Aminoacyl Nucleosides. IV. The Rearrangement of N^6-(α-Aminoacyl)adenines to N-(6-Purinyl)amino Acids. Application to Adenosine Analogs[1]

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Studies in the rearrangement of N^6-(α-aminoacyl)adenines into N-(6-purinyl)amino acids have revealed that N^6-glycyl-9-methyladenine in aqueous solution undergoes facile transformation into a cyclic intermediate, 3-methyl-3*H*-imidazo-[2,1-*i*]purin-8-(7*H*)-one (**5**). This key intermediate, when heated in water or base, undergoes ring opening to give 1-carboxymethyl-9-methyladenine (**6**), which, on further heating in the same solution, rearranges into N-[6-(9-methylpurinyl)]glycine (**7**). The structure of the cyclic compound **5** has been confirmed by its synthesis from *t*-butyl-6-imino-9-methylpurine-1-acetate (**11**) and also from N^6-chloroacetyl-9-methyladenine (**9**). The structures of **6** and **7** were verified by synthesis. The reaction products obtained from **5** under acidic, as well as neutral and alkaline, conditions are described. This study establishes that the 9-substituted N^6-(α-aminoacyl)adenines undergo the rearrangement in a manner similar to that of the N^6-(α-aminoacyl)-adenines.

N^6-Glycyladenine is readily converted in aqueous solution into N-(6-purinyl)glycine through a cyclic intermediate.[1] Evidence[1] indicates that the reaction is common to all N^6-(α-aminoacyl)adenine derivatives. The present paper reports that the corresponding 9-alkyl-N^6-(α-aminoacyl)adenine derivatives undergo a similar set of reactions.[3] One of the purposes of this research was to explore the chemistry of the N^6-(aminoacyl)adenine derivatives in aqueous solution under conditions of temperature and pH which approximate those in the physiological state.

The principal compound used in this study, N^6-glycyl-9-methyladenine, **4**, was prepared in a two-step reaction sequence. The condensation of 9-methyladenine, **1**, with N-benzyloxycarbonylglycine *p*-nitrophenyl ester **2**, in a hot mixture of dimethyl sulfoxide–dimethylformamide gave N^6-(N-benzyloxycarbonylglycyl)-9-methyladenine, **3**, in 44% yield.[4,5] The removal of the benzyloxycarbonyl group from the material to give N^6-glycyl-9-methyladenine trihydrobromide, **4**, was achieved by treatment of **3** with a 30% solution of hydrogen bromide in acetic acid. The benzyloxycarbonyl group could also be removed by hydrogenation over palladium on charcoal in ethanol, but under these conditions part of the N^6-glycyl-9-methyladenine formed was converted into the cyclic intermediate, 3-methyl-3*H*-imidazo[2,1-*i*]purin-8(7*H*)-one, **5**. A trace of 9-methyladenine was also obtained. The position of the acyl substituent at N^6 in compounds **3** and **4** was established by comparing the properties of **4** with those of N^6-acetyl-9-methyladenine, **10**, prepared by the acylation of 9-methyladenine with acetic anhydride. The structure of **10** was in turn established by its reduction to the known N^6-ethyl-9-methyladenine, **13**, prepared from 6-chloro-9-methylpurine, **14**, and ethylamine.[6] The ultraviolet spectra of N^6-(N-benzyloxycarbonylglycyl)-9-methyladenine, **3**, and of N^6-glycyl-9-methyladenine trihydrobromide, **4**, were similar to those of N^6-acetyl-9-methyladenine, **10**. These data indicate, therefore, that the product of acylation of 9-methyladenine with N-benzyloxycarbonylglycine *p*-nitrophenyl ester is N^6-(N-benzyloxycarbonylglycyl)-9-methyladenine, **3**. See Chart I.

N^6-Glycyl-9-methyladenine, **4**, is readily degraded to 9-methyladenine, **1**, in alkaline solution (pH 11.5) at 25°. Compound **4** is more stable in aqueous solution at pH 5.5; although, after a solution of **4** (pH 5.5) stands at 25° for 24 hr, appreciable conversion to the cyclic intermediate, **5**, occurs. When an aqueous solution of **4** at pH 7.0 is heated for 35 min at 100°, a 65% yield of **5** is obtained. N^6-Glycyl-9-methyladenine, analogous to N^6-glycyladenine, undergoes rearrangement and degradation in 0.1 N hydrochloric acid at 100° to give 1-carboxymethyl-9-methyladenine, **6**, N-[6-(9-methylpurinyl)]glycine, **7**, 1-methyl-5-aminoimidazol-4-carboxamide,[7] **8**, and presumably other imidazole compounds. See Chart II.

The N-benzyloxycarbonyl derivative, **3**, like N^6-glycyl-9-methyladenine, **4**, is very unstable in alkaline solutions and gives rise to 9-methyladenine, but is stable in neutral and acidic solutions. An acyl group on the α-amino group of N^6-(α-aminoacyl)adenines or N^6-(α-aminoacyl)-9-methyladenines prevents these compounds from undergoing the conversion to the cyclic compound and/or to any of its degradation products.

In neutral aqueous solution, compound **4** eliminates the elements of ammonia and rearranges to give the cyclic intermediate **5**. The structure of compound **5** was confirmed by synthesis *via* three separate routes. (1) Alkylation of 9-methyladenine with *t*-butyl bromoacetate gave a good yield of *t*-butyl 6-imino-9-methylpurine-1-acetate, **11**. When an aqueous solution of **11** was made alkaline, an instantaneous intramolecular displacement to the cyclic intermediate **5** occurred. (2) Condensation of 9-methyladenine with chloroacetic

(1) Paper III: G. B. Chheda and R. H. Hall, *Biochemistry*, **5**, 2082 (1966).

(2) To whom inquiries should be addressed.

(3) A preliminary report of this work was presented by G. B. Chheda and R. H. Hall, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., p 061, 1966.

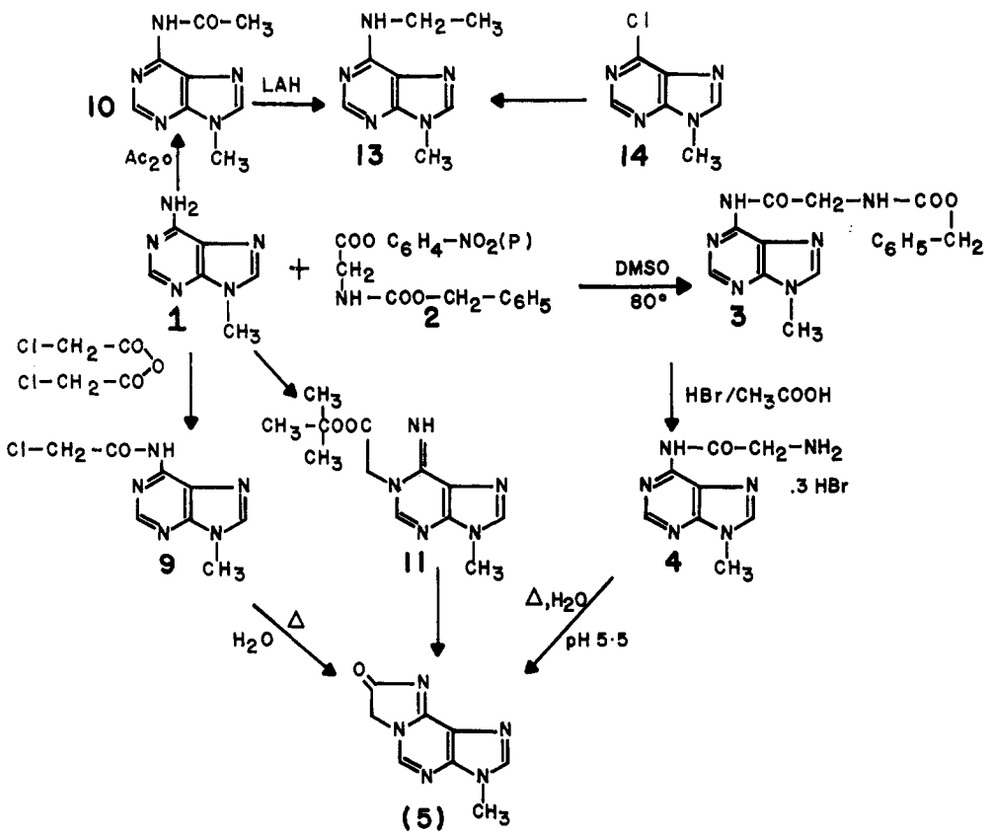
(4) Condensation of 9-methyladenine and N-benzyloxycarbonylglycine using N,N-dicyclohexylcarbodiimide as the coupling agent did not occur, whereas the same reaction with adenine gave a 25% yield of the desired product. The reaction of adenine with N-benzyloxycarbonylglycine *p*-nitrophenyl ester gave an 86% yield of N^6-(N-benzyloxycarbonylglycyl)adenine,[1] compared with a 44% yield of the corresponding material with 9-methyladenine.

(5) These results suggest a poor nucleophilic character of the amino group of 9-methyladenine compared with that of adenine.

(6) R. K. Robins and H. H. Lin, *J. Amer. Chem. Soc.*, **79**, 490 (1957).

(7) A sample of 1-methyl-5-aminoimidazole-4-carboxamide was obtained from Dr. Leon Goldman and his associates: J. W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965).

CHART I



anhydride at 70° in toluene afforded N^6-chloroacetyl-9-methyladenine, **9**, which, when warmed in aqueous solution at pH 5.5, underwent cyclization to yield **5**.[8] (3) 1-Carboxymethyl-9-methyladenine, **6**, was prepared by alkylation of 9-methyladenine with iodoacetic acid. The resulting hydriodide salt was converted into the free base **6** by treatment with dilute ammonia. The compound **6** was also prepared by hydrolysis of 1-*t*-butyl 6-imino-9-methylpurine-1-acetate, **11**. Treatment of **6** with *N*,*N*-dicyclohexylcarbodiimide in a mixture of dimethylformamide and dimethyl sulfoxide solution also gave product **5**. The products prepared by the three synthetic routes were also identical with the product obtained from N^6-glycyl-9-methyladenine. The criteria of identity were melting points, ultraviolet and infrared absorption spectra, mobility on paper chromatography, and electrophoresis. The fact that the compounds **6** and **7** were 1-alkyl-9-methyladenine and 6-alkyl-9-methyladenine, respectively, was further supported by their ultraviolet spectra when compared with those of the corresponding dibenzyladenines.[9]

Additional support for the structure of compound **5** comes from the fact that, when a solution of **5** in 0.1 *N* sodium hydroxide is kept at 25°, 1-carboxymethyl-9-methyladenine, **6**, is obtained, with only traces of its 6 isomer, **7**. Formation of **6** thus confirms that the cyclic intermediate must have the structure **5** and excludes the alternative 7-oxo structure. Compound **6**, in analogy to other 1-alkyadenine derivatives, on further treatment with alkali rearranges to yield *N*-[6-(9-methylpurinyl)]glycine,[10] **7**. The structure of **7** was confirmed by its synthesis from 6-chloro-9-methylpurine, and by rearrangement of the synthetic 1-carboxymethyl-9-methyladenine, **6**. X-Ray crystallographic studies clearly support the structure **5** and indicate that, in the crystalline state, **5** is in the keto form.[11]

The cyclic intermediate is relatively stable in neutral aqueous solution. Examination of the ultraviolet spectra of a solution of **5** kept for 1 month at 25° showed that the $OD_{303m\mu}$ had decreased by only 5.6%. When the cyclic compound was heated in water at pH 5.2 and 100° for 24 hr, it yielded 46% 1-carboxymethyl-9-methyladenine, **6**, 20% *N*-[6-(9-methylpurinyl)]glycine, **7**, 9% 9-methyladenine, and 4% an unidentified material; 7% of the starting material remained unchanged (Table I).

Treatment of **5** with dilute hydrochloric acid yields 1-carboxymethyl-9-methyladenine, **6**, and an imidazole derivative, 1-methyl-5-aminoimidazole-4-carboxamide,[7] **8** (see Table I).

The sequence of events in the conversion and rearrangement of N^6-glycyl-9-methyladenine, **4**, to *N*-[6-(9-methylpurinyl)]glycine, **7**, is evidently similar to that of the reaction of N^6-glycyladenine described

(8) When 9-methyladenine and chloroacetic anhydride were heated under reflux in toluene for 30 min, compound **12** was obtained as a major product, and N^6-chloroacetyladenine as a minor product. When heated in water at 100° for 3 hr, **12** gave the cyclic compound, **5**, and 1-carboxymethyl-9-methyladenine, **6**. Treatment of **12** with 0.1 *N* NaOH gave 9-methyladenine, 1-carboxymethyl-9-methyladenine, **6**, and *N*-[6-(9-methylpurinyl)]-glycine, **7**. The elemental analysis of **12** corresponded to the empirical formula $C_{10}H_9N_5O_2Cl$. Degradation studies suggest that compound **12** may be the *N*-chloroacetyl derivative of the cyclic compound **5**. [Paper chromatography (R_f × 100) for **12** showed D, 41.]

(9) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocycl. Chem.*, **2**, 291 (1965).

(10) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).

(11) R. Parthasarthy and G. B. Chheda, Abstracts, American Crystallographic Association Meeting, Buffalo, N. Y., Aug 1968.

CHART II

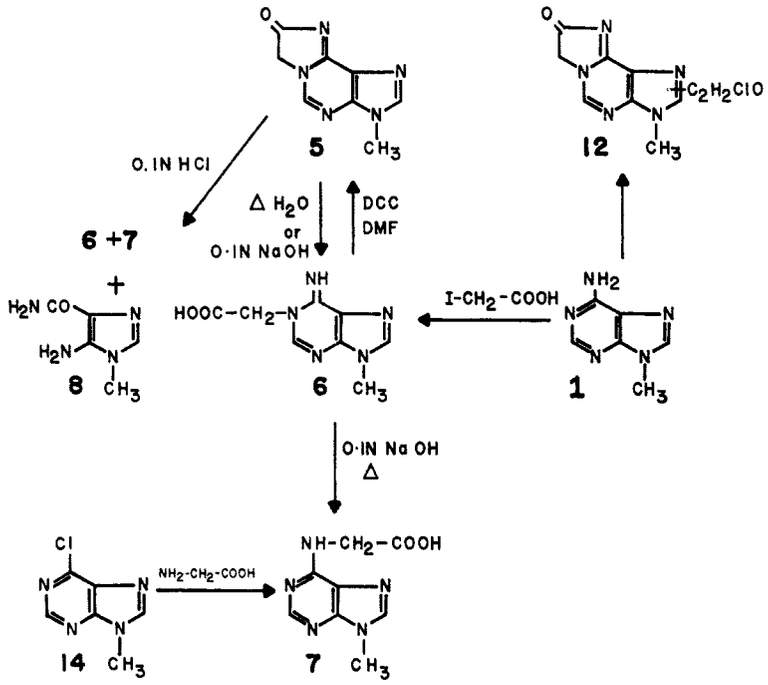


TABLE I

PERCENTAGE YIELDS OF DEGRADATION AND REARRANGEMENT PRODUCTS OBTAINED FROM THE "CYCLIC INTERMEDIATE," 5, AND 1-CARBOXYMETHYL-9-METHYLADENINE, 6, UNDER VARIOUS CONDITIONS

Compd	Reaction conditions	*N*-[6-(9-Methylpurinyl)]-glycine, 7	9-Methyladenine, 1	1-Carboxymethyl-9-methyladenine, 6	Cyclic intermediate	1-Methyl-5-aminoimidazole-4-carboxamide, 8	Unknown compd X	Unidentified material Y
5	0.1 *N* NaOH, 100°, 45 min[a,b]	72.0	11.4		...			
5	0.1 *N* HCl,[a,c] 100°, 45 min	6.1	1.43	40.4	...	6.65		2.51[d]
5	Water at 100°, 24 hr[a]	19.9	8.90	45.5	7.6[e] Unchanged	...	3.98[f]	
6	0.1 *N* NaOH, 100°, 45 min[a,g]	100						
6	0.1 *N* HCl,[a]	8.5		12.5 Unchanged				
6	0.5 *N* HCl,[a]	18.5		...		16.8		
6	Excess 0.1 *N* NaOH, t_R, 2.5 hr[a]	15.7	11.4	49.0				

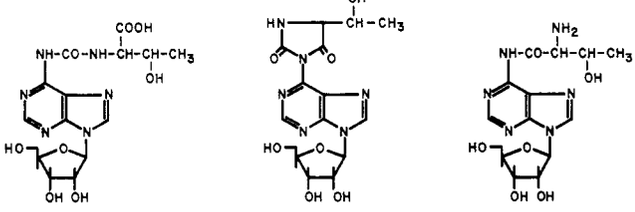
[a] The reactions were carried out in a sealed tube using 3–5 mg of the starting material dissolved in 0.5 ml of acid, base, or water. The sealed tube was immersed in a boiling water bath for the stated period. Progress of these reactions was followed by means of ultraviolet spectrophotometry and paper chromatography. The products of the reaction were isolated by streaking the reaction mixture on 3-mm paper and developing for 24 hr in solvent system A. The bands were detected and marked under a short-wave uv lamp and then cut out and eluted with water. The yields of the products were estimated spectrophotometrically at neutral pH using the predetermined λ_{max} and $\epsilon \times 10^{-3}$ values (see Experimental Section for values of each compound). [b] The solution was first allowed to stand at room temperature for 90 min and was then heated for 15 min at 60° before heating at 100°. The reaction turned reddish brown when heated for 24 hr at 100°. [c] The solution was allowed to stand for 1 hr at room temperature before heating; no degradation occurred under these conditions. [d] Compound Y was quantitated using the same values as 1-methyl-5-aminoimidazole-4-carboxamide, 8; $\lambda_{max}^{pH\ 2.6}$ 267, $\lambda_{max}^{pH\ 7.0}$ 267, and $\lambda_{max}^{pH\ 11.8}$ 266. [e] In order to estimate the amount of unchanged 5, the chromatogram was run in solvent system C instead of system A, since 5 decomposes readily in solvent A. [f] Compound X was estimated using an arbitrary value of ϵ_{max} and mol wt, 13,000 and 180, respectively; $\lambda_{max}^{pH\ 1}$ 265, $\lambda_{max}^{pH\ 7}$ 266, and $\lambda_{max}^{pH\ 13}$ 266. [g] A light pink solution was immediately obtained, which slowly turned darker in color. The solution was kept for 90 min at room temperature, and was then heated at 65° for 15 min and finally at 100° for 45 min. Aliquots of the reaction mixture, when taken at intermediate times and chromatographed, showed that 5 initially formed 1-carboxymethyl-9-methyladenine, 6, which rearranged quantitatively to *N*-[6-(9-methylpurinyl)]glycine, 7, when the solution was heated at 100°.

earlier.[1] The mechanism of this unique rearrangement is reported in detail in the accompanying paper.[12]

The results described in the present paper show no direct involvement of the imidazole portion of the molecule in the rearrangement. Apparently, the N^9 hydrogen can be substituted without significantly modifying the course of the reaction; hence, the reactions of the N^6-(α-aminoacyl)adenines would presumably apply to N^6-(α-aminoacyl)adenosine derivatives.

(12) G. B. Chheda, R. H. Hall, and P. M. Tanna, *J. Org. Chem.*, **34**, 3498 (1969).

These reactions may have some significance for the chemistry of certain molecular species of transfer ribonucleic acid (tRNA). We have isolated a nucleoside from tRNA[13] identified as structure **15**. Under some circumstances, this nucleoside might exist as the corresponding hydantoin, **16**, which in turn could undergo ring opening to form an N^6-threonyladenosine derivative, **17**. It is apparent from the chemistry described here that, if this should happen, a spontaneous conversion into an *N*-(6-purinyl)threonine derivative would occur.



15 16 17

Experimental Section

General.—Melting points were determined in capillary tubes on Mel-Temp and are corrected. Infrared spectra were determined in KBr disks with a Perkin-Elmer 137B "Infracord" spectrophotometer. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer. Nuclear magnetic resonance spectra were determined with a Varian A-60 nmr spectrometer.

Paper Chromatography and Electrophoresis.—The following solvent systems, measured by volume, were used: (A) 2-propanol–water–concentrated ammonium hydroxide (7:2:1); (B) 2-propanol–concentrated hydrochloric acid–water (680:176:144); (C) ethyl acetate–1-propanol–water (4:1:2); (D) 2-propanol–1% aqueous ammonium sulfate (2:1); (E) 1-butanol–water–concentrated ammonium hydroxide (86:14:5); (F) dimethylformamide–chloroform–water (50:40:10); and (G) ethyl acetate–2-ethoxyethanol–16% formic acid (4:1:2).

Electrophoresis was carried out in a Gilson Electrophorator tank at pH 3.5 in 0.05 *M* ammonium formate buffer using 3-mm Whatman papers.

The chromatograms were run in a descending manner on Whatman No. 1 paper for 16–20 hr in systems A, B, D, and E, and for 8 hr in systems C, F, and G. The spots were detected by viewing the chromatogram in short-wave ultraviolet light.

N^6-Acetyl-9-methyladenine, 10.—A stirred suspension of 299 mg (2.00 mmol) of 9-methyladenine and 2.04 ml (20.0 mmol) of acetic anhydride in 15 ml of toluene was heated under reflux for 1 hr. The solution was clarified by filtration and was then allowed to crystallize. A white crystalline product was collected on a filter; yield 309 mg, mp 174–176°. The filtrate was evaporated to dryness, and the white residue was triturated with toluene and filtered; yield 49 mg, mp 170–172°; the total yield was 359 mg (93.6%). Recrystallization of the total initial product from hot toluene gave 272 mg (70.4%) of the crystalline, white, analytically pure product, mp 173–174°. The ultraviolet absorption spectra are similar to the ones shown in Figure 1: λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 275–281 mμ (broad, 15.6); pH 6.6, 274 mμ (15.7); pH 13, 288 mμ (11.5); the material degraded in alkaline solution to 9-methyladenine; ir $\bar{\nu}$ (KBr) 3400 (NH), 3150 (CH_3), 1700 (C=O), 1630, 1600 (C=C, C=N), and 1550 cm^{-1} (weak, amide II). Other absorption peaks were at 1740 (weak), 1475, 1300, and 1235 cm^{-1}; nmr (DMSO-d_6) 10.55 (NH), 8.6, 8.34 (C_2 H, C_8 H), 3.75 (NCH_3) and 2.20 ppm ($C-CH_3$). Paper chromatography ($R_f \times 100$) showed A, 53-elongated spot; B, 38, elongated spot; D, 76; E, 43; G, 47.

Anal. Calcd for $C_8H_9N_5O$: C, 50.26; H, 4.74; N, 36.63. Found: C, 50.41; H, 5.00; N, 36.47.

In neutral aqueous solution (pH 6.3), N^6-acetyl-9-methyladenine was stable for at least 24 hr at room temperature. In 0.1 *N* HCl, the compound underwent about 5% degradation in 6 hr at room temperature, but, in 0.1 *N* alkali, the material was quite unstable, and rapid hydrolysis gave 9-methyladenine.

N^6-(*N*-Benzyloxycarbonylglycyl)-9-methyladenine, 3.—A hot solution (110°) of 897 mg (6.00 mmol) of 9-methyl-adenine in 30 ml of a 1:1 mixture of dimethylformamide and dimethyl sulfoxide was added to a solution of 3.96 g (12.0 mmol) of *N*-benzyloxycarbonylglycine *p*-nitrophenyl ester[14] in 10 ml of dimethylformamide at 40°. The reaction mixture was stirred for 4 hr at 95° and then for 18 hr at room temperature. Traces of unreacted 9-methyladenine were removed by filtration, and the filtrate was evaporated to dryness at 70° *in vacuo*. The solid residue was treated with 60 ml of hot chloroform, and the product was collected on a filter washed with 10 ml of cold 0.1 *N* hydrochloric acid; yield 466 mg, mp 190–193°. The chloroform filtrate, on cooling, deposited additional white product; yield 664 mg, mp 190–191°. Recrystallization of the combined crops from hot methanol gave shiny white needles of the pure product in two crops; yield 898 mg (44%), mp 199–201°; in some runs, additional impure product, mp 170–190° (5–10%), was obtained from the filtrate. An analytical sample was prepared by recrystallization from methanol; white needles, mp 200–202°; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 276 mμ (14.0); pH 5.6, 274 mμ (15.6); pH 13, 290 mμ (11.0); in alkaline solution, the compound slowly degraded to 9-methyladenine; ir $\bar{\nu}$ (KBr) 3300 (NH), 1735 (C=O urethan, C=O amide), 1620, 1590 1575 (C=C, C=N of purine), 1550 (amide II), 1230 (C—O urethan), and 745 cm^{-1} (phenyl); nmr (DMSO-d_6) 10.6 (NH), 8.6, 8.34 (C_2H, C_8H), 7.25 (phenyl), 4.1, 3.95 (CH_2 next to NH), 3.72 (NCH_3), 4.95, and 3.26 ppm. Paper chromatography ($R_f \times 100$) showed A, 50; B, 54, elongated spot; D, 90; E, 42; G, 76.

Anal. Calcd for $C_{16}H_{16}O_3H_6$: C, 56.46; H, 4.74; N, 24.69. Found: C, 56.41; H, 4.76; N, 24.52.

N^6-Ethyl-9-Methyladenine, 13.—To a slurry of 171 mg (4.50 mmol) of lithium aluminum hydride in 20 ml of tetrahydrofuran at room temperature was added dropwise a solution of 287 mg (1.50 mmol) of N^6-acetyl-9-methyladenine in 10 ml of tetrahydrofuran over 1 hr. The reaction mixture was refluxed for 6 hr and then allowed to stir at room temperature for 18 hr. Cold water was added to the reaction mixture and then stirred for 10 min at room temperature. The insoluble inorganic material was filtered and the filtrate was evaporated to dryness. The solid residue was dissolved in hot benzene, cooled, and filtered to remove 37 mg of 9-methyladenine along with salts and the desired product. The filtrate, when concentrated to 2 ml, crystallized to give 104 mg of product, mp 149–153°. The crude product was recrystallized from 10 ml of hot heptane to give a pale white product, mp 157–158°. This material (**13**) prepared from N^6-acetyl-9-methyladenine, was identical with the N^6-ethyl-9-methyladenine, **13**, prepared from 6-chloro-9-methylpurine[4,5] in uv spectra and paper chromatography. The mixture melting point of the two samples was not depressed; uv λ_{max} pH 1, 263 mμ; pH 6.0, 268 mμ; pH 11.8, 268 mμ. Paper chromatography ($R_f \times 100$) showed A, 77; B, 58; C, 76; D, 80; G, 52.

Anal. Calcd for $C_8H_{11}N_5$: C, 54.2; H, 6.25; N, 39.5. Found: C, 54.1; H, 5.96; N, 39.8.

N^6-Glycyl-9-methyladenine Trihydrobromide, 4.—A solution of 850 mg (2.50 mmol) of N^6-(*N*-benzyloxycarbonylglycyl)-9-methyladenine in 9.0 ml of 30% HBr in acetic acid was stirred for 1 hr at 5°. The mixture was diluted with 35 ml of anhydrous ether and centrifuged. The precipitate was triturated three times with 2 ml of ether, recentrifuged three times, and dried *in vacuo* at room temperature for 72 hr over P_2O_5; yield 1.02 g (91.0%), mp 171–177° dec. This material is extremely hygroscopic. The analysis suggests retention of moisture absorbed during transfer and handling. It had uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 273 and 282 mμ (shoulder); pH 6.4, 273 mμ (12.8); pH 11.5, 280–285 mμ; in alkaline solution, rapid degradation occurred to give 9-methyladenine, as can be seen from uv spectra (see Figure 1). The shape of this spectra was similar to N^6-acetyl-9-methyladenine. Paper chromatography ($R_f \times 100$) showed A, 51; B, 32; D, 56, elongated spot; E, 41; G, 30.

Anal. Calcd for $C_8H_{10}N_6O \cdot 3HBr \cdot 2H_2O$: C, 19.80; H, 3.53; N, 17.31; Br, 49.52. Found: C, 19.76; H, 3.32; N, 17.20; Br, 50.40.

N^6-Chloroacetyl-9-methyladenine, 9.—A suspension of 150 mg (1.00 mmol) of 9-methyladenine and 171 mg (1.00 mmol) of chloroacetic anhydride in 20 ml of toluene was stirred for 20 min at ambient temperature. A white fluffy material was filtered

(13) M. P. Schweizer, G. B. Chheda, R. H. Hall, L. Baczynskyj, and K. Biemann, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968.

(14) Purchased from Cyclo Chemical Corp., San Francisco, Calif.

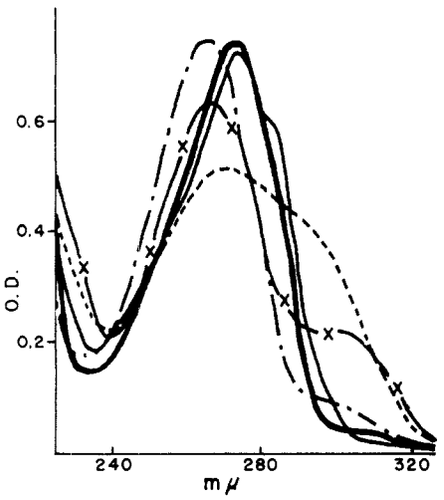


Figure 1.—Ultraviolet spectra of N^6-glycyl-9-methyladenine trihydrobromide, **4**: ——, pH 7.1; ——, pH 1.6; ----, pH 11.5; — — —, pH 11.5, 15 min t_R; ×—×, pH 7.1, 24 hr t_R.

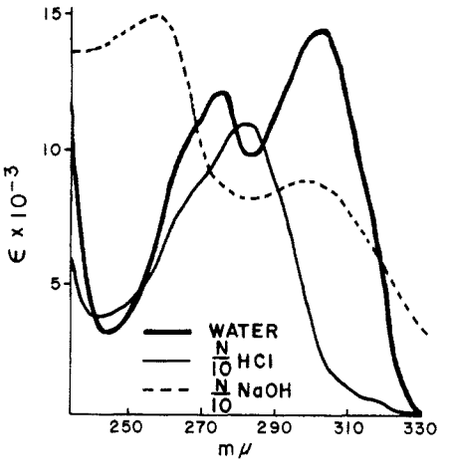


Figure 2.—Ultraviolet spectra of "cyclic intermediate" **5**.

from the thick suspension; yield 267 mg, mp 118–120° dec. This material contained chloroacetic acid and a little 9-methyladenine. The product was dissolved in 8 ml of chloroform and filtered to remove traces of insoluble 9-methyladenine. The chloroform solution was diluted with 3 ml of ether, and the mixture was allowed to crystallize. The crystalline white product was collected on a filter; yield 122 mg (54.2%), softened at 139–141°, but then slowly decomposed over a wide range up to 290°. This material was recrystallized twice by dissolving in chloroform and then adding an equal volume of ether; yield 43 mg (19.1%) of pinkish white product, which softened at 163–167° and then gradually decomposed up to 200–290°. This material has more than one melting point and its melting behavior is dependent upon the rate of heating. This compound is very unstable in presence of moisture and thus cannot be recrystallized from hydroxylic solvents. After prolonged standing at room temperature, it is partially converted into the cyclic compound. The uv spectra of freshly prepared material are similar to N^6-glycyl-9-methyladenine. Paper chromatography (R_f × 100) showed A, streaked; B, 36; E, streaked; G, 15.

Anal. Calcd for $C_8H_9N_5ClO$: C, 42.58; H, 3.57; N, 31.04; Cl, 15.71. Found: C, 41.84; H, 3.57; N, 30.27; Cl, 15.83.

***t*-Butyl 6 Imino-9-methylpurine-1-acetate, 11.**—A milky suspension containing 450 mg (3.0 mmol) of 9-methyladenine and 640 mg (3.30 mmol) of *t*-butyl bromoacetate in 10 ml of dimethyl sulfoxide was stirred in an oil bath at 65°. A clear solution resulted after 30 min. After being stirred for 24 hr at 65°, the yellow reaction mixture was evaporated to dryness, and the residue was triturated with methylene chloride. Upon filtration a white solid, 710 mg (68.7%), of the hydrobromide of *t*-butyl 9-methyladenine-1-acetate, mp 225–230° dec., was obtained. Recrystallization of the product from a methanol–ethanol mixture gave 470 mg (45.5%) of **11**, mp 235–240° dec. Further recrystallizations did not change the melting point of this material. Paper chromatography (R_f × 100) showed A, streaked; B, 73; C, 80; D, 80; E, streaked; F, 75; G, 61. The compound **11** changed to **5** on chromatography solvent A; uv λ_{max} pH 1, 261 mμ; pH 5.8, 261 mμ; pH 11.5, instantaneous conversion to the cyclic intermediate, as can be seen by an appearance of a peak around the 303–305-mμ region. The uv spectra in neutral and acidic pH were characteristic of N^1,N^9-dialkylated adenines.

Anal. Calcd for $C_{12}H_{18}N_5O_2Br$: N, 20.4; Br, 23.2. Found: N, 20.2; Br, 23.0.

3-Methyl-3*H*-imidazo[2,1-*i*]purin-8(7*H*)-one "Cyclic Intermediate," 5. A. From N^6-Glycyl-9-methyladenine, 4.—A solution of 300 mg (0.688 mmol) of N^6-glycyl-9-methyladenine trihydrobromide in 15 ml of water was brought to pH 5.5 by dropwise addition of 1 *N* sodium hydroxide. The solution was heated in a boiling water bath for 30 min. The ultraviolet absorption spectra of the reaction mixture showed the presence of **5**. The reaction mixture, which had turned reddish brown, was evaporated to dryness, and the residue was crystallized from 7 ml of a 2:5 mixture of water and ethanol; yield 105 mg (80%), mp 270–275°. An additional 29 mg of the crude product, mp 275–280°, was obtained from the filtrate. Recrystallization of the initial material from the water–alcohol mixture gave 70 mg (57.2%) of product; mp 297–302°; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 282 mμ (10.9); pH 7, 303 and 274 mμ (14.5, 12.1); pH 13, 303 mμ (12.0); in alkaline solution, the material degraded, as can be seen by following the uv spectra (also see Figure 2 for uv spectra). Paper chromatography (R_f × 100) showed A, streaked; B, 32; C, 4; D, 36; E, streaked; F, 17; G, 13. Paper electrophoresis at 4800 V/cm for 1.75 hr gave a value of −21.5. The cyclic intermediate **5** stains the paper brown. The infrared spectrum (KBr) showed $\bar{\nu}$ 1740 (weak, C=O and 1650 cm^{-1} (C=C, C=N); other absorptions were at 1500, 1390, 1210, 1180, 1040, 1010, 785, and 720.

Anal. Calcd for $C_8H_7N_5O$: C, 50.79; H, 3.73; N, 37.02. Found: C, 50.52; H, 3.88; N, 37.18.

B. From 1-Carboxymethyl-9-methyladenine, 6. Using *N*,*N*-Dicyclohexylcarbodiimide.—A suspension of 110 mg (0.531 mmol) of 1-carboxymethyl-9-methyladenine, **6**, and 416 mg (2.00 mmol) of *N*,*N*-dicyclohexylcarbodiimide in a mixture of 20 ml of dimethyl sulfoxide and 5 ml of dimethylformamide was stirred for 24 hr at 80°. The mixture was cooled to 25°, and precipitated dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness *in vacuo*. The solid residue was triturated with 10 ml of a 1:1 mixture of chloroform and ether, and filtration yielded 101 mg of crude material. The crude product was triturated with 7 ml of water, and the resulting solution was filtered to remove insoluble material. The filtrate contained 32.6 mg (32.2%) of **5** [spectrophotometric estimation, uv λ_{max} (pH 6.5) 303 mμ (ϵ 14,500)]. The residue obtained after evaporation of the filtrate was crystallized from a 5:1 mixture of ethanol and water, giving 14 mg of **5**. The ultraviolet spectra of this material were similar to those of the cyclic intermediate **5**, obtained from N^6-glycyl-9-methyladenine. The paper chromatographic mobilities of the two samples were identical in six solvent systems. (For uv and chromatography data, see above in method A.)

C. From *t*-Butyl 6-Imino-9-methylpurine-1-acetate, 11.—A solution of 172 mg (0.500 mmol) of *t*-butyl 6-imino-9-methylpurine-1-acetate hydrobromide in 5 ml of water was adjusted to pH 11.5 by dropwise addition of 0.1 *N* NaOH. The alkaline solution was stirred at room temperature for 5 min, and was then acidified to pH 6.5 with 0.1 *N* HCl. This solution contained 77.5 mg (82.0%) of **5** [spectrophotometric analysis, uv λ_{max} (pH 6.5) 303 (ϵ 14,500)]. The reaction solution was concentrated to approximately 1 ml *in vacuo*, and the solution was diluted with 4 ml of ethanol and cooled. A red crystalline material was collected on a filter; yield 53 mg (56.3%), mp 280–282° dec. After concentration to dryness and trituration with alcohol–water, 25 mg of additional crude material, mp 280–287°, was obtained from the filtrate. This material exhibited three spots in paper chromatography. The initial crops were crystallized from a mixture of hot ethanol and water, giving fine red needles, mp 300–302°. Infrared and ultraviolet absorption spectra and the chromatographic and electrophoretic mobility of this product in five solvent systems were identical with those of compound **5**

obtained from N^6-glycyl-9-methyladenine. (For ir, uv, and paper chromatography data, see above in method A.)

Anal. Calcd for $C_8H_7N_5O$: C, 50.79; H, 3.73; N, 37.02. Found: C, 51.01; H, 3.85; N, 36.84.

D. From N^6-Chloroacetyl-9-methyladenine, 9.—A solution of 65 mg (0.290 mmol) of N^6-chloroacetyl-9-methyladenine in 3 ml of water was brought to pH 5.5 by dropwise addition of 0.1 *N* NaOH. The reaction mixture was then heated at 100° for 30 min. Analysis of the reaction mixture by means of paper chromatography revealed the formation of 17 mg (32.0%) of **5** (estimated spectrophotometrically, as mentioned above). Mobility of this sample on paper chromatography in several solvent systems was identical with compound **5** obtained from N^6-glycyl-9-methyladenine. When the same reaction was run in tris buffer at pH 5.5 or in a 2:1 mixture of dimethylformamide and water, the yield of **5** did not increase. (For uv and paper chromatography data, see above in method A.)

1-Carboxymethyl-9-methyladenine, 6. A. Alkylation with Iodoacetic Acid.—A solution of 299 mg (2.00 mmol) of 9-methyladenine and 409 mg (2.20 mmol) of iodoacetic acid in 6 ml of dimethyl sulfoxide was stirred for 1 hr at 70° and then for 48 hr at room temperature. After evaporation to dryness at 60° *in vacuo*, the brown residue was triturated with ethanol and filtered; yield, 308 mg of the crude hydriodide. The filtrate was evaporated to a dry brown mass, triturated with 10 ml of chloroform, and filtered, giving additional crude product (180 mg). The crude sample was dissolved in water, and the solution was made alkaline (pH 10) with ammonium hydroxide and was then adjusted to pH 4.6 with formic acid. This solution was heated with charcoal to 70°, filtered, concentrated to a small volume (4 ml), and kept at 4°. A pinkish white product was collected on a filter; yield 114 mg (27.5%), mp 253–255°. Two crystallizations of this product from a hot 1:2 water–ethanol mixture gave an analytically pure pinkish white product, which was dried *in vacuo* at 100° for 22 hr; yield 41 mg (10.0%), mp 274–276°; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 261 mμ (15.8); pH 7, 261 mμ (16.6); pH 11.5, 260 and 267 mμ (shoulder, 16.7, 15.0). The maxima and the shape of the uv spectra were similar to those of N^1,N^9-dialkyladenines;[9] ir (KBr) $\bar{\nu}$ 3300 (NH), 3100 (broad, OH), 1725 (C=O), 1650 (C=C, C=N), and 1600 cm^{-1}; other absorptions were at 1375, 1310, and 780 cm^{-1}. Paper chromatography ($R_f \times 100$) showed A, 24; B, 32; C, 0.5; D, 34; E, 2; F, 4; G, 8. Paper electrophoresis at 3000 V/45 cm for 2 hr gave a value of −15.0. Compound **6** stains the paper a brown color after 3 days.

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.80. Found: C, 46.55; H, 4.21; N, 33.56.

B. From *t*-Butyl 6-Imino-9-methylpurine-1-acetate, 11.—A solution of 300 mg of *t*-butyl 6-imino-9-methylpurine-1-acetate hydrobromide, **11**, in 7 ml of 0.3 *N* NaOH was allowed to stand for 8 hr at room temperature. During this period, the solution developed a pinkish color, which became dark purple. The reaction mixture was acidified to pH 4, treated with charcoal at 65°, and filtered. The solution was concentrated to 3 ml and allowed to stand for 2 hr at 4°. The crystalline product was collected on a filter; yield 77 mg; mp 245–250°. From the filtrate an additional 90 mg of the crude material, mp 220–235°, was obtained. Recrystallization of the first crop of 77 mg from a water–ethanol mixture gave 47 mg of **6**, mp 273–276°. The infrared and ultraviolet spectra, paper chromatographic behavior, and electrophoretic behavior of this product were identical with those of 1-carboxymethyl-9-methyladenine synthesized by method A. (For ir, uv, and paper chromatography and electrophoresis data, see above in method A.)

Conversion of "Cyclic Intermediate," 5, into 1-Carboxymethyl-9-methyladenine, 6.—A solution of 133 mg (0.705 mmol) of **5** in 7.0 ml (0.700 mmol) of 0.1 *N* sodium hydroxide was kept at 25°. After 6 hr, spectrophotometric analysis showed the presence of **6** in 76.8% yield. Formic acid (90%) was added dropwise to the dark purple solution to reduce the pH to 4. The solution was treated with neutral charcoal to remove colored material. Upon cooling, the clear, colorless solution gave a white crystalline product; yield 62 mg, mp 272–273°. The filtrate provided additional product; yield 18 mg (total yield 54.5%), mp 260–265°. The analytical sample, a white crystalline solid, mp 277–278°, was prepared by crystallization of the first crop from 10 ml of a 1:1 mixture of water and alcohol. The product was identical with the 1-carboxymethyl-9-methyladenine acid obtained by alkylation of 9-methyladenine by iodoacetic acid. The criteria used for establishing the identity of the two materials were infrared and ultraviolet spectra, paper chromatography in six solvent systems, paper electrophoresis, and mixture melting point, which was depressed. (For ir, uv, and paper chromatography and electrophoretic data, see above in method A.)

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.35; H, 4.38; N, 33.80. Found: C, 46.45; H, 4.41; N, 33.79.

***N*-[6-(9-Methylpurinyl)]glycine, 7.**—To a solution of 225 mg (3.00 mmol) of glycine and 336 mg (2.00 mmol) of 6-chloro-9-methylpurine in 4 ml of water was gradually added 1 *N* NaOH, until the pH of the solution reached 9. The mixture was refluxed for 2 hr; during this period, the pH was readjusted to 9 (from 5–5.5) every 30 min. The solution was poured into 75 ml of ethanol and kept at 4° for 12 hr. The resulting crystalline precipitate, the sodium salt of the product, was collected on a filter and dried; yield 379 mg (82.6%). It darkened at 240°, and then slowly decomposed. The sodium salt was dissolved in 6.5 ml of water, and the pH of the solution was adjusted to 3 with 88% formic acid. The product separated out; yield 258 mg (62.5%), mp 264–266° dec. Additional product, 55 mg (13.3%), mp 248–250°, was obtained from the filtrate. Crystallization of the precipitated product (first crop) from a hot 1:3 water–alcohol mixture gave material with the same mp 264–266°; uv λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 267 mμ (15.8); pH 5.6, 268 mμ (17.6); pH 13, 268 mμ (18.5); ir (KBr) $\bar{\nu}_{max}$ 3460 (NH), 3190 (OH), 1720 (weak C=O), and 1625 cm^{-1} (COO^-, NH, C=C, C=N); other absorptions were at 1495, 1295, and 770 cm^{-1}. Paper chromatography ($R_f \times 100$) showed A, 39; B, 43; C, 1.5; D, 46; E, 6; F, 50; G, 45. Paper electrophoresis at 3000 V/45 cm for 2 hr gave a value of −27.8.

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.4; H, 4.37; N, 33.8. Found: C, 45.79; H, 4.67; N, 33.58.

Conversion of "Cyclic Intermediate," 5, into *N*-[6-(9-Methylpurinyl)]glycine, 7.—A solution of 120 mg (0.635 mmol) of **5** in 6.5 ml of 0.1 *N* sodium hydroxide was heated under reflux for 45 min. (The solution turned purple in color, grew darker with heating, and finally turned gray.) The reaction solution contained 114 mg (87.5%) of *N*-[6-(9-methylpurinyl)]glycine, **7** (spectrophotometric analysis). The hot solution was decolorized with charcoal, concentrated to 4 ml, and acidified with 90% formic acid to pH 2.8. The pale yellow product weighed 90 mg; mp 255–260° dec. Crystallization of the crude product from 7 ml of a hot 4:3 water–alcohol mixture gave 81 mg (61.2%) of product, mp 260–263° dec. Recrystallization of this material from water–alcohol gave a crystalline white product which darkens at 250° and melts with decomposition at 264–265°. The sample of *N*-[6-(9-methylpurinyl)]glycine was identical with the sample synthesized from 6-chloro-9-methylpurine. The criteria used for establishing the identity of the two products were infrared and ultraviolet spectra, paper electrophoresis, and paper chromatography in six solvent systems. (For ir, uv, paper chromatography, and electrophoresis, see above for the synthetic material.)

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.4; H, 4.37; N, 33.8. Found: C, 46.6; H, 4.18; N, 33.8.

Conversion of 1-Carboxymethyl-9-methyladenine, 6, into *N*-[6-(9-Methylpurinyl)]glycine, 7.—To a solution of 250 μg of 1-carboxymethyl-9-methyladenine acid in 250 μl of water was added 5 μl of 5 *N* NaOH. The reaction solution was heated in a sealed tube for 45 min at 100°. Examination of the reaction mixture by ultraviolet spectrophotometry and paper chromatography revealed a quantitative conversion into *N*-[6-(9-methylpurinyl)]-glycine. No other products were detected in this conversion.

Reactions of Cyclic Intermediate 5 and 1-Carboxymethyl-9-methyladenine, 6.—The reactions **5** and **6**, in water, acid, and base are summarized in Table I.

1-Methyl-5-aminoimidazole-4-carboxamide, 8.—The acid degradation product, 1-methyl-5-aminoimidazole-4-carboxamide, **8**, obtained from the cyclic intermediate and N^6-glycyl-9-methyladenine, was identified by comparison of uv spectra and paper chromatographic behavior with an authentic sample;[6] uv λ_{max} pH 1.9, 265 mμ; pH 7.1, 265 mμ; pH 11.6, 265 mμ. Paper chromatography ($R_f \times 100$) showed A, 46; B, 44; C, 21; D, 53; E, 30; G, 23. Paper electrophoresis at 3000 V/45 cm for 3.5 hr gave a value of −23.5. The compound stains the paper pink 8 hr after application.

Registry No.—**3**, 21342-99-4; **4**, 21343-00-0; **5**, 21343-01-1; **6**, 21343-02-2; **7**, 21343-03-3; **8**, 21343-

04-4; **9**, 21343-05-5; **10**, 21343-06-6; **11**, 21343-08-8; **13**, 5400-01-1.

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Aminoacyl Nucleosides. V. The Mechanism of the Rearrangement of N^6-(α-Aminoacyl)adenines into N-(6-purinyl)amino Acids[1]

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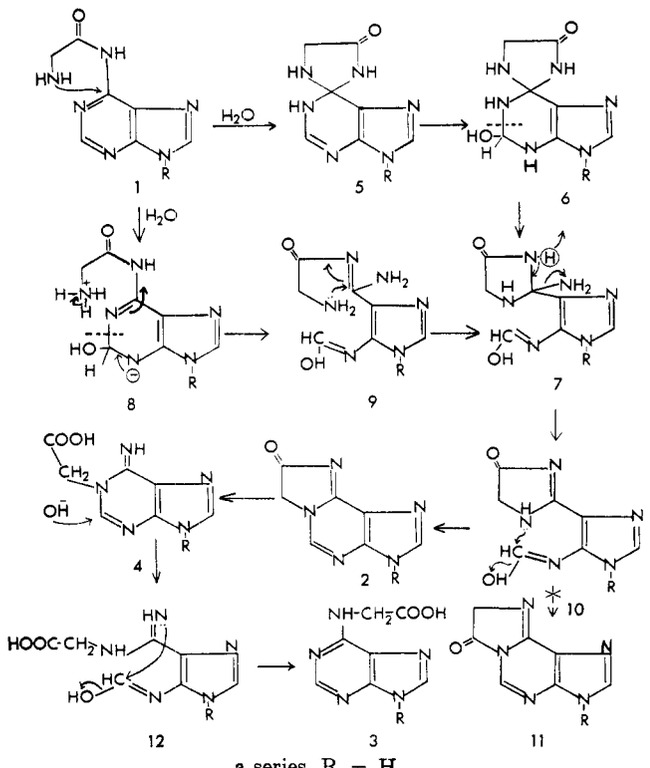
N^6-(Glycyl-α-^{15}N)adenine (**1**), on standing in water at neutral pH, loses the elements of ammonia to yield the cyclic intermediate 3*H*-imidazo[2,1-*i*]purin-8(7*H*)-one (**2**) with complete retention of the ^{15}N. Compound **2**, on warming in water, rearranges to form N-(6-purinyl)glycine (**3**) through an intermediate, 1-carboxymethyladenine (**4**). The same reaction was carried out on N^6-sarcosyladenine-6-^{15}N (**15**). A single product, N-(6-purinyl)sarcosine (**16**), containing 100% of the atom excess ^{15}N, was obtained. On the basis of these results, a mechanism for the rearrangement of N^6-(α-aminoacyl)adenines to N-(6-purinyl)amino acids is proposed. The α-amino group of **1** adds across the C^6–N^1 double bond, and the pyrimidine ring opens. The N^1 of the purine is expelled as ammonia, and the pyrimidine ring re-forms to yield **2**. After lactam opening, **2** behaves as an N^1-alkyladenine, and undergoes the well-known $N^1 \rightarrow N^6$ rearrangement to form **3**.

This paper reports a further study of a rearrangement in which N^6-glycyladenine, **1a**, is converted into N-(6-purinyl)glycine, **3a** (Chart I), with elimination of the elements of ammonia.[2] The rearrangement is general for N^6-(α-aminoacyl)adenines.[4] In addition, N^6-glycyl-9-methyladenine, **1b**, undergoes a similar conversion, which indicates that N^9 hydrogen of purine is not essential for the rearrangement.[1] The studies with **1b** also indicate that the rearrangement could occur at a nucleoside level. The blocked aminoacyladenines like N^6-(N-benzyloxycarbonylglycyl)adenine and N^6-(N-formylglycyl)adenine, when heated in water, do not undergo this rearrangement, which suggests that the presence of a free amino nitrogen is necessary. N^6-(N,N-Dimethylglycyl)adenine and N^6-[α-N-(piperidyl)acetyl]adenine[5] undergo hydrolysis to adenine when subjected to the rearrangement conditions. There appears to be no direct involvement of the imidazole portion of a purine molecule in this rearrangement.

CHART I



a series, R = H
b series, R = CH_3

The first step in the reaction for both N^6-glycyladenine, **1a**, and N^6-glycyl-9-methyladenine, **1b**, involves the elimination of ammonia and the formation of the isolable intermediates, **2a** and **2b**, respectively (Chart I). The structure of **2a** was previously established by comparing its properties with those of a sample synthesized from N^6-chloroacetyladenine.[2] In the latter case, **2a** results from nucleophilic displacement of the chloride by the N^1 nitrogen of N^6-chloroacetyladenine. It is obvious that **2a** could not result (from **1a**) from an analogous intramolecular displacement. The α-amino group must participate in the reaction, because under identical conditions, N^6-acetyladenine undergoes simple hydrolysis to yield adenine.

In order to explain this rearrangement, we had earlier proposed a mechanism based on an addition–elimination reaction, suggesting loss of the N^1 nitrogen of adenine.[2] We now wish to consider this mechanism in more detail and to offer experimental evidence in support of the proposed mechanism. In the initial

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