aryloxypropylene oxides. The



amines when crystalline were characterized and are listed in Table III. As an alternative to hydrogenolysis the benzyl group could be removed by treatment with ethyl chloroformate followed by alkaline hydrolysis of the *N*-ethoxycarbonyl derivative.[4] The *o*-allylphenoxymethyl compound VI (Ar = *o*-allylphenyl; R = H) was prepared via the N-benzylated derivative using the ethyl chloroformate–alkali fis-