Synthesis of 8-Amino-9-β-D-ribofuranosylpurin-6-thione and Related 6-Substituted-8-aminopurine Nucleosides (1)

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Acetylation of 8-amino-9-β-D-ribofuranosylpurin-6-one (III), followed by chlorination of the tetraacetyl derivative 8-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one (IV) with phosphorus oxychloride yielded 8-acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (V). The 6-chloro substitutent of V was readily displaced with thiourea to give, after treatment with sodium methoxide 8-acetamido-9-β-D-ribofuranosylpurine-6-thione (VIII). Chlorination of 8-bromo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one (IX) yielded 6,8-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (X), which underwent nucleophilic displacement with ethanolic ammonia selectively in the 8 position. The resulting 8-amino-6-chloro-9-β-D-ribofuranosylpurine (VII) was converted to 8-amino-9-β-D-ribofuranosylpurine-6-thione (I), 8-amino-6-methylthio-9-β-D-ribofuranosylpurine (II), and to 8-amino-6-hydrazino-9-β-D-ribofuranosylpurine (XI).

The biological activity of purine-6-thione (6-mercaptopurine), 9-β-D-ribofuranosylpurine-6-thione and of 6-methylthio-9-β-D-ribofuranosylpurine is well documented (2). The biochemical transformations which these antimetabolities undergo in the living cell have been largely elucidated, and the mode of action of these compounds has been extensively studied at the molecular level. In addition to the antitumor activity exhibited by purine-6-thione and the corresponding nucleosides, purine-6-thione and 9-β-D-arabinofuranosylpurine-6-thione have also shown significant immunosuppresive activity. Among the many *S*-alkylated derivatives of purine-6-thione which have been synthesized and evaluated, 6-(1-methyl-4-nitro-5-imidazolyl)thiopurine (Azathioprine, Imuran) has found wide application as a clinically useful immunosuppressant (3). Certain 8-amino substituted purines and purine nucleosides such as 8-amino-6-thioguanine (4) and 8-aminoadenosine (5) have also exhibited significant antitumor properties.

In view of these data concerning the biological activity of 6-thio and 8-amino substituted purines and purine nucleosides, a program was initiated in our laboratories for the synthesis and biological evaluation of a number of 6-thio-8-amino substituted purine nucleosides and nucleotides. This paper describes a portion of this work which was directed toward the synthesis of 8-amino-9-β-D-ribofuranosylpurine-6-thione, (I) and 8-amino-6-methylthio-9-β-D-ribofuranosylpurine (II).

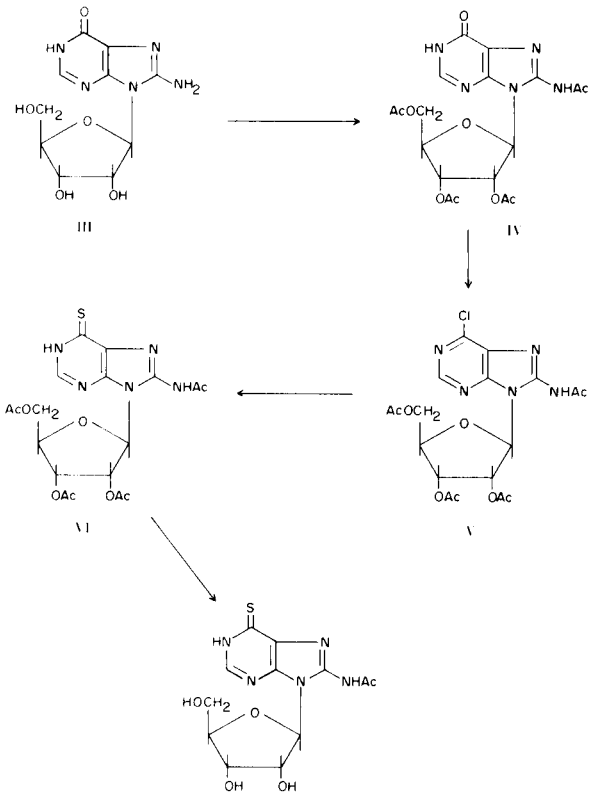
The initial approach toward this synthetic goal involved the use of 8-amino-9-β-D-ribofuranosylpurin-6-one (8-aminoinosine, III) as a starting material (Scheme I). 8-Aminoinosine was acetylated to give 8-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purin-6-one (IV). The fully protected nucleoside IV was chlorinated in the 6-position according to the method of Gerster *et al.* (6), to yield 8-acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl-purine (V). The intermediate V, which was not isolated in analytically pure state, proved to be rather reactive towards nucleophilic substitution. It was readily converted to 8-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine-6-thione (VI) by treatment with thiourea. The ease of the nucleophilic displacement of the 6-chloro substituent in V, readily accomplished by refluxing with thiourea in ethanol, is in contrast with the displacement of the chloro substituent in 8-amino-6-chloro-9-β-D-ribofuranosylpurine (VII) which required harsher conditions (*vide infra*). The enhanced reactivity of V over VII toward nucleophilic displacement is particularly noteworthy in view of the close structural similarity of the two compounds, and is attributed to the electron withdrawing effect of the *N*-acetyl blocking group in V and to the absence of this amide function in VII.

Attempts to deblock VI by treatment with sodium methoxide in methanol resulted only in the removal of the acetyl blocking groups from the ribofuranose moiety, and failed to deacetylate the 8-amino function. When either

VI or 8-acetamido-9-β-D-ribofuranosylpurine-6-thione (VIII) was treated with aqueous base or acid, extensive decomposition took place and no product could be isolated.

As an alternative approach to the synthesis of I, 8-bromo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one (IX) was subjected to chlorination in refluxing phosphoryl chloride (Scheme II). The syrupy intermediate obtained from this reaction was assigned the 6,8-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (X) structure. This structural assignment rested on the basis of analogy with

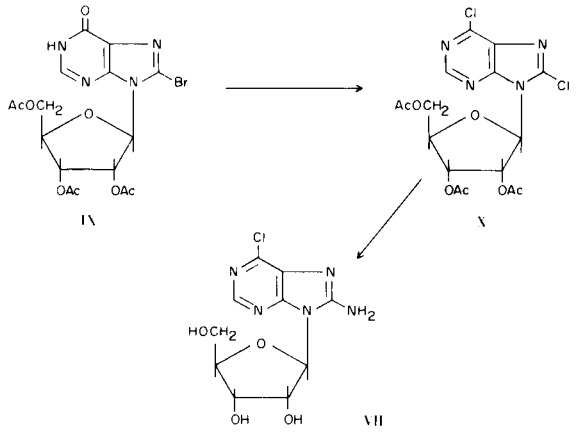
Scheme I



similar chlorination reactions (6, 7, 8), and on the behavior of this substance in subsequent reactions. Definite proof that in addition to chlorination in the 6-position the 8-bromo function of IX has also been replaced by a chloro group, was obtained in the reaction of X with ethanolic ammonia which resulted in the formation of 8-amino-6-chloro-9-β-D-ribofuranosylpurine (VII) and ammonium chloride as a side product.

Compound VII was obtained in good yield (78%), and there was no evidence for the formation of the other theoretically possible isomer, 6-amino-8-chloro-9-β-D-ribofuranosylpurine. The observed selectivity of the displacement of the 8-chloro function over the 6-chloro function of X is at variance with the results of Yamaoka *et* al. (9), who obtained 6-amino-8-chloro-9-β-D-glucopyranosylpurine on treatment of 6,8-dichloro-9-(2,3,4,6-tetraacetyl-β-D-glucopyranosyl)purine with ethanolic ammonia at elevated temperature. Our result is, however, in accordance with the data of Sutcliffe and Robins (10), who found in the course of a detailed investigation that 7- and 9-methyl-2,6,8-trichloropurines and 9-(tetrahydro-2′-pyranyl)-2,6,8-trichloropurine undergo nucleophilic displacement preferentially at the 8-position. The observation concerning selectivity of the aminolysis in the 8-position of X, described here, has been corroborated in our laboratories by results obtained in the reactions of other 9-glycosylated 6,8-dichloropurines with ethanolic ammonia (8).

Scheme II



Scheme III

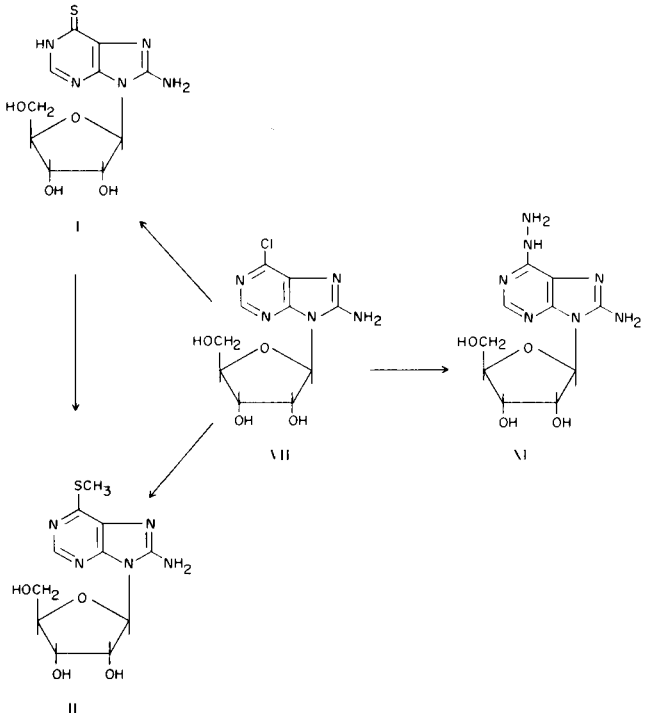
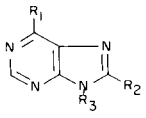


TABLE I

Uv Spectra of Some 6,8-Disubstituted Purine Nucleosides (a).



R_1	R_2	R_3	Compound or Reference No.	λ max *p*H 1	ε	λ max *p*H 11	ε
-SH	$-NHCOCH_3$	β-D-ribofuranosyl	VIII	303	11,800	242 315 345sh	10,950 11,140 8,220
-Cl	$-NH_2$	β-D-ribofuranosyl	VII	278	15,370	257sh 288	7,270 13,500
$-NH_2$	-Cl	β-D-ribofuranosyl	(12)	260	19,300	262	19,800
-SH	$-NH_2$	β-D-ribofuranosyl	I	236 331	14,600 20,500	241 314	18,300 25,900
$-NH_2$	-SH	β-D-ribofuranosyl	(11)	240 308	12,000 27.600	230 297	20,900 25,100
CH_3S-	$-NH_2$	β-D-ribofuranosyl	II	232 296	15,530 20,870	233 303	14,440 19,100
NH_2NH-	$-NH_2$	β-D-ribofuranosyl	XI	268	17,300	278	19,260
-SH	$-NH_2$	-H	(13)	238 332	17,900 25,400	240 312	21,100 26,900

(a) Uv spectra were determined on a Cary 15 ultraviolet spectrophotometer.

TABLE II

PMR Data of Some 6,8-Disubstituted Purine Nucleosides (a)

Compound No.	H_2	8-NH_2	8-NH-C(=O)-CH_3	H_1'	6-S-CH_3
VIII (b)	S (1H) 8.71	–	S (3H) 2.61	d (1H) 6.52 J = 6 Hz	–
VII (c)	S (1H) 8.37	broad S (2H) 7.68	–	d (1H) 6.05 J = 7.5 Hz	–
I (c)	S (1H) 8.10	broad S (2H) 7.13	–	d (1H) 5.95 J = 7.5 Hz	–
II (c)	S (1H) 8.45	broad S (2H) 7.32	–	d (1H) 6.05 J = 7.2	S (3H) 2.62
XI (c)	S (1H) 8.12	broad S (2H) 7.25	–	d (1H) 5.98 J = 7.8	–

(a) PMR spectra were recorded on a Hitachi Perkin Elmer R-20A spectrometer with DSS internal standard, and the chemical shifts are given in δ ppm. (b) In deuterium oxide. (c) In DMSO-d_6.

Nucleophilic displacement of the 6-chloro function of VII to give I, required prolonged heating with aqueous hydrogen sulfide at alkaline *p*H; similar treatment of VII with methanethiol furnished II (Scheme III). The latter could also be obtained by methylation of I *albeit* in a lower overall yield. The displacement reaction of VII is not limited to sulphur nucleophiles. Treatment of VII with aqueous hydrazine yielded 8-amino-6-hydrazino-9-β-

D-ribofuranosylpurine (XI).

The physical characteristics of the known 6-amino-9-β-D-ribofuranosylpurine-8-thione (11) (8-thioadenosine) and 6-amino-8-chloro-9-β-D-ribofuranosylpurine (12) (8-chloroadenosine) differ substantially from the physical characteristics of I and VII. As it is shown in Table I, the uv spectrum of 8-chloroadenosine shows maxima at 260 nm in acidic, and at 262 nm in alkaline medium. In contrast the absorbtion curve of 8-amino-6-chloro-9-β-D-ribofuranosylpurine (VII) shows a significant dependence on *p*H; the latter compound exhibits maxima at 278 nm in acidic, and at 288 nm in alkaline medium. The uv spectrum of 8-thioadenosine exhibits maxima at 240 nm and 308 nm in acidic, and at 230 and 297 nm in alkaline medium, while 8-amino-9-β-D-ribofurosylpurine-6-thione (I) shows absorbtion maxima at significantly higher wavelengths, *i.e.* at 236 and 331 nm in acidic and at 241 and 314 nm in alkaline medium. Since 8-thioadenosine and 8-chloroadenosine are isomeric with I and VII respectively, the possibility of an erroneous assignment of the substitution patterns of I, II and VII is eliminated. The fact that the uv spectrum of I exhibited absorption maxima at the same wavelengths as the known 8-aminopurine-6-thione (13) furnished additional proof regarding the structures of the nucleosides described here.

EXPERIMENTAL

General.

Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus, and are uncorrected. Evaporations were performed under reduced pressure on a rotary evaporator. Thin layer chromatography was performed on Analtech precoated (250 μ) silica gel GF plates, and the spots were visualized by irradiation with a Mineralight uv lamp. Column chromatography was carried out utilizing the method of Loev and Goodman (14) in plastic tubes (purchased from J. T. Baker) transparent to uv light. The tubes were packed with silica gel powder (Baker catalog #3405) containing 1% zinc silicate fluorescent indicator (Baker catalog #2101). The compounds were applied to the column preabsorbed on silica gel. This was accomplished by adding silica gel to a solution of the compounds followed by evaporation to dryness. The columns were then eluted with the appropriate solvent. The position of the bands on the column was visualized by irradiation with Mineralight uv lamp. These columns are referred to in the text as "dry columns." Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee 37921.

8-Acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one (IV).

To a solution of 8-amino-9-β-D-ribofuranosylpurin-6-one (15) (4.24 g., 15 mmoles) in anhydrous pyridine (150 ml.) was added acetic anhydride (25 ml.) and the resulting mixture was refluxed for 3.5 hours. The pyridine was then concentrated *in vacuo* to about 1/3 of its orginal volume, and the mixture was poured on ice. The aqueous solution was extracted with chloroform (3 x 100 ml.), and the combined chloroform extracts were washed with 1*N* hydrochloric acid (3 x 200 ml.) and finally with water. After drying (sodium sulfate) the chloroform was concentrated to dryness. The residual syrup was chromatographed on a dry silica gel column, eluted with the upper phase of ethyl acetate:*n*-propanol: water 4:1:2 (SSE). After combining the fractions corresponding to the fastest moving material (Rf ≈0.85, silica plates, SSE) and repeated dry column chormatography, pure product was obtained (4.80 g.; 71%); this syrupy material solidified on prolonged heating at 70° *in vacuo*.

Anal. Calcd. for $C_{18}H_{21}N_5O_9$: C, 47.89; H, 4.68; N, 15.51. Found: C, 47.70; H, 4.93; N, 15.28.

8-Acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-6-thione (VI) *via* 8-Acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosylpurine (V).

To a suspension of IV (1.700 g., 3.77 mmoles) in phosphorus oxychloride (10 ml.) was added *N,N*-diethylaniline (0.7 ml.), and the mixture was refluxed for 6 minutes. After rapid cooling, the mixture was poured on ice, and the aqueous phase was extracted with chloroform (3 x 100 ml.). The chloroform solution was washed once with 1*N* hydrochloric acid (150 ml.) and then with water until the aqueous phase was neutral. The syrupy V (λ max (methanol) 263 mμ) obtained by evaporation of the chloroform solution, was dissolved in ethanol (100 ml.). Thiourea (0.600 g.) and 2 drops of glacial acetic acid were added and the resulting solution was refluxed for 3 hours. After evaporation of ethanol, the residue was partitioned between water and chloroform (150 ml. each) and the chloroform phase was washed once more with water (100 ml.). After drying (sodium sulfate) the chloroform was evaporated. The residue was chromatographed on a dry column of silica gel, eluted with chloroform:ethyl acetate-7:3. Fractions containing chromatographically homogeneous material (Rf ≈0.21 silica plates, chloroform:ethyl acetate-7:3) were combined and evaporated to give product (0.480 g., 27%, based on IV). A sample was rechromatographed as described above, in order to obtain an analytical sample. The syrupy material solidified on heating *in vacuo*.

Anal. Calcd. for $C_{18}H_{21}N_5O_8S$: C, 46,24; H, 4.52; N, 14.98; S, 6.85. Found: C, 46.53; H, 4.70; N, 14.71; S, 6.70-

8-Acetamido-9-β-D-ribofuranosylpurin-6-thione (VIII).

To a solution of VI (0.670 g., 1.43 mmoles) in anhydrous methanol (80 ml.) was added sodium methoxide (0.324 g., 6.00 mmoles), and the mixture was stirred at room temperature for 3 hours. Amberlite IRC-50 ion exchange resin (H^+ form) was then added until the solution was neutral. The resin was removed by filtration and the methanol was evaporated to dryness. The residue was crystallized from ethanol-cyclohexane to give product, 0.128 g. Concentration of the mother liquor yielded an additional 0.050 g. of product, combined yield 36.2%. Recrystallization from ethanol-cyclohexane furnished a sample of analytical purity, dec., 215-225°. Single spot on silica plates Rf ≈0.1, SSE; and on cellulose plates Rf ≈0.5, 1*M* ammonium acetate:ethanol-3:7.

Anal. Calcd. for $C_{12}H_{15}N_5O_5S$: C, 42.22; H, 4.42; N, 20.51; S, 9.37. Found: C, 42.41; H, 4.74; N, 20.77; S, 9.25.

8-Bromo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one (IX) from 8-Bromo-9-β-D-ribofuranosylpurin-6-one.

To a suspension of 8-bromo-9-β-D-ribofuranosylpurin-6-one (11) (13.88 g., 40 mmoles) in anhydrous pyridine (400 ml.) was added acetic anhydride (30 ml.). After stirring overnight, to the resulting clear solution was added water (20 ml.), and the solution

was evaporated to dryness. Three times water (80 ml.) was added and evaporated, in order to remove residual pyridine. The syrupy residue was dissolved in chloroform (400 ml.) and the chloroform was extracted 3 times with water (400 ml.), 3 times with 1*N* hydrochloric acid solution (400 ml.) and once again with water (400 ml.). The chloroform layer was dried (sodium sulfate) and evaporated to dryness. The residual foam was dissolved in hot ethanol. On cooling the solution deposited a semi-syrupy mass, which on recrystallization from ethanol gave platelets, 13.675 g. (72.5%), m.p. 189-191°. This sample was identical in its spectral properties with the material obtained by Holmes and Robins (11) *via* bromination of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-one. Recrystallization of the sample from ethanol raised the m.p. to 191-192°.

8-Amino-6-chloro-9-β-D-ribofuranosylpurine (VII) *via* 6,8-Dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (X).

To a suspension of IX (8.50 g., 18 mmoles) in phosphorus oxychloride (60 ml.) was added *N,N*-diethylaniline (3.6 ml.), and the resulting clear solution was refluxed in an oil bath for 10 minutes, with the exclusion of moisture. The phosphorus oxychloride was then evaporated *in vacuo* and the residue was poured into ice-water. The aqueous layer was extracted 3 times with chloroform (100 ml. each), and the combined chloroform layers were washed 3 times with ice cold saturated sodium bicarbonate solution and finally with water. After drying (sodium sulfate) the chloroform was evaporated, the residual syrup had λ max (pH = 1) 267-268 mμ. This residue was dissolved in ethanolic ammonia (saturated at 0°) and kept in a pressure bottle overnight at room temperature. The ethanol was then evaporated to dryness, the residue suspended in ethanol (50 ml.) and after standing at -15° for 2 hours crude product was isolated by filtration, weight 4.24 g., 78.5%, m.p. 185-190°. This substance was suitable as an intermediate for further reactions. An analytical sample was obtained by two recrystallizations from water, m.p. 215-217°.

Anal. Calcd. for $C_{10}H_{12}ClN_5O_4$: C, 39.80; H, 4.01; N, 23.21. Found: C, 39.65; H, 3.98; N, 23.21.

8-Amino-9-β-D-ribofuranosylpurin-6-thione (I).

Hydrogen sulfide gas was bubbled into an ice cold suspension of VII (4.52 g., 15 mmoles) in 300 ml. of water containing sodium carbonate (2.50 g.). The mixture was slowly heated to 100°. After 12 hours of heating and continuous bubbling of hydrogen sulfide gas, the solution was cooled and the water was evaporated to dryness. The residue was treated with water (500 ml.), the insoluble fine precipitate, elemental sulphur, was removed by filtration on a Celite pad. The clear aqueous solution was neutralized by addition of IRC-50 ion exchange resin, H^+ form. After removal of the ion exchange resin by filtration the aqueous solution was evaporated to dryness, and the solid residue was crystallized from 95% ethanol to give crude product (4.412 g., 98%). Two recrystallizations from 95% ethanol furnished a sample of analytical purity, dec., >220°.

Anal. Calcd. for $C_{10}H_{13}N_5O_4S$: C, 40.12; H, 4.37; N, 23.40; S, 10.71. Found: C, 40.10; H, 4.18; N, 23.20; S, 10.91.

8-Amino-6-methylthio-9-β-D-ribofuranosylpurine (II).

Compound VII (2.190 g., 7.3 mmoles) was added to a two-phase mixture of water (300 ml.) and methanethiol (30 ml.) containing 1.120 g. of sodium carbonate. The excess methanethiol was allowed to evaporate and then the mixture was heated at 60° for 18 hours. The solvent was evaporated to dryness and the residue columned on a dry column of silica gel eluted with SSE. Fractions containing product were combined, evaporated to dryness and the residue was crystallized from *n*-propanol:water (71.8:28.2, azeotropic mixture) to give 1.714 g. of pure product (75%), m.p. 152-155°.

Anal. Calcd. for $C_{11}H_{15}N_5O_4S$: C, 42.16; H, 4.82; N, 22.35; S, 10.23. Found: C, 42.12; H, 4.85; N, 21.88; S, 10.16.

Alternatively, to a suspension of I (0.300 g., 1 mmole) in methanol (50 ml.) was added concentrated ammonium hydroxide (2 ml.) and then methyl iodide (0.156 g., 1.1 mmoles). After stirring at room temperature for 22 hours the solvent was evaporated to dryness *in vacuo*, twice ethanol was added and evaporated. The residual yellow foam was columned on a dry silica gel column, eluted with SSE. The chromatographically homogeneous material (silica plates Rf = 0.59 SSE) was freeze dried (0.124 g., 39.5%). This substance was identical in every respect (uv, ir, nmr and tlc) with the analytical sample obtained above.

8-Amino-6-hydrazino-9-β-D-ribofuranosylpurine (XI).

To a suspension of VII (1.204 g., 4 mmoles) in water (160 ml.) was added hydrazine hydrate (1.0 ml.) and the resulting mixture was refluxed for 4 hours. After cooling, the clear solution was concentrated to dryness *in vacuo*, to the residue ethanol was added 3 times and evaporated. The final residue was then suspended in ethanol (30 ml.) and after standing at -15° for 2 hours the product was collected by filtration. Crystallization from aqueous ethanol yielded product (0.734 g., 58.2%) m.p. 236-238°, dec. One recrystallization from aqueous ethanol furnished a sample for analysis, m.p. 236-238°.

Anal. Calcd. for $C_{10}H_{15}N_7O_4 \cdot H_2O$: C, 38.09; H, 5.43; N, 31.11. Found: C, 38.28; H, 5.34; N, 31.07.

REFERENCES

(1) This work was presented in part before the Fourth International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July 1973.

(2) C. R. Rhoads, Ed., "Symposium on 6-Mercaptopurine", *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

(3) For a review of various aspects of the biochemistry of purine-6-thione derivatives see "The Biochemical Basis for the Drug Actions of Purines" by John A. Montgomery in "Progress in Medicinal Chemistry," Vol. 7, p. 69, G. P. Ellis and G. B. West, Ed., Butterworth and Co. Ltd., London, 1970.

(4) R. K. Robins, *J. Med. Chem.*, 7, 186 (1964).

(5) A. Bloch, E. Mihich, C. A. Nichol, R. K. Robins, and R. H. Whistler, *Proc. Am. Assoc. Cancer Res.*, 7 (1966), Abstract #25.

(6) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963).

(7) R. B. Meyer, Jr., D. A. Shuman, R. K. Robins, R. J. Bauer, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, 11, 2704 (1972).

(8) K. H. Boswell, L. F. Christensen, D. A. Shuman, and R. K. Robins, *J. Heterocyclic Chem.*, **12**, 1 (1975).

(9) N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965).

(10) E. Y. Sutcliffe and R. K. Robins, *ibid.*, **28**, 1662 (1963).

(11) R. E. Holmes and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 1242 (1964).

(12) C. L. Schmidt and L. B. Townsend, *J. Org. Chem.*, **37**, 2300 (1972).

(13) R. K. Robins, *J. Am. Chem. Soc.*, **80**, 6671 (1958).

(14) B. Loev and M. Goodman, *Chem. Ind.* (London), 2026 (1967).

(15) R. A. Long, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, **32**, 2751 (1967).