151. *The Permanganate Oxidation of Uracil and Cytosine and their 1-Substituted Derivatives.*

By B. Chatamra and A. S. Jones.

Oxidation of uracil with potassium permanganate at pH 7 or 9 at 37° gave urea, oxalurate, oxalate, formate, carbon dioxide, and ammonia. 1-Substituted uracils when similarly oxidised at pH 9 gave *N*-substituted ureas, oxalate, and formate. Cytosine and 1-substituted cytosines gave urea or *N*-substituted urea, biuret or *N*-substituted biuret, oxalate, formate, and ammonia.

It has been established [1] that potassium permanganate at pH 9 converts thymine and 1-substituted thymines into urea or an *N*-substituted urea, acetol, pyruvate, and formate. That study has now been extended to two other pyrimidines found in nucleic acids, namely, uracil and cytosine. Earlier work has shown that uracil, when oxidised at pH 6 gives oxaluric acid [2,3] and that cytosine gives biuret,[3] but no oxidation was carried out at pH 9, and 1-substituted derivatives were not studied.

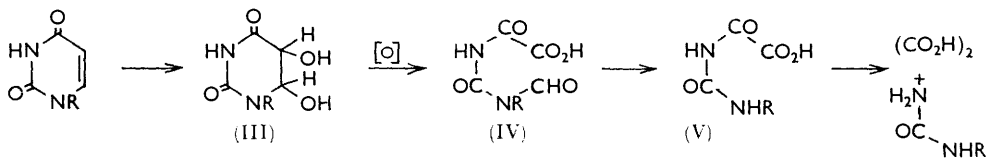
[1] Benn, Chatamra, and Jones, *J.*, 1960, 1014.
[2] Heinrich and Wilson, *J. Biol. Chem.*, 1950, **186**, 447.
[3] Bendich in "Nucleic Acids," Vol. I, by Chargaff and Davidson, Academic Press, Inc., New York, 1955, p. 120.

1-Methyl- and 1-phenyl-uracil (I; R = Me or Ph, respectively) were synthesised as described by Shaw and Warrener.[4] A similar procedure also afforded the hitherto unrecorded 1-benzyluracil (I; R = Ph·CH_2). 1-Methylcytosine (II; R = Me) had been

(I) (II)

synthesised by Johnson and Clapp [5] by methylation of cytosine. They concluded that it was the 1-methyl derivative because it did not give a red colour with *p*-diazobenzenesulphonic acid. This conclusion has now been confirmed by the unambiguous synthesis of the same material by Shaw and Warrener's procedure. 1-Benzylcytosine (II; R = Ph·CH_2) was also obtained by both methods. Deamination of 1-methyl- and 1-benzylcytosine gave 1-methyl- and 1-benzyl-uracil, respectively.

Oxidation of uracil (I; R = H) with potassium permanganate at 37° and pH 7 gave crystalline oxaluric acid (V; R = H) as one of the products; at pH 9, the products were urea, oxalate, and formate, and a little oxalurate and ammonia. Oxidation of 1-methyl-, 1-phenyl-, and 1-benzyl-uracil at pH 9 gave oxalate, formate, and an *N*-substituted urea (*N*-phenyl- and *N*-benzyl-urea were obtained crystalline).

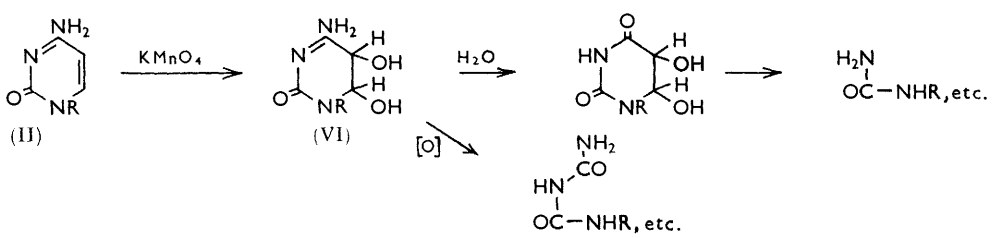


(III) (IV) (V)

The reaction probably proceeded through a glycol (III), as with thymine. This would then be further oxidised to an *N*-substituted *N*-formyl-*N'*-oxalourea (IV), which by hydrolysis would afford an oxaluric acid (V) and formic acid. The oxaluric acid upon further hydrolysis would give oxalic acid and a urea.

In the oxidation of uracil at pH 7 and 9 a small amount of ammonia was detected, and in the absence of bicarbonate buffer carbon dioxide was also found. The former probably arose by hydrolysis of urea and the latter by oxidation of formate and oxalate.

A similar oxidation of the cytosine derivatives gave ammonia, oxalate, formate, urea or *N*-substituted urea, and biuret or *N*-substituted biuret. The molecular proportion of urea derivative to biuret derivative in the products was 1 : 1·5 in the case of cytosine, and 1 : 1·2 in the case of 1-methylcytosine. The ammonia was evolved during the first few minutes of the reaction and therefore probably came from the 4-amino-group. The



(II) (VI)

urea derivatives were not produced by degradation of the biuret derivatives because it was found that biuret was not converted into urea by potassium permanganate in these conditions. It follows, therefore, that some oxidation product of the cytosine derivative

[4] Shaw and Warrener, *J.*, 1958, 157.
[5] Johnson and Clapp, *J. Biol. Chem.*, 1908, **5**, 62.

must have been degraded by alternative pathways. No intermediates have been detected, but it appears probable that a " cytosine glycol " (VI) would be involved as shown.

Experimental

Paper chromatograms were developed by the descending method with Whatman No. 1 paper and butan-1-ol–ethanol–water (4 : 1 : 5). The compounds were located with the silver nitrate spray.[6] Urea derivatives were located with the fructose–hydrogen chloride reagent.[1]

1-Substituted Uracils.—These were prepared by Shaw and Warrener's method [4] and had the following properties: 1-Methyluracil, m. p. 232°; $\lambda_{max.}$ (in water), 265 mμ; $\lambda_{min.}$ 232 mμ (Found: C, 47·8; H, 4·9; N, 22·5. Calc. for $C_5H_6N_2O_2$: C, 47·6; H, 4·8; N, 22·2%). 1-Phenyluracil, m. p. 246°, $\lambda_{max.}$ (in H_2O) 270 mμ, $\lambda_{min.}$ 237 mμ (Found: C, 63·8; H, 4·4; N, 14·8. Calc. for $C_{10}H_8N_2O_2$: C, 63·8; H, 4·3; N, 14·9%).

1-Benzyluracil.—β-Ethoxyacryloyl isocyanate [4] (12 g.), benzylamine (10 g.) and ether (100 ml.) were boiled under reflux for 30 min. After removal of the solvent the residue was heated at 60° with 2N-sodium hydroxide (50 ml.) for 20 min. The oily layer was separated and the aqueous layer was cooled to 0° and then acidified with ice-cold 5N-hydrochloric acid. The pale yellow, crude *1-benzyluracil* was filtered off. A second treatment of the oily layer with 2N-sodium hydroxide gave an additional small amount. The total crude product (12·2 g.) recrystallised from ethanol (charcoal) as needles, m. p. 174—176°, $\lambda_{max.}$ (in H_2O) 267 mμ, $\lambda_{min.}$ 234 mμ (Found: C, 65·3; H, 5·2; N, 13·6. $C_{11}H_{10}N_2O_2$ requires C, 65·3; H, 5·0; N, 13·9%).

1-Methyl-2,4-dithiouracil.—A suspension of 1-methyl-2-thiouracil [7] (15·8 g.) and phosphorus pentasulphide (12 g.) in xylene (250 ml.) was boiled under reflux for 8 hr. After 18 hr. at 0° the solid was filtered off, washed with benzene, and dissolved in dilute aqueous ammonia at 90°. The solution was decolorised with charcoal (5 g.), then neutralised with dilute acetic acid. The resulting precipitate was filtered off and crystallised from ethanol to give *1-methyl-2,4-dithiouracil* (4·8 g.), m. p. 261° (Found: C, 37·9; H, 4·1; N, 17·7. $C_5H_6N_2S_2$ requires C, 37·9; H, 3·8; N, 17·7%).

1-Methyl-2-thiocytosine.—1-Methyl-2,4-dithiouracil (4 g.) and ammonia (*d* 0·88; 24 ml.) were heated in a sealed tube at 120° for 16 hr. After cooling, *1-methyl-2-thiocytosine* was filtered off, washed with concentrated aqueous ammonia, and recrystallised from ethanol as needles (1·9 g.), m. p. 268° (Found: C, 42·5; H, 4·9; N, 29·6. $C_5H_7N_3S$ requires C, 42·5; H, 5·0; N, 29·8%).

1-Methylcytosine.—A solution of 1-methyl-2-thiocytosine (1·6 g.) and chloroacetic acid (1·3 g.) in water (30 ml.) was boiled under reflux for 90 min. 10N-Hydrochloric acid (50 ml.) was then added and boiling continued for 2 hr. The solution was evaporated to dryness, the residue moistened with dilute hydrochloric acid, and evaporation repeated. A solution of the residue was dissolved in a minimum of water, filtered, treated with ammonia (*d* 0·88) to pH 8—9, and left at 0° for 18 hr. The solid was filtered off, washed with water and then with ethanol, and crystallised from methanol to give 1-methylcytosine (1·24 g.) as needles, m. p. 303° (Found: C, 47·7; H, 5·5; N, 33·6. Calc. for $C_5H_7N_3O$: C, 48·0; H, 5·6; N, 33·6%.

Methylation of Cytosine.—Anhydrous cytosine (6·8 g.) was methylated with methyl iodide (10 g.) by Johnson and Clapp's procedure.[5] The product (1·4 g.) was identical with 1-methylcytosine synthesised as described above, with regard to m. p., chromatographic behaviour, and ultraviolet and infrared absorption spectra (Found: C, 48·1; H, 5·9; N, 33·6%).

1-Benzyl-2-thiouracil.—A solution of β-ethoxyacryloyl isothiocyanate (4·7 g.) and benzylamine (3·5 g.) in ether (100 ml.) was boiled under reflux for 20 min. The ether was distilled off and the residue was heated at 50—60° with 2N-sodium hydroxide (50 ml.) for 25 min., cooled to 0°, and acidified with dilute hydrochloric acid. *1-Benzyl-2-thiouracil* was filtered off and recrystallised from ethanol as yellow needles (1·6 g.), m. p. 230—232° (Found: C, 60·4; H, 4·8 N, 12·9. $C_{11}H_{10}N_2OS$ requires C, 60·5; H, 4·6; N, 12·8%).

1-Benzyl-2,4-dithiouracil.—1-Benzyl-2-thiouracil (1·5 g.) and phosphorus pentasulphide (3·5 g.) in xylene (50 ml.) were boiled for 8 hr., then left at 0° for 18 hr. The resulting suspension was filtered and the solid washed with benzene, dried, and dissolved in dilute aqueous ammonia at 90°. The solution was decolorised (charcoal) and neutralised with acetic acid. Upon cooling, *1-benzyl-2,4-dithiouracil* separated. This recrystallised from ethanol as pale yellow needles

[6] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[7] Shaw and Warrener, *J.*, 1958, 153.

(0·9 g.), m. p. 169° (Found: C, 55·6; H, 4·5; N, 12·1. $C_{11}H_{10}N_2S_2$ requires C, 56·4; H, 4·3; N, 12·0%).

1-*Benzyl-2-thiocytosine*.—A solution of 1-benzyl-2,4-dithiouracil (0·8 g.) in ammonia (*d* 0·88; 10 ml.) was heated at 120° in a sealed tube for 16 hr. and left at 0° for 18 hr. 1-*Benzyl-2-thiocytosine* was filtered off, washed with ammonia (*d* 0·88), and crystallised from ethanol, to give plates (0·61 g.), m. p. 252° (Found: C, 60·6; H, 5·0; N, 19·2. $C_{11}H_{11}N_3S$ requires C, 60·8 H, 5·1; N, 19·3%).

1-*Benzylcytosine*.—A solution of 1-benzyl-2-thiocytosine (0·56 g.) and chloroacetic acid (0·45 g.) in water (10 ml.) was boiled under reflux for 1 hr., treated with 10N-hydrochloric acid (10 ml.), and boiled for a further 2 hr., evaporated to dryness, treated again with 10N-hydrochloric acid (10 ml.), and boiled for a further 2 hr. The solution was then evaporated to dryness, 10N-hydrochloric acid (10 ml.) added, and the evaporation repeated. The residue was dissolved in a minimum of hot water, ammonia (*d* 0·88; 2 ml.) added, and the solution decolorised (charcoal). On cooling, 1-*benzylcytosine* separated; it recrystallised from water as needles (0·48 g.), m. p. 286° (Found: C, 65·9; H, 5·7; N, 20·6. $C_{11}H_{11}N_3O$ requires C, 65·7; H, 5·5; N, 20·9%).

Benzylation of Cytosine.—A solution of anhydrous cytosine (2·5 g.), potassium hydroxide (1·7 g.) and redistilled benzyl chloride (6 g.) in ethanol (50 ml.) was boiled under reflux for 2 hr., then evaporated to dryness, and water (100 ml.) was added. The resulting suspension was filtered and the solid residue washed with water and then with ethanol. It then crystallised from water, to give 1-benzylcytosine (3·4 g.), m. p. 288° (Found: C, 65·8; H, 5·5; N, 20·9%).

Deamination of 1-*Methyl- and* 1-*Benzyl-cytosine*.—Samples of the 1-substituted cytosines, prepared by both methods, were dissolved in water and treated with an excess of sodium nitrite and hydrochloric acid. The mixtures were heated at 60—70° for 15 min., then evaporated to a small volume. Paper chromatography showed that each sample of 1-methyl- and 1-benzyl-cytosine gave 1-methyl- and 1-benzyl-uracil, respectively, as the only products.

Permanganate Oxidation of Uracil at pH 7.—A solution of uracil (0·5 g.) in phosphate buffer (pH 7; 100 ml.) was mixed with one of potassium permanganate (1·41 g.) in water (80 ml.) and kept at 37° for 19 hr. Ammonia and carbon dioxide were evolved. Manganese dioxide was centrifuged off and the supernatant liquid concentrated and set aside for 18 hr. Crystals which separated were recrystallised twice from water, redissolved in water, and added to a column of Amberlite IR-120 (H^+ form). The acidic eluate was evaporated to dryness and the residue crystallised from aqueous ethanol to give oxaluric acid, m. p. 208° (Found: N, 21·1. Calc. for $C_3H_4N_2O_4$: N, 21·2%). This acid was treated with sodium hydrogen carbonate at pH 9 at 37° for 19 hr.; oxalic acid and urea were identified chromatographically as products. Urea was recognised by its blue colour with the fructose–hydrochloric acid spray,[1] and oxalic acid as its insoluble calcium salt and by formation of a blue colour with diphenylamine and phosphoric acid.[8]

Permanganate Oxidation of Uracil and 1-*Substituted Uracils at* pH 9.—To the solution of the uracil derivative (1 g. of uracil; 0·1 g. of substituted uracil) and potassium permanganate (2 mol.) in water (300 ml. for uracil; 100 ml. for substituted uracils) there was added solid sodium hydrogen carbonate until the pH reached 9, and the solution was kept at 37° for 19 hr. Manganese dioxide was filtered off and the filtrate concentrated *in vacuo* to about one-tenth of its volume. After the oxidation of uracil, crystals which separated were filtered off and identified as sodium oxalate by reaction with permanganate, formation of an insoluble calcium salt, reaction with diphenylamine and phosphoric acid,[8] and conversion into oxalic acid, m. p. and mixed m. p. 189°. Crystals also separated from the oxidation mixtures from 1-phenyl- and 1-benzyl-uracil. These were identified as *N*-phenyl- and *N*-benzyl-urea, respectively, by m. p., mixed m. p., and paper chromatography.

All the filtrates were subjected to paper chromatography. The tabulated results give the

Uracil	1-Methyluracil	1-Phenyluracil	1-Benzyluracil
A_1 0·0 (white)	A_2 0·0 (white)	A_3 0·0 (white)	A_4 0·0 (white)
B_1 0·051 (white)	—	—	—
C_1 0·086 (brown)	C_2 0·084 (brown)	C_3 0·086 (brown)	C_4 0·085 (brown)
D_1 0·33 (white)	D_2 0·48 (white)	D_3 0·73 (white)	D_4 0·78 (white)
E_1 0·44 (white)	E_2 0·55 (white)	E_3 0·84 (white)	E_4 0·87 (white)

[8] Feigl and Frehden, *Mikrochemie*, 1935, **18**, 272.

R_F values and the colours obtained when the chromatograms were sprayed with silver nitrate.

Components A_1 to A_4 were a mixture of sodium hydrogen carbonate and sodium oxalate, the latter identified as described above.

Component B_1 was chromatographically identical with sodium oxalurate.

Components C_1 to C_4 were sodium formate (R_F; reduction to formaldehyde, detected with chromotropic acid).

Components D_1 to D_4 absorbed ultraviolet light strongly and were chromatographically identical with the starting materials.

Components E_1 to E_4 were chromatographically identical with urea (E_1) or the corresponding *N*-substituted ureas. They all gave blue spots with the fructose–hydrochloric acid spray.

Permanganate Oxidation of Cytosine at pH 7.—Cytosine was oxidised with potassium permanganate in the presence of phosphate buffer (pH 7) as described above. Ammonia and carbon dioxide were evolved. The other products were similar to those described below for the oxidation at pH 9.

Permanganate Oxidation of Cytosine and 1-Substituted Cytosines at pH 9.—These reactions were performed as for the substituted uracils. Evolution of ammonia was noted 5 min. after the reaction had started. After removal of the manganese dioxide, the filtrates were concentrated *in vacuo*. From the oxidation products of cytosine, sodium oxalate separated; from those of 1-benzylcytosine (0·5 g.), *N*-benzylurea (0·122 g.; m. p. and mixed m. p. 147—148°) was obtained.

The filtrates were subjected to paper chromatography; R_F values and colours formed with silver nitrate are tabulated.

Cytosine		1-Methylcytosine		1-Benzylcytosine	
A_1 0·0 (white)	E_1 0·33 (white)	A_2 0·0 (white)	E_2 0·55 (white)	A_3 0·0 (white)	E_3 0·75 (white)
B_1 0·03 (brown)	F_1 0·44 (white)	B_2 0·04 (brown)	F_2 0·65 (white)	B_3 0·04 (brown)	F_3 0·86 (white)
C_1 0·08 (brown)	G_1 0·54 (white)	C_2 0·08 (brown)		C_3 0·08 (brown)	G_3 0·89 (white)
D_1 0·30 (white)		D_2 0·43 (white)		D_3 0·52 (white)	

Components A_1 to A_3 were mixtures of bicarbonate and oxalate, and C_1 to C_3 were formate. Components D_1, D_2, and E_3 were unoxidised cytosine derivatives; E_1, E_2, and G_3 were urea, *N*-methylurea, and *N*-benzylurea, respectively; F_1, F_2, and F_3 were biuret, *N*-methylbiuret, and *N*-benzylbiuret, respectively. The urea derivatives were identified by production of a blue colour with the fructose–hydrochloric acid spray, and the biuret derivatives by production of the violet colour with alkaline copper sulphate. They were also compared chromatographically with synthetic specimens. Components B_1 to B_3 and G_1 were not identified. In experiments with cytosine and 1-methylcytosine the urea spots and the biuret spots were eluted and their nitrogen contents were determined.

Action of Permanganate on Biuret.—Biuret (0·2 g.) was treated with potassium permanganate (0·6 g.) at pH 9 in the conditions used for cytosine. Chromatography of the products showed the presence of unchanged biuret and traces of two unidentified components. Urea was not present.

The authors thank Professor M. Stacey, F.R.S., for his interest, and the British Empire Cancer Campaign for part of the expenses.

CHEMISTRY DEPARTMENT, THE UNIVERSITY,
BIRMINGHAM, 15.

[*Received, August* 31*st*, 1962.]