Carbamates Derived from Aminopurines[1,2]

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Adenine was acylated by ethyl chloroformate and by ethyl chlorothiolformate not at the 6-amino group, but at the 9 (or 7) nitrogen atom. 9-Methyladenine was unreactive to ethyl chloroformate and to diethyl pyrocarbonate, although the pyrimidine analog 4-aminopyrimidine was acylated by the pyrocarbonate. In attempts to prepare a 6-carbamate of 9-methylpurine the 6-iodo-, 6-cyano-, 6-carboxamide and 6-isopropylidene carbohydrazide derivatives of 9-methylpurine were prepared. The hydrazide was not convertible to an azide or to a carbamate. The 6-carboazide of the unmethylated purine was easily converted to 6-N-(*n*-butoxycarbamoyl)purine.

The purpose of this work was to prepare purinyl carbamates as possible inhibitors of cancerous growth and to add to the existing information on acylation of adenine. Ethyl carbamate has some activity[3] in retarding leukemia, and purines, such as 6-mercaptopurine,[4] are useful antimetabolites. No purinyl carbamates were described in detail until 6-N-(ethoxycarbamoyl)purine and 6-N-(methoxycarbamoyl)purine were reported[5] after the present work was started.

Treatment of adenine with ethyl chloroformate gave a compound (I) which was different from the 6-N-(ethoxycarbamoyl)purine of Giner-Sorolla and Bendich.[5] Compound I decomposed at 184–185°, the previously described compound at 225–230°. Differences in ultraviolet absorption are shown in Table I. Hence acylation of adenine by ethyl chloroformate did not occur at the 6-position; the most probable places of attack are the 7- or 9-positions.

A definite choice between the 7- and 9-positions cannot be made at the present time, although the 9-position is preferred because the ultraviolet absorption of compound I in acid (λ_{max} 258) is closer to that of 9-methyladenine (λ_{max} 261) than to that of 7-methyladenine (λ_{max}, 272).[6] Attempts were made to determine the position of the carboxylate group in compound I by reduction with lithium aluminum hydride, which was expected to give the known 9- or 7-methyladenine. However, these products were not obtained.

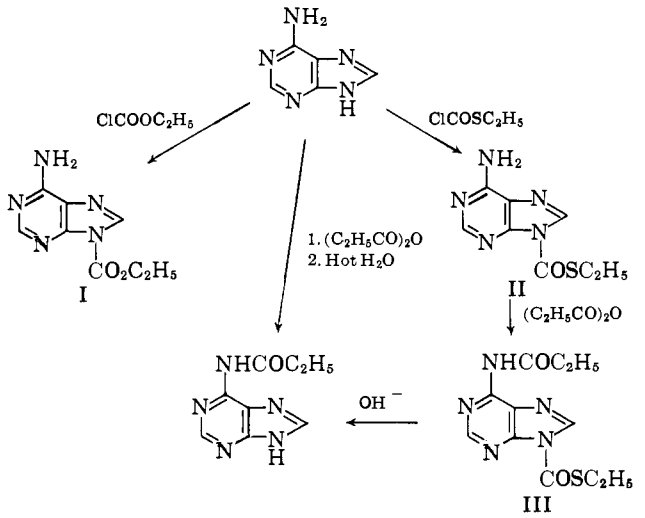


Table I

Ultraviolet Absorption and Chromatographic Data

	pH	λ_{max}	$E_m \times 10^{-3}$	R_f cpd./R_f adenine A[a]	B[b]
A. Purine Derivatives					
Ethyl 6-amino-9(7) carboxylate	0.13	258	10.2	1.15	2.51
	7.07	254	10.2		
6-N-(ethoxycarbamoyl)[c]	0.13	274	15.6		
	7.07	273	13.3		
Adenine[d]	1	262	13.8	1.00	1.00
	7.04	261	12.9		
6-N-(*n*-Butoxycarbamoyl)-	1	274	15.2		
	13	282	12.2		
S-Ethyl 6-amino-9(7)carbothioate	1	252	16.6	1.71	2.66
6-Iodo-9-methyl-	1	275	10.4	1.39	3.18
	7	275	11.0		
	13	275	10.2		
6-Cyano-9-methyl-	1	290	9.00	1.64	3.14
	7	289	8.85		
	13	267	7.07		
6-Iodo-2-methyl-	1	279	9.63	1.40	3.34
	7	278	9.53		
	13	283	8.30		
6-Cyano-2-methyl-	1	296	8.03	1.40	3.43
	7	296	7.60		
	13	296	7.00		
9-Methyl-6-carboxamide	1	277	8.25	1.00	1.25
	7	282	7.50		
	13	282	7.77		
Isopropylidene-(9-methyl-6)-carbohydrazide	1	282	8.15		
	7	281	8.45		
B. Pyrimidine Derivatives					
4-Amino-2-methyl-5-nitro-6-(1H)-pyrimidinone	1	327	8.40	0.92	1.35
	7	327	8.50		
4-Benzylamino-6-chloro-5-nitropyrimidine				1.52	3.30

[a] Solvent A, 1-butanol and 5 *N* acetic acid, 2:1 (vol.:vol.). [b] Solvent B, 1-butanol, formic acid and water, 77:10:13. [c] See ref. 5. [d] See ref. 25.

Treatment of adenine with ethyl chlorothiolformate gave the S-ethyl ester of 6-aminopurine-9(or 7)-carbothioic acid (II). Proof that acylation did not occur at the 6-position was obtained by the reaction of II with propionic anhydride to give III, which on careful hy-

(1) This investigation was supported by PHS Grant No. CY-3477 from the National Cancer Institute, Public Health Service.

(2) From the Ph.D. Thesis of John M. Reitz, University of Delaware, 1962.

(3) A. Haddow and W. A. Sexton, *Nature*, **157**, 500 (1946).

(4) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

(5) A. Giner-Sorolla and A. Bendich, *ibid.*, **80**, 3932 (1958).

(6) G. B. Elion in Ciba Foundation Symposium, "Chemistry and Biology of Purines," ed. by G. E. W. Wolstenholme and C. M. O'Connor, Little Brown and Co., Boston, 1957, p. 46.

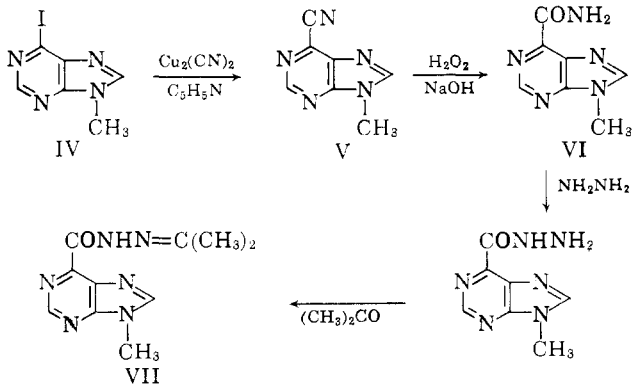
drolysis formed the known 6-(N-propionyl)adenine,[7] prepared from adenine.

Compounds I and II are among the first acylation products of adenine in which substitution did not occur at position 6. Acylation with acetic or benzoic anhydride[8] gives the 6-acetyl and 6-benzoyl derivatives, the structure of which was proved[9] by hydrogenation to 6-ethyl- and 6-benzyladenine. Diacylation products have been obtained[7] from acetic, propionic and butyric anhydrides in which one group is at the 6-position and the other in an unknown location. Very recently Altman and Ben-Ishai reported[10] that the acylation of adenine with benzoyl chloride under Schotten-Baumann conditions gave two monobenzoyladenines, neither of which was identical with the known 6-benzamidopurine. These were postulated to be 7- and 9-acylation products. Similar products were obtained with benzyl chloroformates. These results are parallel to those of the present work, except that only one isomer was obtained with ethyl chloroformate and with ethyl chlorothiolformate.

Compound I was rapidly hydrolyzed by dilute base at room temperature to give adenine and carbonate ions. Compound II was also hydrolyzed to adenine, but much more slowly because of its slight solubility at room temperature.

Attempts were made to prepare a 9-methyl derivative of 6-N-(ethoxycarbamoyl)purine by treating 9-methyladenine with ethyl chloroformate. No reaction occurred under a variety of conditions. Moreover, diethyl pyrocarbonate did not give the carbamate, although it reacted with 4-aminopyrimidine to form the known[11] 4-N-(ethoxycarbamoyl)pyrimidine in 54% yield. The ability of the pyrocarbonic ester to convert amino compounds to carbamates has been largely overlooked since the first preparation and use of the ester by Boehm and Mehta.[12]

The preparation of a carbamate derived from 9-methyladenine was also attempted through the following sequence of reactions



6-Iodo-9-methylpurine (IV) was converted to the cyano derivative (V) by the method[13] used for the unmethylated iodopurine. Conversion to the amide (VI) was practically quantitative with alkaline hydrogen peroxide. When VI was treated with hydrazine, the hydrazide could not be isolated, although its condensation product with acetone (VII) was obtained. Attempts to convert the hydrazide or its isopropylidene derivative (VII) to the azide to complete the Curtius synthesis were unsuccessful.

Although not completed for derivatives of 9-methyl purine, the azide synthesis was satisfactory for the unsubstituted purine. 6-Purinylcarboazide[5] reacted smoothly with *n*-butyl alcohol to give 6-N-(*n*-butoxycarbamoyl)purine (VIII), analogous to the reaction products of the azide with methanol and ethanol.[5]

Another possible method for securing carbamates might be the reaction of 6-halopurines with a metallic cyanate, since this halogen atom is displaced by various nucleophilic reagents. However, when 6-iodopurine was heated with potassium cyanate or silver cyanate in various solvents, including tetrahydrofuran and pyridine, and the mixture subsequently treated with ethanol, no carbamate was obtained.

New compounds which were made with a view to further syntheses were 6-iodo-2-methylpurine (IX), 6-cyano-2-methylpurine (X), 4-amino-2-methyl-5-nitro-6-(1H)pyrimidinone (XI), and 4-benzylamino-6-chloro-5-nitropyrimidine (XII).

Pharmacological Tests.[14]—Toward the KB cell culture compound I showed slight activity (ED_{50} 4.7 μg./ml. in dimethylformamide), but II was inactive. Toward Adenocarcinoma 755, I did not show significant activity (T/C 56 at 25 mg./kg.). II showed activity, T/C 18 at 100 mg./kg. after daily injection in methylcellulose for 11 days, with 9 out of 10 survivors. Toward Sarcoma 180 and toward Lymphoid Leukemia L-1210, II was inactive.

Experimental[15]

Ethyl 6-Aminopurine-9(or 7)-carboxylate (I).— A solution of 266 mg. (2 mmoles) of adenine in 0.41 ml. of 4.811 *N* sodium hydroxide and 3.36 ml. of water was added dropwise to a stirred and ice-cooled 0.20 ml. portion (2 mmoles) of ethyl chloroformate. After stirring for 1 hr. longer, the reaction mixture was checked for neutrality and the precipitated product was filtered and washed with cold water. The product consisted of 293 mg. (72% yield) of a white powder, dec. 177°, which, after two crystallizations from tetrahydrofuran, decomposed at 184–185°. Paper chromatography from two different solvent systems (Table I) gave only one spot. The same compound was obtained by treatment of adenine with ethyl chloroformate in dimethyl formamide in the presence of potassium carbonate.[16]

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.80. Found: C, 46.61; H, 4.62; N, 33.56.

By treatment with 2 equivalents of base at room temperature the 9-carbethoxyadenine was rapidly decomposed to adenine and sodium carbonate. The adenine, obtained in 70% yield, was identified by mixture melting points with specimens of the free base and its picrate.

S-Ethyl 6-Aminopurine-9(or 7)-carbothioate (II).—This was prepared from adenine and ethyl chlorothiolformate[17] in the same way as the oxygen analog (I). The crude product (45% yield) was recrystallized from purified tetrahydrofuran or ethyl acetate to give needles which decomposed slowly above 250° to a yellow solid.

(7) L. Birkofer, *Ber.*, **76**, 769 (1943).
(8) A. Kossel, *Z. Physiol. Chem.*, **12**, 246 (1888).
(9) H. Lettré and H. Ballweg, *Chem. Ber.*, **91**, 345 (1958).
(10) I. Altman and D. Ben-Ishai, *Bull. Res. Council Israel, Sect. A*, **11**, 4 (1962).
(11) E. Dyer, M. L. Gluntz, and E. J. Tanck, *J. Org. Chem.*, **27**, 982 (1962).
(12) T. Boehm and D. Mehta, *Ber.*, **71**, 1797 (1938).
(13) L. B. Mackay and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 3511 1956).
(14) Carried out by the Cancer Chemotherapy National Service Center.
(15) Melting and decomposition points are corrected, taken in a silicon oil bath.
(16) H. S. Bender, Ph.D. Thesis, University of Delaware, 1962, p. 66.
(17) Kindly supplied by Stauffer Chemical Co.

Anal. Calcd. for $C_8H_9N_5OS$: C, 43.04; H, 4.06; S, 14.36. Found: C, 43.11; H, 3.83; S, 13.73.

The compound was easily soluble at room temperature in 6 *N* hydrochloric acid (0.056 g. in 1.0 ml.); after standing 45 min., 51% of the compound was recovered by bringing the pH to 7.0. The compound was slowly soluble with decomposition in 3 *N* sodium hydroxide (0.0032 g. in 5.0 ml. after stirring for 3 hr. at room temperature). Neutralization yielded adenine in 79% yield. In 5.0 ml. of 3 *N* sodium hydroxide at 100°, 0.0046 g. of II dissolved in less than 2 min.; neutralization gave adenine (61% yield).

S-Ethyl 6-Propionylaminopurine-9(or 7)-carbothioate (III).—A mixture of 6.0 ml. (0.047 mole) of propionic anhydride and 0.213 g. (0.001 mole) of II was heated for 8 min. at 112°. Precipitates of the product separated on cooling and also on evaporating the filtrate to 2 ml. at room temperature. After trituration with ether, 0.159 g. (60% yield) of pure III resulted, m.p. 169–170°.

Anal. Calcd. for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07; S, 11.46. Found: C, 47.54; H, 4.85; N, 24.77; S, 11.35.

Conversion to 6-N-(propionyl)adenine[7] was done by heating a mixture of 0.0353 g. of III, 3 ml. of water and 0.64 ml. of 0.39 *N* sodium hydroxide at 90° for 5 min. The resulting solution was brought to a pH of 7. The precipitate which settled on cooling at 10° overnight was 0.0062 g. (26% yield) of 6-N-(propionyl)adenine, identified by comparison of melting point (231–233°) and infrared spectrum with that of a specimen prepared from adenine and propionic anhydride.

6-Iodo-9-methylpurine (IV).—The compound was prepared from 0.026 mole of 6-chloro-9-methylpurine[18] by the method used for 6-iodopurine[19] except that the final purification was done by extraction of the dried precipitate with chloroform, evaporation of the chloroform extract and recrystallization of the residue from a benzene–hexane mixture. The product, obtained in 83% yield, decomposed at 212–215°.

Anal. Calcd. for $C_6H_5N_4I$: C, 27.71; H, 1.94. Found: C, 27.98; H, 2.04.

6-Cyano-9-methylpurine (V).—The compound was prepared from 6-iodo-9-methylpurine (0.0058 mole) and dry cuprous cyanide (0.0058 mole) in pyridine by the method used[13] for 6-cyanopurine. The crude product was purified by solution in benzene and boiling with charcoal to give a 71% yield of yellow crystals, which became colorless needles, m.p. 153–154.5°, after two recrystallizations from a benzene–hexane mixture. Sublimation did not change the melting point.

Anal. Calcd. for $C_7H_5N_5$: C, 52.83; H, 3.17; N, 44.01. Found: C, 53.15; H, 3.19; N, 44.11.

9-Methylpurine-6-carboxamide (VI).—To a solution of 150 mg. (0.0008 mole) of 6-cyano-9-methylpurine in 2.0 ml. of acetone was added 0.045 ml. of 4.811 *N* sodium hydroxide, and then slowly during stirring, 0.314 ml. of 30% hydrogen peroxide, keeping the temperature below 50°. After 1 hr. at room temperature the mixture was chilled and the product was filtered and washed with ethanol. This solid (151 mg., 95%) was recrystallized from ethanol–water twice to give pure material which decomposed at 288–291°.

Anal. Calcd. for $C_7H_7N_5O$: C, 47.45; H, 3.98; O, 9.04. Found: C, 47.34; H, 4.18; O, 8.77.

Isopropylidene-(9-methyl-6-purine)carbohydrazide (VII).—To a solution of 0.10 ml. of 95% hydrazine in 2 ml. of water, adjusted to pH 8 with acetic acid, was added 54 mg. (0.0003 mole) of 9-methylpurine-6-carboxamide. The mixture was refluxed for 15 min., cooled and treated with excess acetone. The precipitate (46.4 mg., 66%) was recrystallized from boiling ethanol, m.p. 230–232° dec.

Anal. Calcd. for $C_{10}H_{12}N_6O$: C, 51.71; H, 5.21; N, 36.19. Found: C, 51.09; H, 5.44; N, 35.82.

Acidic hydrolysis of this compound and treatment with 2,4-dinitrophenylhydrazine gave a 73% yield of acetone 2,4-dinitrophenylhydrazone. The free hydrazide was not obtained.

6-N-(*n*-Butoxycarbamoyl)purine (VIII).—This compound was obtained in 86% yield (crude) from 0.0015 mole of purine-6-carboazide[5] and 1-butanol by the method reported[5] for the ethyl analog. After recrystallization from a mixture of ethyl acetate and ethanol, the compound melted at 235–237° dec.

Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77. Found: C, 50.94; H, 6.16; N, 29.50.

6-Iodo-2-methylpurine (IX).—This compound (dec. 195–197°) was obtained in 66% yield (pure) from 6-chloro-2-methylpurine[20] by the method for 6-iodopurine.[19]

6-Cyano-2-methylpurine (X).—This substance was prepared in 24% yield (crude) from 0.0078 mole of 6-iodo-2-methylpurine by the method used for 6-cyanopurine.[13] The pure product decomposed at 239–241°.

Anal. Calcd. for $C_7H_5N_5$: C, 52.83; H, 3.17. Found: C, 53.18; H, 3.16.

4-Amino-2-methyl-5-nitro-6-(1H) Pyrimidinone (XI).—This compound was prepared in 86% yield (crude) from 0.016 mole of 4-amino-2-methyl-6-(1H) pyrimidinone[21] by the method[22] used for a similar compound. The white crystalline product did not melt or decompose below 333°.

Anal. Calcd. for $C_5H_6N_4O_3$: C, 35.30; H, 3.55; N, 32.93. Found: C, 35.70; H, 3.66; N, 33.00.

4-Benzylamino-6-chloro-5-nitropyrimidine (XII).—This substance, m.p. 127–131°, was prepared in 88% yield (crude) from 4,6-dichloro-5-nitropyrimidine (0.0052 mole) and benzylamine by the method used for a similar compound.[18]

Anal. Calcd. for $C_{11}H_9N_4O_2Cl$: C, 49.92; H, 3.43; N, 21.17. Found: C, 49.73; H, 3.44; N, 21.61.

Use of Diethyl Pyrocarbonate to Prepare Carbamates.—Diethyl pyrocarbonate was prepared by a known method,[23] except that metallic sodium was used[24] instead of potassium. The use of sodium is safer and more convenient even though the yield is low (25%).

Cyclohexylamine was converted to ethyl N-cyclohexylcarbamate[25] in 58% yield by treatment with diethyl pyrocarbonate at 10–20°. 4-Aminopyrimidine was converted to ethyl 4-pyrimidinecarbamate[11] in 54% yield by refluxing a solution of 0.252 g. (0.00264 mole) of 4-amino pyrimidine and 0.57 ml. (0.00396 mole) of diethyl pyrocarbonate in 4 ml. of acetonitrile for 15 min. On cooling 0.194 g. of product separated, and 0.046 g. was obtained by evaporating the filtrate and treating the residue with methanol. One recrystallization from ethyl acetate yielded material melting at 167–168°, which was identical with that previously obtained[11] by a different method.

Attempts to acylate 9-methyladenine with diethyl pyrocarbonate, using dioxane, dimethylsulfoxide and chloroform as solvents, gave unidentified white solids, which were not the expected purine carbamate.

(18) R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957).
(19) G. B. Elion and G. H. Hitchings, *ibid.*, **78**, 3508 (1956).
(20) R. K. Robins, J. W. Jones, and H. H. Lin, *J. Org. Chem.*, **21**, 695 (1956).
(21) A. Maggiolo, A. P. Phillips, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 106 (1951).
(22) D. J. Brown, *J. Appl. Chem.*, **5**, 358 (1955).
(23) E. F. Degering, G. L. Jenkins, and B. E. Sanders, *J. Am. Pharm. Assoc.*, **39**, 624 (1950).
(24) J. V. Miller, M.S. Thesis, University of Delaware, 1962, p. 39.
(25) A. Skita and H. Rolfes, *Ber.*, **53**, 1249 (1920).