of an α-1,4-glucosidic bond by an amylase the residue is always in the same conformation, and (2) that conformation is the B1 boat form of Reeves. The solvolysis or carbonium ion mechanism is depicted in Fig. 1.

In the figure (A) represents a linear α-1,4-linked chain with the glucose residues in the B1 conformation and with R = H for β-amylase action and R = H or n glucose units as an α-amylase substrate. After the substrate has been oriented on the protein surface, it is protonated by the enzyme to form the oxonium ion B. The bond is broken on the C-1 carbon side, leaving the carbonium (oxonium) ion C which is represented by two limiting structures. So far, the addition of the proton and cleavage of the bond are the same for both types of amylases although the orienting surfaces would be different.

Once the α-1,4-link is broken, the conformation of the reducing ring is disrupted. The first ring of the hybrid ion C can now assume a half-chair conformation. However, in the α-amylase case the ion C is permitted to turn only in the direction which leads to the half-chair D with the substituents at carbon atoms 2, 3, 4 and 5 in axial positions. For the β-amylases the first ring of ion C rotates in the opposite direction to give E in which the substituents at carbon atoms 2, 3, 4, and 5 are in equatorial positions. The direction of rotation of the potential reducing ring of the intermediate C depends upon the surface of the enzyme and the steric restrictions imposed by it.

The intermediates D and E, still under the influence of the catalytic protein, undergo solvolysis in the manner indicated. The intermediate D yields F, products with an α-configuration (α-amylases), while intermediate E yields G, β-maltose (β-amylases). The reducing ring of the maltose (or maltooligosaccharides) assumes the chair conformation with the substituents at carbon atoms 2, 3, 4 and 5 in equatorial positions, but the non-reducing ring(s) still retain their original conformation.[39]

The above mechanism is in agreement with the chemical behavior of starch-like polysaccharides (acetals) and with the cleavage point of the α- and β-amylases. It does not account for the mode of action of these enzymes in terms of products, since the sequence depicted takes place *after* the substrate becomes oriented on the enzymes' surface. The α-amylases from different sources evolve their products at different rates,[40] but it is suggested that once the bond to be cleaved has been selected, the cleavage always proceeds by the same mechanism.

Acknowledgments.—We wish to thank Mrs. J. Thomas and Mrs. E. D. Ihnen for performing the mass spectrometer analyses. Special thanks are due to Dr. R. F. Nystrom for his help and for the use of his high vacuum line. We thank Dr. P. E. Yankwich for many valuable discussions. The effort Dr. Howard Clark expended in modifying his oxygen train for the pyrolyses is appreciated. This work was supported in part by a grant from the National Science Foundation (G-1270).

(39) The half-chair conformations used in intermediates D and E and the direction of solvolysis of them are similar to those presented by R. U. Lemieux and G. Huber, *Can. J. Chem.*, **33**, 128 (1955).

(40) J. T. Kung, V. M. Hanrahan and M. L. Caldwell, THIS JOURNAL, **75**, 5548 (1953); R. Bird and R. H. Hopkins, *Biochem. J.*, **56**, 86 (1954).

URBANA, ILL.

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, NEW MEXICO HIGHLANDS UNIVERSITY AND ARIZONA STATE COLLEGE]

Potential Purine Antagonists. XVI. Preparation of Some 2-, 6- and 8-Methylpurines[1]

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RECEIVED JULY 18, 1958

The preparation of 2-methylpurine (VIII), 2,6-dimethylpurine (XII), 2,8-dimethylpurine (VII), 6,8-dimethylpurine (XV) and 2,6,8-trimethylpurine (XVI) has been accomplished. All the methyl derivatives of the purine nucleus possessing a methyl group attached to a carbon atom are now known. The synthesis of several new 2-substituted-6-methylpurines is described.

Recently reported anti-tumor activity and toxicity of 6-methylpurine[4] prompted us to investigate the preparation of related homologs with a methyl substituent at positions 2, 6 and 8 as candidate anti-tumor agents. Of the possible homologs of purine with a methyl group attached to a carbon atom of the purine nucleus, only 6-methylpurine[5] and 8-methylpurine[6,7] previously have been reported.

Recent studies involving a new method of synthesis of 8-methylpurine[7] from 6-chloro-8-methylpurine suggested similarly the preparation of 2-methylpurine (VIII) from 2-methyl-6-chloropurine (III).[8] This was accomplished directly by reduction of III with hydrogen and palladium-on-charcoal catalyst in the presence of ammonium hydroxide. 2-Methylpurine (VIII) also was obtained from 2-methyl-6-purinethiol[8] by removal of the thiol group with Raney nickel. Bendich, Russell

(1) This investigation was supported in part by research grant C-2845 from the National Cancer Institute of the National Institutes of Health, Public Health Service. Presented in part before the Division of Medicinal Chemistry at the 133rd Meeting of the American Chemical Society, April 18, 1958, in San Francisco, Calif.

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(4) D. A. Clarke, F. S. Philips, S. S. Sternberg and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954).

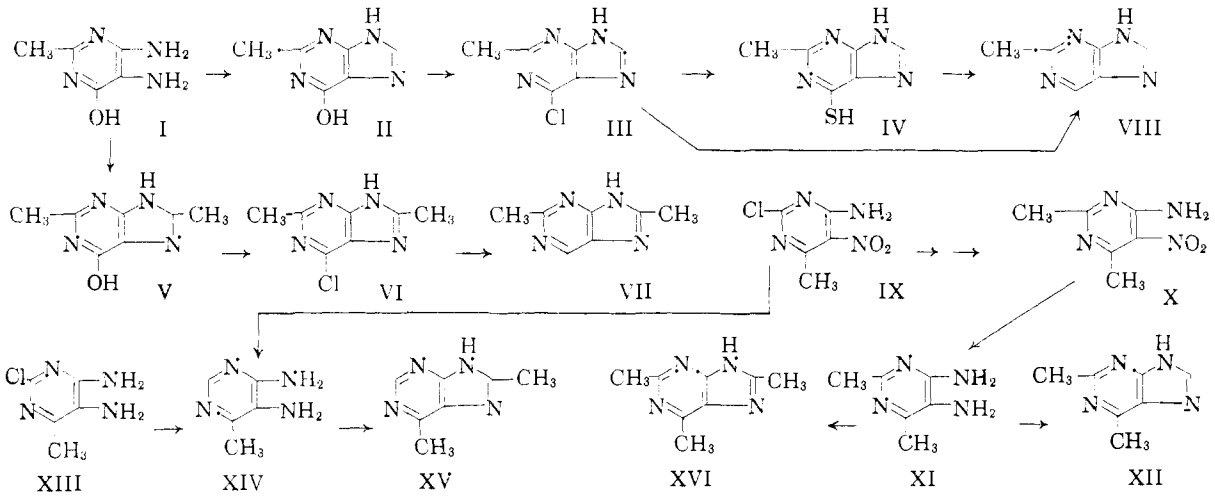
(5) S. Gabriel and J. Colman, *Ber.*, **34**, 1246 (1901).

(6) O. Isay, *ibid.*, **39**, 250 (1906); A. Albert and D. J. Brown, *J. Chem. Soc.*, 2070 (1954).

(7) H. C. Koppel and R. K. Robins, Part XIII, *J. Org. Chem.*, in press.

(8) R. K. Robins, J. W. Jones and H. H. Lin, *ibid.*, **21**, 695 (1956).

REACTION SCHEME I



and Fox[9] have utilized these general methods for preparation of the parent nucleus.

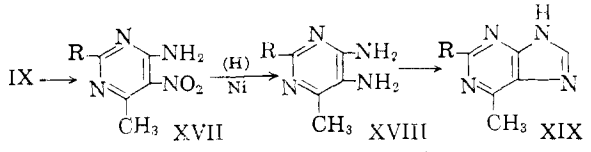
For the synthesis of 2,8-dimethylpurine (VII), 4,5-diamino-6-hydroxy-2-methylpyrimidine[10] was treated with acetic anhydride to give 2,8-dimethyl-6-hydroxypurine (V).[11] 8-Methyl-6-hydroxypurine[7] recently has been prepared similarly from 6-hydroxy-4,5-diaminopyrimidine. Chlorination of V with phosphorus oxychloride gave 2,8-dimethyl-6-chloropurine (VI). Treatment of VI with palladium-on-carbon in the presence of hydrogen gave 2,8-dimethylpurine.

The preparation of 6,8-dimethylpurine (XV) was accomplished from 4,5-diamino-6-methylpyrimidine (XIV)[5] and a mixture of ethyl orthoacetate and acetic anhydride. A new and improved synthesis of 4,5-diamino-6-methylpyrimidine (XIV) has been accomplished in one step from 4-amino-2-chloro-5-nitro-6-methylpyrimidine with palladium-on-charcoal catalyst in the presence of hydrogen.

For the preparation of 2,6,8-trimethylpurine (XVI) and 2,6-dimethylpurine (XII) the intermediate 4,5-diamino-2,6-dimethylpyrimidine (XI) was required. Andersag and Westphal[12] prepared XI in several steps from 5-amino-2,6-dimethyl-4-hydroxypyrimidine. Difficulties encountered in attempts to repeat this work led to the investigation of a new synthetic route to 2,6-dimethyl-4,5-diaminopyrimidine (XI). Rose[13] recently described the preparation of 2-amino-4,6-dimethyl-5-nitropyrimidine from 2-amino-4-chloro-6-methyl-5-nitropyrimidine *via* the intermediate 2-amino-4-dicarbethoxymethyl-6-methyl-5-nitropyrimidine. Following this lead 4-amino-2,6-dimethyl-5-nitropyrimidine (X) was prepared similarly from 4-amino-2-chloro-6-methyl-5-nitropyrimidine[5] (IX). Catalytic reduction of X gave a good yield of 4,5-diamino-2,6-dimethylpyrimidine (XI). Cyclization to 2,6-dimethylpurine (XIII) was accomplished with ethyl orthoformate and acetic anhydride.[14,15] When ethyl orthoacetate and acetic anhydride were used, 2,6,8-trimethylpurine (XVI) was obtained in good yield.

As indicated in reaction scheme II several 2-substituted-6-methylpurines were obtained in a stepwise fashion from 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX). Catalytic reduction of IX with Raney nickel in the presence of hydrogen readily gave 2-chloro-4,5-diamino-6-methylpyrimidine (XVIII, R = Cl) which was cyclized with ethyl orthoformate and acetic anhydride[13] to yield 2-chloro-6-methylpurine (XIX, R = Cl).

REACTION SCHEME II

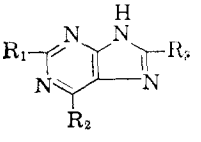


R = Cl, SCH_3, OC_2H_5, $HNCH_3$

As compared with 6-chloro-2-methylpurine (III),[8] 2-chloro-6-methylpurine proved to be rather inert with regard to the nucleophilic displacement of the 2-chloro atom. Thiourea in refluxing ethanol, as well as various amines in alcoholic and aqueous

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF 2,6- AND 8-METHYLPURINES



R_1	R_2	R_8	M.p., °C.	λ_{max}, mμ pH 1	ε	λ_{max}, mμ pH 11	ε
CH_3	H	H	286	266	5400	275	8000
CH_3	CH_3	H	271	270	6960	275	9180
CH_3	CH_3	CH_3	222	273	5020	278	6330
H	CH_3	CH_3	263–265	269	7700	278	8900
H	CH_3	H	236–237[6]	264	6970	272	8160
H	H	CH_3	271–273[6]	264	8760	275	9960
CH_3	H	CH_3	217	269	7200	278	9600

(9) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

(10) R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, *ibid.*, **75**, 263 (1953).

(11) (a) Preparation of V from aminomalonamidamidine has recently been reported by E. C. Taylor, *et al.*, "The Chemistry and Biology of Purines," edited by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown and Co., Boston, Mass., 1957, p. 22; (b) F. Craveri and G. Zoni, *Chimica*, **33**, 473 (1957).

(12) H. Andersag and K. Westphal, *Ber.*, **70**, 2045 (1937).

(13) F. L. Rose, *J. Chem. Soc.*, 4122 (1954).

(14) J. A. Montgomery, THIS JOURNAL, **78**, 1928 (1956).

(15) R. Richter and E. C. Taylor, *Angew. Chem.*, **67**, 303 (1955).

solution, heated on the steam-bath did not effect replacement of the chlorine atom. These reactions[8] proceed smoothly with the isomeric 2-methyl-6-chloropurine. 2-Methoxy-6-methylpurine (XIX, R = OCH_3) was prepared from 2-chloro-6-methylpurine (XIX, R = Cl), however, only when special conditions were employed. Other 2-substituted-6-methylpurines prepared by cyclization of the appropriate 4,5-diaminopyrimidines were 2-methylthio-6-methylpurine (XIX, R = SCH_3) and 2-methylamino-6-methylpurine (XIX, R = NCH_3). The ultraviolet absorption spectra of the "C"-methylated purines are compared in Table I.

Experimental[16]

A. Pyrimidines. 2-Chloro-4,5-diamino-6-methylpyrimidine (XIII).—A suspension of 10 g. of 4-amino-2-chloro-6-methyl-5-nitropyrimidine[5] (IX) in 150 ml. of absolute methanol was shaken for 3 hr. with 5 g. of Raney nickel catalyst at a pressure of 2.7 atm. of hydrogen. The reaction mixture was then filtered and the residue extracted several times with absolute methanol. The combined solution was evaporated to dryness under reduced pressure. The residue then was recrystallized from boiling water to give 5 g. of long, colorless needles, m.p. 265° dec.

Anal. Calcd. for $C_6H_7N_4Cl$: C, 37.9; H, 4.4. Found: C, 38.3; H, 4.5.

4-Amino-6-methyl-2-methylamino-5-nitropyrimidine (XVII, R = $HNCH_3$).—Five grams of 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX) was added to 150 ml. of 95% ethanol; 30 ml. of a 40% aqueous solution of methylamine was added. The reaction mixture then was heated on the steam-bath for 45 min. and cooled. Fine yellow needles separated on cooling. The product was filtered and recrystallized from 75% ethanol to give 4.5 g. of yellow crystals, m.p. 198–199°.

Anal. Calcd. for $C_6H_9N_5O_2$: C, 39.3; H, 4.9. Found: C, 39.7; H, 4.9.

4,5-Diamino-6-methylpyrimidine (XIV).[5]—A mixture of 5 g. of 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX), 100 ml. of absolute ethanol, 2 g. of 5% palladium-over-charcoal and 1 ml. of strong aqueous ammonium hydroxide was shaken with hydrogen under an initial pressure of 3 atm. Absorption was complete in 15 min. The reaction mixture was filtered and the solution evaporated to dryness to yield a white solid, m.p. 207–208°.[5] This material was used without further purification for the preparation of 6-methyl- and 6,8-dimethylpurines.

4,5-Diamino-6-methyl-2-methylaminopyrimidine Dihydrochloride (XVIII, R = $HNCH_3$).—Three and six-tenths grams of crude 4-amino-6-methyl-2-methylamino-5-nitropyrimidine (XVII, R = $HNCH_3$) was shaken with 150 ml. of absolute ethanol, containing 3 g. of moist sponge nickel catalyst, at an initial pressure of 2.7 atm. of hydrogen. The reduction was complete in approximately 45 min. The solution was filtered and cooled, and dry hydrogen chloride was passed into the solution. The hydrochloride separated as a white crystalline solid (3.5 g.), which was recrystallized from a mixture of ethanol and benzene to give an analytically pure sample, m.p. 248–250°.

Anal. Calcd. for $C_6H_{11}N_5 \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 30.6; H, 6.0. Found: C, 30.7; H, 6.1. Calcd. for $C_6H_{11}N_5 \cdot 2HCl$ (sample dried at 110°): N, 31.1. Found: N, 31.3.

4-Amino-6-methyl-2-methylthio-5-nitropyrimidine (XVII, R = SCH_3).—Ten grams of thiourea was mixed with 5.6 g. of 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX) in 160 ml. of absolute ethanol. The solution was refluxed for 30 min. The mixture then was allowed to remain at room temperature overnight, and the yellow crystalline product which separated was washed with ethanol to yield an almost quantitative yield of 4-amino-6-methyl-5-nitro-2-thiopyrimidine, m.p. 220–223° dec. On recrystallization from boiling dilute ethanol the product melted at 220–221° dec.

Anal. Calcd. for $C_6H_6N_4SO_2 \cdot 1\frac{1}{2}H_2O$: C, 28.1; H, 4.2. Found: C, 28.2; H, 3.5.

(16) All melting points are uncorrected and were taken on a Fisher-Johns melting point block, unless otherwise stated.

Twenty grams of the 4-amino-6-methyl-5-nitro-2-thiopyrimidine thus prepared was methylated with 16 ml. of methyl iodide by refluxing for 6 hr. with 10 g. of sodium carbonate (anhydrous) in 10% ethanol. The solvent was removed on a steam-bath under reduced pressure and the mixture cooled and filtered. The yellow product was washed several times with cold water and dried. The product, m.p. 155–156°, was recrystallized from 70% ethanol to yield 14 g. of yellow shiny crystals, m.p. 155.5° sharp.

Anal. Calcd. for $C_6H_8N_4O_2S$: C, 36.0; H, 4.0; N, 28.0. Found: C, 35.8; H, 4.4; N, 27.7.

4-Amino-2,6-dimethyl-5-nitropyrimidine (X).—Twenty-four grams of 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX) and 100 ml. of acetone were added to 30 g. of diethyl malonate. The mixture was stirred and 40 ml. of 11 *N* sodium hydroxide solution carefully added over a period of about 40 min. The temperature was not allowed to rise above 50°. At the end of this period 200 ml. of water was added and the mixture stirred. The mixture then was filtered and the filtrate acidified with glacial acetic acid and cooled. The deep brownish-red precipitate obtained on cooling was filtered and washed with water. The crude sample, which melted between 180–185°, was heated slowly on a hot-plate with 200 ml. of 3 *N* hydrochloric acid for 3 hr., with continuous stirring. At the end of this period the clear yellowish-brown solution was treated with charcoal, filtered and made strongly alkaline with ammonium hydroxide solution. Upon cooling, light-brown needles (5 g.) separated. After one recrystallization from water an analytically pure sample was obtained, m.p. 159–160°.

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.9; H, 4.8; N, 33.3. Found: C, 42.8; H, 4.7; N, 33.5.

4,5-Diamino-6-methyl-2-methylthiopyrimidine (XVIII, R = SCH_3).—A mixture of 4 g. of 4-amino-6-methyl-2-methylthio-5-nitropyrimidine (XVII, R = SCH_3), 3 g. of sponge nickel and 60 ml. of absolute ethanol was placed in a low pressure Parr hydrogenation apparatus at an initial pressure of 3.1 atm. of dry hydrogen. Absorption was complete in 1 hr. The nickel was removed by filtration and the residue washed with 30 ml. of hot ethanol. The combined filtrate was evaporated to dryness under reduced pressure to yield a yellowish-white solid, m.p. 186–190°. Recrystallization from ethanol–water yielded 3 g. of very light-brown needles, m.p. 194–195°.

Anal. Calcd. for $C_6H_{10}N_4S$: C, 42.4; H, 5.9; N, 33.0. Found: C, 42.5; H, 6.2; N, 32.8.

4-Amino-2-ethoxy-6-methyl-5-nitropyrimidine (XVII, R = OC_2H_5).—To a solution of 1 g. of sodium metal in 120 ml. of absolute methanol was added slowly, with shaking, 5 g. of 4-amino-2-chloro-6-methyl-5-nitropyrimidine (IX). After a vigorous reaction the mixture turned deep brownish-red. The reddish mixture was heated on the steam-bath for 1 hr. and finally cooled and filtered; 1.9 g. of pink platelets was isolated, m.p. 168°. For analysis a small amount was sublimed to give white crystals, m.p. 168°.

Anal. Calcd. for $C_7H_{10}N_4O_3$: C, 42.4; H, 5.1. Found: C, 42.2; H, 5.4.

4,5-Diamino-2,6-dimethylpyrimidine (XI).—Three grams of the 4-amino-2,6-dimethyl-5-nitropyrimidine (X) obtained above was mixed with 50 ml. of absolute ethanol and 3 g. of moist sponge nickel catalyst. The mixture was shaken with hydrogen at an initial pressure of 3.4 atm. for 20 min. at which time the reaction was complete. The reaction mixture was filtered and the residue extracted with 3 × 20 ml. of warm absolute ethanol. The combined filtrate was evaporated to dryness under reduced pressure and a whitish-brown residue obtained, m.p. 250–254°. Recrystallization from benzene yielded 1 g. of a white crystalline substance, m.p. 252–254°, reported[12] 248°.

Anal. Calcd. for $C_6H_{10}N_4$: N, 40.6. Found: N, 40.6.

B. Purines. 2-Chloro-6-methylpurine (XIX, R = Cl).—A solution of 25 g. of triethyl orthoformate and 25 g. of acetic anhydride was heated under reflux for 2 hr. with 5 g. of 2-chloro-4,5-diamino-6-methylpyrimidine (XIII). The solvent then was removed under reduced pressure. The brownish residue which solidified on cooling was heated gently with dilute potassium hydroxide and the solution treated with charcoal, filtered and acidified with glacial acetic acid. The solution on cooling yielded 4 g. of 2-chloro-6-methylpurine (XIX, R = Cl). The product is very soluble in hot methanol, ethanol and water and sparingly soluble in ben-

zene and toluene. Recrystallization from an ethanol–benzene mixture yielded 2.5 g., m.p. 275° dec.

Anal. Calcd. for $C_6H_5N_4Cl$: C, 42.7; H, 3.0; N, 33.2. Found: C, 42.8; H, 3.1; N, 33.5.

2-Methylamino-6-methylpurine (XIX, R = $HNCH_3$). Method 1.—A mixture of 4.5 g. of 4,5-diamino-6-methyl-2-methylaminopyrimidine dihydrochloride (XVIII, R = $HNCH_3$) and 15 ml. of formamide was refluxed for 35 min. The hot solution was then poured into 100 ml. of cold water and cooled overnight. A crude, brown product was filtered and re-dissolved in hot dilute hydrochloric acid. The solution was treated with charcoal and filtered. The filtrate was adjusted to *p*H 8 with ammonium hydroxide. Upon cooling, a yellowish product separated which was purified further by reprecipitation to yield 0.5 g., m.p. > 300°.

Anal. Calcd. for $C_7H_9N_5$: C, 51.5; H, 5.5; N, 42.9. Found: C, 51.4; H, 5.8; N, 43.2.

Method 2.—A solution of 3.7 g. of 4-amino-6-methyl-2-methylamino-5-nitropyrimidine (XVII, R = $HNCH_3$) was reduced in absolute ethanol and the solvent evaporated to dryness under reduced pressure. The residue was treated with 40 g. of an equimolar mixture of acetic anhydride and ethyl orthoformate and the solution refluxed 1 hr. The solvent was removed under reduced pressure and the residue warmed on the steam-bath with 2 *N* hydrochloric acid for 45 min. The solution was then treated with charcoal and filtered. The filtrate was made alkaline with ammonium hydroxide to yield 1.6 g. of brown crystalline product, m.p. > 300°. Mixed m.p. determination and ultraviolet absorption data indicated this product to be identical with that obtained by method 1.

2-Methoxy-6-methylpurine (XIX, R = OCH_3).—A solution of 0.5 g. of sodium dissolved in 100 ml. of absolute methanol was heated with 1.8 g. of 6-methyl-2-chloropurine (XIX, R = Cl) on a steam-bath. The solvent was allowed to escape until a viscous mass resulted. Toluene then was added and the mixture boiled gently for 2 hr. The toluene now was allowed to evaporate and the dry, white residue dissolved in 80 ml. of water. The solution was filtered and the filtrate acidified with acetic acid to give a white precipitate. The product was filtered and recrystallized from absolute ethanol to yield 0.8 g. of white crystalline product, m.p. 283–284°.

Anal. Calcd. for $C_7H_8N_4O$: C, 51.2; H, 4.9; N, 34.2. Found: C, 50.8; H, 4.9; N, 34.2.

2,6,8-Trimethylpurine (XVI).—Thirty ml. of an equimolar mixture of acetic anhydride and triethyl orthoacetate was heated under reflux with 1 g. of 4,5-diamino-2,6-dimethylpyrimidine (XI). After 3 hr. the solvent was removed under reduced pressure and the residue dissolved in 10 ml. of absolute ethanol to which had been added 0.5 g. of potassium hydroxide. The solution was warmed 15 min. on a steam-bath and then the solution neutralized with glacial acetic acid and evaporated to dryness. The residue was extracted with boiling benzene. The benzene solution was evaporated to dryness and the residue recrystallized from a benzene–heptane mixture to yield 0.8 g. of a white, crystalline product, m.p. 220°. After purification by sublimation the m.p. was raised to 222°.

Anal. Calcd. for $C_8H_{10}N_4$: C, 59.2; H, 6.2; N, 34.6. Found: C, 58.8; H, 6.9; N, 34.3.

6-Methyl-2-methylthiopurine (XIX, R = SCH_3).—A mixture of 2 g. of 4,5-diamino-6-methyl-2-methylthiopyrimidine (XVIII, R = SCH_3) and 20 ml. of dimethoxymethyl acetate[17] was heated under reflux for 1 hr. The solvent was then removed under reduced pressure and the residual solid extracted with 95% ethanol. The solution upon cooling yielded 1.3 g. of cream colored product, m.p. 290–294°. A small sample, sublimed for analysis, did not change the m.p.

Anal. Calcd. for $C_7H_8N_4S$: C, 46.7; H, 4.4; N, 31.3. Found: C, 46.7; H, 4.1; N, 31.3.

6,8-Dimethylpurine (XV).—Three grams of 4,5-diamino-6-methylpyrimidine (XIV) was added to a mixture of 20 g. of triethyl orthoacetate and 20 g. of acetic anhydride. The solution was refluxed for 2 hr. and then evaporated to dryness under reduced pressure using a steam-bath as a source of heat. To the residue was added 30 ml. of 10% ethanolic potassium hydroxide. The solution was heated for 30 min. on a steam-bath and then neutralized with acetic acid and evaporated to dryness under reduced pressure. The residue was continuously extracted in a soxhlet extractor several times with boiling benzene. The benzene extract on evaporation gave 0.5 g. of light-brown product, m.p. 255–260°. Recrystallization from toluene yielded a white product, m.p. 263–265°. Further purification by sublimation did not change the melting point.

Anal. Calcd. for $C_7H_8N_4$: C, 56.8; H, 5.4. Found: C, 56.8; H, 5.6.

2,6-Dimethylpurine (XII).—The crude product, 4,5-diamino-2,6-dimethylpyrimidine (XI), isolated by evaporation of the solvent from the reduction of 2 g. of 4-amino-2,6-dimethyl-5-nitropyrimidine (X), was added to 70 ml. of a 1:1 (by volume) mixture of acetic anhydride and triethyl orthoformate. The solution was refluxed for 4 hr., the solvent removed under reduced pressure and the residue treated as for the preparation of 6,8-dimethylpurine. The residue obtained from the benzene extract was recrystallized from a benzene-heptane mixture to yield 2 g. of 2,6-dimethylpurine (XII), m.p. 260–263°. A second recrystallization from benzene–heptane raised the m.p. to 266–268°. A sublimed sample melted at 271°.

Anal. Calcd. for $C_7H_8N_4$: C, 56.8; H, 5.4; N, 37.8. Found: C, 56.6; H, 5.9; N, 38.2.

2-Methylpurine. Method 1.—Nine grams of 2-methyl-6-chloropurine (III)[8] and 3 g. of 5% palladium–charcoal catalyst were added to 200 ml. of water to which had been added 3 ml. of concentrated ammonium hydroxide. The mixture was shaken at room temperature at 1 atm. pressure of hydrogen. After 3 hr. the mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The solid was continuously extracted with approximately 200 ml. of boiling toluene in a soxhlet apparatus for 4 days. The cooled toluene yielded 3.8 g. of product, m.p. 282–285°. Recrystallization from a benzene and methanol solution raised the m.p. to 286°.

Anal. Calcd. for $C_6H_6N_4$: C, 53.7; H, 4.5; N, 41.8. Found: C, 54.1; H, 4.4; N, 42.1.

Method 2.—Five grams of 2-methyl-6-purinethiol (IV)[8] was covered with 250 ml. of water, and 12 g. of Raney nickel catalyst (wet weight) was added. This mixture was then refluxed for 1.5 hr., filtered and the filtrate evaporated to dryness under reduced pressure with a steam-bath as source of heat. Two grams of crude product, m.p. 265–280°, was obtained. Two recrystallizations from a benzene–methanol solution raised the m.p. to 286°. A mixed m.p. with that product prepared by method 1 showed no depression.

2,8-Dimethyl-6-hydroxypurine (V).[11]—4,5-Diamino-6-hydroxy-2-methylpyrimidine (I) (38.0 g.) was covered with 500 ml. of acetic anhydride and the mixture refluxed for 5 hr. The excess acetic anhydride was removed by vacuum distillation with a steam-bath as the source of heat. To the solid residue was then added 100 ml. of water. Potassium hydroxide was added until the solution was *p*H 14. The solution next was treated with Norite and filtered. The filtrate was acidified with acetic acid. The solution was cooled and filtered and the precipitate washed with a small portion of water. The white crystals then were dried at 140° to yield 25 g. of product. Recrystallization from water and finally from methanol provided a pure product, m.p. > 300°.

Anal. Calcd. for $C_7H_8N_4O \cdot H_2O$: C, 46.1; H, 5.5; N, 30.8. Found: C, 45.8; H, 5.8; N, 31.2.

6-Chloro-2,8-dimethylpurine (VI).—Ten grams of 2,8-dimethyl-6-hydroxypurine (V) was covered with 250 ml. of phosphorus oxychloride and the mixture refluxed for 3 hr. or until all solid had dissolved. The excess phosphorus oxychloride was removed by vacuum distillation with a steam-bath as the source of heat. The residue was then poured, with stirring, onto chopped ice and allowed to stand 10 min. The solution was kept cold by addition of ice and then made strongly basic by addition of concentrated sodium hydroxide solution. By careful addition of concentrated hydrochloric acid the solution was adjusted to *p*H 5. This aqueous solution was then extracted with 300 ml. of ether on a continuous extractor for 36 hr. The ether yielded 4.5 g. of product, m.p. 234–237°. After recrystallization from benzene–methanol the m.p. was raised to 242°.

Anal. Calcd. for $C_7H_7N_4Cl$: C, 46.0; H, 3.8; N, 30.7. Found: C, 46.2; H, 3.7; N, 30.7.

(17) J. A. Montgomery and C. Temple, Jr., THIS JOURNAL, **80**, 409 (1958).

2,8-Dimethyl-6-purinethiol.—To 50 ml. of absolute ethanol and 1.8 g. of 6-chloro-2,8-dimethylpurine was added 2 g. of thiourea. This mixture then was refluxed for 3 hr., treated with Norite, filtered and the filtrate allowed to cool. The precipitate was filtered and dried at 110° to yield 1.2 g. of an analytically pure product; ultraviolet absorption spectra exhibited at *p*H 1: λ_{max} 232 mμ, ε 14,200; λ_{max} 329 mμ, ε 14,200; at *p*H 11: λ_{max} 235 mμ, ε 20,000; λ_{max}, 314 mμ, ε 14,600.

Anal. Calcd. for $C_7H_8N_4$: C, 46.7; H, 4.5; N, 31.1. Found: C, 46.2; H, 4.6; N, 31.1.

2-Ethoxy-6-methylpurine (XIX, R = OC_2H_5). Method 1.—4,5-Diamino-2-ethoxy-6-methylpyrimidine (XVIII, R = OC_2H_5), obtained after reducing 4 g. of 4-amino-2-ethoxy-6-methyl-5-nitropyrimidine (XVII, R = OC_2H_5) by sponge nickel catalyst in absolute ethanol, was used directly for cyclization after removal of the nickel by filtration and the evaporation of the ethanol under reduced pressure. The dry powdered residue of 4,5-diamino-2-ethoxy-6-methylpyrimidine (XVIII, R = OC_2H_5) was mixed with about 15 ml. of an equimolar mixture of triethyl orthoformate and acetic anhydride and refluxed for 4 hr. The solvent was removed under reduced pressure using a steam-bath as the source of heat. The residue was warmed with dilute potassium hydroxide on the steam-bath. The filtrate, after neutralization and cooling, deposited a brown crystalline product which was filtered, dried and extracted with 5 × 30 ml. of boiling benzene. The nearly colorless benzene extract was concentrated to 100 ml. and allowed to cool to yield 0.8 g. of cream-colored product, m.p. 236°.

Anal. Calcd. for $C_8H_{10}N_4O \cdot \frac{1}{2}H_2O$: C, 51.3; H, 5.9. Found: C, 51.7; H, 5.8.

Method 2.—Five-tenths gram of sodium was dissolved in 60 ml. of absolute ethanol. Then 1 g. of 2-chloro-6-methylpurine (XIX, R = Cl) in 25 ml. of absolute ethanol was added. The solution was refluxed for 24 hr. Toluene now was added, and the ethanol was replaced slowly by toluene in the refluxing mixture. The excess toluene was boiled off to give a deep-brown substance which was treated with about 50 ml. of boiling water. The solution was treated with charcoal, filtered and acidified with glacial acetic acid. On cooling, the product which separated was recrystallized from an alcohol–benzene mixture to yield white crystals, m.p. 230°. A mixed m.p. determination with the product obtained by method 1 showed no depression.

Anal. Calcd. for $C_8H_{10}N_4O \cdot \frac{1}{2}H_2O$: C, 51.3; H, 5.9. Found: C, 51.1; H, 5.9.

2,8-Dimethylpurine (VII).—One gram of 6-chloro-2,8-dimethylpurine was shaken at room temperature with 0.5 g. of 5% palladium–charcoal catalyst in 40 ml. of water and 0.5 ml. of 28% ammonium hydroxide under 1 atm. of hydrogen for 3 hr. The isolation and purification procedure was identical to that employed for the preparation of 2-methylpurine. The yield of 2,8-dimethylpurine was 0.3 g., m.p. 217°.

Anal. Calcd. for $C_7H_8N_4$: C, 56.8; H, 5.4; N, 37.8. Found: C, 56.8; H, 5.4; N, 37.6.

TEMPE, ARIZ.

[CONTRIBUTION FROM THE KETTERING–MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE]

Synthesis of Potential Anticancer Agents. XVII. Preparation of 9-(Substituted-cycloaliphatic)-purines[2]

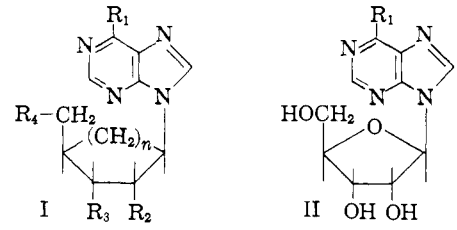
BY HOWARD J. SCHAEFFER AND RICHARD D. WEIMAR, JR.

RECEIVED JULY 24, 1958

The syntheses of 6-chloro-9-(2-cyclohexenyl)-purine, *cis*- and *trans*-2-[9-(6-chloropurinyl)]-cyclohexanols have been completed. From these compounds, several 6-substituted analogs have been prepared. The stereochemical relationship of these compounds with nucleosides is discussed.

In earlier papers of this series,[3,4] we discussed the reasons for our interest in the area of purine ribonucleosides as potential anticancer agents and reported the preparation of a wide variety of these ribonucleosides. Preliminary screening results on some of these ribonucleosides against Adenocarcinoma 755 have shown unpredictable differences in antitumor activity between them and the corresponding free purines.[5] The differences observed were variations in the chemotherapeutic index, in the toxicity, or in both. Nevertheless, the results are encouraging since the differences in activity between the ribonucleosides and the free purines establish that the ribonucleosides remain at least partially intact in the test animals.

Since we were concerned that the sugar moiety of a ribonucleoside might easily be removed hydrolytically or enzymatically in the test animals, we have devised a novel approach for the preparation of compounds related to nucleosides that should be stable under the test conditions. This novel class of compounds are purines which are substituted at the 9-position with a substituted cyclohexyl or cyclopentyl nucleus (I).



n = 1 or 2
R_1 = H, Cl, OH, NH_2, SH or $NHNH_2$
R_2, R_3, R_4 = H or OH

Because this new series of compounds will have a normal C–N bond from the 1-position of the cycloaliphatic group to the 9-position of the purine, it is obvious that this bond will be stable toward hydrolysis in the test animals and, in addition, the cycloaliphatic group should not easily be removed enzymatically. Furthermore, it may be concluded[6] that the substitution of a carbon atom for

(1) Affiliated with Sloan–Kettering Institute. This work was supported by funds from the C. F. Kettering Foundation and the National Institutes of Health, Contract Number SA-43-ph-1740.

(2) For Paper XVI of this series, see T. P. Johnston, L. B. Holum and J. A. Montgomery, to be published.

(3) (a) J. A. Johnson, Jr., H. J. Thomas and H. J. Schaeffer, THIS JOURNAL, **80**, 699 (1958); (b) H. J. Schaeffer and H. J. Thomas, *ibid.*, **80**, 3738 (1958).

(4) H. J. Schaeffer and H. J. Thomas, *ibid.*, in press.

(5) H. E. Skipper and J. R. Thomson, private communication.

(6) A. Maccoll, in W. Klyne, ed., "Progress in Stereochemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1954, pp. 361–365.