TABLE III

ADDITION SALTS OF COMPOUNDS VIII

R	Formula	M.P.			Nitrogen, % Calcd.	Nitrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found
			Dihydrochlorides					
H	$C_7H_{16}N_2Cl_2$	314–315 (dec.)[a]			14.07	14.13	35.57	35.59
CH_3	$C_8H_{18}N_2Cl_2$	260–262[b]			13.14	13.05	33.6	33.4
n-C_4H_9	$C_{11}H_{24}N_2Cl_2$	245–247[b]			10.98	11.05	27.86	27.69
C_6H_5	$C_{13}H_{20}N_2Cl_2$	180–182[c]			10.18	10.30	25.81	25.74
$CH_2C_6H_5$	$C_{14}H_{22}N_2Cl_2$	213–215[b]			9.68	9.58	24.58	24.25
			Methyl Iodides					
H	$C_8H_{17}N_2I$	224–225			10.44	10.55	47.38	47.25
CH_3	$C_9H_{19}N_2I$	290–292			9.93	9.98	45.03	44.75
n-C_4H_9	$C_{12}H_{25}N_2I$	218–220			8.64	8.51	39.14	38.95
C_6H_5	$C_{14}H_{21}N_2I$	262–264			8.14	7.98	36.92	36.79
$CH_2C_6H_5$	$C_{15}H_{23}N_2I$	250–251			7.82	7.78	35.47	35.25
			Dipicrates					
H	$C_{19}H_{20}N_8O_{14}$	247–250	39.04	39.15	3.42	3.48	19.18	18.90
CH_3	$C_{20}H_{22}N_8O_{14}$	242–245	40.01	40.06	3.68	3.85	18.74	18.71
n-C_4H_9	$C_{23}H_{28}N_8O_{14}$	220–222	43.12	43.15	4.37	4.35	17.5	17.45
C_6H_5	$C_{19}H_{21}N_5O_7$[e]	172–174	52.9	52.85	4.87	5.01	16.26	16.19
$CH_2C_6H_5$	$C_{26}H_{26}N_8O_{14}$	228–230	46.3	46.52	3.86	4.00	16.62	16.62

[a] Crystallized from 80% ethanol. [b] Washed with acetone. [c] Crystallized from ethanol. [d] Crystallized from 80% alcohol. [e] Monopicrate.

palladium-on-charcoal. After 2 hr. the temperature was allowed to decrease to room temperature, the catalyst was filtered and the solution was distilled at atmospheric pressure collecting the fraction boiling at 193–198°; yield 2.8 g. (89%). The product was hygroscopic, quickly absorbed carbon dioxide and was characterized through the dihydrochloride, the dipicrate, and the methyl iodide. Analyses and melting points are given in Tables II and III.

8-Methyl-3,8-diazabicyclo[3.2.1]octane methyliodide (IX). To a solution of 1 g. of 8-methyl-3,8-diazabicyclo[3.2.1]-octane in 10 ml. of anhydrous ether 1 g. of methyl iodide (0.44 ml.) was added with cooling. The temperature was maintained at room temperature for 2 hr., the separated product was filtered and 1.3 g. of crystals melting at 222–224° were obtained. After crystallization from ethyl alcohol they melted at 224–225°.

Anal. Calcd. for $C_8H_{19}N_2I$: N, 10.44; I, 47.38. Found: N, 10.55; I, 47.25. Tertiary N calcd.: 522. Found: 5.15.

The ethereal mother liquors of IX were treated with 0.2 ml. of methyl iodide and allowed to stand one night in a refrigerator. The product which separated (1.15 g.) melted at 288–290°. A sample recrystallized from ethanol (m.p. (290–292°) did not depress the melting point in admixture with a sample of 3,8-dimethyl-3,8-diazabicyclo[3.2.1]octane methyl iodide.

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MILAN, ITALY

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, THE HEBREW UNIVERSITY—HADASSAH MEDICAL SCHOOL]

Synthesis and Properties of 3-Methylpurines[1]

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3-Methylpurines are obtained by desulfuration of appropriate mono- and dithio intermediates. However, the synthesis of 3-methylpurine itself failed. The 3-methylated derivatives, which possess a fixed double bond at position 1,2, exhibit a large bathochromic shift of λ_{max} and R_f values, *smaller* than those of the nonmethylated mother substances. The 3-methyl derivative of 6,8-dihydroxypurine is slowly converted into 3-methyluric acid by xanthine oxidase, whereas 3-methyl-8-hydroxypurine is not attacked.

In recent studies on the mechanism of enzymatic oxidation of purines, 3-methylated derivatives have played a major role, because the substituent in the 3-position imposes on the pyrimidine moiety a fixed distribution of double bonds and thus prevents structural changes from taking place in the activated enzyme–substrate complex.[3] In this

(1) This work was supported in part by grant No. RG-6631 from the National Institutes of Health.

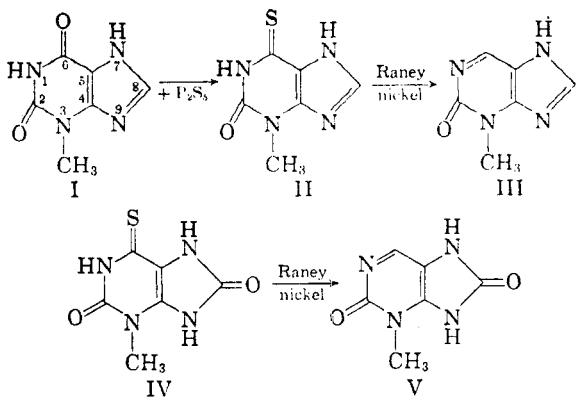
(2) Part of a Ph.D. thesis, submitted to the Faculty of Science, The Hebrew University, Jerusalem, 1960.

(3) F. Bergmann, H. Kwietny, G. Levin, and D. J. Brown, *J. Am. Chem. Soc.*, **82**, 598 (1960).

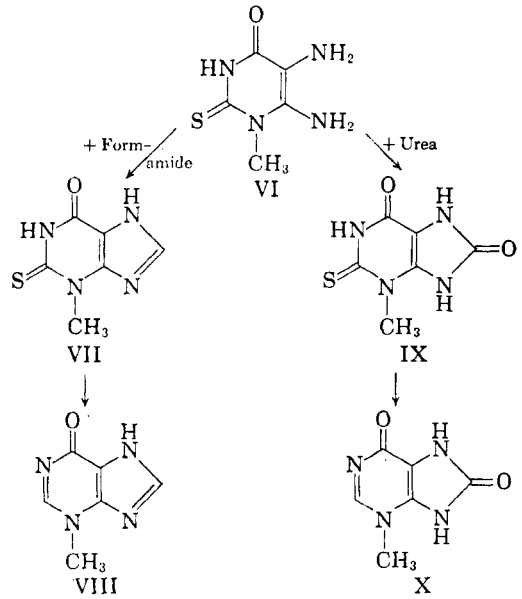
paper, we describe the synthesis of various 3-methylpurines, compare their physical properties in relation to their structure and examine the susceptibility of some of the new derivatives to attack by mammalian xanthine oxidase.

3-Methyl-2-purinone (III). This compound was obtained some sixty years ago by Tafel and Weinschenk[4] by electrolytic reduction of 3-methylxanthine and subsequent dehydrogenation with bromine. The present synthesis starts again with 3-methylxanthine (I), which—like many other xanthines and uric acids—is thiated preferentially at position 6, when reacting with phosphorus pentasulfide.[5-7] Catalytic desulfuration of the intermediate II gives the desired purine derivative III in 12% yield, when the free base is isolated. The yield can however be doubled by crystallizing the neutral sulfate instead.[4]

8-Hydroxy-3-methyl-2-purinone (V). This compound has been synthesised recently by Brown[8] in moderate yield by cyclization of 3-methyl-5-aminocytosin, the latter being the reduction product of 3-methyl-5-nitrocytosin. The purine V can, however, be obtained much more easily and in good yield by catalytic desulfuration of 2,8-dihydroxy-3-methylpurine-6-thione (IV), which is readily available by a new synthetic route.[9]



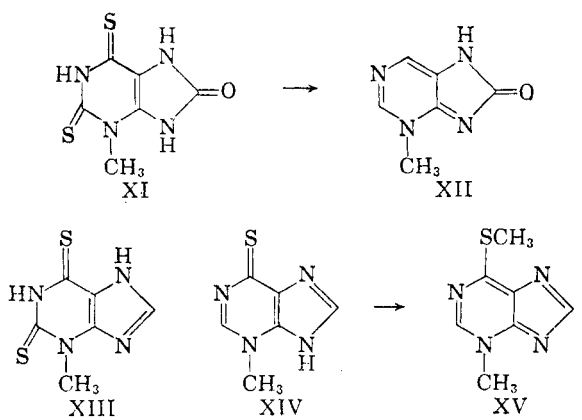
3-Methylhypoxanthine (VIII). This compound has been obtained previously by Traube and Winter[10] and by Elion,[11] using the diamine VI as starting material. An improved method for the synthesis of the latter has been described recently.[9]



Cyclization of VI with formamide to VII and finally desulfuration to VIII both proceed with very satisfactory results. Cyclization of VI with urea yields the unknown 6,8-dihydroxy-3-methylpurine-2-thione (IX). Subsequent catalytic removal of sulfur leads to 8-hydroxy-3-methyl-6-purinone (X).

3-Methyl-8-hydroxypurine (XII). For the synthesis of this compound, 2,6-dimercapto-3-methyl-8-purinol (XI), which has become available recently,[9] is a suitable starting material. Both sulfur atoms can be removed in a single operation.

In an analogous way, attempts were made to synthesize 3-methylpurine itself by catalytic desulfuration of 2-mercapto-3-methylpurine-6-thione (XIII).[11] However, the latter proved to be extremely resistant to removal of the sulfur atoms and when drastic conditions were tried, tars were obtained, which contained some unchanged starting material. Similar decomposition was observed when the desulfuration of 3-methylpurine-6-thione (XIV) or its *S*-methyl derivative (XV) was attempted.



(4) J. Tafel and A. Weinschenk, *Ber.*, **33**, 3372 (1900).
(5) A. G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).
(6) T. L. Loo, M. E. Michael, A. G. Garceau, and J. C. Reid, *J. Am. Chem. Soc.*, **81**, 3039 (1959).
(7) G. B. Elion, S. Mueller, and G. H. Hitchings, *J. Am. Chem. Soc.*, **81**, 3042 (1959).
(8) D. J. Brown, *J. Appl. Chem.*, **9**, 203 (1959).
(9) G. Levin, A. Kalmus, and F. Bergmann, *J. Org. Chem.*, **25**, 1252 (1960).
(10) W. Traube and F. Winter, *Arch. Pharm.*, **244**, 18 (1906).
(11) G. B. Elion, *Ciba Foundation Symposium on Chemistry and Biology of Purines*, J. & A. Churchill Ltd., London, (1957) p. 39.

TABLE I

COMPARISON OF THE SPECTRAL CHARACTERISTICS OF THE NEUTRAL FORMS[a] OF MONO- AND DIHYDROXYPURINES AND THEIR 3-METHYL DERIVATIVES

Purines	λ_{max}	log ϵ_{max}	3-Methylpurines	λ_{max}	log ϵ_{max}	$\Delta\lambda_{max}$
2-Hydroxy[b]	238	3.46	2-Oxo (III)	252	3.54	
	315	3.69		318	4.00	+3
6-Hydroxy[b]	249	4.02	6-Oxo (VIII)	264	4.06	+15
8-Hydroxy[b]	235	3.51	8-Hydroxy (XII)	227	3.85	
	277	4.05		299	4.24	+22
2,8-Dihydroxy[b]	230	3.90	2-Oxo-8-hydroxy (V)	224	4.10	
	310	3.70		254	3.77	
				311	4.11	+1
6,8-Dihydroxy[b]	257	4.08	6-Oxo-8-hydroxy (X)	274	4.24	+17
6-Mercapto	225	3.88	6-Thio (XIV)	243	3.92	
	325	4.28		340	4.38	+15
6-Methylthio[c]	291	4.32	6-Methylthio (XV)	236	4.09	
				312	4.32	+21

[a] The *p*H range at which these compounds exist as uncharged molecules, was determined from the curve, representing λ_{max} as function of *p*H: F. Bergmann, G. Levin, and H. Kwietny-Govrin, in preparation. [b] S. F. Mason, *J. Chem. Soc.*, 2071 (1954). [c] G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

Spectral properties. The data in Table I reveal that those 3-methyl derivatives, in which the double bond distribution in the pyrimidine ring is abnormal, exhibit an unusually large bathochromic shift when compared with other 3-methylated purines of "normal" structure (*e.g.* III or V). That tautomerization as such is not responsible for this phenomenon, can be deduced from a comparison of 1-methyl-2-purinone and 3-methyl-2-purinone (III).[3] Both these isomers possess the same conjugated system in the pyrimidine ring, *viz.* C=C—C=N—C=O, and exhibit the same bathochromic shift of 4 mμ with respect to 2-hydroxypurine. Therefore, the large shifts of λ_{max}, represented by compounds like VIII, X, or XII in Table I, must be ascribed to the fixation of a double bond at $\overset{1}{N} = \overset{2}{C}$. This is true, whether the 1,2-bond is cross-conjugated with the ethylenic group $\overset{4}{C} = \overset{5}{C}$, as in VIII or X, or forms part of a linear conjugated system, as in XII and XIV.

It should be noted that *S*-alkylation of XIV produces a pronounced hypsochromic shift of λ_{max} of 28 mμ. This is in general accord with observations on other thiopurines (see Table I, footnote *c*) and supports the structure assigned (XV). If methylation would take place at a nitrogen atom in position 7 or 9, a small bathochromic shift would be expected.[3]

Unequivocal proof for the structure of XV can be derived from the fact, that decomposition with hot alkali produces methylmercaptan.

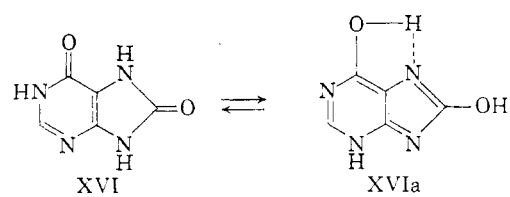
R_f values. In Table II, the R_f values of 3-methylpurines are compared with those of their nonmethylated mother substances. Fixation of a double bond at 1,2 produces R_f values, considerably smaller than for the unmethylated compounds possessing a normal double bond distribution. This is in sharp contrast to previous experiences showing methylation always to *increase* the R_f.[3,12] The abnormal cases, registered in Table II, are also characterised by a much increased water solubility, a property that makes isolation of these purines quite difficult. The unusual chromatographic behavior of these derivatives with a fixed 1,2-double bond is so characteristic as to serve as a diagnostic criterion for structural assignment.

TABLE II

COMPARISON OF R_f VALUES OF VARIOUS PURINES AND THEIR 3-METHYL DERIVATIVES

Substance	R_f A[a]	R_f B[b]	Fluorescence[c]
2-Hydroxypurine	0.25	0.22	Sky blue
3-Methyl (III)	0.48	0.39	Sky blue
6-Hydroxypurine	0.47	0.40	Dark blue
3-Methyl (VIII)	0.35	0.33	Dark blue
8-Hydroxypurine	0.55	0.63	Dark blue
3-Methyl (XII)	0.42	0.41	Dark blue
Xanthine	0.27	0.31	Dark blue
3-Methyl	0.42	0.49	Dark blue
2,8-Dihydroxypurine	0.22	0.20	Blue
3-Methyl (V)	0.39	0.42	Blue
6,8-Dihydroxypurine	0.30	0.34	Dark blue
3-Methyl (X)	0.20	0.06	Dark blue
6-Thiopurine	0.52	0.60	Blue-yellowish
3-Methyl (XIV)	0.40	0.43	Yellow
6-Methylthiopurine	0.80[d]		Green-yellow
3-Methyl (XV)	0.71[d]		Green-yellow

[a] 95% ethanol, 85 vol.; glacial acetic acid, 5 vol.; water, 10 vol. [b] 95% ethanol, 70 vol.; pyridine, 20 vol.; water, 10 vol. [c] Observed under a Mineralight UV lamp, emitting radiation of about 255 mμ. [d] These R_f values were determined in the following solvent: Isopropyl alcohol, 65 vol.; dimethylformamide, 25 vol.; 25% ammonia, 10 vol.



XVI XVIa

(12) S. Dikstein, F. Bergmann, and M. Chaimovitz. *J. Biol. Chem.*, **221**, 239 (1956).

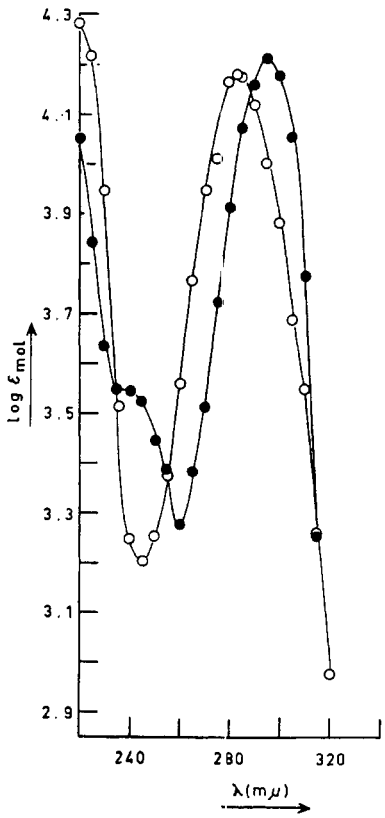


Fig. 1. Ultraviolet absorption spectra at *p*H 8.0 of 3,6-dihydro-3-methyl-6-oxo-8-hydroxypurine (X) (○—○) and 3-methyluric acid (●—●)

Enzymatic oxidations. Some of the new 3-methylpurines, described in the present paper, were tested as substrates of milk xanthine oxidase (XO). In a recent article,[3] it was assumed that conversion of 6,8-dihydroxypurine (XVI) to uric acid involves an "active" form XVIa, because of the inertness of the 1- and 9-methyl derivatives toward enzymatic oxidation. In X, a methyl group is as close to the point of attack (C-2) as in the 1-methyl derivative of XVI, but a double bond is now fixed in position 1,2. The reaction of X with XO is extremely slow, *i.e.* less than $\frac{1}{2000}$ of the rate for xanthine. Therefore, even after six days' incubation with the highest enzyme concentration feasible, the oxidation of X is still incomplete. However, the spectral changes during the reaction reveal a slow shift of λ_{max} towards higher wave lengths, as required by the curves, represented in Fig. 1. Unequivocal proof for the formation of 3-methyluric acid was obtained by paper chromatography (see Table III). The oxidation product of X was further characterised by the shift of λ_{max} as function of *p*H.

This result is in agreement with the observation that 3-methylhypoxanthine is oxidized slowly to 3-methylxanthine, whereas the 1-methyl derivative is refractory.[3]

3-Methyl-8-hydroxypurine (XII) is resistant to enzymatic attack, although it possesses a fixed double bond at 1,2. This shows that additional structural requirements are necessary for the attack at C-2, a problem that will be discussed elsewhere. However, it should be borne in mind that 3-methylhypoxanthine is oxidized at about $\frac{1}{500}$ the rate of hypoxanthine. If a similar reduction should apply to XII, then—in view of the already quite low rate of oxidation of 8-hydroxypurine[3]—the reaction of its 3-methyl derivative with XO might be immeasurably slow.

TABLE III

COMPARISON OF THE PROPERTIES OF 8-HYDROXY-3-METHYL-6-PURINONE (X) AND ITS OXIDATION PRODUCT, 3-METHYLURIC ACID

Compound	R_f[a]	λ_{max}(mμ) at *p*H 2.3	8.0	9.6	Fluorescence[b]
X	0.3	275	284	284	Dark blue
3-Methyluric acid	0.4	288	295	295[c]	Violet

[a] Determined in solvent A of Table II. [b] See footnote *c* in Table II. [c] See F. Bergmann and S. Dikstein, *J. Am. Chem. Soc.*, **77**, 691 (1955).

EXPERIMENTAL

2-Hydroxy-3-methylpurine-6-thione (II).[11] 3-Methylxanthine (I)[13] (3 g.) and phosphorus pentasulfide (15 g.) were refluxed in pyridine (150 ml.) for 2 hr. After removal of the solvent *in vacuo*, the excess sulfide was decomposed by heating with water for 15 min. Then sufficient ammonia was added to bring the *p*H to 9 and the mixture was cooled. After 0.5 hr., the precipitate of ammonium phosphate was filtered off, and the filtrate concentrated *in vacuo* to 50 ml. Acidification to *p*H 5.5 precipitated a brown material, which was dissolved in 5% sodium hydroxide. This solution was treated with charcoal and acidified with acetic acid. Finally, the thio derivative II was recrystallized from water as yellowish needles of dec. p. >300°; yield 2.2 g. (67%).

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.3; N, 30.8; S, 17.6. Found: C, 39.8; H, 3.3; N, 30.5; S, 17.5.

3-Methyl-2-purinone (III). A solution of II (1.2 g.) in 1*N* sodium hydroxide (25 ml.) was refluxed in the presence of Raney nickel (4 g.) for 2 hr. The catalyst was filtered off, the filtrate acidified with glacial acetic acid and treated with charcoal. The solution was then concentrated *in vacuo* to a small volume (2–3 ml.) and left for 2 days at 0°. The white crystals which had appeared (170 mg.), were recrystallized from ethanol as colorless needles of dec. p. 297–300°; yield 120 mg. (12%).

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.3. Found: C, 48.3; H, 3.9; N, 37.1.

The yield can be doubled by altering the isolation procedure as follows: The reaction mixture, freed of the catalyst, was brought to dryness *in vacuo* and the residue dissolved in ethanol, containing 5% sulfuric acid. Water was added dropwise, until a clear solution resulted. The latter was decolorized with charcoal and left at 0°. In this way, the neutral sulfate of III crystallized in large, colorless plates. The product showed an ultraviolet spectrum and R_f values, identical with those of the free base, described above; yield 23%.

Anal. Calcd. for $C_6H_6N_4O \cdot {}^1/_2H_2SO_4$[4]: C, 36.2; H, 3.5; N, 28.1; S, 8.0. Found: C, 36.0; H, 3.8; N, 27.9; S, 8.4.

(13) H. Bredereck, H. Schuh, and A. Martini, *Chem. Ber.*, **83**, 201 (1950).

8-Hydroxy-3-methyl-2-purinone (V). A mixture of 3-methyl-6-thiouric acid (IV)[9] (0.2 g.) and Raney nickel (0.8 g.) in 5% ammonia (20 ml.) was refluxed for 70 min. The filtrate was acidified and cooled to deposit 90 mg. (55%) of V, dec. p. >300°. The product crystallized from water in flat rods and proved identical with a sample, provided by Dr. D. J. Brown, National Australian University, Canberra, Australia.[8]

6-Hydroxy-3-methylpurine-2-thione (VII).[11] The diamine VI (3.3 g.) and formamide (12 ml.) were heated for 1.5 hr. to 180–190°. Initially, a clear solution was obtained. Then the purine, formed by cyclization, crystallized progressively during the reaction. After cooling, 3.2 g. (91%) of a yellowish precipitate were deposited, which were removed by filtration. The product (VII) crystallized from water in pointed, elongated prisms of dec. p. >300°.

Anal. Calcd. for $C_6H_6N_4OS$: N, 30.8. Found: N, 31.15.

3-Methylhypoxanthine (VIII). To a solution of 6-hydroxy-3-methylpurine-2-thione (VII) (3.0 g.) in 5% ammonia (70 ml.), which was stirred and heated to 90°, Raney nickel (9 g.) was added. After refluxing the mixture for 2 hr., the catalyst was filtered off and the filtrate cooled overnight. The first crop of VIII (1.5 g.) crystallized directly. A second crop (0.4 g.) was isolated after concentrating the mother liquor; total yield, 77%. Recrystallization from 50% ethanol gave colorless needles of dec. p. >300°. The product proved identical with an authentic sample, kindly supplied by Dr. G. B. Elion of the Wellcome Research Laboratories, Tuckahoe, N. Y. Analyses of VIII after drying at 110°, indicated the presence of one-third molecule of water.

Anal. Calcd. for $C_6H_6N_4O \cdot {}^1/_3\ H_2O$: C, 46.15; H, 4.3; N, 35.9. Found: C, 46.0; H, 4.4; N, 35.8.

6,8-Dihydroxy-3-methylpurine-2-thione (IX). An intimate mixture of the diamine VI[9] (1 g.) with an equal weight of urea was heated to 195° for 20 min. The solid cake was dissolved in 5% sodium hydroxide and the solution treated with charcoal. Acidification with 20% sulfuric acid precipitated the yellow product, which was purified by repeated reprecipitations, dec. p. >300°; yield, 90%.

Anal. Calcd. for $C_6H_6N_4O_2S$: C, 36.3; H, 3.0. Found: C, 36.2; H, 3.0.

8-Hydroxy-3-methyl-6-purinone (X). The foregoing compound (IX) (0.5 g.) in 1*N* sodium hydroxide (10 ml.) was desulfurated by refluxing with Raney nickel (1.5 g.) for 1.5 hr. The filtrate, when acidified with 20% sulfuric acid, gave 0.3 g. of a white precipitate. The product (X) crystallized from water in colorless plates, dec. p. >300°; yield 18%.

Anal. Calcd. for $C_6H_6N_4O_2 \cdot {}^1/_2H_2O$: C, 41.1; H, 4.0; N, 32.0. Found: C, 40.9; H, 4.3; N, 32.3.

3-Methyl-8-hydroxypurine (XII). 2,6-Dimercapto-3-methyl-8-purinol (XI)[9] (1.0 g.) was dissolved in 2.5% sodium hydroxide (10 ml.) and refluxed in the presence of Raney nickel (2 g.) under continuous stirring. After 45 min., 2 g. of fresh catalyst was added and the refluxing continued for 70 more min. The filtrate was adjusted to *p*H 7.5 by addition of glacial acetic acid and evaporated to dryness. From the residue, the sodium acetate was extracted with cold ethanol. The insoluble portion was then recrystallized from hot 90% ethanol to give colorless, polyhedric prisms, which start to sublime at about 250° and melt above 300°; yield, 250 mg. (36%).

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.3. Found: C, 47.9; H, 4.0; N, 37.8.

Attempted synthesis of 3-methylpurine. 1. *2-Mercapto-3-methylpurine-6-thione* (XIII). This compound was obtained by thiation of IX (0.8 g.) with phosphorus pentasulfide (2.5 g.) in pyridine (45 ml.). The mixture was treated as described for II. The crude product was dissolved in 5% sodium hydroxide and, after treatment with charcoal, reprecipitated by addition of glacial acetic acid. An analytically pure sample was obtained from a mixture of dimethylformamide and water as yellowish elongated prisms; yield, 68%; dec. p. > 300°.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.0. Found: C, 36.55; H, 3.4.

Desulfuration of this product with Raney nickel under a variety of conditions led to intractable tars, which still contained small maounts of starting material.

2. *3-Methylpurine-6-thione* (XIV).[11] A mixture of the hypoxanthine derivative (VIII) (1.1 g.) and phosphorus pentasulfide (5 g.) in pyridine (60 ml.) was refluxed for 4 hr. under continuous stirring. After removal of the solvent and decomposition with hot water, 0.8 g. of a yellow powder were deposited. The product crystallized from water in yellowish, pointed prisms of dec. p. >300°; yield 0.8 g. (65%).

Anal. Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.65; N, 33.8. Found: C, 43.3; H, 3.7; N, 34.05.

3. *3-Methyl-6-methylthiopurine* (XV). The foregoing compound (XIV) (0.4 g.) was dissolved in 2.5% sodium hydroxide (5 ml.) and stirred at room temperature with methyl iodide (0.3 ml.). After a few minutes, a white precipitate started to separate. After 2 hr., the oil had disappeared. Filtration gave 0.4 g. (95%) of colorless needles. Purification from water yielded colorless prisms of m.p. 166°.

Anal. Calcd. for $C_7H_8N_4S \cdot 2H_2O$: C, 38.9; H, 5.6; N, 25.9. Found: C, 38.6; H, 5.3; N, 26.5.

Neither of the last two compounds could be desulfurated successfully to the desired 3-methylpurine.

Enzyme experiments. Highly purified xanthine oxidase from cow's milk was a gift of Prof. F. Bergel and Dr. R. C. Bray, Chester Beatty Institute of Cancer Research, London, England. When diluted 1:4800, the enzyme produced at *p*H 8.0 and 28° 1γ of uric acid/ml./min. with $6.5 \times 10^{-5}M$ xanthine as substrate. Catalase (Worthington) was added in all experiments at a final dilution of 1:500.

Incubation with XO was carried out at *p*H 8.0 (0.01*M* phosphate buffer), with a substrate concentration of $6.5 \times 10^{-5}M$. The progress of the reaction was measured spectrophotometrically against a blank, containing all components besides XO.

Paper chromatography was carried out on Whatman paper No. 1, using the descending method.

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