Synthesis and Biological Activity of Selected 2,6-Disubstituted-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purines†,‡

Leon F. Christensen, Arthur D. Broom,*

Department of Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112

Morris J. Robins,

Department of Chemistry, The University of Alberta, Edmonton 7, Canada

and Alexander Bloch

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203.
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A number of 2-substituted-α- and -β-2′-deoxyadenosines have been prepared, and their growth inhibitory activity against leukemia L-1210, *Streptococcus faecium*, and *Escherichia coli* has been evaluated *in vitro*. Fusion of 2,6-dichloropurine with 1,3,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranose or with methyl 3,5-di-*O*-*p*-toluyl-2-deoxy-D-*erythro*-pentofuranoside gave the anomeric mixtures of the acetyl- (80%) or *p*-toluyl- (30%) blocked deoxynucleosides. The acetylated mixture was treated with liquid ammonia, and the resulting 2-chloro-2′-deoxy-α- and -β-adenosines were reacylated with *p*-toluyl chloride and resolved by alumina column chromotography. Resolution of the *p*-toluyl-blocked deoxynucleosides of 2,6-dichloropurine by silica gel chromatography was effected directly. Reaction of the anomerically pure 2-chloro-2′-deoxyadenosines with the appropriate nucleophiles gave the 2-methoxy, 2-methylthio, 2-hydrazino, and 2-amino derivatives. The α anomers were generally less active inhibitors of cell growth than were the corresponding β derivatives. At 1×10^{-4} *M*, the inhibition of L-1210 growth ranged from 10% for 2-amino-α-D-2′-deoxyadenosine to 30% for the 2-chloro-substituted analog. The β anomers inhibited 50% of the tumor cell growth at concentrations ranging from 7×10^{-8} *M* for 2-chlorodeoxyadenosine to 8×10^{-6} *M* for the 2-methoxy derivative. For the same extent of inhibition, 5×10^{-6} *M* 2-chloroadenosine was required. The 2-hydrazino-substituted α-adenosine was active in *E. coli* K 12, 50% growth inhibition occurring at 4×10^{-5} *M*.

A substantial number of 2,6-disubstituted purines, their nucleosides, and nucleotides exert inhibitory activity in various biological systems.[1] For example, 2-fluoroadenosine and 2′-deoxyfluoroadenosine are potent inhibitors of the growth of a number of experimental tumors.[2,3] In addition, 2-chloroadenosine interferes with the aggregation of blood platelets and exerts a variety of cardiovascular effects,[4-8] which are also exerted by 2-methylthioadenosine and some homologous 2-alkylthio analogs.[9]

In an interesting study, LePage and his coworkers[10,11] showed that both the α and β anomers of 2′-deoxy-6-thioguanosine are inhibitors of certain mouse tumors, but that the α anomer is less toxic to the host because it is converted to the active nucleotide by the tumor but not by the bone marrow cells. In the study presented here, the synthesis of the α and β anomers of a number of 2′-deoxyribonucleosides of 2,6-disubstituted purines was undertaken, in order to evaluate their comparative activity in various test systems.

Results and Discussion

Chemistry. Fusion coupling of 2,6-dichloropurine (**2**) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranone (**1**), as previously described,[12] gave the anomeric mixture (**3**) in about 80% yield. The α:β ratio obtained was similar to that reported by Montgomery and Hewson.[3] When the mixture **3** was treated with methanolic ammonia to give the corresponding 2-chloro-2′-deoxyadenosines (**4**) the product was found to be contaminated with 2-chloro-6-methoxy-9-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purine. This accompanying nucleophilic displacement of 6-chloro by the methoxy group is, probably, also responsible for the contaminating nucleoside observed by Goodman and coworkers[13] in their preparation of 2,6-diaminopurine deoxyribonucleosides by reaction of blocked 6-chloro-2-acetamidopurine deoxyribonucleosides with hot methanolic ammonia.

The clean conversion of **3** to **4** was, however, successfully accomplished by the use of liquid ammonia at room temperature. An early attempt to resolve the anomeric mixture **4** with Dowex 1-X2 (OH^-) and methanol-water[14] as the eluent resulted in substantial conversion of **4** to the corresponding 2-methoxy-2′-deoxyadenosines (**11** and **16**). Thus convenient separation by the "Dekker Column"[14] was precluded, and alternative resolution of the anomers had to be sought.

The mixture **4** (obtained in 77% yield by treatment of **3** with liquid ammonia) was acylated with *p*-toluyl chloride, and the blocked mixture was resolved, using an alumina column. Pure **7** and **9** were obtained in 36 and 24% yields, respectively. Removal of the *p*-toluyl groups with methanolic sodium methoxide at room temperature gave good yields of 2-chloro-2′-deoxyadenosine§ (**10**) and its α anomer (**15**).

Fusion of 2,6-dichloropurine (**2**) with methyl 2,5-di-*O*-*p*-toluyl-2-deoxy-D-*erythro*-pentofuranoside[16] (**5**), as previously described[17] for the L enantiomer of **5**, gave a 32% yield of a mixture of blocked deoxyribonucleosides which was resolved by silica gel column chromatography into pure 2,6-dichloro-9-(3,5-di-*O*-*p*-toluyl-2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purines (**8** and **6**, respectively). Treatment of **8** and **6** with liquid ammonia was used as an

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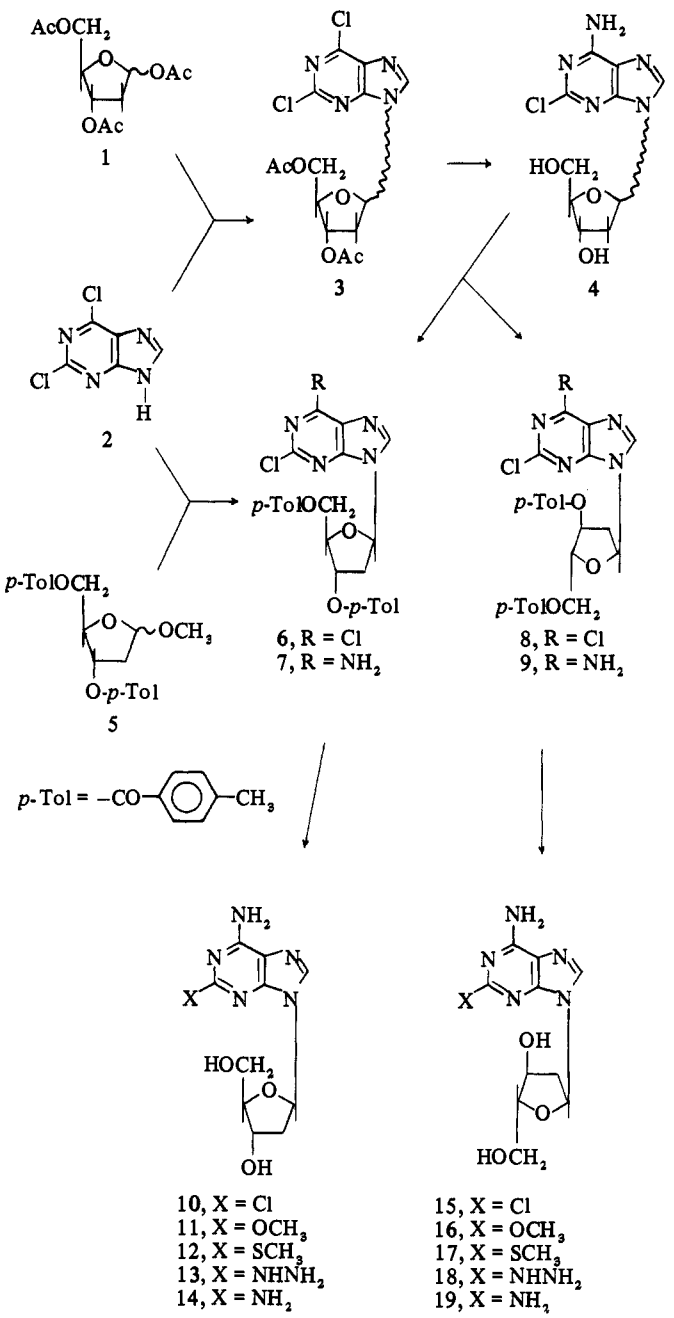
§This compound has been reported as a noncharacterized intermediate by Venner[15a] and by Kehara and Tada.[15b]

Table I. Nmr Data for Some 2,6-Disubstituted-9-(2-deoxy-D-*erythro*-pentofuranosyl)purines

Compound	Anomeric Configuration	H_1' peak shape[a]	$\delta_{H_1'}$, ppm	$J_{H_1'}$,[b] Hz	H_1' peak width, Hz	H_8[c]	Solvent[d]
10	β	t	6.24	6.5	13.0	8.12	W
11	β	t	6.34	6.8	13.7	8.22	D
12	β	t	6.32	6.6	13.3	8.18	W
14[e]	β	q	6.10	6.7	13.5	7.70	W
15	α	q	6.28	7.0 2.8	9.4	8.38	W
16	α	q	6.30	7.4 3.2	11.0	8.13	W
17	α	q	6.25	6.9 3.9	11.2	8.12	W
18	α	q	6.22	7.4 2.9	10.8	8.02	W
19	α	q	6.27	7.3 2.6	9.8	8.15	D + W

[a]For convenience t = triplet, q = quartet for ABX spin system.[17] [b]Values are first-order "apparent" coupling constants. [c]All sharp singlets. [d]D = DMSO-d_6, W = D_2O. [e]See Discussion.



alternative route for the synthesis of 2-chloro-2′-deoxy-α- and -β-adenosines (**15** and **10**, respectively).#

Reaction of **10** or **15** with the appropriate nucleophiles gave the 2-methoxy- (**11** and **16**), 2-methylthio- (**12** and **17**), and 2-hydrazino- (**13** and **18**) 2′-deoxy-β- or -α-adenosines. Hydrogenation of the 2-hydrazino-2′-deoxy-α- or -β-adenosine (**13** and **18**) over Raney nickel provided an alternative route to the known[3,13] 2,6-diamino-9-(2-deoxy-β- or -α-D-*erythro*-pentofuranosyl)purine (2-amino-2′-deoxy-β- or -α-adenosine) (**14** and **19**).

All of these anomeric 2′-deoxynucleoside pairs obey Hudson's rule of isorotation.[19] The pmr signal for the anomeric proton of the deblocked compounds follows the "triplet-quartet" splitting pattern for the α and β anomers, respectively, as previously observed.[12,17] An exception is 2,6-diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**14**), the anomeric proton signal of which exhibits a small splitting (1.8 Hz) of the center peak of the ABX triplet.** However, the peak width of 13.9 Hz corresponds to the usual β values[12] (Tables I and II).

Biology. The two principal questions considered in evaluating the growth inhibitory activity of the newly prepared compounds concerned the contribution which the anomeric structure and the substituent at the 2 position of the heterocycle make to the extent of biological activity. The data in Table III show that the β anomers are uniformly more inhibitory than are the corresponding α derivatives. This difference is particularly apparent in the tumor system, where the β anomers interfered with cell growth at concentrations ranging from 10^{-8} to 10^{-5} *M*, whereas, at 1×10^{-4} *M*, the α anomers inhibited the tumor by only 9–30%. This finding parallels the observation by LePage and his coworkers who found that 6-thio-2′-deoxy-α-guanosine was markedly less effective against a mammary tumor in C_3H mice than was the β analog.[10,11] However, since the toxicity of the α anomer was far below that of the β nucleoside, higher and multiple doses could be employed, resulting in enhanced inhibitory activity. Similarly, the survival of mice bearing Mecca lymphosarcoma was increased more

#It is recognized that a designation such as "α-2′-deoxyadenosine" is not systematic nomenclature for 9-(2-deoxy-α-D-erythropentofuranosyl)adenine. The former is, however, much shorter and there is precedent for such usage.[18]

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Table II. Ultraviolet Spectral Data for Some 2,6-Disubstituted-9-(2-deoxy-D-*erythro*-pentofuranosyl)purines

Compd	λ_{max}, nm ($\epsilon_{max} \times 10^{-3}$)		
	EtOH	pH 1	pH 11
6	273 (11.2)		
7	264 (18.6)		
8	273 (11.6)		
9	269 (15.8)		
10	265 (15.9)	264 (15.2)	264 (15.7)
11	267 (14.5)	246 (9.9)	267 (15.7)
		274 (14.4)	
12	276 (14.8)	269 (15.6)	276 (15.3)
14	257 (10.4)	253 (12.5)	256 (10.7)
	281 (11.4)	289 (10.6)	279 (11.4)
15	264 (14.6)	265 (14.7)	264 (17.2)
16	253 (9.3)	247 (7.7)	253 (9.3)
	267 (12.9)	274 (12.2)	267 (12.4)
17	280 (17.4)	269 (19.4)	276 (20.0)
18	255 (10.8)	283 (12.2)	255 (10.8)
	288 (19.2)		285 (9.6)
19	256 (8.3)	251 (9.3)	248 (9.2)
	280 (9.2)	289 (8.5)	279 (9.6)

extensively by the α than by the β anomer, and this result, too, was attributed to the fact that the low toxicity of the α anomer permitted the use of higher doses. This low toxicity was held to be a result of the inability of the bone marrow cells to phosphorylate the α anomer, whereas the tumor carried out the phosphorylation effectively.

The observation that some of the α anomers prepared show, albeit limited, inhibitory activity against L-1210, would suggest that they may undergo phosphorylation. Other tumors, such as the Mecca lymphosarcoma which is quite sensitive to 6-thio-2′-deoxy-α-guanosine, might be more sensitive to inhibition of their growth by these compounds than is L-1210. Since the base analog 2-chloroadenine is more inhibitory against L-1210 than is 2-chloro-2′-deoxy-α-adenosine (**15**), the relatively low activity of the α anomer cannot be attributed to extensive cleavage of its glycosidic bond.

The difference in the extent of growth inhibition exerted by the α and β anomers can also be seen in the microbial systems. Where inhibition occurs, it is exerted by the β anomer. There are two exceptions, namely, 2-hydrazino-2′-deoxy-α-adenosine (**18**) which inhibits *Escherichia coli* growth by 50% at 4×10^{-5} *M*, and 2-chloro-2′-deoxy-α-adenosine (**15**) which interferes with *Streptococcus faecium* growth at 4×10^{-4} *M*. Whether this activity of the two α anomers in the bacterial system is a reflection of the extent of their phosphorylation remains to be determined.

A comparison of the relationship between structure and biological activity shows that 2-chloro-2′-deoxy-β-adenosine (**10**) is a markedly more active inhibitor of L-1210 growth than is the corresponding ribonucleoside or the base analog. It is unclear whether this fact denotes a more extensive conversion of the deoxynucleoside to the active intermediate, or a difference in the site of action. Deoxyadenosine itself, at 10^{-4} *M*, does not interfere with the growth of the L-1210 cells.

The presence of a methoxy group at the 2 position of β-deoxyadenosine leads to good inhibitory activity in the tumor but not in the bacterial cells, whereas the 2-methylthio derivative is only marginally active in the three test systems. Of interest is the fact that 2′-deoxy-β-adenosine *per se* inhibits the growth of *E. coli* K 12 by 50% at 4×10^{-7} *M*, and that the introduction of substituents at the 2 position of this metabolite decreases or abolishes this inhibitory activity.

In view of the observed *in vitro* activity of some of the α anomers, their potential antitumor activity deserves further evaluation *in vivo*.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Pmr spectra were recorded on a Varian A-60 or a Jeolco C6OH spectrometer. Optical rotations were determined using a Perkin-Elmer Model 141 polarimeter with a 1 cm³, 1 dm cell. Uv spectra were obtained using a Cary 15 spectrometer. Evaporations were effected with a Büchi flash evaporator with aspirator vacuum and bath temperature of 50° or lower. Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the theoretical values.

2-Chloro-6-amino-9-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purine (4). The crystalline anomeric fusion product **3** (4.74 g, 0.0122 mole) was sealed in a stainless steel bomb with 100 ml of liquid NH_3 at room temperature for 26 hr. The NH_3 was allowed to evaporate and the residue was dissolved in EtOH, 12.2 ml of aqueous 1 *N* NaOH was added, and the solution was evaporated to dryness. Crystallization of this residue from 50 ml of H_2O gave 2.67 g (76.5%) of anomeric **4**, suitable for acylation without further purification.

2-Chloro-6-amino-9-(3,5-di-*O*-*p*-toluyl-2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purine (9 and 7). A solution of 3 g (0.0105 mole) of the above product **4** in 90 ml of dry pyridine was evaporated to dryness, and the residue was redissolved in 90 ml of dry pyridine. This magnetically stirred mixture was cooled to −20°, and 4 ml (0.0315 mole) of *p*-toluyl chloride was added slowly. After solution was complete, the reaction mixture was allowed to stand for 20 hr at −20° and was then poured into 150 ml of ice-cold satd aqueous $NaHCO_3$. This mixture was shaken vigorously and was then extracted with three 100-ml portions of $CHCl_3$. The combined organic phase was washed with 40 ml of H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in a minimal volume of $CHCl_3$–PhH (7:3) and applied to a neutral alumina column (400 g) packed in PhH. The column was washed with 3 l. of $CHCl_3$–PhH (1:1), and the wash discarded. The α anomer **9** was eluted with 3 l. of

Table III. Biological Activity of Some 2,6-Disubstituted-9-(2-deoxy-D-*erythro*-pentofuranosyl)purines

Compd	Concentration (*M*) for 50% growth inhibition of			
	E. coli (K_{12})	*S. faecium*	Leukemia L-1210	% inhibition of L-1210 at 1×10^{-4} *M* compound
2-Chloroadenine	2×10^{-5}	1×10^{-4}	3×10^{-5}	
2-Chloroadenosine	5×10^{-5}	3×10^{-5}	5×10^{-6}	
15	1×10^{-3}	4×10^{-4}	1×10^{-4}	31
10	2×10^{-5}	3×10^{-5}	7×10^{-8}	
16	1×10^{-3}	1×10^{-3}	1×10^{-4}	12
11	1×10^{-3}	1×10^{-3}	8×10^{-6}	
17	1×10^{-3}	1×10^{-3}	1×10^{-4}	11
12	1×10^{-3}	8×10^{-4}	1×10^{-4}	21
18	4×10^{-5}	1×10^{-3}	1×10^{-4}	0
19	$>10^{-3}$	1×10^{-3}	1×10^{-4}	9

PhH–EtOAc (1:1). This solution was evaporated to dryness and the residue crystallized from 100 ml of Me_2CO in two crops to give 2.50 g (38.3%) of 9, mp softens 205°, melts 219–221°, resolidifies 225°, $[\alpha]^{26}D$ – 27.9° (*c* 1, $CHCl_3$). *Anal.* ($C_{26}H_{24}ClH_5O_5$) C, H, N.

Elution of the column with 4 l. of EtOAc removed the β anomer 7 which was isolated as for 9 above and crystallized from 150 ml of EtOH to give 1.54 g (23.6%) of 7, mp 192–194° with softening at 170–180°, $[\alpha]^{26}D$ 63.1° (*c* 1, $CHCl_3$). *Anal.* ($C_{26}H_{24}ClH_5O_5$) C, H, N.

2,6-Dichloro-9-(3,5-di-*O*-*p*-toluyl-2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)purine (8 and 6). A finely divided mixture of 12.3 g (0.0678 mole) of 2 and 50 g (0.12 mole) of 5 was heated in an oil bath (138°) until completely melted. Dichloroacetic acid (5 drops) was added and the mixture fused under aspirator vacuum for 17 min. The melt was allowed to cool to about 100° and was then dissolved in EtOAc. This solution was washed with two 100-ml portions of ice-cold satd aqueous $NaHCO_3$ and 100 ml of H_2O, dried over Na_2SO_4, filtered, and evaporated to a heavy syrup. This material was dissolved in a minimal volume of PhH, and applied to a silica gel column (1200 g, 6 × 140 cm) packed in PhH. The column was developed with 80 l. of 0.1% EtOAc in PhH, and evaporation of this solution gave 33.85 g of unreacted 5. Elution was continued with 35 l. of 1% EtOAc in PhH. The early fractions contained a small amount of 5 and the latter fractions were blank. The elutant was changed to 2% EtOAc in PhH and from the first 20 l. was obtained 3.60 g (15.8% based on unrecovered 5) of needles of the α anomer 8 (from EtOH), mp 124–125°, $[\alpha]^{28}D$ – 26.5° (*c* 1, $CHCl_3$). *Anal.* ($C_{26}H_{22}Cl_2N_4O_5$) C, H, N.

The following 3 l. from the column contained 6 and 8 from which 0.67 g (3%) of pure 6 was obtained by fractional crystallization from EtOH. From the following 25 l. of 2% EtOAc in PhH was obtained 2.33 g (10%) of 6 (after crystallization from EtOH); total crystalline 6 was 3.0 g (13%), mp 155–157°, $[\alpha]^{28}D$ – 37.9° (*c* 1, $CHCl_3$). *Anal.* ($C_{26}H_{22}Cl_2N_4O_5$) C, H, N.

2-Chloro-6-amino-9-(2-deoxy-β- and -α-D-*erythro*-pentofuranosyl)purine (10 and 15). Method A. To 75 ml of MeOH containing 0.44 g (0.0115 mole) of NaOMe was added 1 g (0.0019 mole) of 7 and the resulting solution was stirred for 1.5 hr at room temperature. The solution was then neutralized with 10 ml of Amberlite IRC-50 (H^+), filtered, and evaporated to dryness, and the resulting residue was triturated several times with Et_2O and then crystallized from 7 ml of H_2O to give 0.24 g (62%) of 10, mp softens at 210–215° and then solidifies and turns brown, $[\alpha]^{26}D$ – 18.8° (*c* 1, DMF). *Anal.* ($C_{10}H_{12}ClN_5O_3$) C, H, N.

Treatment of the α anomer 9 in a similar manner and crystallization from 5 ml of H_2O gave 0.37 g (67%) of 15, mp softens from 200° and resolidifies by 215°; $[\alpha]^{28}D$ + 85.6° (*c* 1, DMF). ($C_{10}H_{12}ClN_5O_3$) C, H, N.

Method B. To 150 ml of liquid NH_3 in a stainless steel bomb was added 2.46 g (0.0045 mole) of 6. The mixture was sealed in and allowed to stand for 25 hr at room temperature. The NH_3 was then allowed to evaporate. The residue was treated with EtOH and 4.6 ml of aqueous 1 *N* NaOH, and this mixture was evaporated to dryness. To the residue was added 10 ml of EtOH and 7 g of silica gel and this mixture was evaporated to dryness and the residue, adsorbed on silica gel, was applied to a dry packed column (100 g) of silica gel. The column was washed with 1 l. of $CHCl_3$ and 1 l. of $CHCl_3$–EtOAc (1:1), and the washes were discarded. The product was eluted with EtOAc–MeOH (8:2) and evaporation of this solution followed by crystallization of the residue from 15 ml of EtOH gave 0.77 g (59%) of 10 which was identical with that prepared *via* method A above.

Treatment of 1.21 g (0.0023 mole) of 8 by method B gave 0.51 g (80%) of 15 identical with that from method A.

2-Methoxy-6-amino-9-(2-deoxy-β- and -α-D-*erythro*-pentofuranosyl)purine (11 and 16). Method A. To 12 ml of abs MeOH containing 0.45 g (0.0083 mole) of NaOMe was added 0.50 g (0.0013 mole) of 7 and the solution was refluxed for 15 hr while protected from moisture. The cooled solution was added to 50 ml of MeOH containing Amberlite IRC-50 (H^+) and stirred until neutral. The resin was removed by filtration, 3 g of neutral alumina was added to the filtrate, and this mixture was evaporated to dryness. The charged adsorbant was applied to the top of a column (25 g packed in $CHCl_3$) of neutral alumina and the column was washed with 200 ml of EtOAc. The product was eluted with 400 ml of EtOAc–MeOH (3:1) and this solution was evaporated to dryness. The residue was crystallized from 10 ml of EtOH at –20° in two crops to give 0.19 g (71%) of 2′-deoxyspongosine (11), mp 173–174.5°, $[\alpha]^{26}D$ – 17.0° (*c* 1, DMF). *Anal.* ($C_{11}H_{15}N_5O_4$) C, H, N.

Similar treatment of 0.30 g (0.00058 mole) of 9 and crystallization from 5 ml of Me_2CO gave 0.080 g (49%) of 16, mp 161–163°, $[\alpha]^{29}D$ + 78.6° (*c* 1.9, DMF). *Anal.* ($C_{11}H_{15}N_5O_4$) C, H, N.

Method B. To 10 ml of abs MeOH containing 0.27 g (0.005 mole) of NaOMe was added 0.26 g (0.0092 mole) of 15 and the resulting solution was refluxed for 18 hr with exclusion of moisture. The product 16 (0.12 g, 46%) was isolated and purified as in method A and was identical with that product.

2-Methylthio-6-amino-9-(2-deoxy-β- and -α-D-*erythro*-pentofuranosyl)purine (12 and 17). Method A. To a solution of 5 ml of MeSH in 15 ml of dry DMF cooled to –20° was added 0.23 g (0.010 g-atom) of Na. The reaction was allowed to warm to room temperature, by which time the Na was reacted, and 0.25 g (0.00087 mole) of 10 was added. The reaction was stirred for 6 hr at 55° with exclusion of moisture and was then neutralized with Amberlite IRC-50 (H^+) in MeOH. The resin was removed by filtration and 3 g of neutral alumina was added to the filtrate. This mixture was evaporated, and the charged adsorbant was applied to a column (20 g, packed in PhH) of neutral alumina. The column was washed with 200 ml of EtOAc–MeOH (95:5), and the wash discarded. The product was eluted with 400 ml of EtOAc–MeOH (3:1) and evaporation of this solution followed by crystallization of the residue from 15 ml of Me_2CO gave 0.13 g (50%) of 12, mp 194–196°, $[\alpha]^{29}D$ – 9.6° (*c* 1, DMF). *Anal.* ($C_{11}H_{15}N_5O_3S$) C, H, N.

When 0.29 g (0.0011 mole) of 15 was used in the above reaction, 0.19 g (62%) of crystalline 17, mp 181–183°, $[\alpha]^{28}D$ + 57° (*c* 1, H_2O) was obtained. *Anal.* ($C_{11}H_{15}N_5O_3S$) C, H, N.

Method B. Reaction of 7 or 9 by method A gave 58% of 12 or 49% of 17.

2-Hydrazino-6-amino-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-purine (18). To 20 ml of 97% anhydrous N_2H_4 was added 0.40 g (0.0014 mole) of 15. The reaction mixture was stoppered and allowed to stand for 18 hr at room temp. The reaction mixture was then evaporated, 1.4 ml of aqueous 1 *N* NaOH was added, and the residue was coevaporated several times with *i*-PrOH and crystallized from 10 ml of H_2O. The crystals of 18 (0.36 g, 86%), mp 205–207° with softening at 192–194°, $[\alpha]^{26}D$ + 81.3° (*c* 0.94, DMF), were dried over P_2O_5 and analyzed as the monohydrate. *Anal.* ($C_{10}H_{15}N_7O_3 \cdot H_2O$) C, H, N.

2,6-Diamino-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (19). A solution of 0.20 g (0.00071 mole) of 18 in 150 ml of EtOH–H_2O was hydrogenated at 45 psi for 17 hr over 0.5 g of Raney nickel. The catalyst was removed by filtration using a Celite pad and the filtrate was evaporated. The residue was crystallized from 5 ml of H_2O to give 0.093 g (49%) of 19, mp 234–236°, $[\alpha]^{30}D$ + 79.6° (*c* 0.79, H_2O). See ref 4 for comparable physical constants and ref 11 for data on a questionably pure product. *Anal.* ($C_{10}H_{14}N_6O_3$) C, H, N.

2,6-Diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (14). To 30 ml of 95% anhydrous N_2H_4 was added 0.395 g (0.00138 mole) of 10 and the stoppered reaction mixture was allowed to stand for 16 hr at room temp. The N_2H_4 was evaporated *in vacuo* (bath temp <35°); the residue was treated with 1.4 ml of aqueous 1 *N* NaOH and then coevaporated several times with *i*-PrOH. The resulting product, 13, was rather unstable and became colored upon further manipulation. Therefore, it was dissolved in MeOH–H_2O and hydrogenated at 45 psi for 16 hr at room temp over 1 g of Raney nickel. The mixture was filtered and 2 g of silica gel was added to the filtrate. This mixture was filtered and the charged adsorbant was applied to a dry packed silica gel column (50 g). The column was washed with 400 ml of EtOAc and 300 ml of EtOAc–MeOH (95:5), and the product was eluted with EtOAc–MeOH (9:1). The appropriate fractions were combined and evaporated, and the residue was crystallized from Me_2CO–MeOH to give 0.075 g (21%) of 14, mp 147–149°, $[\alpha]^{26}D$ – 39.2° (*c* 0.83, H_2O). See ref 11 for data on a questionably pure sample. *Anal.* ($C_{10}H_{14}N_6O_3$) C, H, N.

Biological Assays. The procedures used have been published previously.[20]

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Antitumor and Mutagenic Properties of a Variety of Heterocyclic Nitrogen and Sulfur Mustards†

Hugh J. Creech,* Robert K. Preston, Richard M. Peck, Anna P. O'Connell,

The Institute for Cancer Research, Philadelphia, Pennsylvania 19111

and Bruce N. Ames

Department of Biochemistry, the University of California, Berkeley, California 94720. *Received January 5, 1972*

Nitrogen and sulfur mustard derivatives of a variety of quinolines, acridines, azaacridines, benzacridines, and azabenzacridines have been synthesized for studies of the roles played by their polycyclic and alkylating components in the development of antitumor and mutagenic properties. For a display of exceptionally high activity in both the bis- and monoalkylating series of compounds, it appears that the polynuclear component must be an aromatic fused 3- or 4-ring system connected to the mustard moiety by an aminoalkyl linkage. Such nitrogen half-mustards with an ethyl substituent on the amino nitrogen containing the 2-chloroethyl group (the ICR 170 type) display a pronounced degree of activity against ascites tumors and are highly mutagenic for *Drosophila* and *Neurospora*, but not for *Salmonella*. Homologs in which the ethyl group is replaced by hydrogen to give a secondary amine (the ICR 191 type) are extremely potent frameshift mutagens for *Salmonella* and *Escherichia coli*, but not for *Drosophila* and *Neurospora*, and are relatively ineffective as antitumor agents. The sulfur mustard derivatives are active against ascites tumors, but rarely display mutagenic activity. It is considered that activity is imparted to the half-mustards because of the ability of an appropriate polynuclear component to intercalate into the nucleic acids of the ascites tumors or of the bacteria accompanied by the characteristic base alkylating reaction of the 2-chloroethyl group. The ability to exert a bifunctional action is apparently a necessary requirement for both antitumor and frameshift mutagenic activity.

It has been generally assumed[1] that the manifestation of antitumor activity by alkylating agents requires the presence of two alkylating groups, as in nitrogen mustard [methylbis(2-chloroethyl)amine], to permit cross-linking of two nucleophilic centers of biological macromolecules, *e.g.*, deoxyribonucleic acids. In our initial studies involving replacement of the methyl group of nitrogen mustard, it was discovered that compounds containing certain heterocycles joined through an aminoalkyl chain to the bisnitrogen mustard moiety were considerably more active on a molar basis than nitrogen mustard itself in tests with ascites tumor.[2,3] This demonstration that certain heterocyclic components have a potentiating influence on the nitrogen mustard moiety led us to synthesize a series of compounds containing a single 2-chloroethyl group (the nitrogen half-mustards).[4-6] Several of these compounds were found to be almost as active as their corresponding bisnitrogen mustards, thus indicating that certain heterocyclic nuclei are essentially equivalent to a 2-chloroethyl group in providing antitumor activity. Hence, some of the heterocyclic nitrogen half-mustards possess a mixed, or hybrid, bifunctionality. In many instances, treatment of mice bearing ascites tumors with these compounds caused at least a fivefold increase in survival time over that of the control mice. Since, in several long-term experiments, the tumors did not recur, it is clear that all of the ten million ascites tumor cells present in the mouse were destroyed by certain compounds. It was found that a definite degree of complexity is apparently required in the heterocyclic structure, namely, at least a 3-ring linear or angular structure, such as acridine or phenanthridine. In addition, the length of the aminoalkylamino side chain and the type of amine to which the 2-chloroethyl group is attached are important. It appears probable that the active heterocyclic nitrogen half-mustards exert their antitumor influence because of the intercalation of the heterocyclic component into the nucleic acids of the ascites tumor cells accompanied by the usual chemical reaction of the single 2-chloroethyl group. This view is supported by the finding

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