Diazo, Bromo, and Mesyloxy Ketones as Biological Alkylating Agents[1a]

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Thirteen diazo, bromo, and methanesulfonoxy ketones and diketones were synthetized from 3-indolylglyoxylic acid, indole-2-carboxylic acid, and mucic acid. Their *in vitro* alkylating activity was assessed by measurements of hydrolysis rate, reaction with thiosulfate and nitrobenzylpyridine. *In vivo* antitumor activity was investigated and compared with *in vitro* results. No practical cytostatic activity was found.

Partly due to some dissatisfaction with the practical cytostatic activity of nitrogen mustards, an increasing number of investigations deal with "nonclassical" alkylating agents. These are represented by methanesulfonates, halo ketones, diazo ketones, and nitrosomethylamines, *i.e.*, compounds generally capable of furnishing carbonium ions. The results are encouraging.

On the other hand, the activity of a cytostatic group may be greatly influenced by the carrier molecule. This concept, introduced first by Ing,[2] is being exploited to a great extent, with special emphasis on carrier molecules not cell alien. However, little attention has been paid to the preparation of series of compounds with varying alkylating groups but identical carriers.[3]

There are few papers dealing with indole as a carrier molecule. Elderfield and Wood[4] described indole mustards and Diugiud, *et al.*,[5a] dealt with similar compounds, but without publishing pharmacological data. Only Goodman and DeGraw[5b] describe indole mustards together with their pharmacology.

A number of contradictory papers deal with the activity of serotonin and indolic acids on malignant cells. Pukhalskaya[6a–c] claims an inhibitory action of serotonin base on various tumors (*e.g.*, 97% at 10 mg./kg. on Jensen sarcoma). Araya, *et al.*,[7] on the other hand, state that serotonin accelerates the rate of growth of Ehrlich ascites tumor, while Jenkins[8] disclaims any effect in the same system. Moreover, Scott[9] describes the inhibitory action of serotonin *antagonists* on ascites tumors. Ethyl 3-indolylacetate is highly toxic for Ehrlich ascites, while the corresponding acid prolongs the *in vitro* survival of these cells.[10] The situation is very confusing; one may say only that there is activity of some kind shown by indole compounds on malignant cells.

Carbohydrate carriers were used extensively in cancer chemotherapy.[11–13] Diazo ketones derived from aldaric acids are known from the work of Wolfrom[14] and Vargha,[15] and hexitol mesylates are well known and used clinically.

Synthesis.—We undertook to synthetize a series of diazo, bromo, and mesyloxy ketones derived from indole and 5-methoxyindole. The properties of these compounds are shown in Table I.

Although the compounds are mostly new, their synthesis was carried out by conventional methods. The acid chlorides were treated with diazomethane, and the diazo ketones so obtained decomposed with HBr or methanesulfonic acid in aprotic solvents. The amount of acid used is very critical, since an excess leads to the formation of intractable tars.

The 1,8-disubstituted 2,7-dioxo-3,4,5,6-galactotetraacetoxyoctane derivatives, listed in Table II, were prepared from tetraacetylmucic acid[16] through the acid chloride.[17] The known[14] 1,8-bisdiazo compound X is obtained easily and reacts smoothly with HBr and methanesulfonic acid.

The deacetylation of this compound proved to be more difficult. The standard procedure[18] with 0.1% sodium methoxide in methanol suspension gave unchanged starting material only. With higher concentrations (1%) deacetylation occurred but failed again in larger batches. Finally, the method chosen consisted in adding the acetoxydiazo ketone slowly to a stirred 0.33% $NaOCH_3$ solution, giving the diazo ketone XIII in a reasonable yield. However, no bromo or mesyloxy ketone could be obtained from this compound by the standard procedures. The attempted deacetylation of the bromo and mesyloxy compounds XI and XII was equally unsuccessful.

In vivo **Cytostatic Activity.**—All the compounds were tested for antitumor activity on Sarcoma 180 and lymphatic leukemia L1210 by standard CCNSC pro-

(1) (a) Presented at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964. (b) To whom all communications should be addressed.

(2) H. R. Ing, *Trans. Faraday Soc.*, **39**, 372 (1943).

(3) R. C. Elderfield, R. N. Prasad, and T.-K. Liao, *J. Org. Chem.*, **27**, 573 (1962).

(4) R. Elderfield and J. R. Wood, *J. Org. Chem.*, **27**, 2463 (1962).

(5) (a) L. I. Diugiud, E. Dawson, and C. L. Thomas, Abstracts of Papers, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963, p. 12-O; (b) J. DeGraw and L. Goodman, *J. Med. Chem.*, **7**, 213 (1964).

(6) (a) E. C. Pukhalskaya, M. F. Petrova, and G. P. Menshikov, *Byul. Eksperim. Biol. i Med.*, **50**, 105 (1960); *Cancer Chemotheray Abstr.*, **2**, 35 (1961); *Chem. Abstr.*, **55**, 13657 (1961); (b) E. C. Pukhalskaya, *Vestn. Akad. Med. Nauk SSSR.*, **12**, 61 (1960); (c) E. C. Pukhalskaya, Abstracts of Papers, VIIIth International Cancer Congress, Moscow; Medgiz Publishing House, 1962, p. 335; *Cancer Chemotherapy Abstr.*, **3**, 3068 (1962).

(7) H. Araya, A. Nakagawa, and T. Munakata, *Folia Pharmacol. Japan*, **58**, 138 (1962); *Cancer Chemotherapy Abstr.*, **3**, 4393 (1962).

(8) S. G. Jenkins, R. Zeppa, and C. G. Thompson, *Surg. Forum*, **13**, 71 (1962); *Cancer Chemotherapy Abstr.*, **4**, 35 (1963).

(9) K. G. Scott, *Ann. N. Y. Acad. Sci.*, **103**, 285 (1963).

(10) R. D. Schultz and D. Norman, *Nature*, **198**, 553 (1963).

(11) L. Vargha, Ö. Fehér, and S. Lendvai, *Acta Chim. Acad. Sci. Hung.*, **19**, 307 (1959); *Chem. Abstr.*, **54**, 3212 (1960).

(12) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2821 (1961).

(13) (a) T. Nogrady, *ibid.*, **26**, 4177 (1961); (b) K. M. Vagi, V. W. Adamkiewicz, and T. Nogrady, *Can. J. Chem.*, **40**, 1049 (1962).

(14) M. L. Wolfrom, S. W. Waisbrot, and R. L. Brown, *J. Am. Chem. Soc.*, **64**, 2329 (1942).

(15) L. Vargha, L. Toldy, Ö. Fehér, T. Horváth, E. Kasztreiner, J. Kuszman, and S. Lendvai, *Acta Physiol. Acad. Sci. Hung.*, **19**, 305 (1961); *Chem. Abstr.*, **60**, 9794 (1964).

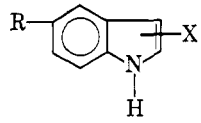
(16) M. Adelman and J. G. Beckenridge, *Can. J. Res.*, **24B**, 297 (1946).

(17) O. Diels and F. Löflund, *Ber.*, **47**, 2351 (1914).

(18) L. Vargha, personal communication; *cf.* also ref. 15.

TABLE I

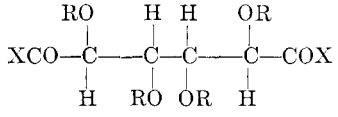
INDOLE DERIVATIVES



No.	R	X	M.p., °C.	Formula		% calcd.	% found	Infrared, μ NH	N≡N	C=O	$ROSO_2CH_3$
I	H	3-COCOCHN$_2$	148–150 dec.	$C_{11}H_7N_3O_2$	N:	19.71	19.52	3.08 s	4.70 s	6.30 s	...
II	H	3-COCOCH$_2$Br	148–149	$C_{11}H_8BrNO_2$	Br:	30.04	30.29	3.07 s	...	5.80 w	...
										6.25 s	
III	H	3-COCOCH$_2$OSO$_2$CH$_3$	197–198	$C_{12}H_{11}NO_5S$	S:	11.40	11.16	3.06 s	...	5.78 m	7.38 s
										6.29 s	8.58 s
IV	CH_3O	3-COCOCHN$_2$	150 dec.	$C_{12}H_9N_3O_3$	N:	17.35	17.54	3.10 s	4.72 s	6.42 s	...
V	CH_3O	3-COCOCH$_2$Br	177–178	$C_{12}H_{10}BrNO_3$	Br:	26.99	27.20	3.05 s	...	5.82 w	...
									...	6.30 s?	...
VI	CH_3O	3-COCOCH$_2$OSO$_2$CH$_3$	205–206 dec.	$C_{13}H_{13}NO_6S$	S:	10.31	10.70	3.10 s	...	5.80 w	7.39 s
									...	6.37 s?	8.60 s
VII	H	2-COCHN$_2$	166–167 dec.	$C_{10}H_7N_3O$	N:	22.69	22.51	3.07 s	4.77 s	6.05 m	...
VIII	H	2-COCH$_2$Br	130–131	$C_{10}H_8BrNO$	Br:	33.56	33.36	3.02 s	...	6.09 s	...
IX	H	2-COCH$_2$OSO$_2$CH$_3$	148–150	$C_{11}H_{11}NO_4S$	S:	12.66	12.62	2.98 s	...	5.98 s	7.45 s
											8.55 s

TABLE II

MUCIC ACID DERIVATIVES



No.	R	X	M.p., °C.	Formula		% calcd.	% found	Infrared, μ N≡N	C=O	$ROSO_2CH_3$
X	CH_3CO	CHN_2	180–181 dec.	$C_{16}H_{18}N_4O_{10}$	N:	13.14	12.95	4.72 s	5.72 s	...
XI	CH_3CO	CH_2Br	164–166	$C_{16}H_{20}Br_2O_{10}$	Br:	30.04	30.08	...	5.75 s	...
XII	CH_3CO	$CH_2OSO_2CH_3$	193–194	$C_{18}H_{26}O_{16}S_2$	S:	11.41	11.31	...	5.75 s	7.35 s
										8.35 s
XIII	H	CHN_2	163–165 dec.	$C_8H_{10}N_4O_6$	N:	21.72	21.30	4.72 s	6.20 s	...

cedures.[19] In addition compounds I–III were screened for *in vitro* cytostatic activity on He-La cell cultures. Results for the indoles are shown in Table III. All compounds were inactive *in vivo* at near-toxic doses. *In vitro* cytostatic activity was shown only by the bromo ketone II at 1 g./ml.

TABLE III

In Vivo AND *in Vitro* ACTIVITY OF INDOLE DERIVATIVES

No.	Tumor inhib. T/C	He-La inhib., γ/ml.	$k_{H_2O} \times 10^{-4}$ M sec.$^{-1}$ (°C.)	$k_{S_2O_3} \times 10^{-4}$ M sec.$^{-1}$ (°C.)	50% T with nitrobenzylpyridine, 10^{-4} M
I	0	10	2.3 (20)	410 (20)	<0.01
II	0	1	8.9 (20)	No reaction	0.81
III	0	10	9.0 (50)	3.3 (50)	0.07
IV	0	..			0.01
V	0	..			1.10
VI	0	..			<0.01
VII	0	..			<0.01
VIII	0	..			2.30
IX	0	..			0.33

The bromo ketone VIII was found to have a weak muscle relaxant activity at the 300-mg./kg. dose level, but this did not justify further tests.

(19) J. Leiter, B. J. Abbot, and S. A. Schepartz, *Cancer Res.*, **25**, 207 (1965).

The mucic acid derivatives are shown on Table IV. Compounds X and XII had a limited activity on S180 (T/C is 0.73 and 0.66, respectively, which is usually regarded as not statistically significant on this tumor) and no activity on L1210 at toxic levels (250 mg./kg.). Vargha, *et al.*,[15] reported on average activity of T/C = 0.5 for XIII on the Yoshida, Ehrlich ascites, and Guerin tumors at 100 mg./kg. In our test on S180 and L1210 the compound was inactive in doses as high as 250 mg./kg.

TABLE IV

In Vivo AND *in Vitro* ACTIVITY OF MUCIC ACID DERIVATIVES

No.	Tumor inhib. T/C	$k_{H_2O} \times 10^{-4}$ M sec.$^{-1}$ (°C.)	$k_{S_2O_3} \times 10^{-4}$ M sec.$^{-1}$ (°C.)	50% T with nitrobenzylpyridine, 10^{-4} M
X	0.73	0.9 (20)	0.9 (20)	0.02
		29.0 (66)	29.0 (66)	
XI	0	2.7 (50)	2.8 (50)	0.25
XII	0.66	7.8 (5)	3.7 (50)	0.12
		92.0 (20)		
XIII	0.50	280.0 (20)	200.0 (20)	0.11

In Vitro **Alkylating Activity.**—The search for cytostatic agents is still very empirical. To put it on a more secure basis, comparison of biological activity and alkylating potency is desirable. In biological alkylations oxygen-, nitrogen-, and sulfur-containing

functional groups of proteins and DNA are involved. Compounds reacting by an SN2 mechanism will react fast and are likely to be "mopped up" in irrelevant reactions on their way to the target cells. In SN1 reactions the rate-determining step is the slow ionization. Compounds reacting in this way will be able to diffuse with relatively little change and react mainly in the malignant cell. Therefore, the relative reactivity of potential cytotoxic agents with various nucleophiles and with water can be utilized to predict their possible *in vivo* fate. These ideas were reviewed recently by Ross[20] and by Warwick.[21]

A well-studied example[22] is the following hydrolysis of a diazo ketone. The hydronium ion catalyzed pre-

$$RCOCHN_2 + H_3O^+ \rightleftharpoons RCO\overset{+}{C}H_2N_2 + H_2O$$

$$RCO\overset{+}{C}H_2N_2 \longrightarrow RCOCH_2^+ + N_2$$

$$RCOCH_2^+ + H_2O \longrightarrow RCOCH_2OH + H^+$$

equilibrium protonation will furnish the diazonium ion, which then decomposes in the rate-determining step to a carbonium ion. This is followed by a fast reaction with any nucleophile, in this case water. The reaction is usually pseudo first order and follows the A1 mechanism of Ingold.

We determined hydrolysis rates and rates of reaction with thiosulfate for compounds I–III and X–XIII only. N-Alkylation was measured on all compounds, using the method of Epstein.[23] The data obtained for nitrogen do not represent direct reaction rates, but simply concentrations which give a 50% transmittance at 560 mμ of the dye formed with 4-(4-nitrobenzyl)-pyridine.

The results for indoles are summarized in Table III. As already mentioned, none of the compounds show *in vivo* activity. All the diazo ketones react poorly with the pyridine derivative (N-alkylation), while the bromo ketones are active in this respect. The diazo ketone I reacts at a markedly fast rate with thiosulfate (S-alkylation) but hydrolyzes very slowly.

The mucic acid derivatives in Table IV give more significant results. Here again, the bromo ketone is the most active as an N-alkylating agent, but a very poor S- and O-alkylator. The acetylated diazo ketone and mesyloxy ketone are moderately active in every respect. The deacetylated diazo ketone, however, shows a very high rate of reaction with *both* water and thiosulfate, in the order of 10^{-2} moles l./sec. and is the only compound showing reasonable biological activity in some tumors. This seems to indicate that diazo ketones are primarily OH and SH reagents. The environment of the functional group is crucial, and electron donor groups seem to be important.

It is realized that a much more extensive series of compounds would be necessary to make any general statements. Also, the role of stereochemical factors ought to be investigated in the same way as has been done for other carbohydrate cytostatics.

(20) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co., London, 1962.

(21) G. P. Warwick, *Cancer Res.*, **23**, 1315 (1963).

(22) H. Dahn and H. Gold, *Helv. Chim. Acta*, **46**, 983 (1963).

(23) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).

Experimental

All melting points were taken in capillaries in a Gallenkamp aluminum block and are corrected. Infrared spectra were recorded on a Beckman IR-8 instrument in KBr disks. Analyses were performed by Dr. C. Daessle, Montreal.

Indole-2-carbonyl Chloride.—Indole-2-carboxylic acid (1.60 g.) was suspended in 20 ml. of dry ether and 20 ml. of acetyl chloride, and 2.25 g. of PCl_5 was added. The acid dissolved at room temperature in 10 min. The solution was refluxed for 1 hr. and evaporated under reduced pressure, leaving 1.75 g. (97%) of yellow crystals. Recrystallized from heptane, it melted at 107–108°. (Kermack, *et al.*,[24] and Matell[25] do not give a definite melting point.)

Diazo Ketones I, IV, and VII.—The appropriate indolylglyoxylyl chlorides[26,27] or indole-2-carbonyl chloride were finely ground and added as solids to an ethereal solution of 4 moles of diazomethane at 0° with stirring. The solids dissolved immediately with brisk N_2 evolution, and the diazo ketones crystallized overnight in yields of 95, 98, and 75%, respectively. I and IV were crystallized from methanol–water, VII from dioxane–water.[25]

Bromo Ketones II, V, and VIII.—The diazo ketones were suspended in ether and cooled to 0°, and dry HBr gas was bubbled in very slowly until the N_2 evolution ceased. The pH was checked from time to time and not allowed to fall below 4. The red solution was treated with Norit and evaporated at room temperature under reduced pressure. The crude products were recrystallized from benzene (II) or benzene–petroleum ether (V and VIII). Yields were 96, 61, and 70%, respectively. All the compounds are light sensitive.

Methanesulfonates III and IX.—The diazo ketones were dissolved in dry ether (130 and 20 vol.) and the calculated amount of methanesulfonic acid, diluted with 20 vol. of dry ether, was added rapidly. Gas evolution was vigorous and immediate. The solution was left at room temperature, and some dark precipitate, formed initially, was removed by treatment with Norit and filtration. The clear yellow solution deposited crystals in the case of III in a yield of 45%, which was recrystallized from 1-butanol. IX was obtained by precipitation with petroleum ether in a yield of 48%. It was recrystallized from benzene. Both compounds are light sensitive.

Methanesulfonate VI was prepared as described above, in 20 vol. of dioxane, being kept at room temperature for 3 hr. The crystallization was completed by precipitation with petroleum ether, and the dark compound was recrystallized from toluene; yield 80%.

1,8-Dibromo-2,7-dioxo-3,4,5,6-galactotetraacetoxyoctane (XI).—To a solution of 1.7 g. of the 1,8-bisdiazo derivative[14] in 17 ml. of dioxane was added 0.50 ml. of 48% aqueous HBr. There was a vigorous gas evolution at room temperature. The solution was heated for 5 min. on the steam bath and evaporated under reduced pressure. The solid residue was suspended in water, filtered, washed with water and methanol, and finally recrystallized from 1-butanol. The yield was 1.80 g. (84.1%).

1,8-Dimethanesulfonoxy-2,7-dioxo-3,4,5,6-galactotetraacetoxyoctane (XII).—To a solution of 1.7 g. of bisdiazo compound in 17 ml. of dioxane was added a solution of 0.66 ml. of methanesulfonic acid in 2 ml. of dioxane. The solution was heated on the steam bath for 30 min. and diluted with water. The precipitated crystals were filtered off, washed with water, and recrystallized from 2-pentanone; yield 1.66 g. (74.1%).

1,8-Bisdiazo-2,7-dioxo-3,4,5,6-galactotetrahydroxyoctane (XIII).—To a stirred solution of 4 ml. of 2% sodium methoxide and 20 ml. of dry methanol, 1.0 g. of the finely powdered tetraacetate X was added in portions over 10 min. The suspension was stirred at room temperature for 30 min. more, filtered, and washed with methanol. Colorless crystals (0.35 g., 58.0%) were obtained, m.p. 163–165° dec. The melting point was unchanged after recrystallization from water.

(24) W. O. Kermack, W. H. Perkin, and R. Robinson, *J. Chem. Soc.*, 1602 (1921).

(25) The diazoketone I is mentioned by F. Lingus and H. Hellmann [*Angew. Chem.*, **69**, 97 (1957)] without physical data, and VII by M. Matell [*Arkiv Kemi*, **10**, 179 (1956)] with a wide melting range.

(26) M. E. Speeter and W. C. Anthony, *J. Am. Chem. Soc.*, **76**, 6208 (1954).

(27) J. A. Ballantyne, C. B. Barrett, R. J. S. Beer, B. G. Boggiano, K. Clarke, S. Eardley, B. E. Jennings, and A. Robertson, *J. Chem. Soc.*, 2222 (1957).

Reaction Rate Measurements. Diazo Ketones. Hydrolysis.—The substance (1 mmole) was dissolved in 10 ml. of dioxane and placed into an erlenmeyer flask equipped with a side arm (leading to a gas buret) and a separatory funnel with a pressure-equalizing connection. Sulfuric acid (9.5 ml. of 14% w./w., 13 mmoles, final concentration 3 *N*) was run in within 1–2 sec., with rapid magnetic stirring. The temperature of the solution was kept constant within ±1°, and the evolved N_2 was plotted against time.

Thiosulfate.—The reaction was run as in the hydrolysis experiments, but 10 mmoles of $Na_2S_2O_3 \cdot 5H_2O$/mmole of diazo group, dissolved in 3 ml. of water, was added prior to the addition of the acid. The very fine precipitate of thiosulfate dissolved immediately on addition of the acid. The evolved N_2 was measured as a function of time.

Bromo Ketones. Hydrolysis.—Since no Br^- could be detected on reaction with water, the hydrolysis was run in the presence of NaOH. The substance (0.5 mmole for difunctional compounds or 1 mmole) was dissolved in 10 ml. of tetrahydrofuran, and 10 ml. of 0.1 *N* NaOH and 20 ml. of water were added. Six to eight identical samples were prepared and were kept in the same constant temperature bath, being titrated with 0.1 *N* HCl against methyl orange after suitable time intervals.

Thiosulfate.—The substance (0.5 or 1 mmole) in 5 ml. of acetone and 10 ml. of 0.1 *N* $Na_2S_2O_3$ was kept at constant temperature, and each sample was titrated with standard iodine solution using starch indicator.

Mesyloxy Ketones. Hydrolysis was measured in the same way as in the case of the bromo ketones.

Thiosulfate was measured as with bromo ketones, but acetone or acetic acid was used as the solvent.

Calculations.—All the reaction rate data fitted a first-order plot well. The rate constants were calculated from half-times, according to the equation $k = (\ln 2)/t_{1/2}$.

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3′-Deoxynucleosides. II. Purine 3′-Deoxynucleosides

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The 9-(3-deoxy-β-D-ribofuranosides) of adenine, 2,6-diaminopurine, purine, purine-6-thiol, 6-methylaminopurine, 6-ethylaminopurine, and 6-dimethylaminopurine have been synthesized in order to compare their properties in certain biological systems.

3′-Deoxyadenosine (cordycepin),[1] an inhibitor of the growth of KB cells in culture,[1] *B. subtilis*,[2] an avian tubercle bacillus,[2] and Ehrlich ascites carcinoma[3] in mice, has more recently been shown[4] to be a potent inhibitor of RNA synthesis.

In a recent communication[5] we reported a brief description of a synthesis of 3′-deoxyadenosine. This synthetic scheme was designed to permit the synthesis of large amounts of 3′-deoxyadenosine as well as to supply a synthetic approach to analogs of 3′-deoxyadenosine which might have interesting biological properties.

The present paper describes the detailed experimental procedure for this synthesis of 3′-deoxyadenosine as well as the synthesis of 3′-deoxyadenosine-8-C^{14} by minor modifications of these procedures. In addition, six new related purine 3′-deoxynucleosides have been prepared to permit a comparison of some of their biological properties with those of cordycepin.

It is known[6a,b] that 3′-deoxyadenosine is metabolized *via* deamination to 3′-deoxyinosine. 3′-Deoxyinosine is not an inhibitor of cell growth nor is it an inhibitor of RNA synthesis. It seemed desirable, therefore, to synthesize compounds which would not be inactivated by deamination[6c] and which might retain the inhibitory properties of 3′-deoxyadenosine. It has been reported[6a,7] that, although 2′deoxyadenosine is deaminated by adenosine deaminase, 6-N-methyl-2′-deoxyadenosine is not a substrate for this enzyme; moreover, it is actually an inhibitor of adenosine deaminase. On the other hand, it has been demonstrated that 6-methylaminopurine is demethylaminated to hypoxanthine and methylamine by both bacterial[8a] and mammalian[8b,c] cells, and that 6-dimethylaminopurine is resistant to a similar enzymatic degradation. Information concerning the stability of 6-ethylaminopurine in these systems does not appear to be available. In the light of these findings, 6-methylamino-, 6-dimethylamino-, and 6-ethylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**4**, **5**, and **6**) were synthesized. Both the 6-N-methyl and 6-N-ethyl derivatives retain a proton on the 6-nitrogen of the adenine moiety, a desirable feature if the biological activity of 3′-deoxyadenosine depends on hydrogen bonding with enzymes at this position. The similarity of the dimethyl derivative

(1) E. A. Kaczka, E. L. Dulaney, C. O. Gitterman, H. B. Woodruff, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 452 (1964).

(2) K. G. Cunningham, S. A. Hutchinson, W. Manson, and F. S. Spring, *J. Chem. Soc.*, 2299 (1951).

(3) D. V. Jagger, N. M. Kredich, and A. J. Gaurino, *Cancer Res.*, **21**, 216 (1961).

(4) (a) H. Klenow and S. Frederickson, Abstracts, the VIth International Congress of Biochemistry, New York, N. Y., July 1964, p. 66; (b) H. T. Shigeura and C. N. Gordon, *J. Biol. Chem.*, **240**, 806 (1965).

(5) E. Walton, R. F. Nutt, S. R. Jenkins, and F. W. Holly, *J. Am. Chem. Soc.*, **86**, 2952 (1964).

(6) (a) H. Klenow, *Biochem. Biophys. Acta*, **76**, 347 (1963). (b) G. Weinbaum, J. C. Cory, R. J. Suhadolnik, P. Meyers, and M. A. Rich, ref. 4a, Abstract No. IV-195, p. 343. (c) Studies of the effects of structural modifications on the activity of variously substituted purines as inhibitors and substrates of adenosine deaminase have been reported. See, for example, H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965), as well as G. A. LePage and I. G. Junga, *Cancer Res.*, **25**, 46 (1965).

(7) A. Coddington and M. Bagger-Sorensen, *Biochim. Biophys. Acta*, **72**, 598 (1963).

(8) (a) C. H. Reny, *J. Biol. Chem.*, **236**, 2999 (1961); (b) D. E. Duggan and E. Titus, *Biochim. Biophys. Acta*, **55**, 273 (1962); (c) C. E. Jamison, J. W. Huff, and M. P. Gordon, *Cancer Res.*, **22**, 1256 (1962).