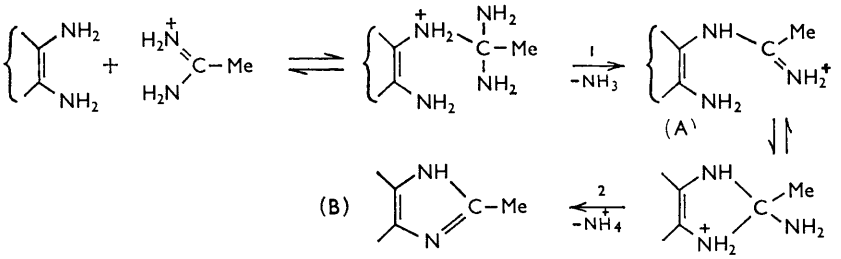
880. *The Synthesis of 8-Substituted Purines.*

By F. Bergmann and M. Tamari.

4,5-Diaminopyrimidines condense with amidine salts, giving 8-substituted purines. High yields are obtained with 4,5-diaminouracil and its mono- and di-thio-derivatives, but they are lower with 6-substituted diaminopyrimidines. 2-Hydroxy-8-methylpurine is unstable in dilute sulphuric acid, and 3,8-dimethyl-6-thioxanthine is unstable under all conditions, so that it could not be isolated.

Introduction of a methyl group into position 8 of the purine ring has been effected previously by condensation of the appropriate 4,5-diaminopyrimidines with acetic anhydride [1,2] or with a mixture of this reagent and ethyl orthoacetate.[3] A similar principle underlies the method of Richter, Loeffler, and Taylor, who condensed aminomalonamidamidine with orthoacetate.[4] These procedures require drastic conditions and the yields are often unsatisfactory.

An alternative method, in which ammonia is eliminated instead of water or alcohol, has proved in our hands to be superior in most cases. Condensation of an acetamidine salt with an appropriate derivative of 4,5-diaminopyrimidine in the absence of a solvent leads directly and in high yields to the desired 8-methylpurines. The conditions used are analogous to those applied by Galat and Elion to the amidation of esters by molten ammonium salts.[5]



A number of 8-methyl derivatives, obtained in this way, are listed in Table 1. Likewise, benzamidine gave 8-phenylpurines, which indicates that the method may be generally applicable.

The detailed mechanism of the condensation may be formulated as a series of acid-catalysed transamidations, in analogy with the mechanism of acid hydrolysis of amides [6] or of the conversion of orthoformate into formamidines.[7] The reaction illustrated takes place only between an amidine salt and a free diaminopyrimidine, but fails with the sulphates of the latter. This observation indicates that in the intermediate phases of the reaction the proton of the amidinium ion is redistributed between the various basic groups present, in order to make possible the stepwise elimination of two molecules of ammonia. In the first step, ammonia is liberated, while the proton remains on the intermediate amidine (A), which is the stronger base. In the second step, however, ammonium ion is removed, because now ammonia (pK 9·2) surpasses the basicity (pK ~2) of the imidazole ring [8,9] in (B).

[1] Isay, *Ber.*, 1906, **39**, 250.
[2] Johns, *J. Biol. Chem.*, 1912, **67**, 11.
[3] Koppel and Robins, *J. Org. Chem.*, 1958, **23**, 1457.
[4] Richter, Loeffler, and Taylor, *J. Amer. Chem. Soc.*, 1960, **82**, 3144.
[5] Galat and Elion, *J. Amer. Chem. Soc.*, 1943, **65**, 1566.
[6] Ingold, "Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, Ithaca, New York, 1953, p. 786.
[7] Roberts, *J. Amer. Chem. Soc.*, 1950, **72**, 3603.
[8] Albert and Brown, *J.*, 1954, 2060.
[9] Bergmann and Dikstein, *J. Amer. Chem. Soc.*, 1955, **77**, 691.

The best yields were obtained in the condensation of 4,5-diaminouracil and its thio-derivatives. If only a 6-hydroxyl group was present condensation with acetamidine or benzamidine hydrochloride gave inferior results, but these were somewhat improved by using the amidine acetates. The latter also reacted faster than the hydrochlorides, in

TABLE 1.

(*a*) 8-Substituted purines, obtained by condensation of amidines and 4,5-diaminopyrimidines.

No.	Substituent at position 2	4	8	Reaction time (min.)	Yield (%)	$\lambda_{max.}$ (mμ) at pH 8·0	R_F in solvent [a] A	B	C	Fluorescence
1 [c]	H	OH	Me	60	56 (67) [b]	252	0·57	0·70	—	Violet
2 [c]	OH	OH	Me	30	94	240, 275	0·54	0·60	0·42	,,
3	OH	SH	Me	35	83 [d]	251, 344	0·50	0·56	0·57	Blue
4 [e]	SH	OH	Me	30	77	235, 280	0·45	0·74	0·61	Violet
5 [e]	SH	SH	Me	25	65 (86)	247, 285, 351	0·53	0·59	0·71	Blue
6	SH	NH_2	Me	30	66	230, 251, 280	0·61	0·67	—	Blue
7	H	OH	Ph	70	50 (78)	291	0·58	0·79	—	Blue
8 [e]	OH	OH	Ph	40	80	228, 309	0·52	0·66	—	Blue

No.	Found (%) C	H	N	Formula	Required (%) C	H	N
3	39·3	3·3	30·5	$C_6H_6N_4OS$	39·6	3·3	30·8
6	29·2	3·8	27·8	$C_6H_7N_5S,\frac{1}{2}H_2SO_4,H_2O$	29·0	4·0	28·2
7	62·4	3·6	26·4	$C_{11}H_8N_4O$	62·3	3·8	26·4

[a] Solvents: A, 95% EtOH–H_2O–AcOH 85 : 10 : 5 (v/v); B, 95% EtOH–pyridine–H_2O 70 : 20 : 10 (v/v); C, propan-2-ol–dimethylformamide–10% NH_3 65 : 25 : 10 (v/v). [b] Figures in parentheses are the yields obtained with acetamidine acetate. [c] Koppel and Robins, ref. 3. [d] This compound was also prepared by reaction of 8-methylxanthine with phosphorus pentasulphide in 90% yield. [e] Cook and Thomas (*J.*, 1950, 1884) obtained this purine in 20% yield by a different method. [f] Nos. 1—6 needles, 7 and 8 rods; nos. 1—5 from H_2O; no. 6 from 5% H_2SO_4; no. 7 reprecipitated; no. 8 from dil. NH_3.

(*b*) 3,8-Dimethylpurines obtained by direct condensation.

No.	2-Subst.	Yield (%)	$\lambda_{max.}$ (mμ) at pH 8·0	R_F in solvent A	B	C	Fluorescence
9	O	92	275	0·64	0·79	0·68	Blue
10	S	88	233, 288	0·60	0·84	—	Violet

No.	Form and solvent	Found (%) C	H	N	Formula	Required (%) C	H	N
9	Needles H_2O	46·2	4·5	30·6	$C_7H_8N_4O_2$	46·7	4·4	31·1
10	Prisms ,,	43·0	3·6	28·7	$C_7H_8N_4OS$	42·9	4·1	28·6

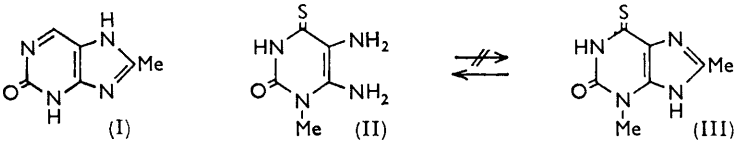
contradiction to the relative strengths of acetic and hydrochloric acid in water. A similar reversed order of catalytic activity has been reported for amidations of esters in liquid ammonia;[10,11] but the present experiments have not been extended to embrace the whole series of acids studied by previous authors.

Condensation failed with 4,5-diamino-6-mercaptopyrimidine. Therefore, 6-mercapto-8-methylpurine was prepared by thiation of the corresponding hypoxanthine (no. 1 in Table 1).

[10] Fellinger and Audrieth, *J. Amer. Chem. Soc.*, 1938, **60**, 579.
[11] Shatenshtein, *J. Amer. Chem. Soc.*, 1937, **59**, 432.

The reaction with 4,5-diamino-2-hydroxypyrimidine was attempted under a variety of conditions. The best results were achieved by condensation with acetamidine acetate at 140—145°. Even then, as shown by paper chromatography, a mixture of compound (I) and the original pyrimidine was obtained, from which pure 2-hydroxy-8-methylpurine could not be isolated. Therefore, we have used an alternative route, namely, desulphuration of the corresponding 6-thio-derivative (no. 3 in Table 1) in ammoniacal solution. Compound (I) cannot be isolated as its sulphate. Even 1% sulphuric acid in absolute ethanol at 0° opened the imidazole ring. Clearly this compound is considerably more sensitive to acids than its parent 2-hydroxypurine: >90% of the latter is decomposed in N-sulphuric acid at 100° in 1 hr.[8] It therefore appears remarkable that Johns [2] isolated compound (I) as its nitrate from a 30% solution of nitric acid: in our hands, such concentrated acid caused ring opening, whereas dilute nitric acid (pH 2) produced the crystalline nitrate after 2 months' storage at room temperature.

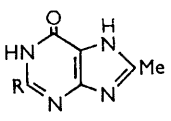
An even more surprising observation is that in an attempted synthesis of 1,2-dihydro-3,8-dimethyl-2-oxopurine the desired intermediate (III) proved unstable. The compound (III) could not be prepared either by condensing 4,5-diamino-3-methyl-6-thiouracil (II) with various salts of acetamidine or by selective thiation of 3,8-dimethylxanthine (no. 9 in Table 1) at carbon atom 6. In the latter procedure, the reaction mixture was decomposed with cold dilute ammonia and the filtrate brought to pH 6 by addition of acetic acid. Only the original thiouracil (II) was obtained crystalline even by this cautious method. It is thus evident that the reaction had proceeded as desired, but that the primary product (III) is unstable and decomposes during either the reaction or the attempted isolation. Similar ring opening occurred in attempts to thiate 3-methyl-8-phenylxanthine. The xanthines (nos. 2 and 8) themselves are, however, perfectly stable under conditions which lead to degradation of 2-hydroxypurine.[8]

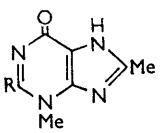


Physical Properties.—The 8-methyl group exerts a small bathochromic effect on the absorption maximum. Such slight shifts are well known for methyl substitution in aromatic rings. Apparently the purine skeleton behaves like an aromatic system in transmitting inductive effects, although *N*-methylation may produce either batho- or hypso-chromic changes of $\lambda_{max.}$.[9]

It is noteworthy that 3-methylpurines, in which alkylation has caused fixation of a double bond in position 1,2, exhibit a large bathochromic shift (14—15 mμ) owing to transition from the *ortho*-quinonoid system of hypoxanthine to a *para*-quinonoid structure.[12] This shift is not influenced by a univalent substituent in position 2 (cf. IV—V, where $\lambda_{max.}$ at pH 8 are indicated).

(IV)		$\lambda_{max.}$
a:	R = H	252
b:	R = SMe	265





(V)		$\lambda_{max.}$
a:	R = H	266
b:	R = SMe	280

Table 1 (nos. 2—5) illustrates how in some solvents replacement of a hydroxyl by a mercapto-group decreases and in others increases the R_F value. Comparison of R_F values is possible only if corresponding molecular structures are being considered. *E.g.*, 8-methylxanthine (no. 2) has p*K* 7·5. In solvent C, it is certainly present as monoanion, while in A it forms neutral molecules. On the other hand, 8-methyl-6-thioxanthine (no. 3), with

[12] Bergmann, Levin, Kalmus, and Kwietny-Govrin, *J. Org. Chem.*, 1961, **26**, 1504.

pK ~12·5, exists mainly as neutral molecules even in solvent C. The 2-thio-analogue has pK ~11·5 and the corresponding 2,6-dithio-derivative pK 10·7. A reversal in the order of R_F values within this group of 8-methylpurines, when passing from acid to basic solvents, may therefore be attributed to the different distribution of ionised forms.

Experimental

Ultraviolet spectra were measured in 0·1M-phosphate buffer of pH 8·0, on a Beckman DU spectrophotometer. Paper chromatograms were developed by the descending method, with the solvents specified in Table 1. Spots were located by their fluorescence under a Mineralight ultraviolet lamp (λ ~255 mμ). pK Values were determined by the spectrophotometric method, as modified by Bergmann and Dikstein.[9]

General Condensation Procedure.—A mixture of a 4,5-diaminopyrimidine and 2 equivalents of acetamidine hydrochloride was heated to 180—190°. At this temperature, a homogeneous melt was usually formed and evolution of ammonia started. After reaction had ceased, the dark cake was dissolved in N-sodium hydroxide, the solution decolorised with charcoal, and the product precipitated by acetic acid (to pH ~6). All purines synthesised in the present investigation decomposed above 310°, unless stated otherwise.

The yields of 8-methyl- (no. 1) and 8-phenyl-hypoxanthine (no. 3) and of 2,6-dithio-8-methylxanthine (no. 5) were considerably improved by addition of 2 equivalents of anhydrous sodium acetate to the reaction mixture (see Table 1).

2-*Hydroxy*-8-*methylpurine* (cf. I).—(*a*) 4,5-Diamino-2-hydroxypyrimidine [13] (1 g.), acetamidine hydrochloride (1 g.), and anhydrous sodium acetate (0·8 g.) were heated at 140—145° for 20 min. Thereafter the mass solidified. The cake was dissolved in 10% aqueous ammonia and boiled with charcoal. The filtrate was acidified with acetic acid and left in the cold room for 24 hr., whereupon 0·65 g. of crystals was deposited, exhibiting $\lambda_{max.}$ 307 mμ at pH 8·0. Chromatography in solvent A gave 2 spots: (1) R_F 0·32: an extract of this spot had $\lambda_{max.}$ 286—287 mμ (pH 8·0), characteristic of the starting material. (2) R_F 0·43: this material had $\lambda_{max.}$ 313 mμ, corresponding to compound (I). The components were not separated by crystallisation.

(*b*) A suspension of 8-methyl-6-thioxanthine (5 g.) and Raney nickel (1·5 g.; wet weight) in 5% aqueous ammonia (25 ml.) was refluxed for 80 min. The catalyst was filtered off and the solution brought to pH 2 by addition of nitric acid. After 2 months at room temperature, large prisms with a brown tint had crystallised. The substance was identified unequivocally by its absorption spectrum, which is very similar to that of 2-hydroxypurine, and by its conversion into 8-methylxanthine under the influence of mammalian xanthine oxidase.[14] If the solution was acidified with sulphuric acid, the product decomposed quantitatively to the starting material. The result was the same when an ammoniacal solution of compound (I) was evaporated to dryness, the residue extracted with absolute alcohol, and the mixture acidified with 1% of sulphuric acid in absolute alcohol.

8-*Methyladenine.*[3]—2-Mercapto-8-methyladenine (no. 6) (580 mg.) and Raney nickel (1·5 g.; wet weight) in 5% aqueous ammonia (100 ml.) were refluxed for 2 hr. The solution was filtered while hot. On cooling, prisms (300 mg., 62%) were deposited. The physical properties were identical with those reported by Koppel and Robins.[3] The substance had R_F 0·57, 0·67, and 0·64 in solvents A, B, and C, respectively.

6-*Mercapto*-8-*methylpurine.*—8-Methylhypoxanthine (1·3 g.), phosphorus pentasulphide (5 g.), and dry pyridine (50 ml.) were refluxed for 4 hr. The solvent was removed *in vacuo* and the residue extracted with N-sodium hydroxide. After filtration, the solution was brought to pH 6 by glacial acetic acid, boiled with charcoal, again filtered, concentrated *in vacuo*, and left at 0° overnight. The precipitate (1·1 g., 76%) was removed and recrystallised from water as needles, decomp. >310°. This product has been obtained previously by Koppel and Robins [3] by a more devious route; it had $\lambda_{max.}$ (pH 8·0) 232, and 316 mμ and R_F 0·64 in solvent A and 0·71 in solvent C (reddish fluorescence).

3,8-*Dimethyl*-2-*methylmercaptohypoxanthine.*—3,8-Dimethyl-2-thioxanthine (1 g.) and methyl iodide (0·7 ml.) were stirred in 0·5N-sodium hydroxide (10 ml.) at room temperature for 30 min., precipitation beginning in 10 min. The *compound* crystallised from water in yellowish lancets,

[13] Kalmus and Bergmann, *J.*, 1961, 760.
[14] Unpublished results.

decomp. 312—315° (0·95 g., 88%), R_F 0·73 in solvent B (Found: C, 45·7; H, 4·6; N, 27·1. $C_8H_{10}N_4OS$ requires C, 45·7; H, 4·8; N, 26·7%).

3,8-*Dimethylhypoxanthine*.—3,8-Dimethyl-2-thioxanthine (2 g.) and Raney nickel (6 g.; wet weight) in 5% ammonia (50 ml.) were refluxed for 2 hr. The filtrate was left at 0° overnight without depositing crystals. After concentration *in vacuo*, the *hypoxanthine* (1·4 g., 83%) was obtained; it crystallised from ethanol as prisms, decomp. ~300°, R_F 0·6 in solvent B (Found: C, 51·1; H, 5·2; N, 33·8. $C_7H_8N_4O$ requires C, 51·2; H, 4·9; N, 34·1%).

Attempted Synthesis of 3,8-*Dimethyl*-6-*thioxanthine*.—(a) 4,5-Diamino-3-methyl-6-thiouracil (II) and acetamidine hydrochloride or acetate at temperatures between 150° and 200° yielded only starting material and tars. (b) Reaction of 3,8-dimethylxanthine with phosphorus pentasulphide in pyridine, removal of the solvent *in vacuo*, decomposition with cold aqueous ammonia, and adjustment of the pH to 6 produced only the diamine (II). The latter was identified with authentic material by its spectrum ($\lambda_{max.}$ at pH 8·0, 249 and 344 mμ) and its R_F value (0·33 in solvent A).

Conversion of 8-*Methyl*-2,6-*dithioxanthine into* 8-*Methylpurine*.—8-Methyl-2,6-dithioxanthine (1 g.) and Raney nickel (2·5 g.) in 5% aqueous ammonia (50 ml.) were refluxed for 70 min. The catalyst was removed and the filtrate concentrated *in vacuo*. On being left overnight, the solution deposited needles (150 mg., 22%). 8-Methylpurine has $\lambda_{max.}$ at pH 8·0, 266 mμ, R_F 0·75 in solvent A (violet fluorescence). The yield by this method is only about half that reported by Albert and Brown,[8] but the product is at once in pure form.

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