the following manner. Oxytocin (100 μg.) and D-leucine-oxytocin (100 μg.) as well as a mixture of 100 μg. of each of the two peptides were applied separately to a strip of Whatman No. 1 paper and chromatographed for 16 hr. at room temperature with the solvent system butanol–acetic acid–water (4:1:5) (descending). The R_f values of the ninhydrin spots obtained were 0.67 for oxytocin and 0.73 for D-leucine-oxytocin. The mixture of the two peptides had been separated into two distinct spots according to the R_f values reported.

Countercurrent Distribution of a Mixture of Oxytocin and D-Leucine-oxytocin.—A mixture of oxytocin (10 mg.), D-leucine-oxytocin (9 mg.) and synthetic tritium-labeled oxytocin[3] (1 mg.) was introduced into the first 10 tubes of the 6-ml. 200-tube Craig countercurrent machine and submitted to 450 transfers in the solvent system butanol–propanol–0.05% acetic acid (2:1:3) at 4°. The resulting distribution of the material was analyzed by measurement of the Folin–Lowry color and of the radioactivity by liquid scintillation counting.[15] Oxytocin and radioactive oxytocin, exhibiting a K value of 0.32, were separated from D-leucine-oxytocin with a K value of 0.49, as shown in Fig. 1.

Acknowledgments.—The authors wish to thank Mr. Joseph Albert for the microanalyses, Mrs. Lorraine Abrash for the amino acid analyses, and Miss Maureen O'Connell, Miss Catharine Smith and Mr. Hans Holzhauser for the bioassays.

(15) Aliquots of the lower phase (0.1 ml.) were counted in a Tracerlab scintillation counter with the dioxane–xylene solution of the phosphor as used by H. J. Jacobson, G. N. Gupta, C. Fernandez, S. Hennix and E. V. Jensen, *Arch. Biochem. Biophys.*, **86**, 89 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Potential Purine Antagonists. XXXIV. The Synthesis of 3-Methylguanine and a Study of the Structure and Chemical Reactivity of Certain 3-Methylpurines[1]

BY LEROY B. TOWNSEND AND ROLAND K. ROBINS

RECEIVED FEBRUARY 23, 1962

The synthesis of 3-methylguanine (I) has been achieved. A study of the chemical properties of I and a number of related 2-amino-3-methyl-6-substituted purines has revealed that the classical, fixed double-bond type structure which can be written for these compounds does not account for the observed chemical reactivity toward nucleophilic substitution. Evidence is presented which suggests that these compounds possess a high degree of aromaticity with an increased electron density in the imidazole ring and an over-all decrease of electron density in the pyrimidine ring.

The superior antitumor activity of 2-amino-1-methyl-6-purinethione[2] over that of 2-amino-6-purinethione (6-thioguanine) against *Adenocarcinoma* 755 prompted us to investigate the synthesis of additional N-methyl derivatives of 6-thioguanine. The preparation of the 9-methyl[3] and 7-methyl[4] derivatives of 2-amino-6-purinethione have already been reported by thiation of the corresponding N-methylguanine with phosphorus pentasulfide in pyridine. Since the required 3-methylguanine (I) has not been reported previously, the synthesis of I was undertaken in our laboratory.

The study of 3-N-substituted purines is presently of considerable interest in view of the work of Leonard and Deyrup[5] who have shown that the naturally-occurring alkaloid, triacanthine, is 6-amino-3-(γ,γ-dimethylallyl)-purine. Also it has been shown[6] recently that uric acid-3-riboside occurs in beef blood.

It is of interest that a previous unsuccessful attempt to prepare 3-methylguanine has been recorded.[7] Brookes and Lawley[8] have recently noted that no 3-substituted guanines are known. The synthesis of 3-methylguanine (I) *via* the classical Traube synthesis was investigated. The condensation of methylguanidine and ethyl cyanoacetate has been described by Roth, Smith and Hultquist[9] as yielding 4-amino-6-hydroxy-2-methylaminopyrimidine and a ring N-methylated isomer which was assigned the structure 2,4-diamino-1-methyl-6-pyrimidone. This structural assignment was re-investigated by Boon and Bratt[10] and Curran and Angier[11] who established the structure as 2,4-diamino-3-methyl-6-pyrimidone. Nitrosation and reduction[9] provided 3-methyl-2,4,5-triamino-6-pyrimidone (IV) sulfate. Ring closure to 3-methylguanine (I) proceeded readily with boiling formamide.[12]

All known N-methylguanine derivatives were compared with I utilizing ultraviolet absorption spectra and paper chromatography and were shown to be different from I. A rigorous structure proof of 3-methylguanine was sought, however, in view of several recorded rearrangements[13,14] of various N-methylpyrimidines. Treatment of 3-methylguanine with mineral acid and sodium nitrite under standard conditions for converting guanine to xanthine gave only unreacted I. Refluxing 6 N hydrochloric acid did not change 3-methylguanine after 3 hours. Refluxing 2 N sodium hydroxide, however, converted I to 3-methylxanthine (II) in good yield. An authentic sample of II was prepared from 2-mercapto-3-methyl-6-purinone[15]

(1) Supported by Research Grant No. T-181 from the American Cancer Society.

(2) C. W. Noell, D. W. Smith and R. K. Robins, *J. Med. Pharm. Chem.*, in press.

(3) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).

(4) R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957).

(5) N. J. Leonard and J. A. Deyrup, *ibid.*, **84**, 2148 (1962).

(6) H. S. Forrest, D. Hatfield and J. M. Lagowski, *J. Chem. Soc.*, 963 (1961).

(7) G. B. Elion, in "The Chemistry and Biology of Purines," Ciba Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, eds., Little, Brown and Co., Boston, Mass., 1957, p. 42.

(8) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 3923 (1961).

(9) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, *J. Am. Chem. Soc.*, **73**, 2864 (1951).

(10) W. R. Boon and G. Bratt, *J. Chem. Soc.*, 2159 (1957).

(11) W. V. Curran and R. B. Angier, *J. Am. Chem. Soc.*, **80**, 6095 (1958).

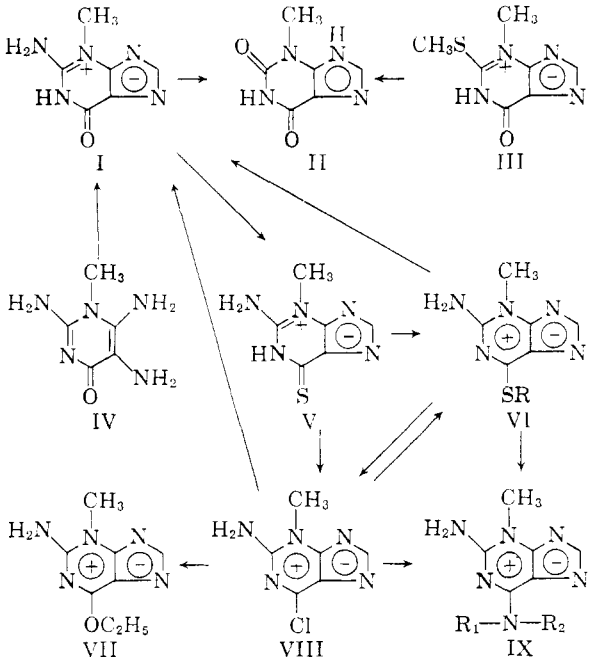
(12) R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, *ibid.*, **75**, 263 (1953).

(13) D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1298 (1961).

(14) D. J. Brown, E. Hoerger and S. F. Mason, *ibid.*, 4035 (1955).

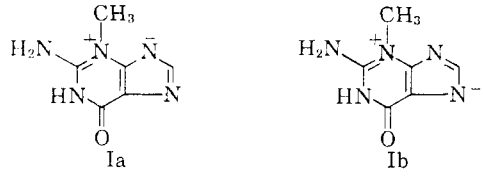
(15) W. Traube and F. Winter, *Arch. Pharm.*, **244**, 16 (1906).

REACTION SCHEME



which was methylated to yield 3-methyl-2-methylthio-6-purinone (III); III was converted to 3-methylxanthine[16-18] with refluxing 6 *N* hydrochloric acid. The structure of 2-mercapto-3-methyl 6-purinone has been verified[19] recently by conversion to 3-methylhypoxanthine. The identity of 3-methylxanthine, prepared from I and III, was established by comparison of ultraviolet and infrared spectra and by the use of paper chromatography in three different solvents.

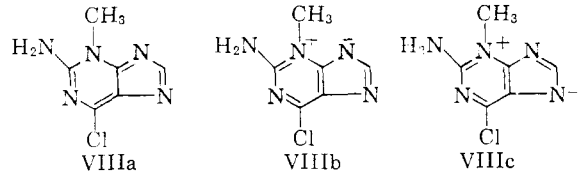
It is of considerable theoretical interest that 3-methylguanine is resistant to attack by nitrous acid and is hydrolyzed in aqueous base to 3-methylxanthine. A previous attempt[7] to prepare 3-methylguanine (I) from 3-methyl-2-methylthio-6-purinone (III) with aqueous ammonia at high temperatures gave only 3-methylxanthine. It would now appear quite probable that 3-methylguanine was prepared in this reaction but was hydrolyzed to II by the base present. It would thus appear that structures Ia and Ib contribute significantly to the state of the molecule.



Treatment of 3-methylguanine with phosphorus pentasulfide in pyridine gave a good yield of 2-amino-3-methyl-6-purinethione (V). Alkylation of V with various alkyl halides in the presence of potassium hydroxide provided a number of 6-alkylthio-2-amino-3-methylpurines (VI) which are listed in Table I. That alkylation had occurred on the sulfur atom was shown by acid hydrolysis of VI, R = CH_3, to I. Treatment of 2-amino-3-methyl-6-methylthiopurine (VI, R = CH_3) with chlorine gas in methanol gave a good yield of 2-amino-6-chloro-3-methylpurine (VIII) which was prepared similarly from V. This type of oxidative replacement of the sulfur atom previously has been reported for the synthesis of 2-amino-6-chloropurine.[20] Treatment of VIII with refluxing 1 *N* hydrochloric acid regenerated 3-methylguanine (I).

A study of the reactivity of 2-amino-6-chloro-3-methylpurine (VIII) toward nucleophilic attack revealed some very interesting facts. 2-Amino-6-ethoxy-3-methylpurine (VII) was prepared readily from VIII and refluxing ethanol containing an excess of sodium ethoxide. It was later discovered that the same compound could be prepared from VIII in less than 30 minutes with sodium ethoxide at room temperature. Similarly, *n*-butanethiol in methanol in the presence of potassium hydroxide at room temperature quantitatively converted VIII to the 2-amino-6-*n*-butylthio-3-methylpurine (VI, R = *n*-C_4H_9) in 15 minutes. Under similar conditions neither sodium ethoxide nor *n*-butyl mercaptide will react with the parent structure, 2-amino-6-chloropurine.

Ammonia passed through refluxing ethanol converted 2-amino-6-chloro-3-methylpurine (VIII) to 2,6-diamino-3-methylpurine (IX, R_1,R_2 = H). 2-Amino-6-chloropurine itself remains unchanged under these conditions. The classical, fixed-bond structure which can be drawn for 2-amino-6-chloro-3-methylpurine (VIIIa) actually predicts a *higher* electron density in the pyrimidine ring due to the aliphatic amine type structure for nitrogen 3 and the general inductive effect of the 3-methyl group. Since the chlorine atom in VIII is more susceptible to nucleophilic displacement than the parent compound lacking the 3-methyl group, structure VIIIa does not adequately represent the compound in question. Structures VIIIb and VIIIc suggest that the compound retains a great deal of aromaticity and therefore probably is



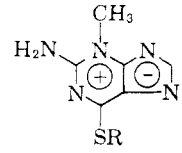
planar, but the adjacent 2-amino group would be expected to help mask the electron-withdrawing effect of the quaternized nitrogen at position 3. It would appear from the observed chemical properties that formula VIII best shows the general electron deficiency in the pyrimidine ring.

This type of electron deficiency is also exhibited by other 2-amino-3-methylpurine derivatives. 2-Amino-3-methyl-6-methylthiopurine (VI, = CH_3) and *n*-butylamine (40% aqueous) warmed on the

(16) H. Bredereck, H. Schuh and A. Martini, *Ber.*, **83**, 201 (1950).
(17) W. Traube, *ibid.*, **33**, 3049 (1900).
(18) J. M. Gulland, E. R. Holiday and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).
(19) F. Bergmann, G. Levin, A. Kalmus and H. Kwietny-Govrin, *J. Org. Chem.*, **26**, 1504 (1961).
(20) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel and A. G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).

TABLE I

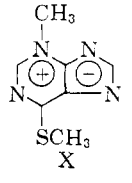
6-ALKYLTHIO-2-AMINO-3-METHYLPURINES



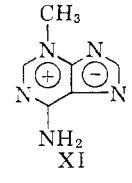
R	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Recrystn. solv.	Yield, %	Alkyl halide employed
$CH_2C_6H_5$	274	57.6	57.8	4.8	4.9	25.8	25.9	Ethanol–water	58	Cl
$CH_2C_6H_4Cl$-*o*	276	51.2	51.3	3.9	4.2	23.0	22.8	Methanol–water	54	Cl
$CH_2C_6H_4F$-*o*	282–283	54.0	53.8	4.2	4.5	24.2	23.9	Ethanol–water	44	Cl
$CH_3 \cdot {}^1/_2H_2O$	289–292	41.2	41.1	4.9	5.0	34.3	34.4	Water	75	I
$C_2H_5 \cdot {}^1/_2H_2O$	261–262	44.0	44.2	5.5	5.2	32.1	31.9	Water	46	I
C_4H_9-*n*	258–259	50.7	50.6	6.4	6.0	29.6	29.2	Water	38	I

steam-bath readily gave 2-amino-6-*n*-butylamino-3-methylpurine. This is to be contrasted with the conditions necessary for the preparation of 2-amino-6-*n*-butylaminopurine[21] from 2-amino-6-methylthiopurine which requires a sealed vessel at 130° for 16 hours. Neither 2-amino-6-methylthiopurine[20] nor 2-amino-9-methyl-6-methylthiopurine[21a] showed any evidence of reaction with 40% aqueous *n*-butylamine after 8 hours of refluxing. 2-Amino-6-chloro-3-methylpurine (VIII) and *n*-butylamine gave the same product (IX, R_1 = H, R_2 = *n*-C_4H_9) at room temperature in 5 minutes. 2,6-Diamino-3-methylpurine (IX, R_1,R_2 = H) did not react with excess sodium nitrite in the presence of aqueous acetic acid heated on the steam-bath. This procedure changes 2,6-diamino-9-β-D-ribofuranosylpurine to crotonoside.[22] Thus, the electron deficiency of the pyrimidine is again demonstrated.

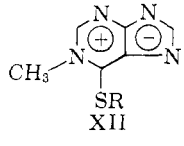
This rather unexpected reactivity toward nucleophilic attack is shared by other 3-methylpurines. The compound, 3-methyl-6-methylthiopurine,[19,23] which it is now proposed should be written as X, already has been shown to exhibit[23] similar properties. 3-Methyladenine (XI) has been observed to be inert to treatment with nitrous acid[23] under conditions which normally change the 6-amino



X



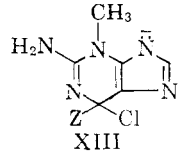
XI



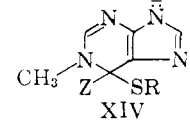
XII

group to hydroxy. Certain 6-alkylthio-1-methylpurines (XII) prepared and studied in our laboratory[24] have also shown extraordinary reactivity.

It would thus appear that this type of compound is quite general and may be predicted to arise when a quaternized nitrogen can be stabilized by a negative charge in an adjacent ring system. The negative charge induced by an attacking anion, Z:⁻, at position 6 can be accommodated readily in the pyrimidine ring by the formation of transition states such as XIII and XIV. The extraordinary stability of such transition states might be an important factor in the unusual reactivity exhibited by these N-methylpurines toward nucleophilic reagents. This interesting phenomenon is presently under study in our laboratory with related heterocyclic systems.



XIII



XIV

Bergmann and co-workers[19] have noted that several 3-methylpurines exhibit an unusually large bathochromic shift in the ultraviolet which was ascribed to "double-bond fixation" in these derivatives. Such a shift in absorption maxima was not observed with the 2-amino-3-methylpurine derivatives described in the present work. In general, the ultraviolet absorption spectra of these compounds (see Table II) were in the same range as the parent purine derivatives possessing no methyl group at position 3.

It is quite possible that the shift in the ultraviolet observed by Bergmann[19] is due to the greater electron movement between the electron-enriched imidazole ring and the electron-deficient pyrimidine ring. This effect is partially nullified by the presence of an amino group at the 2-position of the pyrimidine ring.

The compounds described in this work have been submitted to the Cancer Chemotherapy National Service Center for antitumor testing.

Experimental[25]

2-Amino-3-methyl-6-purinone (3-Methylguanine) (I). Method 1.—3-Methyl-2,4,5-triamino-6-pyrimidone sulfate[9] (50 g.) was added to 250 ml. of formamide and the reaction mixture refluxed for 1.5 hr. The reaction mixture was cooled and the precipitate filtered and washed with water and acetone. The solid was dried at 110° to yield 27 g. of product. A sample was recrystallized from water for analysis; m.p. > 300°.

Anal. Calcd. for $C_6H_7N_5O$: C, 43.6; H, 4.2; N, 42.4. Found: C, 43.2; H, 4.5; N, 42.2.

(21) J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, **80**, 407 (1958).

(21a) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).

(22) J. Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1951).

(23) J. W. Jones and R. K. Robins, *ibid.*, **84**, 1914 (1962).

(24) L. B. Townsend and R. K. Robins, *J. Org. Chem.*, **27**, 990 (1962).

(25) All melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected.

TABLE II

ULTRAVIOLET ABSORPTION OF SOME 2-AMINO-3-METHYLPURINES[a]

Compound	pH 1 λmax, mμ	ε	pH 11 λmax, mμ	ε
3-Methylguanine	244[c]	8,300	273	13,700
	264	11,200		
2-Amino-3-methyl-6-purinethione	237	4,360	235	8,550
	256	6,260	279	12,000
	340	33,600	330	33,600
2,6-Diamino-3-methylpurine	243	11,400	243	6,000
	279	13,800	282.5	16,650
2-Amino-6-chloro-3-methylpurine	257	3,120	230[b]	16,100
	316	10,250	313	9,250
2-Amino-6-methoxy-3-methylpurine	235[c]	5,000	287	14,000
	284	11,800		
2-Amino-6-ethoxy-3-methylpurine	235[c]	4,620	287	12,600
	284	11,600		
2-Amino-3-methyl-6-methylthiopurine	275	14,200	232	10,600
	318	15,900	264	11,400
			319	16,300
2-Amino-6-ethylthio-3-methylpurine	277	13,900	232	10,200
	318	16,400	266	11,300
			319	17,400
2-Amino-6-(*n*-butylthio)-3-methylpurine	278	12,800	232	9,500
	319	16,300	267	9,500
			320	15,900
2-Amino-6-benzylthio-3-methylpurine	278	11,800	232	11,600
	320	16,500	267	10,000
			321	16,800
2-Amino-6-(*o*-chlorobenzylthio)-3-methylpurine	277	12,200	232	10,700
	319	16,800	264	9,600
			321	17,000
2-Amino-6-(*o*-fluorobenzylthio)-3-methylpurine	276	13,000	232	11,000
	319	17,500	265	10,400
			320	17,000
2-Amino-6-(*n*-butylamino)-3-methylpurine	224	14,700	228[b]	22,000
	249	12,700	245[c]	8,150
	280	16,500	284	18,000

[a] The spectra were determined in aqueous solution at buffered *p*H. [b] Shoulder. [c] Inflection.

Method 2.—2-Amino-6-chloro-3-methylpurine (VIII) (0.4 g.) was added to 100 ml. of 1 *N* hydrochloric acid and the solution refluxed for 2 hr. and treated with charcoal. The filtrate was adjusted to *p*H 7; the precipitate was filtered to yield 0.3 g. of product which was recrystallized from water for analysis. The ultraviolet absorption spectra and paper chromatography[26] showed the product to be 3-methylguanine. 3-Methylguanine exhibited an R_f of 0.23 in solvent B, 0.52 in solvent D, and 0.52 in solvent E.

Anal. Calcd. for $C_6H_7N_5O$: N, 42.4. Found: N, 42.1.

2-Amino-3-methyl-6-purinethione (V).—A mixture of 20 g. of 3-methylguanine and 100 g. of phosphorus pentasulfide was suspended in 1400 ml. of pyridine. The solution was refluxed for 16 hr., and the excess pyridine was removed under reduced pressure using a steam-bath as a source of heat. To the residue was added 1 l. of water and the reaction mixture heated on the steam-bath for 3 hr. and finally cooled overnight. The precipitate was filtered, washed with water, and dissolved in hot dilute aqueous ammonia. The solution was treated with charcoal and filtered. The *p*H of the filtrate then was adjusted to 6 with glacial acetic acid and the solution allowed to cool. The precipitate was filtered, washed with water, and dried to yield 19 g. of pale yellow solid, m.p. > 300°. A sample was recrystallized from water for analysis.

Anal. Calcd. for $C_6H_7N_5S \cdot 1/2H_2O$: C, 37.9; H, 4.2; N, 36.8. Found: C, 37.8; H, 4.0; N, 36.2.

3-Methylxanthine (II). Method 1.—One gram of 3-methylguanine (I) was added to 20 ml. of 2 *N* sodium hydroxide. The solution was refluxed for 4 hr. and then evaporated to dryness *in vacuo* using a steam-bath as the source of heat. Water (25 ml.) was added, and the solution was again evaporated to dryness. The residue was dissolved in boiling water, treated with charcoal, and filtered. After cooling overnight at 15°, the precipitate was filtered to yield 0.85 g. of product, m.p. >300°. A sample was recrystallized from water for analysis.

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.7; H, 3.8; N, 33.6.

Method 2.—One gram of 3-methyl-2-methylthio-6-purinone (III) was refluxed for 1 hr. in 100 ml. of 6 *N* hydrochloric acid. The solution was evaporated to dryness *in vacuo* using a steam-bath as the source of heat. Water (50 ml.) was added, and the solution was again evaporated to dryness. The residue was dissolved in 50 ml. of dilute, aqueous ammonia and the solution treated with charcoal and filtered. The filtrate was adjusted to *p*H 7 with dilute glacial acetic acid and allowed to cool. The precipitate was filtered to yield 0.6 g. of product, m.p. >300°. A small sample was recrystallized from water for analysis.

Anal. Calcd. for $C_6H_6N_4O$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.3; H, 3.7; N, 33.4.

The identity of the products prepared by methods 1 and 2 was established by infrared and ultraviolet absorption spectra and paper chromatography[26] in three solvents. 3-Methylxanthine exhibited an R_f of 0.46 in solvent A, 0.37 in solvent B and 0.55 in solvent D.

2-Amino-6-alkylthio-3-methylpurines (VI) (Table I).—To 25 ml. of 1 *N* potassium hydroxide was added 1 g. of 2-amino-3-methyl-6-purinethione (V), and an equimolar quantity of the appropriate alkyl halide, dissolved in 2.5 ml. of *p*-dioxane, then was added slowly over a 15-min. period. The solution was warmed to 35–40° and stirred for 3 hr. The reaction mixture was allowed to cool and the precipitate filtered. The product was purified by recrystallization from the solvents indicated in Table I.

2-Amino-6-chloro-3-methylpurine (VIII). Method 1.—Absolute methanol (30 ml.) was cooled to 10° in an ice-bath, and chlorine gas was passed into the solution at a moderate rate for approximately 10 min. The flow of chlorine was slowed, and 2-amino-3-methyl-6-methylthiopurine (VI, R = CH_3) was added slowly in small portions maintaining the temperature below 10°. After the final addition of solid (2 g. total), the flow of chlorine was discontinued, and the mixture was stirred and cooled for an additional 30 min. The precipitate was then filtered and washed with cold ethanol. The solid was dried at 80° to yield 1.2 g. of the hydrochloride salt which was dissolved in 10 ml. of water at room temperature and the solution treated with charcoal and filtered. The solution was adjusted to pH 7 with aqueous ammonia and the precipitate filtered and dried to yield 1 g. of 2-amino-6-chloro-3-methylpurine. Recrystallization from water gave a purified solid which turned red at 210–220° and gradually decomposed above 280° when heated slowly on the melting point block.

Anal. Calcd. for $C_6H_6N_5Cl$: C, 39.4; H, 3.3; N, 38.2. Found: C, 39.4; H, 3.4; N, 38.2.

Method 2.—The same procedure as outlined above was followed, except that 2 g. of V was used in place of 2-amino-3-methyl-6-methylthiopurine, to yield 1.2 g. of the hydrochloride salt which was converted to the free base (0.8 g.). Recrystallization from water for analysis gave a product identical with that prepared by method 1 as judged on the basis of ultraviolet and infrared spectra and paper chromatography.

2-Amino-6-ethoxy-3-methylpurine (VII).—To 20 ml. of absolute ethanol, containing 0.5 g. of sodium, was added 0.5 g. of VIII and the solution refluxed for 18 hr. The reaction mixture was cooled to room temperature and neutralized with 1.2 ml. of glacial acetic acid. The solution was evaporated to dryness *in vacuo* and the residue dissolved in 25 ml. of boiling water. The solution was treated with charcoal, filtered, and allowed to cool and the *p*H adjusted to 7. The precipitate was filtered and the product dried at 110° to

(26) Chromatography solvents: A, dimethylformamide, 25 vol.–ammonium hydroxide, 10 vol.–isopropyl alcohol, 65 vol.; B, *n*-butyl alcohol, 5 vol.–acetic acid, 2 vol.–water, 3 vol.; C, Venner solvent [H. Venner, *Z. physiol. Chem.*, **322**, 122 (1960)] butyl alcohol, 20 vol.–acetone, 25 vol.–water, 7.5 vol.–ammonium hydroxide, 1.5 vol.; D, 5% ammonium carbonate in water; E, disodium hydrogen phosphate, 5%, in water saturated with isoamyl alcohol.

yield 0.45 g. A small sample was recrystallized from water for analysis to give a product with a melting point of 243–244°.

Anal. Calcd. for $C_8H_{11}N_5O$: C, 49.7; H, 5.7; N, 36.2. Found: C, 49.6; H, 5.6; N, 35.8.

2-Amino-3-methyl-6-methoxypurine was similarly prepared from VIII and sodium methoxide in refluxing methanol. The crude product was recrystallized from water to give a 50% yield, m.p. >300°.

Anal. Calcd. for $C_7H_9N_5O \cdot H_2O$: C, 42.6; H, 5.6; N, 35.5. Found: C, 42.3; H, 5.5; N, 35.2.

3-Methyl-2-methylthio-6-purinone (III).—2-Mercapto-3-methyl-6-purinone[15] (5 g.) was placed in 50 ml. of water, and just enough solid potassium hydroxide added to dissolve all the solid. The solution was cooled to 25° and 7 g. of methyl iodide added. The solution was stirred vigorously for 2 hr. at room temperature and finally treated with charcoal and filtered and the *p*H adjusted to 7 with glacial acetic acid. After cooling, the precipitate was filtered and the solid reprecipitated to yield 4.1 g. of product, m.p. >300°.

Anal. Calcd. for $C_7H_8N_4OS$: C, 42.9; H, 4.1; N, 28.6. Found: C, 43.0; H, 3.8; N, 28.4.

2,6-Diamino-3-methylpurine (IX, R_1, R_2 = H).—2-Amino-6-chloro-3-methylpurine (VIII, 0.25 g.) was added to 100 ml. of absolute ethanol and the solution refluxed for 8 hr. A stream of anhydrous ammonia was passed through the refluxing solution during the entire reaction period. The solution was then set on the steam-bath and the volume reduced to 50 ml. The solution was allowed to cool at 15° for 12 hr. and the solid filtered to yield 0.2 g. of product. A small sample was recrystallized from a methanol–ethyl acetate mixture for analysis and gave a product melting at >300°.

Anal. Calcd. for $C_6H_6N_6 \cdot HCl$: C, 36.0; H, 4.5; N, 42.0. Found: C, 36.2; H, 4.3; N, 41.9.

2-Amino-6-(*n*-butylamino)-3-methylpurine (IX, R_1 = H, R_2 = n-C_4H_9). **Method 1.**—2-Amino-6-chloro-3-methylpurine (VIII, 0.5 g.) was added to 30 ml. of *n*-butylamine and the solution stirred at room temperature for 1 hr. The solution then was evaporated to dryness in a stream of air, and the residue was triturated with 35 ml. of acetone at room temperature. The precipitate was filtered to yield 0.35 g. of a solid which was recrystallized from chloroform for analysis and gave a product melting at 255–258°.

Anal. Calcd. for $C_{10}H_{16}N_6$: C, 54.6; H, 7.3; N, 38.2. Found: C, 55.0; H, 6.5; N, 38.2.

Method 2.—2-Amino-3-methyl-6-methylthiopurine (VI, R = CH_3) (0.5 g.) was added to 25 ml. of *n*-butylamine (40% aqueous), and the solution was refluxed on the steam-bath for 2 hr. The excess *n*-butylamine was removed *in vacuo* on the steam-bath and 10 ml. of water added to the residue. The *p*H of the solution was adjusted to 6 and the solution evaporated to dryness in a stream of air. The residue was recrystallized from chloroform to give a product identical with that prepared by method 1 as judged on the basis of ultraviolet spectra.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, N. Y.]

Hydroxyl Group (V)[1] and Imidazole (X)[2] Catalysis. The General Base Catalysis of Ester Hydrolysis by Imidazole and the Influence of a Neighboring Hydroxyl Group

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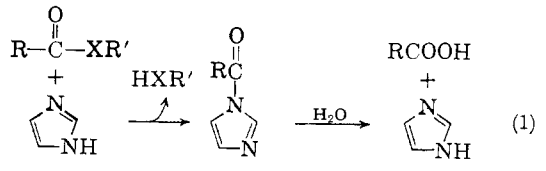
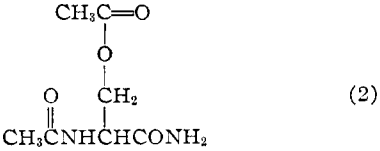
Imidazole has been found to be a catalyst for the hydrolysis of alkyl acetyl esters (CH_3COOR') in which the pK_a' of HOR' is less than pK_w. The catalytic coefficient for imidazole catalysis in these cases has been found to be associated with a deuterium solvent kinetic isotope effect (*i.e.*, k^H/k^D) of 2 to 4. It is suggested that the mechanism represents an imidazole general base or general acid-catalyzed hydrolysis. The rate of alkaline hydrolysis of the cyclopentyl ester of dichloroacetic acid has been shown to be only about half as sensitive to facilitation by a neighboring hydroxyl group as previously found for the corresponding acetyl ester. In addition, the rate of imidazole general base-catalyzed hydrolysis of cyclopentyl dichloroacetate has been found to be completely insensitive to the presence of a neighboring hydroxyl group.

The mechanism of the imidazole catalysis of the hydrolysis of the ester bond (R–CO–XR') has been found to be dependent on the nature of the group R– and the conjugate acid of the leaving group HXR'. When R– represents an alkyl or aryl group and HXR' phenol, a substituted phenol or a thiol then nucleophilic catalysis (1) invariably has been noted.[2,4,5]



(1)

When R– represents a particularly electron withdrawing substituent group as $CHCl_2$–, CH_3OCO– etc.[6,7] and HXR' an alcohol, then the catalysis occurs by way of general base or general acid mechanisms in which N-acetylimidazole is not an intermediate. A general base mechanism has also been noted in one case in which R– is not electron withdrawing and HXR' represents an alkoxide ion of pK_a' less than pK_w. This ester is the enzymologically important N,O-diacetylserine amide[8] (2).



(2)

The pK_a' of the leaving group (N-acetylserine amide) has been established recently as being

(1) For Hydroxyl Group Catalysis. IV, see T. C. Bruice and Fritz-Hans Marquardt, *J. Am. Chem. Soc.*, **84**, 365 (1962).

(2) For Imidazole Catalysis. IX, see T. C. Bruice and J. J. Bruno, *ibid.*, **84**, 2128 (1962).

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