Purine Nucleosides. IX. The Synthesis of 9-β-D-Ribofuranosyl Uric Acid and Other Related 8-Substituted Purine Ribonucleosides[1]

Richard E. Holmes and Roland K. Robins[2]

Contribution from the Department of Chemistry, Arizona State University, Tempe, Arizona. *Received November 30, 1964*

The synthesis of 9-β-D-ribofuranosyl uric acid (IV) has been accomplished in several steps from guanosine. This product is identical with 9-ribosyl uric acid isolated from Lactobacillus plantarum. Comparison of IV and the data available as reported by Falconer and Gulland for 9-ribosyl uric acid isolated from beef blood and liver reveals that these products are very likely the same. Other purine nucleosides synthesized containing a keto group at position 8 are 2-amino-9-β-D-ribofuranosyl-6,8-purinedione (V) and 6-amino-9-β-D-ribofuranosyl-8-purinone (XII). Refluxing aqueous hydrazine and 8-bromoguanosine yields 8-aminoguanosine (VII), the first reported 8-aminopurine nucleoside. 8-Aminoadenosine (IX) was prepared from 8-bromoadenosine via 8-azidoadenosine which was converted to IX by catalytic reduction. The possible biochemical significance of these new purine ribonucleosides is discussed.

The structure of "uric acid riboside" isolated from beef blood has been determined as 3-D-ribosyl uric acid,[3,4] and confirmed by synthesis.[5,6] Falconer and Gulland[7] report that "uric acid riboside" isolated from fresh beef blood and liver is 9-ribosyl uric acid based on comparison of the ultraviolet absorption spectra with the corresponding methylated uric acids. An unsuccessful attempt has been made to synthesize 9-ribosyl uric acid[5] in an effort to clarify the nature of the product described by Falconer and Gulland.[7] The biosynthesis of uric acid 9-ribonucleotide has recently been reported[8] in an extract of *Lactobacillus plantarum*. The nucleotide was converted into a compound which has been assumed to be 9-ribosyl uric acid.[8]

The present work describes the chemical synthesis of 9-β-D-ribofuranosyl uric acid. Since it was desired to obtain uric acid 9-ribonucleoside as the ribofuranosyl derivative of established β-configuration, 8-bromoguanosine[9] was selected as the starting material for the synthesis.

8-Bromoguanosine[9] (I) was treated with sodium benzyloxide in dimethyl sulfoxide to give 8-benzyloxyguanosine (II) in 70% yield. Treatment of II with sodium nitrite in aqueous acetic acid at 5° gave 8-benzyloxyxanthosine (III) which in turn gave 9-β-D-ribofuranosyl uric acid (IV) when III was debenzylated by catalytic hydrogenation with palladium-on-carbon catalyst (Scheme I).

Comparison of the ultraviolet absorption spectra with that recorded by Falconer and Gulland[7] for uric acid 9-riboside has been made in Table I. Although the spectra are not absolutely identical, there is considerable similarity and the small differences noted could well be due to a small amount of impurity since Falconer and Gulland[7] record only a nitrogen analysis as a check on the purity of their product.

Table I. Comparison of Ultraviolet Absorption Spectra of 9-β-D-Ribofuranosyl Uric Acid (IV) and 9-Ribosyl Uric Acid Isolated from Natural Sources

9-β-D-Ribofuranosyl uric acid (IV)[a]		9-Ribosyl uric acid[b]		9-Ribosyl uric Acid[c]	
$\lambda_{max}^{pH\ 1}$	$\lambda_{min}^{pH\ 1}$	$\lambda_{max}^{0.05\ N\ HCl}$	$\lambda_{min}^{0.05\ N\ HCl}$	$\lambda_{max}^{pH\ 1}$	$\lambda_{min}^{pH\ 1}$
236.5	258	238	258	234	257
287.5		288		286	
$\lambda_{max}^{pH\ 14}$	$\lambda_{min}^{pH\ 14}$	$\lambda_{max}^{0.05\ N\ NaOH}$	$\lambda_{min}^{0.05\ N\ NaOH}$	$\lambda_{max}^{pH\ 14}$	$\lambda_{min}^{pH\ 1}$
248	275	251	282	250	274
302		301		303	
$\lambda_{max}^{H_2O}$	$\lambda_{min}^{H_2O}$	$\lambda_{max}^{H_2O}$	$\lambda_{min}^{H_2O}$	$\lambda_{max}^{pH\ 7}$	$\lambda_{min}^{pH\ 7}$
240	222	241	225	238	220
294	265	293	271	293	263

[a] Synthetic. [b] Read from spectra of Falconer and Gulland.[7] [c] Isolated from *Lactobacillus plantarum*.[8,10]

The optical rotation of 9-β-D-ribofuranosyl uric acid (IV) is $[\alpha]^{29}D$ −41.2° (*c* 1.02, 0.1 *N* sodium hydroxide) which compares favorably with the value $[\alpha]^{20}D$ −40.8 (*c* 1 g., 0.1 *N* sodium hydroxide) reported by Falconer and Gulland.[7] This is decidedly different from the value of $[\alpha]^{25}D$ −19.8 recorded by Forrest and co-workers[3] for 3-ribosyl uric acid. 9-β-D-Ribofuranosyl uric acid (IV) was stable to refluxing 1 *N* hydrochloric acid after 2 hr. In contrast, 3-ribosyl uric acid is readily hydrolyzed[3] by normal hydrochloric acid after 1 hr. at 100°. Falconer and Gulland[7] report that 6 hr. in 16% sulfuric acid was required for complete hydrolysis of their product. On the basis of the present evidence it does appear that the product described by Falconer and Gulland as "uric acid 9-riboside" could well have been 9-β-D-ribofuranosyl uric acid. Although recent attempts to reisolate the compound of Falconer and Gulland have not been successful,[3] it is possible that bacterial or enzymatic contamination of the crude blood and/or liver may have been responsible for the results obtained by the earlier workers. The ready availability of 9-β-

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(2) Department of Chemistry, University of Utah, Salt Lake City, Utah.

(3) H. S. Forrest, D. Hatfield, and J. M. Lagowski, *J. Chem. Soc.*, 963 (1961). See this paper for a review of earlier literature on uric acid riboside.

(4) D. Hatfield, R. R. Rinehart, and H. S. Forrest, *ibid.*, 899 (1963).

(5) R. Lohrmann, J. M. Lagowski, and H. S. Forrest, *ibid.*, 451 (1964).

(6) L. Birkofer, A. Ritter, and H. P. Külthau, *Angew. Chem.*, **75**, 209 (1963).

(7) R. Falconer and J. M. Gulland, *J. Chem. Soc.*, 1369 (1939).

(8) D. Hatfield, R. A. Greenland, H. L. Stewart, and J. B. Wyngaarden, *Biochim. Biophys. Acta*, **91**, 160 (1964).

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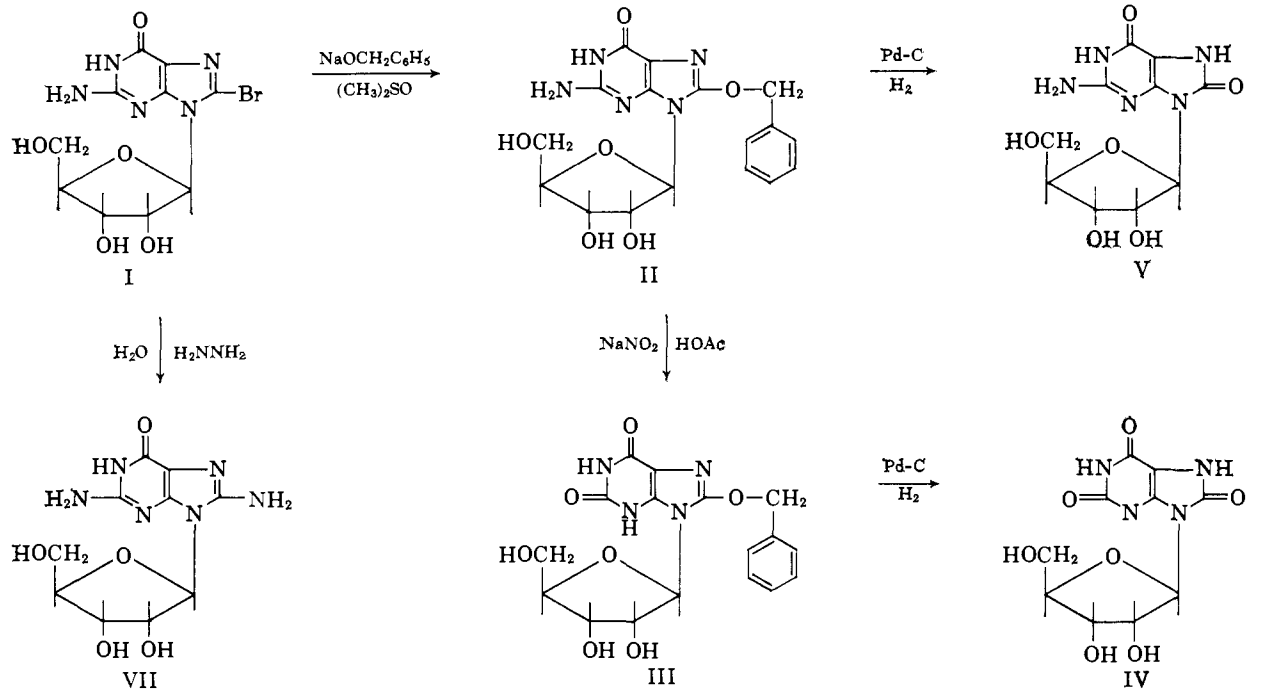
Scheme I



D-ribofuranosyl uric acid provided by the present synthesis should be of assistance in aiding biochemists in the future detection of this interesting purine nucleoside.

A careful comparison (ultraviolet absorption spectra and chromatography in four solvent systems) has established the identity of our product (IV) with that of 9-ribosyl uric acid isolated from *L. plantarum*.[8,10]

The use of the benzyloxy group as a method of introducing a keto function into the purine nucleus has previously been reported[11] in a synthesis of the nucleoside analog 9-(tetrahydro-2′-furyl)guanine. Catalytic debenzylation of 8-benzyloxyguanosine gave an 80% yield of 2-amino-9-β-D-ribofuranosyl-6,8-purinedione (8-hydroxyguanosine, V). Although this purine nucleoside has not yet been isolated from biological sources, the purine base 2-amino-6,8-purinedione has been shown to occur naturally in the sea squirt *Microcosmos polymorphus*.[12]

In accord with a general program begun in our laboratory involving the synthesis of purine nucleosides of potential biological origin,[13,14] a number of additional 8-substituted purine ribosides were prepared. Since the methoxy group has been shown to occur in the natural purine nucleoside spongosine[15,16] (2-methoxyadenosine), the isomeric 8-methoxyadenosine (VI) was prepared from 8-bromoadenosine[9] (VIII) and refluxing methanol containing sodium methoxide. There is a marked difference between the ease of substitution of the 8-bromo group in 8-bromoadenosine and 8-bromoguanosine. 8-Bromoadenosine is much more reactive.

This is probably due in part to the fact that in the case of strongly basic nucleophiles the proton at N-1 of 8-bromoguanosine is removed to give an anion which resists another negative charge introduced into the molecule *via* the transition state due to an attacking nucleophile at position 8. Support for this contention is found in the case of a good nucleophile which is a relatively weak base. In this instance, 8-bromoguanosine is essentially as reactive as 8-bromoadenosine. Thus, thiourea in refluxing ethanol readily provided 2-amino-9-β-D-ribofuranosylpurin-6-one-8-thione (8-mercaptoguanosine).[9] The conditions for the preparation of 8-methoxyadenosine (VI) gave only starting material with 8-bromoguanosine. Although 8-methylthioadenosine was readily prepared from 8-bromoadenosine and sodium methylmercaptide in refluxing ethanol[9] under the same conditions, 8-bromoguanosine was recovered unchanged. The general deactivating effect of an anion toward nucleophilic substitution in the purine ring has already been well documented.[17]

The influence of substituent groups on the ease of reaction of the 8-bromo group is strikingly demonstrated by the fact that 8-bromo-2′,3′,5′,6-N-tetraacetyladenosine reacted with methanolic ammonia (containing a small amount of water) to yield 6-N-acetylamino-9-β-D-ribofuranosyl-8-purinone. Subsequent treatment of 6-N-acetylamino-9-β-D-ribofuranosyl-8-purinone with normal sodium hydroxide provided 6-amino-9-β-D-ribofuranosyl-8-purinone (XII, 8-hydroxyadenosine) in good yield. The 8-"hydroxy" purine nucleosides described in the present study all show a carbonyl band in the infrared region 1730–1750 cm.$^{-1}$ which has been assigned by Mason[18,19] to the cyclic amide struc-

(10) The authors wish to thank Drs. Dolph Hatfield and James B. Wyngaarden for their helpful assistance in making this comparison possible.

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(18) S. F. Mason in "The Chemistry and Biology of Purines," A Ciba Symposium, G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957, p. 67.

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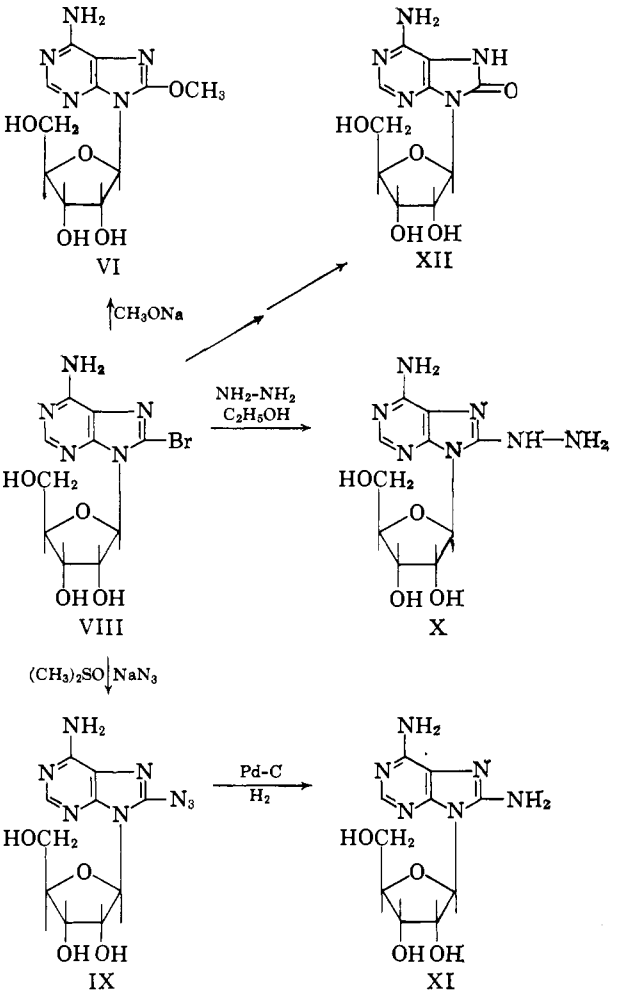
ture of 8-purinones. Acid hydrolysis of XII gave 6-amino-8-purinone which was identified by rigorous comparison with an authentic sample previously prepared in this laboratory.[20] 6-Amino-9-β-D-ribofuranosyl-8-purinone (XII) is of interest due to the antitumor activity of the purine base 6-amino-8-purinone.[21]

The possibility of the introduction of an amino substituent *via* the 8-bromo group was next investigated. Attempts at direct amination of 8-bromoadenosine and 8-bromoguanosine with aqueous and alcoholic ammonia below 125° were unsuccessful. At higher temperatures considerable decomposition was noted. Zimmer and Mettalia report that 8-hydrazinocaffeine,[22] when heated in refluxing dimethylformamide, yields 8-aminocaffeine. When 8-bromoguanosine (I) was refluxed with an aqueous solution of hydrazine for 36 hr., the product isolated was 8-aminoguanosine (VII) in 68% yield. Acid hydrolysis of VII readily gave D-ribose and 2,8-diamino-6-purinone.[23] This certainly provides a most convenient synthesis of 2,8-diamino-9-β-D-ribofuranosyl-6-purinone (8-aminoguanosine, VII). 2,8-Diaminopurine nucleosides are of current interest due to their structural relationship to the paralytic shellfish poison, saxitoxin.[24] Attempts to extend this method to the preparation of 8-aminoadenosine were unsuccessful. The synthesis of 8-hydrazinoadenosine (X) was accomplished with refluxing ethanolic hydrazine but attempts to convert X to 8-aminoadenosine (XI) were not successful. 8-Aminoadenosine was finally obtained *via* 8-azidoadenosine (IX) which was prepared by treating 8-bromoadenosine (VIII) with sodium azide in dimethyl sulfoxide. This is the first recorded nucleophilic displacement of halogen by azide ion in a purine nucleoside.[25] The azido group was identified by the strong infrared absorption band at 2180 cm.$^{-1}$ noted for azidopurines.[26] Catalytic hydrogenation of 8-azidoadenosine (IX) gave an 82% yield of 8-aminoadenosine (6,8-diamino-9-β-D-ribofuranosylpurine, XI). Acid hydrolyses gave D-ribose and 6,8-diaminopurine[20] (see Scheme II).

It is of interest that refluxing 1 *N* hydrochloric acid hydrolyzed 8-aminoadenosine (XI) and 8-aminoguanosine (VII) in less than 15 min. while 2-amino-9-β-D-ribofuranosyl-6,8-purinedione (V) and 6-amino-9-β-D-ribofuranosyl-8-purinone (XII) were essentially unchanged after 2 hr. under the same conditions. One possible explanation of this stability noted could be due to the fact that III, V, and XII exist in the keto form in position 8 so that a proton is at position 7 in the neutral molecule; therefore, further protonation of the imidazole ring is difficult. In the case of the 8-aminonucleosides, position 7 should be even more readily protonated than in adenosine and guanosine. This would appear to lend support to the theory that protonation at position 7 is an important step in the acid cleavage of purine

Scheme II



nucleosides.[27] All products were chromatographically pure in three solvent systems. R_f values are recorded in Table II as an aid to the possible detection of these nucleosides in future biochemical studies. Ultraviolet absorption data are summarized in Tables III and IV.

Table II. R_f Values of Some 8-Substituted Purine Ribonucleosides

Compd.	Solvent[a] A	B	C
8-Aminoguanosine (VII)	0.41	0.46	0.24
2-Amino-9-β-D-ribofuranosyl-6,8-purinedione (V)	0.51	0.57	0.19
6-Amino-9-β-D-ribofuranosyl-8-purinone (XII)	0.46	0.64	0.32
8-Aminoadenosine (XI)	0.30	0.50	0.31
9-β-D-Ribofuranosyl uric acid (IV)	0.55	0.61	0.17

[a] Solvent: A, 5% NH_4HCO_3 in H_2O; B, EtOH–H_2O, 7:3 (v./v.); C, DMF–NH_4OH–*i*-PrOH, 25:10:65 (v./v.). All R_f values were determined on Whatman No. 1 by the descending method.

Experimental[28]

8-Methoxyadenosine (VI). 8-Bromoadenosine[9] (2 g.) was added to a solution of 0.4 g. of sodium dissolved in

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(25) A. Bendich, A. Giner-Sorolla, and J. J. Fox (ref. 18, p. 8) have reported the formation of 6-azidopurine from 6-chloropurine but no experimental details have since appeared.
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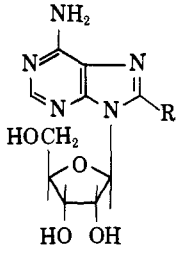
(27) S. G. A. Alivisatos, L. Lamantia, and B. L. Matijevitch, *Biochim. Biophys. Acta*, 58, 209 (1962).
(28) All melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected.

Table III. Ultraviolet Absorption Spectra of Some 8-Substituted Adenosines



R	Compd. no.	$\lambda_{max}^{pH\ 1}$, mμ	ε	$\lambda_{max}^{pH\ 11}$, mμ	ε
OCH_3	VII	261	13,600	259	14,700
$NHNH_2$	X	264	12,800	262	13,500
N_3	IX	281	17,300	281	13,500
				228	13,400
NH_2	XI	270	13,500	273	16,400
OH	XII	284	8,500	280	13,600
		264	8,900		

Table IV. Ultraviolet Absorption Spectra of Some 8-Substituted Purine Ribonucleosides and Guanosines

Compd	$\lambda_{max}^{pH\ 1}$, mμ	ε	$\lambda_{max}^{pH\ 11}$, mμ	ε
8-Benzyloxyxanthosine (III)	284	10,200	286	8,600
	235	11,300	243	15,000
9-β-D-ribofuranosyl uric acid (IV)	287.5	11,300	298	9,600
	236.5	9,600	244	12,000
8-Benzyloxyguanosine (II)	294	8,700	268[a]	8,900
	246	11,700	248	12,300
2-Amino-9-β-D-ribofuranosyl-6,8-purinedione (V)	294	11,100	280	10,200
	246	14,900	246	12,600
8-Aminoguanosine (VII)	289	9,600	271[a]	11,600
	250	16,900	257.5	13,500

[a] Shoulder.

60 ml. of methanol. The resulting solution was refluxed for 18 hr., cooled to room temperature, and neutralized with glacial acetic acid. The solvent was removed *in vacuo* and the residue was stirred in 20 ml. of water. This slurry was filtered and the residue was recrystallized from ethanol–water to yield 0.79 g. (44% yield) of product, m.p. 206–208° dec.

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot 0.5H_2O$: C, 43.2; H, 5.26; N, 22.9. Found: C, 43.6; H, 5.13; N, 23.0.

8-Aminoguanosine (VII). 8-Bromoguanosine[9] (10 g.) was added to a solution of 4 ml. of 95% hydrazine in 200 ml. of water. The resulting solution was refluxed for 48 hr.; after 20 hr. a precipitate began to form. The hot mixture was filtered; the precipitate was set aside and the filtrate was allowed to cool slowly to room temperature during which time additional product precipitated. The mixture was filtered again; the precipitates were combined (7.2 g.) and recrystallized from pyridine–water to yield 5.9 g. (69% yield) of product, m.p. >230° dec. An analytical sample, m.p. >240° dec., was obtained by recrystallization from water. 8-Aminoguanosine slowly darkens when exposed to air.

Anal. Calcd. for $C_{10}H_{14}N_6O_5 \cdot 0.5H_2O$: C, 39.1; H, 4.92; N, 26.4. Found: C, 39.2; H, 5.02; N, 26.5.

8-Hydrazinoadenosine (X). 8-Bromoadenosine[9] (2 g.) was added to a solution of 1.5 ml. of 95% hydrazine in 200 ml. of ethanol. The resulting solution was refluxed for 16 hr.; the solvent was removed *in vacuo* and the residue was recrystallized from a minimum amount of water to yield 0.91 g. (47% yield) of product which darkens at 180°, dec. 209–212°. 8-Hydrazinoadenosine darkens on exposure to air. Two more recrystallizations from water afforded an analytical sample, m.p. 212–214° dec. (darkens 185°).

Anal. Calcd. for $C_{10}H_{15}N_7O_4 \cdot 2H_2O$: C, 36.0; H, 5.75; N, 29.4. Found: C, 36.2; H, 5.08; N, 29.8.

8-Azidoadenosine (IX). Sodium azide (3 g.) was added to a solution of 5 g. of 8-bromoadenosine[9] in 50 ml. of dimethyl sulfoxide. The resulting solution was heated at 75° for 10 hr., cooled to room temperature, and then slowly poured into 1 l. of methylene chloride. This mixture was kept at −15° for 16 hr. and then was filtered. The residue was stirred in 50 ml. of water for 30 min., and the resulting slurry was filtered. The residue was recrystallized from water to yield 2.9 g. (65% yield) of product. 8-Azidoadenosine slowly darkens on exposure to air, and therefore was used immediately. An analytical sample, m.p. 226–229° dec. (Kofler Heizbank), was prepared by one more recrystallization from water.

Anal. Calcd. for $C_{10}H_{12}N_8O_4$: C, 38.95; H, 3.92; N, 36.3. Found: C, 38.34; H, 4.15; N, 36.02.

8-Aminoadenosine (XI). 8-Azidoadenosine (IX, 2.9 g.) was dissolved in 300 ml. of hot water, and the solution was added to a suspension of 2 g. of 5% palladium on carbon in 50 ml. of water. The resulting mixture was hydrogenated at 50 p.s.i. of hydrogen for 3.5 hr. and filtered. The carbon was washed with 200 ml. of hot water; the aqueous solution was evaporated to dryness *in vacuo* and the residue was recrystallized from a minimum amount of water to yield 2.2 g. (82% yield) of product, m.p. softens 180–185°, dec. 225–227°. For analysis a sample was dried at 110° at *ca.* 0.1 mm. for 10 hr.

Anal. Calcd. for $C_{10}H_{14}N_6O_4 \cdot 0.5H_2O$: C, 41.2; H, 5.18; N, 28.8. Found: C, 41.5; H, 5.05; N, 28.8.

The p.m.r. spectrum in dimethyl sulfoxide (TMS external standard) showed a sharp singlet (1H) at δ 8.37 due to the proton on C-2 and a doublet at 7.06 and 6.95 (4H) due to the two amino groups.

6-N-Acetylamino-9-β-D-ribofuranosyl-8-purinone. 8-Bromo-2′,3′,5′-tri-O-acetyladenosine[9] (5 g.) was added to a solution of 10 ml. of pyridine in 20 ml. of acetic anhydride. The resulting solution was heated at 50° for 8 hr. and then cooled to room temperature. Ethanol (50 ml.) was added and then the solvent was removed *in vacuo*. This process was repeated four times. The product was isolated as a glass. The ultraviolet absorption data showed $\lambda_{max}^{pH\ 1}$ 282 mμ, $\lambda_{max}^{methanol}$ 283 mμ, and $\lambda_{max}^{pH\ 11}$ 300 mμ. The crude 8-bromo-6-N-acetylamino-2′,3′,5′-tri-O-acetyladenosine (4.1 g.) was dissolved in 30 ml. of methanol and to this solution was added 100 ml. of methanolic ammonia (which contained a small amount of water). The resulting solution was allowed to remain overnight at room temperature. The solvent was removed *in vacuo* and the residue was recrystallized from water to

yield 1.5 g. of brown product which was dissolved in hot water. The solution was treated with charcoal, filtered, and allowed to cool to yield 0.94 g. (36% yield) of product, m.p. darkens >250°, dec. 262–264°. For analysis a sample was dried at 110° at *ca.* 0.1 mm. for 10 hr. The ultraviolet absorption data exhibited $\lambda_{max}^{pH\ 1}$ 289 mμ (ε 12,800) and 239 mμ (ε 5700); $\lambda_{max}^{pH\ 11}$ 301 mμ (ε 13,500) and 265 mμ (ε 7000).

Anal. Calcd. for $C_{12}H_{15}N_5O_6$: C, 44.3; H, 4.64; N, 21.5. Found: C, 44.4; H, 4.56; N, 21.7.

6-Amino-9-β-D-ribofuranosyl-9H-purine-8-(7H)-one (8-Hydroxyadenosine) (XII). 6-N-Acetylamino-9-β-D-ribofuranosyl-8-purinone (2.4 g.) was added to 50 ml. of 1 *N* sodium hydroxide. The resulting solution was heated on a steam bath for 6 hr., cooled to room temperature, neutralized to pH 7 with dilute hydrochloric acid, and allowed to remain at 5° for 18 hr. The precipitate that formed was removed by filtration and recrystallized from a small amount of water to yield 1.6 g. (76% yield) of product, m.p. 237–238° dec. For analysis a sample was dried at 110° at *ca.* 0.1 mm. for 10 hr.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.63; N, 24.8. Found: C, 42.8; H, 5.00; N, 24.6.

The p.m.r. spectrum in dimethyl sulfoxide (TMS external standard) showed a broad singlet at δ 6.9 (2H) due to the amino group and a broad singlet at 10.9 due to the hydrogen at position 7.

8-Benzyloxyguanosine (II). Dimethyl sulfoxide (200 ml.) was added to a solution of 2 g. of sodium dissolved in 70 ml. of benzyl alcohol. To the resulting solution was added 10 g. of 8-bromoguanosine[9] (I) dissolved in 80 ml. of dimethyl sulfoxide. The solution was heated at 65° for 12 hr. and cooled to room temperature. Glacial acetic acid (10 ml.) was added until the solution was neutral and the resulting solution was then poured slowly into 4 l. of ethyl ether. The ethyl ether layer was decanted and discarded. The oily residue remaining was slowly poured into 1 l. of acetone. The precipitate that formed was removed by filtration and stirred with 60 ml. of water. The slurry was filtered and the residue was recrystallized from water–ethanol to yield 7.6 g. (68% yield) of product, m.p. 168–170° dec. (air-dried). An analytical sample, m.p. 171–173° dec., was obtained by one more recrystallization from water–ethanol and was dried at 78° at *ca.* mm. for 10 hr.

Anal. Calcd. for $C_{17}H_{19}N_5O_6 \cdot H_2O$: C, 50.1; H, 5.19; N, 17.2. Found: C, 50.1; H, 5.50; N, 16.9.

2-Amino-9-β-D-ribofuranosyl-6,8-purinedione (8-Hydroxyguanosine, V). 8-Benzyloxyguanosine (II, 3 g.) was dissolved in a hot solution of 50 ml. of water and 50 ml. of ethanol. The resulting solution was added to 1.5 g. of 5% palladium on carbon in 50 ml. of water and was hydrogenated under 3 atm. of hydrogen for 16 hr. at room temperature. The solution was filtered and the carbon was washed with 150 ml. of hot water. The aqueous solution was evaporated to dryness *in vacuo* and the residue was recrystallized from a minimum amount of water to yield 1.8 g. (80% yield) of product, m.p. 224–228° dec. An analytical sample, m.p. 232–235° dec., was obtained by one more recrystallization from water and was dried at 110° at *ca.* 0.1 mm. for 10 hr.

Anal. Calcd. for $C_{10}H_{13}N_5O_6 \cdot 0.5H_2O$: C, 38.96; H, 4.58; N, 22.7. Found: C, 38.88; H, 4.51; N, 23.0.

The p.m.r. spectrum in dimethyl sulfoxide (TMS external standard) showed a broad singlet at δ 6.82 (2H) due to the amino group and a broad singlet at 10.4 (1H) due to the hydrogen at position 7.

8-Benzyloxyxanthosine (III). 8-Benzyloxyguanosine (II, 3 g.) was dissolved in 100 ml. of glacial acetic acid; then 50 ml. of water was added and the solution was immediately cooled to 5°. Sodium nitrite (3 g.), dissolved in 10 ml. of water, was added slowly over a period of 15 min., during which time the temperature was maintained at 5°. After addition of the sodium nitrite, the solution was stirred for 75 min., and then the solvent was removed *in vacuo* (bath temperature at 15°). A toluene–ethanol mixture was added to the residue and evaporated *in vacuo* in order to remove all traces of acetic acid. The residue was stirred in 20 ml. of water for 20 min.; the mixture was filtered and the residue was recrystallized from methanol to yield 1.1 g. (38% yield) of product, m.p. 151–155° dec. One more recrystallization from methanol gave an analytical sample, m.p. 154–157°, which was dried at 80° at *ca.* 0.1 mm. for 6 hr. The proton magnetic resonance spectrum determined in dimethyl sulfoxide revealed the disappearance of the peak at δ 6.8 due to the 2-amino group.

Anal. Calcd. for $C_{17}H_{18}N_4O_7 \cdot H_2O$: C, 50.0; H, 4.93; N, 13.7. Found: C, 50.1; H, 4.89; N, 13.6.

9-β-D-Ribofuranosyl Uric Acid (IV). 8-Benzyloxyxanthosine (III, 1.1 g.) was dissolved in a hot solution of 50 ml. of water and 50 ml. of ethanol. The resulting solution was added to 0.5 g. of 5% palladium on carbon in 50 ml. of water and hydrogenated under 3 atm. of hydrogen for 6 hr. at room temperature. The solution was filtered and the carbon was washed with 100 ml. of hot water. The aqueous solution was evaporated to dryness *in vacuo* and the residue was dissolved in a minimum amount of hot water. The pH of the solution was adjusted to 2 by addition of dilute hydrochloric acid. The solution was allowed to remain overnight at room temperature and the colorless needles that crystallized were removed by filtration to yield 0.57 g. (70% yield) of product, m.p. dec. >240°. Falconer and Gulland[7] report their compound crystallized from water in colorless needles or square or rectangular plates. For analysis a sample was dried at 110° for 6 hr. The optical rotation value was $[\alpha]^{29}D$ −41.2° (*c* 1.02, 0.1 *N* NaOH).

Anal. Calcd. for $C_{10}H_{12}N_4O_7 \cdot 2H_2O$: C, 35.72; H, 4.89; N, 16.7. Found: C, 35.64; H, 4.96; N, 16.3.

After drying for 16 hr. at 110° at *ca.* 0.1 mm. over phosphorus pentoxide, the analysis was redetermined.

Anal. Calcd. for $C_{10}H_{12}N_4O_7 \cdot 0.5H_2O$: C, 38.9; H, 4.2; N, 18.15. Found: C, 39.1; 4.54; N, 17.95.

The p.m.r. spectrum in dimethyl sulfoxide (TMS external standard) showed broad absorption at two peaks (3H) at δ 11.50 and 11.36 due to the protons at positions 1, 3, and 7. This product gave R_f values essentially the same as reported in four solvent systems[8] for 9-ribosyl uric acid isolated from *L. plantarum*.[8]