Synthesis of 3-Methylisoguanine [6-Amino-3-methylpurin-2(3*H*)-one]

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The first synthesis of 3-methylisoguanine is reported. Methylation of 4,6-diamino-2-hydroxypyrimidine (I) at pH 11·6 gave the 1-methyl derivative (II), which on nitrosation and reduction yielded 4,5,6-triamino-1-methylpyrimidin-2(1*H*)-one (IV). Cyclisation either *via* the 5-formamido-derivative (VII) or directly by use of diethoxymethyl acetate [or, preferably, formic acid–formamide (77% yield)] gave 3-methylisoguanine (V) exclusively. Its structure was established by deamination to 3-methylxanthine, no 1-methylxanthine being obtained.

Isoguanine occurs in nature as the 9-β-D-ribofuranosyl derivative (crotonoside), which has been isolated from the Croton bean.[1] The u.v. spectrum of this material was identical with that of 9-methylisoguanine prepared from 2,6-dichloro-9-methylpurine.[2] 7-Methylisoguanine could not be obtained from 2,6-dichloro-7-methylpurine by an analogous synthesis [3] since rearrangement occurred during the treatment of 6-amino-2-chloro-7-methylpurine with sodium ethoxide, and 7-methylguanine was isolated as the major product. 7-Methylisoguanine has been synthesised in low yield by fusing 4-amino-5-cyano-1-methylimidazole hydrochloride with urea.[4] 1- and 3-Methylisoguanine do not appear to be known, and direct methylation of isoguanine with dimethyl sulphate in dimethylacetamide [5] gives the 3,9-dimethyl derivative as the sole product.

In connection with studies on xanthine and isoguanine nucleosides we required a sample of 3-methylisoguanine. The two obvious synthetic approaches are: (i) direct methylation of isoguanine and (ii) methylation of a suitable pyrimidine precursor followed by cyclisation to the corresponding isoguanine. Since it seemed

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[2] J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 1938, 692.

[3] E. Fischer, *Ber.*, 1897, **30**, 2400.

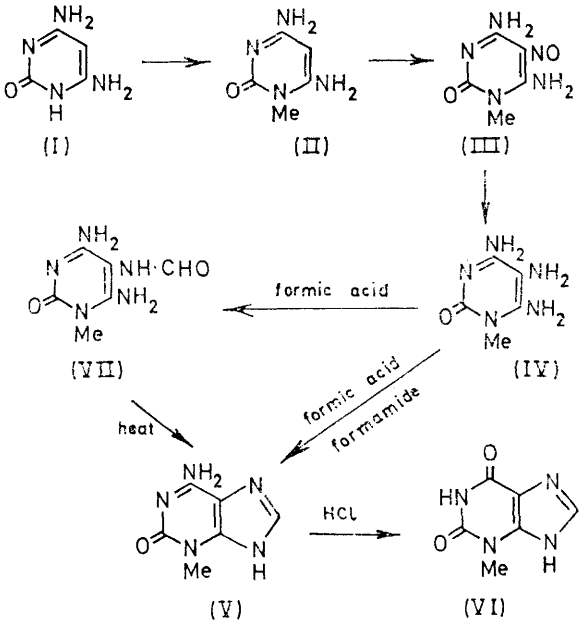
[4] E. Shaw, *J. Org. Chem.*, 1962, **27**, 883.

[5] T. Okano, S. Goya, and T. Kaizu, *J. Pharm. Soc. Japan*, 1967, **87**, 569.

unlikely that direct monomethylation of isoguanine at N-3 could be achieved, the latter approach was investigated.

We decided to use the general method developed by Bendich and his co-workers [6] for the preparation of isotopically labelled isoguanine, despite the fact that cyclisation of the appropriate *N*-methylpyrimidine could theoretically give rise to either 1- or 3-methylisoguanine. However, as the amino-group adjacent to the *N*-methyl group should be more basic than the other one we expected the 3-methyl isomer to be the major product.

Malononitrile was condensed with thiourea by the method of Traube [7] to give 4,6-diamino-2-mercaptopyrimidine, which was converted into the corresponding 2-hydroxypyrimidine (I) by treatment with chloroacetic acid.[6] To obtain 4,6-diamino-1-methylpyrimidin-2-one (II) the 2-hydroxypyrimidine (I) was subjected to



various methylation procedures. Treatment of a solution of compound (I) in 2N-potassium hydroxide solution with dimethyl sulphate at room temperature or at 55° was unsuccessful, and starting material was recovered. However, if the pH of the pyrimidine solution was maintained at 11·6 (pK_a 11·45) during the methylation by the addition of sodium hydroxide solution, methylation proceeded at the 1-position. By conducting the methylation at 70° in a buffer solution [8] at pH 11·6 an acceptable yield (62%) of the 1-methylpyrimidine (II) was obtained. The product precipitated at pH 7·0 from the mixture after removal of unchanged 2-hydroxy-4,6-diaminopyrimidine, and was sufficiently pure for conversion into the 5-nitroso-derivative (III). This was accomplished with nitrous acid, and the 5-nitroso-compound (III) was reduced to the triaminopyrimidine (IV) with sodium dithionite.

Several methods are available for cyclising 4,5-diaminopyrimidines to the corresponding purine derivatives.[9,10] The successful use of formic acid in the presence of sodium formate for the cyclisation of various 4,5,6-triaminopyrimidines was found to depend on the nature of the C-2 group.[6] Thus 4,5,6-triamino-2-mercaptopyrimidine gave the 5-formamido-derivative whereas the corresponding 2-hydroxy-compound did not react. Bendich and his co-workers therefore devised a method which appeared to have general applicability. This involved heating the triaminopyrimidine with formic acid in anhydrous formamide at 160° in a sealed tube.

When the triaminopyrimidine (IV) was treated with 95% formic acid at 100° the 5-formamido-compound (VII) was obtained in 51% yield. The presence of a methyl group in the 1-position therefore enhances the reactivity of the 5-amino-group. When heated under reduced pressure the 5-formamido-derivative was converted into 3-methylisoguanine (V). In view of the reactivity of the 5-amino-group in compound (IV), cyclisation was attempted with a mixture of acetic anhydride and triethyl orthoformate [11] (no purine could be isolated in this case) and then with diethoxymethyl acetate. The latter reagent [9] is often superior to ethyl orthoformate–acetic anhydride, as in the cyclisation of 2,4-diamino-5-benzylamino-6-hydroxypyrimidine to 7-benzylguanine.[12] Treatment of the triaminopyrimidine (IV) with diethoxymethyl acetate in dimethylformamide at 100—120° gave 3-methylisoguanine in 35% yield without isolation of the intermediate formamido-derivative (VII).

The best synthesis of 3-methylisoguanine was achieved by use of the general method of Bendich and his co-workers; it proved unnecessary to carry out the reaction in a sealed tube. When the triaminopyrimidine (IV) was heated at 180° with a solution of formic acid in anhydrous formamide for 3 h, 3-methylisoguanine (V) was obtained in 77% yield. T.l.c. of the reaction mixture after removal of formamide showed only traces of the formamido-compound (VII).

The structure of 3-methylisoguanine was established by deamination to 3-methylxanthine by the method of Spies.[13] The u.v. spectrum of the product was identical with that reported for 3-methylxanthine.[14] 3-Methylxanthine, which does not have a dissociable 2-hydroxy-group, shows only one band in the u.v. spectrum (at 275 nm in alkali). 1-Methylxanthine has a u.v. spectrum different from this and similar to that of

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[7] T. Traube, *Annalen*, 1904, **331**, 64.
[8] T. Teorell and E. Stenhagen, *Biochem. Z.*, 1939, **299**, 416.
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[10] J. A. Montgomery and C. Temple, jun., *J. Amer. Chem. Soc.*, 1958, **80**, 409.
[11] H. W. Post and E. R. Erickson, *J. Org. Chem.*, 1937, **2**, 260.
[12] G. T. Rogers and T. L. V. Ulbricht, 'Synthetic Procedures in Nucleic Acid Chemistry,' eds. W. W. Zorbach and R. S. Tipson, Interscience, New York, 1968, vol. 1, p. 15.
[13] J. R. Spies, *J. Amer. Chem. Soc.*, 1939, **61**, 350.
[14] W. Pfleiderer and G. Nübel, *Annalen*, 1961, **647**, 155.

xanthine, with peaks at 240 and 283 nm in alkali.[15] Chromatographic analysis of the deaminated cyclisation product did not indicate the presence of any 1-methylxanthine; the reaction appears to give 3-methylisoguanine exclusively.

EXPERIMENTAL

U.v. spectra were measured with a Hilger–Watts Ultrascan recording spectrophotometer for solutions in 95% ethanol. T.l.c. was carried out on silica gel (Merck GF 254).

4,6-Diamino-1-methylpyrimidin-2(1H)-one (II).—4,6-Diamino-2-hydroxypyrimidine (II) (4·0 g) (obtained from the sulphate by neutralising with sodium hydroxide solution) was dissolved in pH 11·6 buffer[8] (55 ml) at 60—70°. Dimethyl sulphate (5·7 g, 2 equiv.) was then added dropwise to the hot solution during 1 h. The heating was continued at 100° for a further 2 h. The pH was then adjusted to 10·2 with glacial acetic acid, and a white precipitate appeared. The suspension was stirred at this pH for 10 min and filtered to give starting material (750 mg). More acetic acid was then slowly added to the pale yellow filtrate until the pH reached 6·5; a further precipitate was then obtained. The suspension was concentrated to about 35 ml and left at 5°. Filtration gave the *1-methyl derivative* (II) (2·55 g, 55%). A further crop was obtained from the mother liquors (total yield 2·66 g, 62%). The product was crystallised from dilute sulphuric acid to give a *hemisulphate* which decomposed at 325° (Found: C, 31·65; H, 4·8; N, 29·35. $C_5H_8N_5O,0{\cdot}5H_2SO_4$ requires C, 31·75; H, 4·75; N, 29·65%), $\lambda_{max.}$ 276 (pH 1·0), 282 (pH 7·0), and 278 nm (pH 13·0).

4,5,6-Triamino-1-methylpyrimidin-2(1H)-one (IV).—The diamine (II) (1·4 g) was dissolved in glacial acetic acid (35 ml) containing water (20 ml). The solution, cooled to 5°, was then treated with sodium nitrite (1·35 g) in water (20 ml) and stirred for 2 h. The red precipitate was filtered off, washed with water, ethanol, and ether and dried to give 4,6-diamino-1-methyl-5-nitrosopyrimidin-2(1*H*)-one (III) (1 g, 80%). Without further purification this was suspended in water (40 ml) and sodium dithionite (2 g) was added. The mixture was boiled for 3 min and the red colour disappeared. Sulphuric acid (50%; 6 ml) was added and the mixture was filtered through charcoal. The filtrate was cooled and left at 5° to yield the crystalline *triamine* (IV) *sulphate* (0·960 g, 64%), m.p. >300°, a sample of which was recrystallised from 2N-sulphuric acid (Found: C, 23·45; H, 4·65; N, 27·65. $C_5H_9N_5O,H_2SO_4$ requires C, 23·7; H, 4·35; N, 27·65%), $\lambda_{max.}$ 280 (pH 1·0), 284 (pH 7·0), and 282 nm (pH 13·0).

6-Amino-3-methylpurin-2(3H)-one (V) (*3-Methylisoguanine*).—(*a*) The triaminopyrimidine (IV) (1 g.) was dissolved in 98% formic acid (30 ml) and heated on a steam-bath for 2·5 h. The solvent was removed at 50° under reduced pressure and the residue was treated with ether. A solution of the solid obtained in ethanol (25 ml) was treated with charcoal, filtered, and set aside to yield crystals (700 mg, 51%) of *4,6-diamino-5-formamido-1-methylpyrimidin-2(1H)-one* (VII), R_F (H_2O) 0·68, m.p. 235° (Found: C, 39·4; H, 4·9; N, 38·2. $C_6H_9N_5O_2$ requires C, 39·35; H, 4·95; N, 38·3%), $\lambda_{max.}$ 274 (pH 1·0 and 7·0) and 272 nm (pH 13·0).

This product (100 mg.) was finely powdered and heated at 190° under reduced pressure (0·01 mmHg) in an oil-bath for 3 h. The cooled solid was crystallised from water to give *3-methylisoguanine* (no m.p. <300°) (63 mg.), R_F (H_2O) 0·5 (Found: C, 43·5; H, 4·3; N, 42·35. $C_6H_7N_5O$ requires C, 43·65; H, 4·25; N, 42·4%), $\lambda_{max.}$ 285 (pH 1·0), 286 and 240 (pH 7·0), and 243 and 285 nm (pH 13·0).

(*b*) *Cyclisation with formic acid–formamide.* The triaminopyrimidine (IV) (1·5 g) was mixed with formic acid (95%; 5 ml) in anhydrous formamide (25 ml) and the solution was heated at 180° for 3 h. The solvents were removed under reduced pressure and the residue was mixed with hot water to give 3-methylisoguanine (V) (1·23 g, 77%), R_F (H_2O) 0·5. Crystallisation from aqueous alcohol gave 966 mg of product, identical with 3-methylisoguanine obtained from the formamido-compound (VII).

(*c*) *Cyclisation with diethoxymethyl acetate.* The triaminopyrimidine (IV) sulphate (300 mg.) was neutralised with sodium hydroxide solution and the product was evaporated to dryness. Diethoxymethyl acetate[11] (20 ml) and dimethyl formamide (3 ml) were added and the mixture was heated at 100° for 2 h. It was then evaporated and the residue was digested with 2M-sodium hydroxide solution (20 ml) at 90° for 20 min. The solution was neutralised with acetic acid and concentrated; 3-methylisoguanine (55 mg, 35%) was deposited, R_F (H_2O) 0·5.

Deamination of 3-Methylisoguanine (V).—3-Methylisoguanine (100 mg) was dissolved in 25% hydrochloric acid and heated under reflux for 3 h. The u.v. spectrum of the product was determined after adjusting the pH of the solution: $\lambda_{max.}$ 235sh and 270 (pH 1·0 and 6·0) and 274 nm (pH 13·0), identical to the reported data for 3-methylxanthine.[14]

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