Hochstetter, A. (1912), German Patent 268,452; (1914), *Chem. Abstr. 8*, 1854.
Holy, A. (1965), *Chem. Ind. (London)*, 721.
Hough, L., Priddle, J. E., and Theobald, R. S. (1960), *Advan. Carbohydrate Chem. 15*, 91.
Hough, L., Priddle, J. E., and Theobald, R. S. (1962), *J. Chem. Soc.*, 1934.
Ikehara, M., Ohtsuka, E., and Kodama, Y. (1964), *Chem. Pharm. Bull. (Tokyo) 12*, 145.
Jarman, M., and Reese, C. B. (1964), *Chem. Ind. (London)*, 1493.
Kenner, G. W., Todd, A. R., Webb, R. F., and Weymouth, F. J. (1954), *J. Chem. Soc.*, 2288.
Metzenburg, R. L., and Mitchell, H. K. (1954), *J. Am. Chem. Soc. 76*, 4187.
Michelson, A. M., Szabo, L., and Todd, A. R. (1956), *J. Chem. Soc.*, 1546.
Mizuno, Y., Ikehara, M., Ueda, T., Nomura, A., Ohtsuka, E., Ishikawa, F., and Kanai, Y. (1961), *Chem. Pharm. Bull. (Tokyo) 9*, 653.
Reese, C. B., and Sulston, J. E. (1964), *Proc. Chem. Soc.*, 214.
Ruyle, W. V., Shen, T. Y., and Patchett, A. A. (1965), *J. Org. Chem. 30*, 4355.
Smith, I. (1960), Chromatographic and Electrophoretic Techniques, Vol. 1, New York, N. Y., Interscience, p 253.
Smith, M., Rammler, D. H., Goldberg, I. H., and Khorana, H. G. (1964), *J. Am. Chem. Soc. 84*, 430.
Tener, G. M. (1961), *J. Am. Chem. Soc. 83*, 159.
Wright, R. S., Tener, G. M., and Khorana, H. G. (1958), *J. Am. Chem. Soc. 80*, 2004.
Yung, N. C., and Fox, J. J. (1961), *J. Am. Chem. Soc. 83*, 3060.
Yurkevich, A. M., Kolodkina, I. I., and Preobrazhenskii, N. A. (1965), *Dokl. Akad. Nauk SSSR 164*, 828.
Zemlicka, J. (1964), *Chem. Ind. (London)*, 581.

Aminoacyl Nucleosides. III. A Novel Rearrangement: Conversion of N^6-(α-Aminoacyl)adenines into N-(6-Purinyl)amino Acids*

Girish B. Chheda and Ross H. Hall

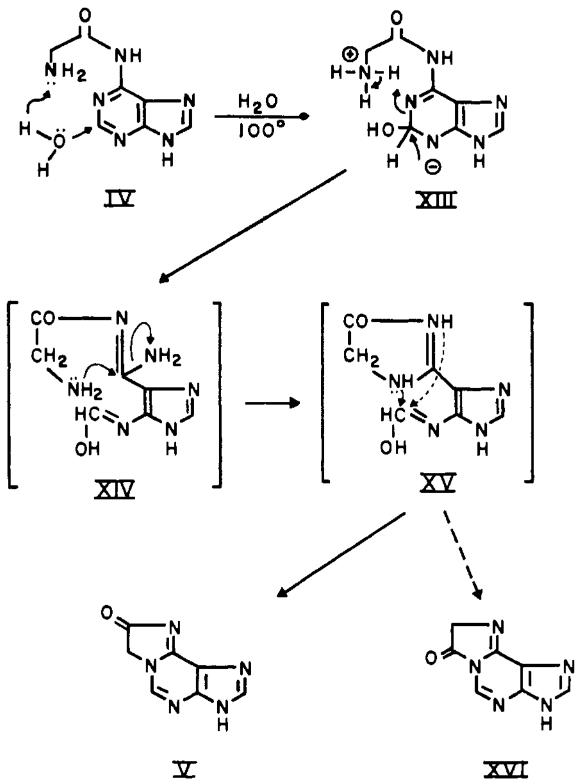
ABSTRACT: A spontaneous conversion of N^6-glycyladenine (IV) to N-(6-purinyl)glycine (VI) is described. N^6-Glycyladenine (IV) in aqueous solution over a period of several hours at room temperature, or in a few minutes at 100°, loses the elements of ammonia and forms a crystalline cyclic intermediate which has been assigned the structure, 3*H*-7,8-dihydro-8-oxoimidazo-[2,1-*i*]purine (V). The elemental analysis and ultraviolet and infrared absorption spectra of the product are consonant with this assignment. In neutral solution, compound V undergoes ring opening and rearrangement to yield N-(6-purinyl)glycine (VI). Treatment of V with 0.5 N hydrochloric acid produces mainly 5-aminoimidazole-4-carboxamide (VIII) and another compound which appears to be a carboxamidine (IX) as well as a small amount of VI. Preliminary studies suggest that these reactions are common to all N^6-(α-aminoacyl)-adenines.

These results led us to investigate the properties of N^6-chloracetyladenine (VII). This compound is also unstable in aqueous solution as N^6-glycyladenine and forms an identical cyclic intermediate V. The significance of these findings is enhanced by recent reports of the isolation of N^6-(N-formyl-α-aminoacyl)-adenosines from an enzymic digest of yeast soluble ribonucleic acid (s-RNA). N^6-(N-Carbobenzoxyglycyl)-adenine (III) is stable to boiling water; thus the possibility arises that a function of the formyl residue on the α amino groups of the natural compounds is to provide chemical stability.

We have reported the isolation of a class of nucleosides from enzymic hydrolysates of yeast soluble ribonucleic acid (s-RNA) which has been identified as N^6-(N-formyl-α-aminoacyl)adenosines (Hall, 1964; Hall and Chheda, 1965). Corroborative evidence for the presence of such amino acid nucleoside derivatives in yeast s-RNA was provided by the isolation of a derivative of N^6-(N-formylthreonyl)adenine from a mild acid hydrolysate of yeast s-RNA (Hall and Chheda, 1966). The N^6-(N-formyl-α-aminoacyl)adenosines are labile compounds which have made it difficult to affect quantitative recovery using the above isolation techniques. Further, the high order of chemical reactivity of the aminoacyladenosine derivatives raises the question

* From the Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203. *Received February 9, 1966.* This investigation has been supported in part by a grant from the National Cancer Institute, U. S. Public Health Service (CA-05697). Papers one and two in this series are Hall (1964) and Hall and Chheda (1965), respectively.

SCHEME I



of whether these compounds reflect correctly the structure of such derivatives in native s-RNA. Clearly, more information concerning the basic chemistry of the N^6-(α-aminoacyl)adenosines is desirable and is a prerequisite to an understanding of the role of these components in the function of yeast s-RNA.

We have, accordingly, undertaken a study of appropriate model compounds and the chemistry of one such model, N^6-glycyladenine, is the subject of this paper. The salient point which emerges from this study is that N^6-(α-aminoacyl)adenines are very unstable in aqueous solution. They undergo spontaneous degradation and rearrangement to form *N*-(6-purinyl)amino acids. This finding suggests that one function of the formyl residue on the α-amino group of the N^6-(*N*-formyl-α-aminoacyl) adenosines may be to stabilize these compounds. A preliminary account of this research has appeared (Chheda and Hall, 1965).

Discussion

N^6-Glycyladenine. N^6-(*N*-Carbobenzoxyglycyl)adenine was prepared in 20% yield by condensing adenine with *N*-carbobenzoxyglycine in the presence of *N,N*-dicyclohexylcarbodiimide according to the method of Brink and Schein (1963).[1] Alternatively, this intermediate was obtained in 86% yield by condensing adenine (I) (Scheme I) with *N*-carbobenzoxyglycine *p*-nitrophenyl ester (II) in hot dimethylformamide–dimethyl sulfoxide. The carbobenzoxy group was removed by treatment with hydrogen bromide in acetic acid and the hydrobromide of N^6-glycyladenine (IV) was obtained in quantitative yield. The salt was converted smoothly into the free base by treatment with triethylamine or tributylamine.

These synthetic methods are nonspecific and attachment of the glycyl residue, in theory, could occur at any one of the nitrogen atoms. Brink and Schein (1963) originally assigned the structure, N^6-glycyladenine (IV), to the synthetic compound on the basis that 1 mole of nitrogen was released when it was treated with nitrous acid. This evidence *per se* is not sufficient to exclude attachment at N^1 or N^3 since deamination of the exocyclic imino group does not appear to occur (Jones and Robins, 1962). In order to assign the correct structure for *N*-glycyladenine, its properties were compared with those of other synthetic *N*-acyladenines. This comparison, while not completely adequate since there is no unequivocal synthesis of N^1-, N^3-, or N^6-acyladenines, does favor the N^6 position as the point of attachment. We prepared, according to literature methods, N^6-acetyladenine (Schein, 1962), N^6-chloroacetyladenine (Craveri and Zoni, 1958), and N^6-

[1] There was obtained a large amount of a by-product which was characterized as the *N*-(carbobenzoxyglycyl)dicyclohexylurea

$$C_6H_{11}NHCON(C_6H_{11})COCH_2NHCOOCH_2C_6H_5$$

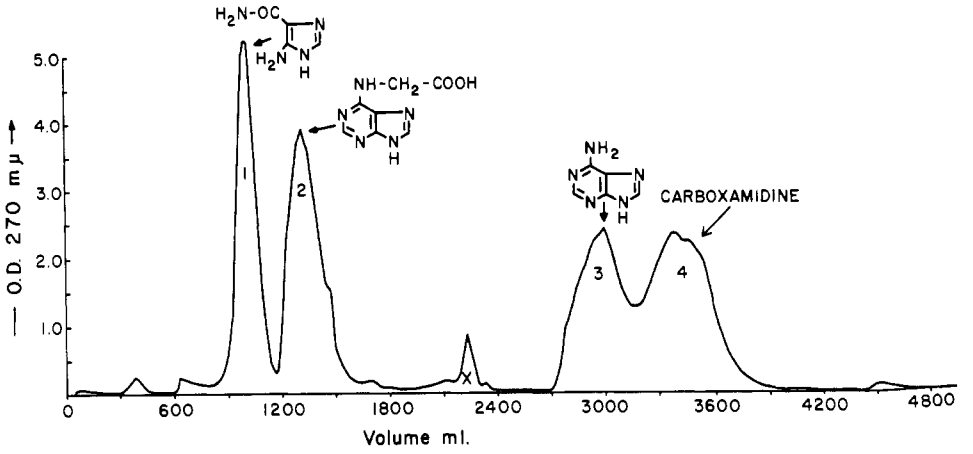


FIGURE 1: Separation of reaction products obtained from N^6-glycyladenine (reaction 1, Table I), on Dowex-50W × 8 [H^+], (200–400 mesh), column size 16 × 1 cm. Column was developed with a linear gradient of 0.2–1.6 M HCl (6000 ml, total volume).

benzoyladenine[2] (Kossel, 1888). The infrared spectra of these model compounds and that of the N^6-glycyladenine are similar, in particular, all four compounds show absorption owing to carbonyl stretching frequencies (1700–1720 cm^{-1}) and no absorption owing to the amino group of adenine (Angell, 1961). The ultraviolet absorption spectra of N^6-glycyladenine, N^6-acetyladenine, and N^6-chloroacetyladenine are also similar, as shown in Table I. In addition, we reduced N^6-glycyladenine with lithium aluminum hydride and obtained a product that shows ultraviolet spectra similar to those of N^6-alkyladenines (Lettré and Ballweg, 1961) rather than N'-alkyladenines (Montgomery and Thomas, 1965) (Table I).

Degradation and Rearrangement of N^6-Glycyladenine. When a neutral aqueous solution of N^6-glycyladenine (IV) was kept at room temperature for several hours or warmed for a few minutes at 100°, the solution became dark purple and the ultraviolet spectrum developed a new peak at 303 mμ. Chromatographic analysis of this solution showed that the starting material had disappeared and three new ultraviolet-absorbing compounds were present, a cyclic intermediate V, *N*-(6-purinyl)-glycine (VI), and adenine, in yields of 38, 13, and 27%, respectively. When an aqueous solution of N^6-glycyladenine was refluxed for 35 hr, compound V which was formed at first disappeared and *N*-(6-purinyl)-glycine (VI) and adenine were obtained, each in 30% yield. Thus in aqueous solution, N^6-glycyladenine rapidly undergoes two competing reactions. In the first, hydrolysis occurs to form adenine and glycine, and in the second, N^6-glycyladenine loses the elements of ammonia and cyclizes to form the intermediate V. This cyclic intermediate in turn undergoes ring opening and rearrangement to form *N*-(6-purinyl)glycine (VI) at a much slower rate. (See Schemes I and II).

A more detailed study of the conversion revealed that other competing reactions occur, depending on the conditions. Thus, when N^6-glycyladenine was heated in water and then in 0.5 N hydrochloric acid at 100°, five products were formed which could be separated on a cation-exchange column of Dowex-50 (see Figure 1 and Table II). Two of the products, adenine and *N*-(6-purinyl)glycine, had been obtained from the neutral reaction mixture. The three new compounds were imidazole derivatives, including 5-aminoimidazole-4-carboxamide. The identity of the other two was not established rigorously, but on the basis of their ultraviolet absorption spectra and the other properties discussed below, they are imidazole derivatives. When a solution of the cyclic intermediate V in 0.5 N hydrochloric acid was heated at 100° for 40 min, a 69% yield of imidazole products was obtained and only a trace amount of *N*-(6-purinyl)glycine was obtained. The yields of the breakdown and rearrangement products obtained from N^6-glycyladenine under a variety of conditions are recorded in Table II. These data can be correlated with three courses of degradation of N^6-glycyladenine which are pH dependent: (1) in dilute sodium hydroxide solution, hydrolysis to adenine and glycine occurs; (2) in water the cyclic intermediate V is formed which rearranges to *N*-(6-purinyl)glycine; (3) in dilute hydrochloric acid adenine and the cyclic intermediate V are formed. Intermediate V in the acid solution undergoes ring opening to form a series of imidazole derivatives.

Identification of Reaction Products Obtained by Breakdown of N^6-Glycyladenine (Figure 1). The five degradation products discussed in the preceding section and listed in Table II were identified as follows: *N*-(6-purinyl)glycine, adenine, and 5-aminoimidazole-4-carboxamide were identified by a comparison of the in-

[2] The structure of N^6-benzoyladenine was suggested by its reduction with lithium aluminum hydride to form N^6-benzyladenine which was identical with the product synthesized from 6-chloropurine and benzylamine (Bullock *et al.*, 1957).

TABLE I: Ultraviolet Spectra of Some Acyl and Alkyl Adenines.

Compd	pH[b]	λ_{max} (mμ)	$\epsilon \times 10^{-3}$
N^6-Glycyladenine trihydrobromide	1	273	
	Methanol	274	
	13	280	
N^6-Glycyladenine (IV)	1	275	
	5.5	280	12.3[a]
	13	279	
N^6-Acetyladenine	1	281	13.5
	7.1	279	11.8
	13	280	10.5
N^6-Chloroacetyladenine (VII)	1	274	13.0
	7	278	11.2
	13	276	8.8
Fraction I (VIII)	1.4	266	
	5.8	266	
	11.5	277	
5-Aminoimidazole-4-carboxamide hydrochloride	1.4	266	9.25
	5.8	266	11.1
	11.5	277	12.3
Fraction 2 (VI)	1	272	15.8
	7	268	15.3
	13	272	15.5
N-(6-Purinyl)glycine[c]	1	273	17.8
	7	268	17.7
	13	274	18.3
Fraction 3 (I)	1	263	
	6.8	260	
	11.1	269	
Adenine	2	263	13.1
	13	269	12.3
Fraction 4 (IX)	1	282 (min 245)	
	7	283 (min 243)	
	11.5	291	
5-Aminoimidazole-4-*N'*-methylcarboxamidine[d]	4	281	
	13	290	
Cyclic intermediate (V)	1	282	10.9
	5.5	303, 269, 220	14.4, 9.0, 20.6
	13	306	15.4
Lithium aluminum hydride reduction product of N^6-glycyladenine	1.8	274	
	6.5	264	
	11.4	272	
N^6-(β-Aminoethyl)adenine hydrochleoride[e]	1	275	
	13	273.5	
1-Mthyladenine[f]	1	257	11.7
	7	264	10.8
	13	269	14.1

[a] Value reported by Brink and Schein (1963). [b] pH 1 and 13 spectra were run in 0.1 N HCl and 0.1 N NaOH, respectively. [c] Values reported by Ballio and Vittorio (1960). [d] Values reported by Brookes and Lawley (1960). [e] Values reported by Lettré and Ballweg (1961). [f] Values reported by Montgomery and Thomas (1965).

frared and ultraviolet absorption spectra and chromatographic mobilities of the isolated products with those of authentic samples. The basic material isolated from the fraction corresponding to peak 4, Figure 1, appears to be a carboxamidine. Its ultraviolet absorption spectra and mobility on the ion-exchange column are similar to those of 5-aminoimidazole-4-*N'*-methylcarboxamidine which was obtained from 1-methyladenine (Brookes

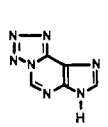
TABLE II: Percentage Yields of Breakdown and Rearrangement Products Obtained from N^6-Glycyladenine under Different Conditions.

Reaction No.	Starting Material	Reaction Condn	Peak 1 5-Aminoimidazole-4-carboxamide Hydrochloride (%)	Peak 2 *N*-(6-Purinyl)-glycine (%)	Peak 3 Adenine (%)	Peak 4 Carboxamidine (%)	Peak X %
1	N^6-Glycyladenine[a]	40 min in water at 100° then 40 min in 0.5 *N* HCl at 100°	21.2	13.0	18.9	~7	1.81
2	N^6-Glycyladenine[a]	20 min in 0.5 *N* HCl at 100°	16.6	2.98	22.9	~14	4.5
3	N^6-Chloroacetyladenine[a]	Same as in 1	16.4	1.67	35.4	~21.4	1.95
4	Cyclic intermediate V[a]	40 min in 0.5 *N* HCl at 100°	30.0	2.0	2.09[b]	~29.9	2.19
5	N^6-Glycyladenine	0.1 N NaOH at 100° for 1 hr			~100		
6	N^6-Glycyladenine	Aq soln at 100° for 35 hr		30	30		

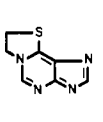
[a] The products from reactions 1–4 were separated on an ion-exchange column of Dowex-50. The separation conditions were identical for each of these four reaction mixtures, and are illustrated in Figure 1. The yield of each product was calculated from the spectrophotometric data and is based on N^6-glycyladenine. The products from reactions 5 and 6 were isolated by precipitation and crystallization. [b] No definite peak was obtained in this case. This probably is owing to an impurity of adenine present in the starting material.

and Lawley, 1960). Treatment of this carboxamidine (IX) with concentrated ammonia in a sealed tube yielded 5-aminoimidazole-4-carboxamide, which is analogous to the degradation of 5-aminoimidazole-4-*N'*-methylcarboxamidine under similar conditions.

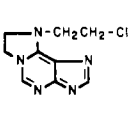
The Structure of the Cyclic Intermediate (V). The proposed structure, 3*H*-7,8-dihydro-8-oxoimidazo[2,1-*i*]purine,[3] for the cyclic intermediate was assigned on the basis of the following evidence: (1) The elemental analysis fits an empirical formula of $C_7H_5N_5O$ which differs from that of N^6-glycyladenine by the elements of ammonia. (2) The shift of the maximum absorption in the ultraviolet spectra to 303 mμ indicates a saturated C^6–N^1 bond (Skulachev, 1964). An analogous fused ring system, the tetrazolopurine (X) has an ultraviolet absorption spectra almost identical with that of compound V (Johnson *et al.*, 1958). (3) The breakdown and rearrangement of compound V described above indicates attachment of an alkyl group at N^1, since this chemistry is analogous to that reported for N^1-methyladenine. Thus, under slightly alkaline conditions N^1-methyladenine rearranges to N^6-methyladenine (Taylor, 1957; Elion, 1962) and under acid conditions it undergoes ring opening (Brookes and Lawley, 1960). (4) In analogous 6-substituted alkylpurines, with a functional group three atoms removed from the 6 position, interaction occurs with the N^1 position; *e.g.*, 6-(2-chloroethylthio)purine undergoes facile conversion to 7,8-dihydrothiazolo[2,3-*i*]purine (XI) and N^6,N^6-bis(2-chloroethyl)adenine spontaneously forms 9-(2-chloroethyl)-8-dihydro-9*H*-imidazo[2,1-*i*]purine (XII) (Johnston *et al.*, 1962).



X



XI

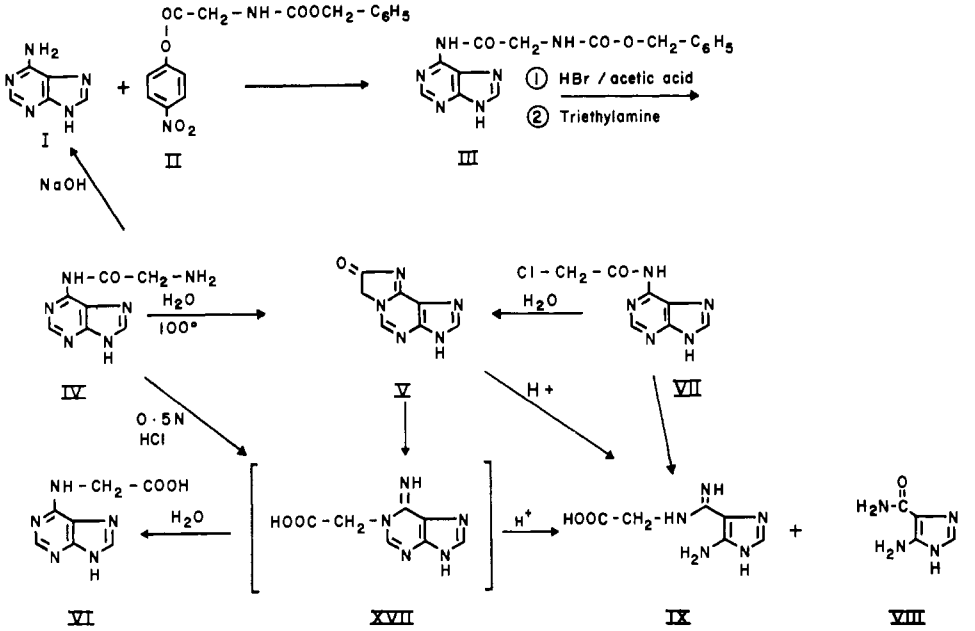


XII

Mechanism of Rearrangement. The unusual nature of the reactions of N^6-glycyladenine prompted us to study the properties of an analogous acyladenine derivative with a different α functional group, N^6-chloroacetyladenine (VII). An aqueous solution of this compound was heated several minutes at 100° and a cyclic intermediate was isolated in 29% yield from the resulting purple solution. This cyclic intermediate is identical with that obtained from N^6-glycyladenine on the basis of infrared

[3] The compound V, though written in keto form, may very well exist predominantly in enol form.

SCHEME II



and ultraviolet spectra, and chromatographic mobility in several solvent systems. On treatment with acid it is degraded to the series of products, including *N*-(6-purinyl)glycine (see Table II and Scheme II) which were obtained from N^6-glycyladenine. The same cyclic intermediate V was formed when dimethylformamide was used as solvent instead of water, suggesting that the product formed indeed was the result of an intramolecular nucleophillic displacement.

A mechanism for the formation of the cyclic intermediate from N^6-chloroacetyladenine (VII) thus appears to involve a nucleophillic displacement in which the chlorine atom serves as a leaving group. An analogous mechanism for the cyclization of N^6-glycyladenine with concomitant elimination of ammonia is not probable, since the amino group is not known to be a leaving group except possibly in the protonated form. It appears more likely that the amino group participates in a cleavage of N^1–C^2 bond probably by intramolecular base catalysis by the addition of water to C_2 of IV as shown in XIII (Scheme II). Now the α amino group as the conjugate acid should facilitate the ring opening and give XIV. The postulated intermediate, XIV could undergo addition–elimination to afford intermediate XV which could undergo ring closure at either nitrogen of the imidazolone to yield compound V or XVI. Compound XVI deserves some consideration as a structure for the cyclic intermediate since the five breakdown products formed from the cyclic intermediate could conceivably arise from this compound. The evidence cited above, however, favors structure V.[4]

Formation of *N*-(6-purinyl)glycine (VI) starting from V in alkaline or neutral media probably occurs by hydrolysis to give first adenine-1-acetic acid (XVII) which then rearranges to compound VI by ring opening and closing as reported for the rearrangement of 1-methyladenine to 6-methylaminopurine (Taylor, 1957; Elion, 1962). The formation of carboxamidine IX and 5-aminoimidazole-4-carboxamide (VIII) from compound V occurs only under strongly acidic conditions most probably *via* adenine-1-acetic acid which could degrade in a fashion similar to that observed for 1-methyladenine (Brookes and Lawley, 1960). Confirmation of these reaction mechanisms may be provided by current studies in our laboratory on suitably labeled glycyladenine and other model compounds.

Conclusions

The sequence of reactions that N^6-glycyladenine undergoes is probably general for all N^6-(α-aminoacyl)-adenines; *e.g.*, we have isolated from an aqueous solution of N^6-phenylalanyladenine a cyclic product with properties corresponding to those of compound V (G. Chheda and R. Hall, unpublished results). N^6-(*N*-carbobenzoxy-α-aminoacyl)adenines are stable in boiling water and the corresponding *N*-formyl derivatives of N^6-phenylalanyladenine and N^6-valyladenine are also stable under these conditions (G. Chheda and R. Hall, unpublished data). Therefore, degradation will not occur when the α amino group is blocked by an amide linkage.

These data are of particular importance to our understanding of the significance of the N^6-(*N*-formyl-α-aminoacyl)adenosines isolated from yeast s-RNA (Hall and Chheda, 1965). It would appear that a function of

[4] In what may very well be an analogous reaction, Johnston and Gallagher (1963) report that, under acidic conditions, 6-(2-aminoethylthio)purine undergoes ring closure with loss of the elements of ammonia to form 7,8-dihydrothiazolo[2,3-*i*]purine (XI). If one considers that the 2-amino group does not serve as a leaving group then in this case the N^1 nitrogen from the ring is the nitrogen eliminated.

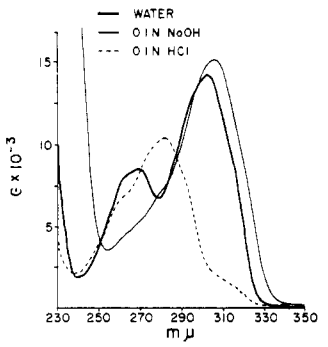


FIGURE 2: Ultraviolet absorption spectra of the cyclic intermediate V.

the formyl group is to provide chemical stability to these compounds. Since the isolated aminoacyl nucleosides were not recovered quantitatively from the enzymic hydrolysates, we cannot conclude that all aminoacyl groups attached to the N^6 position of adenylic acid residues in RNA would have a formyl protecting group, but nevertheless a protecting group on all such derivatives would be a requirement for stability.

Experimental Section

General. Melting points were determined in capillary tubes and are uncorrected. Infrared spectra were determined in KBr disks with a Perkin-Elmer 137B "Infracord" spectrophotometer. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer.

Paper Chromatography. 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) methanol–glacial acetic acid–water (12:3:5); (G) methanol. Chromatograms were run in a descending manner on Whatman no. 1 paper for 16 hr.

N^6-(N-Carbobenzoxyglycyl)adenine (III). To a solution of 3.3 g (10.0 mmoles) of *N*-carbobenzoxyglycine *p*-nitrophenyl ester in 8 ml of dimethylformamide at room temperature was added a hot solution (at 110°) of 675 mg (5.0 mmoles) of adenine in 14 ml of a mixture of dimethyl sulfoxide and dimethylformamide (7 ml each). The reaction mixture was then placed in an oil bath at 90 ± 2° and stirred for 4 hr. The solution was cooled to room temperature and evaporated to dryness at 55° *in vacuo*. The solid residue was triturated first in hot toluene (25 ml) and then in boiling chloroform (30 ml). The white insoluble product was collected on a filter and dried, 1.41 g (86.5%), mp 233–234°. The product was stirred in 10 ml of cold 0.5 N HCl and then filtered, washed with water, and dried, 1.35 g (83.1%), mp 234–235°. An analytical sample was prepared by crystallization from ethanol, mp 234–235°. The infrared and ultraviolet spectra and the chromatographic behavior of this material were identical with those of the N^6-(*N*-carbobenzoxyglycyl)adenine prepared according to the procedure of Brink and Schein (1963). The mixture melting point of the two products was not depressed; γ_{max} in cm^{-1} (KBr): 3375 (NH), 2800 (N_9–H), 1715, 1695 (C=O urethan, C=O amide), 1630, 1580 (C=C, C = N of purine), 1545 (NH), 1220, 1060 (CO-urethan), 885 (N_9–H).

Anal. Calcd for $C_{15}H_{14}N_6O_3$: C, 55.21; H, 4.32; N, 25.76. Found: C, 55.13; H, 4.55; N, 25.65.

N^6-Glycyladenine Trihydrobromide. To 6.52 g (20.0 mmoles) of N^6-(*N*-carbobenzoxyglycyl)adenine (III) was added 45 ml of a solution of 30% HBr in acetic acid. After being stirred for 1 hr, the mixture was diluted with 35 ml of anhydrous ether. The precipitated salt was collected on a filter, washed with ether, and dried *in vacuo* at room temperature over NaOH pellets, 9.08 g. The product was triturated with chloroform, then filtered, and dried *in vacuo* at room temperature for 24 hr over NaOH pellets and then again *in vacuo* at 56° over P_2O_5 for 24 hr, 8.68 g (99.5%); γ_{max} in cm^{-1} (KBr): 3090 (NH_3^+), 2600–2900 (broad) (NH_3^+), 1720 (C = O).

Anal. Calcd for $C_7H_8N_6O \cdot 3HBr$: C, 19.32; H, 2.55; Br, 55.12, N, 19.32. Found: C, 18.94; H, 3.13; Br, 53.83; N, 19.09.

N^6-Glycyladenine (IV). To a stirred suspension of 539 mg (1.24 mmoles) of N^6-glycyladenine hydrobromide salt in 7 ml of chloroform at 8° was added 0.70 ml (5.00 mmoles) of triethylamine. The reaction mixture was stirred for 15 min at 8–13°, and the pale white precipitate which formed was collected on a filter, washed with cold chloroform, ether, and ice-cold water. The precipitate was dried *in vacuo* first at 25 and then at 80° over P_2O_5 for 18 hr at each temperature, 220 mg (84.5%); it slowly decomposed over a range of 180–240°; γ_{max} in cm^{-1} KBr: 3425, 3200 (OH, NH_2, NH), 1720 (C=O), 1630, 1560 (NH, and C=C, C=N purine), 1320 (purine ring), 890 (N_9–H).

Anal. Calcd for $C_7H_8N_6O \cdot H_2O$: C, 40.00; H, 4.80; N, 39.98. Found: C, 39.69; H, 4.78; N, 39.85.

N^6-Chloroacetyladenine (VII) was prepared according to the procedure of Craveri and Zoni (1958); γ_{max} in cm^{-1} (KBr): 3340 (NH), 1710 (C=O), 1640, 1580, 1550 (C = C, C = N, NH), 710 (CCl).

Anal. Calcd for $C_7H_6ClN_5O$: C, 39.73; H, 2.84; Cl, 16.76; N, 33.10. Found: C, 40.01; H, 2.93; Cl, 17.0; N, 32.90.

Formation of the "Cyclic Intermediate" V, 3H-7,8-Dihydro-8-oxoimidazo[2,1-i]purine.

A. FROM N^6-GLYCYLADENINE (IV). A suspension of 375 mg (1.78 mmoles) of N^6-glycyladenine in 20 ml of water was heated at 100° for 40 min, during which time the solution turned a dark purple. The solution was centrifuged in order to remove a colloidal purple matter and the clear liquid was evaporated to dryness. The residue was triturated with 4 ml of cold water, filtered, washed with water, and dried, 120 mg (38.5%). Crystallization from hot water gave solid grains of the

desired product, 56 mg. Additional product was recovered from the filtrate, 27 mg (total 83 mg 26.6%); the product does not have a melting point.

An analytical sample was prepared by triturating the crystalline material with hot water and then filtering the insoluble pure product; it was dried *in vacuo* at 100° for 36 hr over P_2O_5; it starts to decompose at 310–320°, but does not melt when heated to 345°; γ_{max} in cm^{-1} (KBr): 3050 (NH, OH); 2800 (acidic H), 1635 (broad) (may be due to C=O, or C=C, C=N purine), 1200 (CO); R_{Ad} A, 0.64; B, 0.79; C, 2.00; D, 0.61. The ultraviolet absorption spectra is shown in Figure 2 and the values are recorded in Table I. There is no absorption of light in the visible region. Therefore, the dense purple color which appeared in the original reaction mixture appears to be owing to a trace product removed by centrifugation.

Anal. Calcd for $C_7H_5N_5O$: C, 47.98; H, 2.87; N, 40.0. Found: C, 47.63; H, 2.96; N, 39.66.

B. FROM N^6-CHLOROACETYLADENINE (VII). A suspension of 211 mg (1.00 mmole) of N^6-chloroacetyladenine in 15 ml of water (pH adjusted to 6.8 by adding 0.1 N NaOH) was placed in a water bath at 60° for 20 min. The suspension, after readjusting the pH to 6.8 was heated in a boiling water bath for a period of 40 min. During this period the pH was adjusted to 6.8 every 8 min. The purple reaction mixture was centrifuged and the supernatant was evaporated *in vacuo* to dryness. The residue was dissolved in 10 ml of water, centrifuged, and the clear solution was allowed to crystallize. A pale brown solid was obtained in three small crops of 52 mg (29.7%). The infrared and ultraviolet spectra and the chromatographic behavior of this material are identical with those of the cyclic intermediate, V, isolated from N^6-glycyladenine. The product did not melt when heated to 340°.

Conversion of N^6-Glycyladenine (IV) to N-(6-Purinyl)-glycine (VI). A solution of 530 mg (2.52 mmoles) of N^6-glycyladenine in 50 ml of water was refluxed for a period of 35.5 hr during which time the pH was maintained between 7.0 and 8.2. The dark purple reaction solution was filtered through a Celite pad and then evaporated to dryness *in vacuo*. The residue was triturated with 5 ml of cold water and filtered in order to remove adenine, 102 mg (30.0%). The filtrate was concentrated to 3 ml and the pH was adjusted to 3 with 88% formic acid. The precipitated *N*-(6-purinyl)-glycine was collected on a filter and dried; 152 mg (30.2%). This product was purified by dissolving in sodium bicarbonate solution and then precipitating with formic acid at pH 3; 100 mg (20.6%). A sample for analysis was prepared by crystallization from hot water. The pale brown aggregates were dried *in vacuo* at 100° for 66 hr over P_2O_5. The sample began decomposing >300°. The ultraviolet and infrared spectra and the paper chromatographic behavior (systems A–E and G) were identical with those of the authentic sample of *N*-(6-purinyl)glycine (see Table I); γ_{max} in cm^{-1} (KBr): 3350 (NH), 3200–3000 (OH acid), 1670 (C=O), 1625 (NH, C=N, C=C), 1390 (CO).

Anal. Calcd for $C_7H_7N_5O_2 \cdot 0.425\ H_2O$: C, 41.70; H, 3.92; N, 34.80; H_2O, 3.80. Found: C, 41.82; H, 4.08; N, 35.20; H_2O, 4.22.

The Stability of the Cyclic Intermediate V. The cyclic intermediate was stable for months in neutral aqueous solution at room temperature. Solutions of compound V in 0.1 N hydrochloric acid or 0.1 N sodium hydroxide were stable at room temperature, for at least 25 min, before degradation was observed as judged by a change in the ultraviolet spectra.

Reactions of The Cyclic Intermediate V. I. TREATMENT WITH 0.5 N HCL. A reaction mixture containing 35 mg (0.20 mmole) of the cyclic intermediate V in 3 ml of 0.5 N hydrochloric acid was heated in a boiling water bath for a period of 40 min. The degradation products were separated on a column of Dowex-50 resin according to the procedure described below. The results are recorded in Table II (reaction 4). A small amount of adenine was obtained, but this is probably due to contamination of the starting material.

2. TREATMENT WITH WATER. An aqueous solution, 30 ml (9.32 mg/l.) was gently refluxed for a period of 24 hr. The examination of aliquots by ultraviolet spectra revealed that after 3 hr of refluxing 27.7% of the starting material remained. At the end of 18.5 hr the yield of *N*-(6-purinyl)glycine was estimated at 87% and this was the only detectable product of the reaction.

In another experiment a solution of 1.95 mg of the cyclic intermediate in 2 ml of water was gently refluxed and the progress of the reaction was followed by paper chromatography. In 40 min a new spot appeared which was lost within 3.5 hr and *N*-(6-purinyl)glycine was produced. At the end of 7 hr only *N*-(6-purinyl)glycine could be seen; the new spot and starting material had disappeared. The transient intermediate on the basis of chromatographic mobility (in system D) is probably identical with that described in the next section.

3. TREATMENT WITH ALKALI. A solution of 2.16 mg of the cyclic intermediate V in 2 ml of 1 N sodium hydroxide was allowed to stand at room temperature for 110 min and then heated in an oil bath at 100° for 19 hr. The course of the reaction was followed by paper chromatography (in system A). The cyclic material V is first converted into another product as evidenced by a spot adjacent to *N*-(6-purinyl)glycine. This spot finally disappeared as the heating was continued and only *N*-(6-purinyl)glycine remained at the end of 19 hr. The transient intermediate may be adenine-1-acetic acid (XVII) formed by the hydrolysis of the cyclic compound V which in turn rearranges to *N*-(6-purinyl)glycine (VI); no 5-aminoimidazole-4-carboxamide VIII could be detected. The ultraviolet spectra of the reaction solution at the end of 19 hr of heating resembled those of *N*-(6-purinyl)glycine.

Treatment of N^6-Glycyladenine with Water and Hydrochloric Acid and Separation of the Products by Means of an Ion-Exchange Column. A suspension of 507 mg (2.42 mmoles) of N^6-glycyladenine in 35 ml of water was heated at 100° for 40 min during which time the solution turned purple. The solution was cooled to room temperature and diluted with an equal volume of 1 N hydrochloric acid. The mixture was heated at

TABLE III: Paper Chromatographic Data.

Compd	Solvent Systems (R_F Values)						
	A	B	C	D	E	F	G
Fraction 1, Figure 1	0.60	0.47	0.21	0.59	0.28	0.71	0.25
5-Aminoimidazole-4-carboxamide	0.60	0.47	0.21	0.59	0.28	0.71	0.25
Fraction 2, Figure 1	0.37	0.40	0.04	0.50	0.03	0.64	0.27
N-(6-Purinyl)glycine	0.37	0.40	0.04	0.50	0.03	0.64	0.27
Adenine	0.57	0.28	0.33	0.66	0.35	0.66	0.18
N^6-(*N*-Carbobenzoylglycyl)adenine (III)	0.74			0.71	0.49	0.83	
Cyclic intermediate (V)	0.37	0.23	0.66	0.40			
N^6-Chloroacetyladenine (VII)	0.59	0.30	0.88				

100° for 40 min. The red-brown solution was evaporated *in vacuo* (bath temperature 15°). The brown residue was triturated with 5 ml of water and again evaporated *in vacuo*. Finally the residue was dissolved in water and lyophilized to give a brown powder. The powder was dissolved in 10 ml of water and pH was adjusted to 7 by addition of dilute sodium hydroxide solution. The solution was diluted to 18 ml with water and this preparation served as a stock solution for the following experiments.

An aliquot of the stock solution (2.65 ml) (equivalent to 74 mg, 0.357 mmole of N^6-glycyladenine) was absorbed on a column (16 × 1 cm) of Dowex-50W ion-exchange resin (H^+ form, 200–400 mesh). The column was developed with a linear gradient of 0.2 M HCl–1.6 M HCl, total volume, 6000 ml. The elution profile obtained is shown in Figure 1. No additional products were obtained when the elution was continued further with stronger hydrochloric acid solutions.

The fractions corresponding to the five ultraviolet-absorbing peaks of Figure 1 were concentrated to a small volume *in vacuo* (1 mm) employing a flash evaporator with a bath temperature of 15°. Liquid nitrogen was used to cool the condensing flask. Each of the five fractions contained a single ultraviolet-absorbing compound as judged by paper chromatography in seven solvent systems (see Table III). The specific isolation and characterization details are given below. A summary of the results of this experiment is recorded in Table II (reaction 1).

Fraction I, 5-Aminoimidazole-4-carboxamide Hydrochloride (VIII). The solution was evaporated to dryness. The white residue was triturated with three portions of alcohol (2 ml) and dried, mp 240–260° dec. Infrared and ultraviolet spectra of this material were identical with those of an authentic sample of 5-aminoimidazole-4-carboxamide hydrochloride (obtained from Nutritional Biochemical Corp.). The R_F values of this material in the seven solvent systems were the same as those for the standard sample of 5-aminoimidazole-4-carboxamide hydrochloride; γ_{max} in cm^{-1} (KBr): 3600, 3300 (NH_2, NH^+), 2850 (NH_3^+), 1700, 1650, 1600 (NH_3^+, C=O, C=C, C=N); yield was 21.2%, based on N^6-glycyladenine.

Anal. Calcd for $C_4H_7ClN_4O$: C, 29.54; H, 4.34; Cl, 21.81; N, 34.46. Found: C, 29.33; H, 4.46; Cl, 22.03; N, 33.96.

Fraction 2, N-(6-Purinyl)glycine Hydrochloride (VI). The concentrated solution was diluted twice with 15 ml of ice-cold water and again concentrated to a small volume. The white crystalline product was filtered off. The crystals were washed with warm absolute alcohol, and dried, mp 230–245° dec. The ultraviolet spectra and the R_F values in seven solvent systems were same as those for the standard sample of *N*-(6-purinyl)-glycine prepared from 6-chloropurine and glycine according to the procedures of Carter (1956) and Ward *et al.* (1961). The yield was 13% based on N^6-glycyladenine (Table IV).

Fraction 3, Adenine. Adenine was identified from its ultraviolet spectra and by chromatographic comparison with a standard sample. The yield was 18.9% based on N^6-glycyladenine.

Fraction 4, 5-Aminoimidazole-4-N'-carboxymethylcar-

TABLE IV: Paper Electrophoresis.

	Distance Moved from the Origin (cm)	
	Isolated Sample[a]	*N*-(6-Purinyl)-glycine
in 0.05 M Glycine buffer pH 9.2 at 1000 v (22 v/cm) for 4 hr on Whatman 1MM paper	+23.3	+23.3
in 0.05 M Formic acid pH 3 at 4500 v (100 v/cm) for 1 hr on Whatman 3MM paper	−15.3	−15.3

[a] *Anal.* Calcd for $C_7H_7N_5O_2 \cdot HCl$: C, 36.61; H, 3.51; Cl, 15.44; N, 30.50. Found: C, 37.01; H, 4.24; Cl, 16.19; N, 29.77. (Better analytical data was obtained on the free base.)

boxamidine (*IX*). The fraction contained a product which had an ultraviolet absorption spectra very similar to 5-aminoimidazole-4-*N'*-methylcarboxamidine. The yield was 21.2% based on N^6-glycyladenine. To 0.2 ml (0.66 mg) of solution of this material in water was added 0.5 ml of concentrated ammonia. The mixture was heated in a sealed tube at 100° for 24 hr and then evaporated to dryness. Paper chromatography of this reaction mixture in solvent systems A, B, D, E, and G revealed that 5-aminoimidazole-4-carboxamide was the major product of this reaction.

Fraction X. This fraction contained a material which appears to be 5-aminoimidazole-4-carboxamide (VIII) from the ultraviolet spectra and from the chromatographic comparison in four solvent systems. It is formed most probably on the column from the degradation of carboxamidine (fraction 4).

Treatment of N^6-Glycyladenine (IV) with 0.5 N HCl. A solution of 96 mg (0.456 mmole) of N^6-glycyladenine in 6 ml of 0.5 N hydrochloric acid was heated at 100° for 20 min. The red-brown solution was lyophilized, and the brown powder was dissolved in 3 ml of water. The solution was neutralized with sodium hydroxide and the products were separated on a column of Dowex-50 ion-exchange resin as described above. The results are listed in Table II (reaction 2).

Treatment of N^6-Chloroacetyladenine (VII) with Water and Hydrochloric Acid. A suspension of 106 mg (0.50 mmole) of N^6-chloroacetyladenine in 8 ml of water was heated in a boiling water bath for 40 min. The dark purple solution which had a pH of 2.4 was diluted with an equal volume of 1 N hydrochloric acid and the solution was heated again in a boiling water bath for 40 min. The reaction mixture was then analyzed on Dowex-50 ion-exchange resin as described above. The yields of the products obtained are recorded in Table II (reaction 3).

Acid Hydrolysis of N^6-(N-Carbobenzoxyglycyl)adenine (III). A suspension of N^6-(*N*-carbobenzoxyglycyl)-adenine in 0.5 N hydrochloric acid was heated at 100° for 20 min. Examination of the colorless solution by means of ultraviolet absorption spectra and paper chromatography revealed a total conversion to adenine.

References

Angell, C. L. (1961), *J. Chem. Soc.* 504.

Ballio, A., and Vittorio, V. di (1960), *Gazz. Chim. Ital. 90*, 501.

Brink, J. J., and Schein, A. H. (1963), *J. Med. Chem. 6*, 563.

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

Bullock, M., Hand, J., and Stokstad, E. L. R. (1957), *J. Org. Chem. 22*, 568.

Carter, C. E. (1956), *J. Biol. Chem. 223*, 139.

Chheda, G. B., and Hall, R. H. (1965), Abstracts, 149th National Meeting of the American Chemical Society, Detroit, Mich., April, p 12N.

Craveri, F., and Zoni, G. (1958), *Chimica (Milan) 34*, 407.

Elion, G. B. (1962), *J. Org. Chem. 27*, 2478.

Hall, R. H. (1964), *Biochemistry 3*, 769.

Hall, R. H., and Chheda, G. B. (1965), *J. Biol. Chem.* 240, PC2754.

Hall, R. H., and Chheda, G. B. (1966), *Federation Proc. 25*, 776.

Johnston, T. P., Fikes, A. L., and Montgomery, J. A. (1962), *J. Org. Chem. 27*, 973.

Johnston, T. P., and Gallagher, A. (1963), *J. Org. Chem. 28*, 1305.

Johnson, J. A., Jr., Thomas, H. J., Schaeffer, H. J. (1958), *J. Am. Chem. Soc., 80*, 699.

Jones, J. W., and Robins, R. K. (1962), *J. Am. Chem. Soc. 84*, 1914.

Kossel, A. (1888), *Z. Physiol. Chem. 12*, 247.

Lettre', H., and Ballweg, H. (1961), *Ann. 649*, 124.

Montgomery, J. A., and Thomas, H. J. (1965), *J. Org. Chem. 30*, 3235.

Schein, A. H. (1962), *J. Med. Pharm. Chem. 5*, 302.

Skulachev, V. P. (1964), Abstracts, Intern. Congr. Biochem., 6th, New York, N. Y., p 758.

Taylor, E. C. (1957), The Chemistry and Biology of Purines, Boston, Mass., Little, Brown, p 57.

Ward, D. N., Wade, J., Walborg, E. F., and Osdene, T. S. (1961), *J. Org. Chem. 26*, 5000.