The Synthesis of Some 1-Substituted Cytosine and Uracil Derivatives

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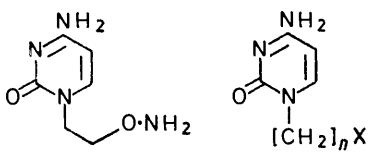
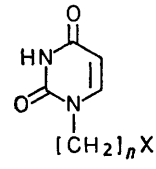
The synthesis of cytosines and uracils carrying the following substituents on N-1 is described: 2-mercaptoethyl (as the disulphide), 2-amino-oxyethyl, 3-carboxypropyl, 3-hydroxypropyl, 3-mercaptopropyl (as the disulphide), 3-amino-oxypropyl, and 3-aminopropyl (cytosine only).

1-(2-AMINO-OXYETHYL)CYTOSINE (I) undergoes reversible ring closure at C-6 to give a bicyclic 5,6-dihydrocytosine derivative. As a result, electrophilic substitution reactions at C-5 and nucleophilic displacements of the amino-function at C-4 are much faster than those shown by simple 1-alkylcytosines.[1] In order to extend these studies a range of related cytosine and uracil derivatives [(II) and (III)] have been synthesized and



(I)

(II)



(III)

$n = 2$ { a; X = CO_2H | b; X = OH | c; X = SH }

$n = 3$ { d; X = CO_2H | e; X = OH | f; X = SH | g; X = NH_2 | h; X = O·NH_2 }

$n = 2$ { a; X = CO_2H | b; X = OH | c; X = SH | d; X = O·NH_2 }

$n = 3$ { e; X = CO_2H | f; X = OH | g; X = SH | h; X = O·NH_2 }

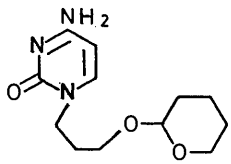
are described here. A detailed discussion of their chemistry will be given later. Structural assignments are consistent with the modes of synthesis and with the spectroscopic data (see Experimental section).

The carboxylic acids (IIa and d) were prepared by alkylation of *N*(4)-acetylcytosine [2,3] with ethyl 3-bromopropionate and ethyl 4-bromobutyrate,[4] respectively, followed by hydrolysis with ethanolic potassium hydroxide. The uracil (IIIa) is known.[5] Alkylation of 4-methylthiopyrimidin-2(1*H*)-one (5-methyl-4-thiouracil),[6] as its sodium salt, with ethyl 4-bromobutyrate gave a mixture of N-1 and O-2 substituted isomers, identified by their u.v. spectra.[6] However, alkylation of the free pyrimidine with ethyl 4-bromobutyrate in the presence of dry potassium carbonate gave the N-1 substituted isomer only and this ester was converted into the acid (IIIe) by treatment with refluxing hydrochloric acid.

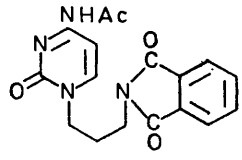
Of the hydroxy-compounds (IIb and e) and (IIIb and f), all but 1-(3-hydroxypropyl)cytosine (IIe) have been described.[7,8] Neither 3-bromopropanol nor its *p*-nitrobenzoate [7] was effective in alkylating *N*(4)-acetylcytosine, although a variety of conditions were tried. Instead the tetrahydropyranyl ether of 3-bromopropanol was prepared [9] and was used to alkylate *N*(4)-acetylcytosine, giving a mixture of N-1 and O-2 substituted isomers. Attempts to effect an O-2 ⟶ N-1 isomerisation by mercury(II) bromide in toluene were unsuccessful. The isomers were not separated by chromatography on silica gel, but with Grade IV neutral alumina as adsorbent the deacetylated compound (IV)

[1] D. M. Brown, P. F. Coe, and D. P. L. Green, *J. Chem. Soc. C*), 1971, 867.
[2] M. T. Doel, A. S. Jones, and N. Taylor, *Tetrahedron Letters*, 1969, 2285.
[3] D. T. Browne in 'Synthetic Procedures in Nucleic Acid Chemistry,' eds. W. W. Zorbach and R. S. Tipson, vol. 1, Interscience, Wiley, New York, 1968, p. 78.
[4] J. Lavety and G. R. Procter, *Org. Synth.*, 1965, **45**, 42.
[5] T. Ueda and J. J. Fox, *J. Org. Chem.*, 1964, **29**, 1767.
[6] G. C. Hopkins, J. P. Jonak, H. Tieckelmann, and H. J. Minnemeyer, *J. Org. Chem.*, 1966, **31**, 3969.
[7] B. R. Baker and T. H. Schwann, *J. Medicin. Chem.*, 1966, **9**, 73.
[8] N. Veda, K. Kondo, M. Kono, K. Takemoto, and M. Imoto, *Makromol. Chem.*, 1968, **120**, 13.
[9] F. Bohlmann, H. Bornowski, and P. Herbst, *Chem. Ber.*, 1960, **93**, 1931.

was eluted with chloroform and identified from its u.v., mass, and n.m.r. spectra. As it was considered that the basic alumina had catalysed the deacetylation, the crude mixture obtained from the acetylation reaction was treated, instead, with hot 0·1N-sodium hydroxide for 30 min. Extraction of the solution with chloroform gave a white, crystalline solid, identical with that obtained from the alumina column. Treatment of compound (IV) with 0·1N-hydrochloric acid in dioxan removed the tetrahydropyranyl group and gave the hydrochloride of (IIe).



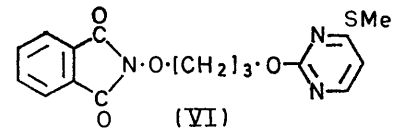
(IV)



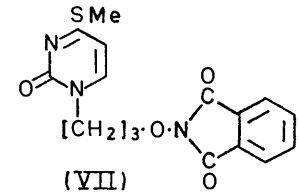
(V)

Phthalimide derivatives are often used to introduce an amino-function into a molecule;[10] the phthaloyl group is rapidly removed by hydrazine hydrate.[11] Unfortunately, this method cannot be used with molecules containing pyrimidine residues as they are attacked by hydrazine, giving a variety of products.[12,13] The phthaloyl group may also be removed by vigorous treatment with acid.[14,15] *N*(4)-Acetyl-1-(3-phthalimidopropyl)cytosine (V) was synthesised by alkylation of *N*(4)-acetylcytosine with 3-phthalimidopropyl bromide;[16] hydrolysis with acetic acid–water–hydrochloric acid (1 : 1 : 1) gave the dihydrochloride of (IIg) in high yield.

1-(2-Amino-oxyethyl)cytosine (I) had been prepared by alkylation of the sodium salt of *S*-methyl-4-thiouracil with *N*-(2-bromoethoxy)phthalimide[17] and subsequent amination and cleavage of the phthaloyl intermediate with methanolic ammonia.[1] A similar procedure using *N*-(3-bromopropoxy)phthalimide[17] gave the O-2 substituted isomer (VI), identified from its u.v. spectrum and easy hydrolysis to uracil. When *S*-methyl-4-thiouracil was used in the presence of potassium carbonate, t.l.c. indicated two major components, identified as the O-2 and N-1 substituted isomers (VI) and (VII), respectively. These were separated by chromatography on silica gel but treatment of the latter (VII) with methanolic ammonia failed to give compound (IIh). Since the foregoing sequence had proved to be unsuccessful, *N*(4)-acetylcytosine was alkylated with both *N*-(2-bromoethoxy)phthalimide and *N*-(3-bromopropoxy)phthalimide, in each case giving the N-1 substituted isomer. Treatment with methanolic ammonia, followed by acidification, gave the dihydrochlorides of



(VI)



(VII)

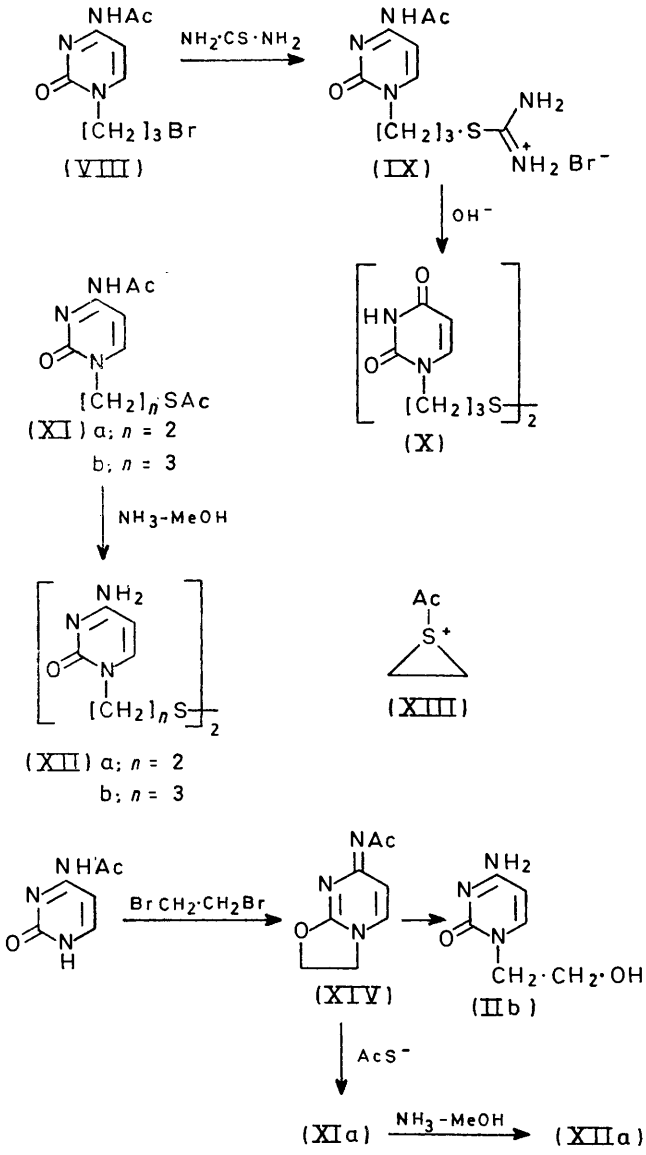
compounds (I) and (IIh), which could also be formed directly by treating the phthaloyl intermediates with a hot acetic–hydrochloric acid mixture. The uracil derivatives (IIId) and (IIIh) were prepared by hydrolysis of the corresponding 1-substituted 4-methylthiouracil derivatives.

The thiols (IIc and f) and (IIIc and g) were of particular interest as there are several examples recorded of pyrimidine thioanhydronucleosides, formed by addition of a thiol function on the sugar residue to the 5,6-double bond.[18–20] The bromide (VIII)[21] reacted with thiourea in acetone to give the isothiouronium salt (IX). However, on treatment with 20% potassium hydroxide solution the uracil disulphide (X) was obtained. An alternative approach involved alkylation of *N*(4)-acetylcytosine with *S*-3-bromopropyl thioacetate.[22] This gave the diacetate (XIb), which yielded the disulphide (XIIb) on treatment with methanolic ammonia. On repeating this procedure with *S*-2-bromoethyl thioacetate as alkylating agent, none of the corresponding diacetate (XIa) was detected. Bauer *et al.*[22] suggest that the episulphonium ion (XIII) is formed from *S*-2-bromoethyl thioacetate and observe complications in alkylation reactions. As an alternative route to the mercaptoethylcytosine (IIc), *N*(4)-acetylcytosine was treated with 1,2-dibromoethane. The product (XIV) was prone to ring-opening and gave rise on attempted crystallization to 1-(2-hydroxyethyl)cytosine (IIb). However when the crude reaction product was treated directly with sodium thioacetate, the diacetate (XIa) was obtained, which was converted into the disulphide (XIIa) with methanolic ammonia.

In order to prepare the uracil analogue (IIIg), attempts were made to alkylate uracil,[2,23] but yields of

[10] S. Gabriel, *Ber.*, 1887, **20**, 2224.
[11] H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 1926, 2348.
[12] F. Baron and D. M. Brown, *J. Chem. Soc.*, 1955, 2855.
[13] F. Lingens and H. Schneider-Bernlohr, *Annalen*, 1965, **686**, 134.
[14] M. S. Dunn and B. W. Smart, *Org. Synth.*, 1963, Coll. Vol. IV, p. 55.
[15] G. Burger and T. E. Weichelsbaum, *Org. Synth.*, 1943, Coll. Vol. II, p. 384.
[16] Ch'eng-Yeh Yuan, Chun Ts'ai Lin, and Jih-Hsin Liu, *Hua Hsueh Hsueh Pao*, 1959, **25**, 183 (*Chem. Abs.*, 1960, **54**, 4405d).
[17] L. Bauer and K. S. Suresh, *J. Org. Chem.*, 1963, **28**, 1604.
[18] B. Bannister and F. Kagan, *J. Amer. Chem. Soc.*, 1960, **82**, 3363.
[19] R. Chambers and V. Kurkov, *J. Amer. Chem. Soc.*, 1963, **85**, 2160.
[20] E. J. Reist, A. Benitez, and L. Goodman, *J. Org. Chem.*, 1964, **29**, 554.
[21] Ref. 3, p. 96.
[22] L. Bauer, K. S. Suresh, and B. K. Ghosh, *J. Org. Chem.*, 1965, **30**, 949.
[23] A. P. Martinez and W. W. Lee, *J. Org. Chem.*, 1965, **30**, 317.

the thioacetate (XV) were low. When, however, sodium hydride in dimethylformamide was used to form the uracil anion, the product was the N-3 substituted

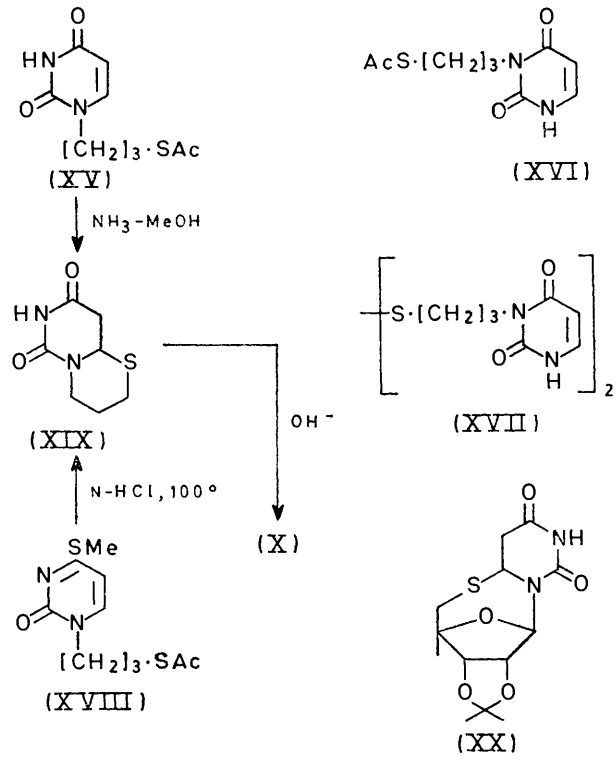


derivative (XVI), identified from its u.v.[24] and n.m.r. spectra, and this gave the 3,3′-linked disulphide (XVII) when treated with methanolic ammonia.

The thioacetate (XVIII) was prepared by alkylation of 4-methylthiouracil; when this was treated with refluxing N-hydrochloric acid, or the uracil derivative (XV) was treated with methanolic ammonia, the same compound was produced. The u.v. spectrum of this product in water showed only end absorption, but on addition of alkali a peak at 267 nm appeared, corresponding to the 1-alkyluracil anion chromophore, and subsequent acidification only partially reversed this change.

[24] J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, 1952, **9**, 119.

These changes are analogous to those observed by Bannister and Kagan [18] and by Chambers and Kurkov [19] in corresponding reactions of the thioanhydronucleoside (XX). Consequently, the product was assigned the bicyclic structure (XIX) with which the spectroscopic data are entirely consistent. When compound (XIX)



was treated with 0·1N-sodium hydroxide for 20 h the disulphide (X) was formed.

Some properties of the foregoing compounds will be described in a subsequent paper.

EXPERIMENTAL

N(4)-Acetyl-1-(2-ethoxycarbonylethyl)cytosine.— *N*(4)-Acetylcytosine (500 mg) was dissolved in dry dimethyl sulphoxide (50 ml) with heating. After cooling, dry potassium carbonate (434 mg) and ethyl 3-bromopropionate (600 mg) were added. The mixture was stirred at room temperature for 16 h, the solution filtered, and the solvent was removed under high vacuum to give a yellow oil. This was taken up in a little water and extracted with chloroform (×3). The extracts were washed with water, dried (Na_2SO_4), and evaporated to a yellow oil which slowly crystallized to give the colourless *ester* (50·5%), m.p. 131—132° (from benzene) (Found: C, 51·7; H, 6·0; N, 16·9%; M^+, 253. $C_{11}H_{14}N_3O_4$ requires C, 52·1; H, 5·9; N, 16·6%; *M*, 253), τ ($CDCl_3$) 8·80 (t, $CO_2CH_2{\cdot}CH_3$), 7·79 (s, $CH_3{\cdot}CO$), 7·19 (t, $CH_2{\cdot}CO_2Et$), 5·92 (m, $CO_2CH_2{\cdot}CH_3$ and $N{\cdot}CH_2$), and 2·31 and 2·76 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 214 (ε 19,050), 247 (14,700), and 300 (6780), $\lambda_{min.}$ 227 (5560) and 271 nm (3000).

1-(2-*Carboxyethyl*)*cytosine* (IIa).—The foregoing ester (200 mg) was refluxed for 30 min with potassium hydroxide

in absolute ethanol (10%; 25 ml). The solution was cooled and evaporated. The white solid which separated was taken up in a little water and applied to a Dowex-50 (H^+) column. The column was washed with water and then eluted with N-ammonia to give the *product* (98%), m.p. 266—268° (from water–acetone) (Found: C, 45·9; H, 4·95; N, 23·0%; M^+, 183. $C_7H_9N_3O_3$ requires C, 45·9; H, 4·95; N, 22·9%; *M*, 183), τ (CF_3·CO_2D) 6·92 (t, CH_2), 5·72 (t, CH_2), and 3·50 and 2·00 (both d, 5- and 6-H, *J* 6 Hz), $\lambda_{max.}$ (H_2O) 279 (ε 11,540), $\lambda_{min.}$ 243 (3238), $\lambda_{max.}$ (0·1N-HCl) 212 (9720) and 281 (12,640), $\lambda_{min.}$ 240 nm (1078).

N(4)-*Acetyl*-1-(3-*ethoxycarbonylpropyl*)*cytosine*.—This *ester* (50%) was prepared like the lower homologue from *N*(4)-acetylcytosine (500 mg), potassium carbonate (434 mg), and ethyl 4-bromobutyrate (613 mg); m.p. 112—113° (from benzene) (Found: C, 54·1; H, 6·55; N, 16·0%; M^+, 267. $C_{12}H_{17}N_3O_4$ requires C, 53·9; H, 6·4; N, 15·7%; *M*, 267), τ ($CDCl_3$) 8·72 (t, CO_2CH_2·CH_3), 7·85 (m, CH_2·CH_2·CH_2), 7·70 (s, CH_3·CO), 7·61 (m, CH_2·CO_2Et), 5·90 (m, CO_2CH_2·CH_3 and N·CH_2), and 2·54 and 2·32 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 215 (ε 20,100), 246 (14,600), and 300 (6890), $\lambda_{min.}$ 228 (6220) and 271 nm (3220).

1-(3-*Carboxypropyl*)*cytosine* (IId).—This *acid* (75%) was prepared like the lower homologue; m.p. 235° (decomp.) (from ethanol–water) (Found: C, 48·8; H, 5·65; N, 21·4%; M^+, 197. $C_8H_{11}N_3O_3$ requires C, 48·7; H, 5·6; N, 21·3%; *M*, 197), τ (CF_3·CO_2D) 7·82 (q, CH_2·CH_2·CH_2), 7·38 (t, CH_2·CO_2H), 5·92 (t, N·CH_2), and 3·63 and 2·14 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (H_2O) 278 (ε 9152), $\lambda_{min.}$ 247 (2769), $\lambda_{max.}$ (0·1N-HCl) 212 (9302) and 282 nm (12,000).

1-(3-*Ethoxycarbonylpropyl*)-4-*methylthiopyrimidine*-2(1H)-*one*.—4-Methylthiouracil (100 mg), potassium carbonate (98 mg), and ethyl 4-bromobutyrate (200 mg) were heated at 70° in dry dimethylformamide (25 ml) for 6 h. The solvent was removed under high vacuum and the residue was taken up in a little water and extracted with chloroform (×3). The extracts were washed with water, dried (Na_2SO_4), and evaporated to give a crystalline mass, homogeneous by t.l.c. Recrystallization from ether gave the *product* (63·5%), m.p. 53·5—54·5° (Found: C, 51·5; H, 6·3; N, 10·8; S, 12·5%; M^+, 256. $C_{11}H_{16}N_2O_3S$ requires C, 51·5; H, 6·1; N, 10·5; S, 12·2%; *M*, 256), τ ($CDCl_3$) 8·76 (t, CO_2CH_2·CH_3), 7·60, 7·50 (m, CH_2·CH_3 and CH_2·CH_2·CH_2), 6·0 (m, N·CH_2·CH_2·CH_2), and 3·81 and 2·64 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 304 (ε 11,810), $\lambda_{infl.}$ 276 (9052), $\lambda_{min.}$ 240 nm (1903).

1-(3-*Carboxypropyl*)*uracil* (IIIe).—The foregoing ester (100 mg) was refluxed for 2·5 h with N-HCl (10 ml). The solution was evaporated to yield the *acid* (90%), m.p. 188—191° (from acetone–water) (Found: C, 48·7; H, 5·06; N, 14·2%; M^+, 198. $C_8H_{10}N_2O_4$ requires C, 48·5; H, 5·05; N, 14·1%; *M*, 198), τ (CF_3·CO_2D) 7·82 (q, CH_2·CH_2·CH_2), 7·38 (t, CH_2·CO_2H), 5·95 (t, N·CH_2), and 3·80 and 2·30 (both d, 5- and 6-H, *J* 8 Hz), $\lambda_{max.}$ (0·1N-HCl) 267 nm (ε 9709).

N(4)-*Acetyl*-1-(3-*tetrahydropyran*-2-*yloxypropyl*)*cytosine*.—*N*(4)-Acetylcytosine (100 mg) was dissolved in dry dimethyl sulphoxide (20 ml), and potassium carbonate (91 mg) and 3-bromopropyl tetrahydropyranyl ether (220 mg) were added. The solution was stirred at room temperature for 6 h and then poured into saturated sodium chloride solution. This was extracted with chloroform (×3). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to leave an oil which did not crystallize. Preparative t.l.c. showed it to be mainly one compound, with $\lambda_{max.}$ (EtOH) 297, 246, and 215, $\lambda_{min.}$ 270 and 229 nm. This corresponds to an N-1 substituted *N*(4)-acetylcytosine. A faster moving component had $\lambda_{max.}$ (EtOH) 300, 278, and 235, $\lambda_{min.}$ 293, 264, 241, and 227 nm and was assumed to be the O-2 substituted *N*(4)-acetylcytosine. The mixture was dissolved in benzene and applied to a silica gel column. Elution with chloroform–benzene (1 : 1) gave the faster-moving component (not fully characterized), and finally elution with chloroform gave the N-1 substituted compound, but this could not be induced to crystallize. T.l.c. showed that it still contained two minor components which were not identified. Attempted chromatography on Grade IV neutral alumina gave a colourless crystalline product which was identified as 1-(3-tetrahydropyran-2-yloxypropyl)cytosine (see later).

1-(3-*Tetrahydropyran*-2-*yloxypropyl*)*cytosine* (IV).—*N*(4)-Acetylcytosine, potassium carbonate, and 3-bromopropyl tetrahydropyranyl ether were treated as in the previous experiment and the crude material obtained from the chloroform extracts was dissolved in ethanol (5 ml) and 0·1N-sodium hydroxide (5 ml). The yellow solution was refluxed for 30 min, cooled, reduced in volume, and extracted with chloroform. Evaporation of the dried (Na_2SO_4) extracts gave a white solid which was shown by t.l.c. to be identical with that obtained before. Recrystallization from absolute ethanol gave 1-(3-*tetrahydropyran*-2-*yloxypropyl*)*cytosine*, m.p. 199—201° (Found: C, 56·8; H, 7·8; N, 16·3%; M^+, 253. $C_{12}H_{19}N_3O_3$ requires C, 56·9; H, 7·7; N, 16·6%; *M*, 253), τ [$(CD_3)_2SO$] 8·49 (6H, m), 8·16 (2H, q), 6·62 and 6·28 (6H, m), 5·47 (1H, s), 3·02 (2H, s), and 4·34 and 2·46 (both d, 5- and 6-H, *J* 8 Hz), $\lambda_{max.}$ (0·1N-HCl) 285 (12,400), $\lambda_{min.}$ 242 nm (1445).

1-(3-*Hydroxypropyl*)*cytosine Hydrochloride* (IIe).—1-(3-Tetrahydropyran-2-yloxypropyl)cytosine (50 mg) was dissolved in dioxan (5 ml) and water (1 ml), and 0·1N-hydrochloric acid (5 ml) was added. The solution was stirred at room temperature for 7 h, after which t.l.c. showed complete disappearance of the starting material. Evaporation left an oil which crystallized on trituration with ethanol and ether to give the *product*, m.p. 165—167° (from ethanol–ether) (Found: C, 40·9; H, 5·8; Cl, 17·1; N, 20·3. $C_7H_{12}ClN_3O_2$ requires C, 40·8; H, 5·8; Cl, 17·3; N, 20·4%), τ (D_2O) 8·05 (q, CH_2·CH_2·CH_2), 6·34 (t, CH_2), 6·04 (t, CH_2), and 3·83 and 2·14 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (0·1N-HCl) 282 nm (ε 12,290).

N(4)-*Acetyl*-1-(3-*phthalimidopropyl*)*cytosine* (V).—*N*(4)-Acetylcytosine (100 mg), potassium carbonate (91 mg), and 3-phthalimidopropyl bromide (350 mg) were stirred in dry dimethyl sulphoxide (20 ml) for 20 h at room temperature. The mixture was poured into saturated sodium chloride solution (20 ml) and extracted with chloroform (×3); the extract was washed with water, dried (Na_2SO_4), and evaporated yielding an oil which crystallized on trituration with ethanol. The *product*, recrystallized twice from absolute ethanol, had m.p. 203—206°; yield 50% (Found: C, 60·2; H, 4·8; N, 16·2%; M^+, 340. $C_{17}H_{16}N_4O_4$ requires C, 60·0; H, 4·7; N, 16·5%; *M*, 340), τ [$(CD_3)_2SO$] 7·97 (m, CH_2·CH_2·CH_2), 7·92 (s, CH_3·CO), 6·33 (m, CH_2·CH_2·CH_2), 2·17 (s, aromatic), and 2·70 and 1·90 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 219 (ε 56,670), 233 (24,680), 241 (24,010), and 300 (8164), $\lambda_{min.}$ 231 (24,610), 237 (22,860), and 270 nm (3698).

1-(3-*Aminopropyl*)*cytosine* (IIg) *Dihydrochloride*.—*N*(4)-Acetyl-1-(3-phthalimidopropyl)cytosine (1·5 g) was treated

with water–conc. hydrochloric acid–acetic acid (1 : 1 : 1; 30 ml) in a sealed tube at 120° for 40 h. After evaporation to a small volume and cooling, phthalic acid was filtered off and the filtrate evaporated to give a white solid, m.p. 293° (decomp.) (from ethanol–water) (Found: C, 35·1; H, 5·8; Cl, 29·0; N, 23·1. $C_7H_{12}Cl_2N_4O$ requires C, 34·9; H, 5·8; Cl, 29·3; N, 23·2%), τ (D_2O) 7·88 (q, $CH_2{\cdot}CH_2{\cdot}CH_2$), 6·90 (t, $CH_2{\cdot}NH_2$), 6·02 (t, $N{\cdot}CH_2$), and 3·79 and 2·13 (both d, 5- and 6-H, *J* 8 Hz), $\lambda_{max.}$ (0·1N-HCl) 283 nm (ε 11,954).

N(4)-*Acetyl*-1-(2-*phthalimido-oxyethyl*)*cytosine*.— *N*(4)-Acetylcytosine (500 mg), *N*-(2-bromoethoxy)phthalimide (1·0 g), and potassium carbonate (451 mg) were stirred at room temperature for 16 h in dimethyl sulphoxide (50 ml). The solution was poured into saturated sodium chloride solution and extracted with chloroform (×3); the combined extracts were washed with water. Drying (Na_2SO_4) and evaporation gave the *product* (50%), m.p. 230—234° (decomp.) (from 95% ethanol) (Found: C, 56·3; H, 4·1; N, 16·1%; M^+, 322. $C_{16}H_{14}N_4O_5$ requires C, 56·1; H, 4·1; N, 16·4%; *M*, 322), τ [$(CD_3)_2SO$] 7·88 (s, $CH_3{\cdot}CO$), 5·54 and 5·82 (m, $CH_2{\cdot}CH_2$), 2·14 (s, aromatic), and 2·75 and 1·75 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 217 (ε 47,420), 241 (21,690), and 300 (8622), $\lambda_{min.}$ 237 (20,260) and 270 nm (3089).

1-(2-*Amino-oxyethyl*)*cytosine* (I) *Dihydrochloride*.—The foregoing phthalimide derivative (100 mg) was refluxed for 20 min with acetic acid–conc. hydrochloric acid (13·5 : 7·5; 20 ml). On cooling and partial evaporation, phthalic acid crystallized out and was removed. The remaining solution was evaporated to dryness and the dihydrochloride was recrystallized from ethanol–water; had m.p. 209° (lit.,[1] 210°).

1-(2-*Phthalimido-oxyethyl*)*uracil*.—Uracil (500 mg), potassium carbonate (616 mg), and *N*-(2-bromoethoxy)-phthalimide (1·21 g) in dry dimethyl sulphoxide (100 ml) were stirred for 3 h at room temperature. Water (100 ml) was added and the solution left at 0° for 14 h. A white solid precipitated which was filtered off and recrystallized from aqueous ethanol to give fine, white *needles* (52%), m.p. 253—255° (Found: C, 56·1; H, 3·9; N, 14·1%; M^+, 301. $C_{14}H_{11}N_3O_5$ requires C, 55·8; H, 3·7; N, 14·0%; *M*, 301), τ [$(CD_3)_2SO$] 5·99 and 5·64 (m, $CH_2{\cdot}CH_2$), 2·22 (s, aromatic), and 4·46 and 2·14 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 220 (ε 46,200) and 261 (7300), $\lambda_{min.}$ 250 (6030), $\lambda_{infl.}$ 296 nm (2170).

1-(2-*Amino-oxyethyl*)*uracil* (IIId) *Hydrochloride*.—(*a*) 4-Methylthio-1-(2-phthalimido-oxyethyl)pyrimidin-2(1*H*)-one [1] (50 mg) was refluxed for 15 min with acetic acid–hydrochloric acid (13·5 : 7·5; 10 ml). On cooling and evaporating to small bulk, phthalic acid crystallized out and was filtered off. N-Hydrochloric acid (10 ml) was added, and the solution refluxed for 30 min. Evaporation left the *product* (65%), m.p. 183—184° (from aqueous ethanol) (Found: C, 34·8; H, 4·95; Cl, 16·9; N, 20·5%; M^+, 171. $C_6H_{10}ClN_3O_3$ requires C, 34·7; H, 4·8; Cl, 17·1; N, 20·3%; *M*, 171), τ (D_2O) 5·85 (t, CH_2), 5·65 (t, CH_2), and 4·13 and 2·32 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (0·1N-HCl) 265 (ε 9638), $\lambda_{min.}$ 232 nm (884).

(*b*) 1-(2-Phthalimido-oxyethyl)uracil was hydrolysed with acetic acid–hydrochloric acid (13·5 : 7·5; 10 ml) for 15 min. Removal of phthalic acid followed by evaporation gave 1-(2-amino-oxyethyl)uracil dihydrochloride.

N(4)-*Acetyl*-1-(3-*phthalimido-oxypropyl*)*cytosine*.— *N*(4)-Acetylcytosine (1·0 g), dry potassium carbonate (905 mg), and *N*-(3-bromopropoxy)phthalimide (2·71 g) were stirred at room temperature in dimethyl sulphoxide (100 ml) for 6 h. The solvent was evaporated off under high vacuum. The resulting yellow oil solidified on trituration with water and the *product* was recrystallized from 95% ethanol; m.p. 205—206°; yield 42% (Found: C, 57·0; H, 4·65; N, 15·5%; M^+, 356. $C_{17}H_{16}N_4O_5$ requires C, 57·2; H, 4·5; N, 15·3%; *M*, 356), τ [$(CD_3)_2SO$] 8·90 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), 7·94 (s, $CH_3{\cdot}CO$), 5·84 and 6·00 (m, $CH_2{\cdot}N$, $CH_2{\cdot}N$, and $CH_2{\cdot}O$), 2·14 (s, aromatic), and 2·62 and 1·86 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 217 (ε 59,330), 242 (22,150), and 301 (9249), $\lambda_{min.}$ 238 (20,420) and 271 nm (3664).

1-(3-*Amino-oxypropyl*)*cytosine* (IIh) *Dihydrochloride*.—The foregoing phthalimide derivative (500 mg) was refluxed with acetic acid–hydrochloric acid (13·5 : 7·5; 15 ml) for 15 min. Phthalic acid was removed and the remaining solution evaporated to give an oil, which crystallized on trituration with ethanol. The *dihydrochloride* (72%) had m.p. 106—108° (from aqueous ethanol) (Found: C, 32·7; H, 5·65; Cl, 27·7; N, 21·7. $C_7H_{14}Cl_2N_4O_2$ requires C, 32·7; H, 4·55; Cl, 27·6; N, 21·8%), τ (D_2O) 7·7 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), 5·94 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), and 3·74 and 2·04 (both d, 5- and 6-H, *J* 8 Hz), $\lambda_{max.}$ (0·1N-HCl) 283 nm (ε 11,690).

1-(3-*Phthalimido-oxypropyl*)-S-*methyl*-4-*thiouracil* (VII).—*S*-Methyl-4-thiouracil (355 mg) and potassium carbonate (345 mg) were stirred in dimethylformamide (20 ml) at room temperature for 15 min. *N*-(3-Bromopropyloxy)phthalimide (900 mg) was added and the solution was heated at 60° for 6 h. The solvent was removed under high vacuum and the residue extracted with chloroform. The extracts were washed with water, dried (Na_2SO_4), and evaporated to give a white crystalline product, which was shown by t.l.c. to contain two major components and several minor ones. This was taken up in benzene, adsorbed on Kieselgur, and applied to a silica gel column (150 g; prepared in benzene). Elution with benzene–chloroform (1 : 1) separated the two major components, which both crystallized on removal of the solvent. Both were recrystallized from ethanol and identified as the N-1 and O-2 substituted derivatives. The 1-*isomer* had m.p. 152—154° (Found: C, 55·7; H, 4·4; N, 12·0; S, 9·3%; M^+, 345. $C_{16}H_{15}N_3O_4S$ requires C, 55·6; H, 4·35; N, 12·2; S, 9·3%; *M*, 345), τ ($CDCl_3$) 7·74 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), 7·46 (s, $CH_3{\cdot}S$), 5·79 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), 2·16 (d, aromatic), and 3·73 and 2·10 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 220 (ε 44,520) and 303 (14,050), $\lambda_{min.}$ 250 nm (5286). The O-2 isomer was identical with that described later.

4-*Methylthio*-2-(3-*phthalimido-oxypropyloxy*)*pyrimidine* (VI).—The sodium salt of *S*-methyl-4-thiouracil (450 mg) and *N*-(3-bromopropoxy)phthalimide (1·0 g) were heated at 60° for 6 h in dry dimethylformamide (150 ml). The solvent was removed under vacuum and the residual oil crystallized from ethanol. The *product* (66%) had m.p. 146—148° (Found: C, 55·3; H, 4·3; N, 12·2; S, 9·5%; M^+, 345), τ ($CDCl_3$) 7·71 (m, $CH_2{\cdot}CH_2{\cdot}CH_2$), 7·44 (s, $CH_3{\cdot}S$), 5·58 and 5·34 (t, $CH_2{\cdot}CH_2{\cdot}CH_2$), 2·20 (d, aromatic), and 3·19 and 1·86 (both d, 5- and 6-H, *J* 5 Hz), $\lambda_{max.}$ (EtOH) 220 (ε 53,540), 241 (15,110), and 288 (12,700), $\lambda_{min.}$ 239 (14,900) and 258 nm (3174).

Hydrolysis of this product in N-hydrochloric acid at 100° for 2 h, gave uracil, which was recrystallized from aqueous ethanol and identified by t.l.c. and its u.v. spectrum.

1-(3-*Amino-oxopropyl*)*uracil* (IIIh) *Hydrochloride*.—This *hydrochloride* was prepared like the lower homologue; m.p. 190—192° (from aqueous ethanol) (Found: C, 38·0; H, 5·65; Cl, 15·9; N, 18·8. $C_7H_{12}ClN_3O_3$ requires C, 37·9; H, 5·42; Cl, 16·0; N, 19·0%), τ (D_2O) 7·70 (m,

CH_2·CH_2·CH_2), 5·80 (t, CH_2), 5·71 (t, CH_2), and 4·08 and 2·21 (both d, 5- and 6-H, J 6 Hz), $\lambda_{max.}$ (0·1N-HCl) 266 nm (ε 10,031).

N(4)-*Acetyl*-1-(3-*isothiouroniopropyl*)*cytosine Hydrobromide* (IX).—*N*(4)-Acetyl-1-(3-bromopropyl)cytosine [21] (VIII) (1·0 g) [m.p. 136—138°, M^+ 273 and 275, $\lambda_{max.}$ (EtOH) 216, 247, and 301 nm] and thiourea (300 mg) were refluxed for 17 h in acetone (100 ml). Pale yellow crystals appeared which were filtered off and recrystallized from aqueous methanol; m.p. 228—230°; yield 75% (Found: C, 34·2; H, 4·5; S, 8·9; Br, 22·7; N, 20·0. $C_{10}H_{16}BrN_5O_2S$ requires C, 34·3; H, 4·55; Br, 22·9; N, 20·0; S, 9·15%), τ (D_2O) 7·9 (m, CH_2·CH_2·CH_2), 7·73 (s, CH_3S), 5·83 (m, CH_2·CH_2·CH_2), and 4·01 and 2·12 (both d, 5- and 6-H, J 7 Hz), $\lambda_{max.}$ (H_2O) 214 (ε 24,322), 244 (13,360), and 298 nm (8960).

Boiling the isothiouronium salt (100 mg) under reflux for 30 min with 20% (w/v) potassium hydroxide solution (20 ml) and neutralizing the solution by passing it through a Dowex-50 (H^+) resin column gave a product with $\lambda_{max.}$ (H_2O) 268, $\lambda_{min.}$ 236 nm, identified as bis-3-(1,2,3,4-tetrahydro-2,4-dioxopyrimidin-1-yl)propyl disulphide (X) (see later).

N(4)-*Acetyl*-1-(3-*acetylthiopropyl*)*cytosine* (XIb).—*N*(4)-Acetylcytosine (153 mg), potassium carbonate (138 mg), and *S*-3-bromopropylthioacetate (400 mg) were stirred for 16 h at room temperature in dimethyl sulphoxide (50 ml). Removal of the solvent under high vacuum, followed by extraction with chloroform, washing, drying, and evaporation gave a pale yellow crystalline solid which was recrystallized from benzene. Two components were revealed by t.l.c. but a second recrystallization removed one of these and gave the *product*, m.p. 148—150° (Found: C, 49·1; H, 5·55; N, 15·9; S, 11·1%; M^+, 269. $C_{11}H_{15}N_3O_3S$ requires C, 49·1; H, 5·55; N, 15·6; S, 11·9%; M, 269), τ ($CDCl_3$) 7·97 (q, CH_2·CH_2·CH_2), 7·71 and 7·74 (s, CH_3·CO), 7·12 (t, S·CH_2), 6·09 (t, N·CH_2), and 2·56 and 2·36 (both d, 5- and 6-H, J 8 Hz), $\lambda_{max.}$ (EtOH) 216 (ε 21,460), 247 (16,480), and 301 (7270), $\lambda_{min.}$ 229 (9269) and 273 nm (3041).

Bis-3-(4-*amino*-1,2-*dihydro*-2-*oxopyrimidin*-1-*yl*)*propyl Disulphide* (XIIb).—The foregoing diacetate (100 mg) was treated with methanolic ammonia (10 ml; pre-saturated at 0°) at room temperature for 15 h. Yellow crystals which separated yielded the *product*, m.p. 264—266° (from methanol), which gave a positive disulphide test (Found: C, 45·8; H, 5·6; N, 22·2; S, 17·7. $C_{14}H_{20}N_6O_2S_2$ requires C, 45·7; H, 5·45; N, 22·1; S, 17·4%), τ (CF_3·CO_2D) 7·96 (m, CH_2·CH_2·CH_2), 7·38 (m, CH_2·S), 5·90 (m, CH_2·N), and 3·66 and 2·15 (both d, 5- and 6-H, J 8 Hz), $\lambda_{max.}$ (H_2O) 275 (ε 13,830), $\lambda_{min.}$ 251 (7321), $\lambda_{max.}$ (0·1N-HCl) 283 (19,810), $\lambda_{min.}$ 242 nm (2207).

1-(3-*Acetylthiopropyl*)*uracil* (XV).—Uracil (2·7 g), potassium carbonate (3·3 g), sodium iodide (1·2 g), and *S*-3-bromopropyl thioacetate (1·58 g) in dimethyl sulphoxide (50 ml) were heated in an oil bath at 90° for 2·5 h. The solution was cooled and poured into ice–water (100 ml), and the pH was adjusted to 2 with N-hydrochloric acid. Extraction with chloroform, drying (Na_2SO_4), and evaporation gave an oil which crystallized on trituration with ether. Three components were revealed by t.l.c., but the major *product* was obtained by recrystallization from benzene–light petroleum (b.p. 60—80°); m.p. 110—112° (Found: C, 47·4; H, 5·05; N, 12·1; S, 13·7%; M^+, 228. $C_9H_{12}N_2O_3S$ requires C, 47·4; H, 5·26; N, 12·3; S, 14·0%; M, 228), τ ($CDCl_3$) 8·03 (q, CH_2·CH_2·CH_2), 7·72 (s, CH_3·CO), 7·12 (t, CH_2·S), 6·22 (t, CH_2·N), and 4·28 and 2·77 (both d, 5- and 6-H, J 8 Hz), $\lambda_{max.}$ (EtOH) 265 (ε 9454), $\lambda_{min.}$ 242 (5188), $\lambda_{infl.}$ 232 nm (5451).

Reaction of Uracil with S-3-*Bromopropyl Thioacetate in the Presence of Sodium Hydride.*—(a) *In dimethyl sulphoxide:* 3-(3-*acetylthiopropyl*)*uracil* (XVI). Uracil (2·24 g) was dissolved in dimethyl sulphoxide (50 ml) and added dropwise to a suspension of sodium hydride (1·08 g; 50% dispersion in oil pre-washed with petroleum) in dimethyl sulphoxide (25 ml). The solution was stirred at room temperature for 0·5 h and then *S*-3-bromopropyl thioacetate (3·94 g) was added. The solution was stirred until it had become clear (2·5 h). Water (50 ml) was added and the mixture extracted with chloroform (×3). The extracts gave an oil which crystallized from benzene; the *product* had m.p. 170—171° (Found: C, 47·6; H, 5·07; N, 12·6; S, 14·2%; M^+, 228), τ ($CDCl_3$) 7·95 (q, CH_2·CH_2·CH_2), 7·60 (s, CH_3·CO), 7·00 (t, CH_2·S), 5·90 (t, CH_2·N), 4·10 and 2·75 (both d, 5- and 6-H, J 7 Hz), $\lambda_{max.}$ (H_2O) 211, 237, and 259, $\lambda_{min.}$ 227 and 248 nm.

(b) *In dimethylformamide:* 1-(3-*acetylthiopropyl*)*uracil* (XV). Uracil (2·24 g) dissolved in dimethylformamide (50 ml) was added dropwise to sodium hydride (1·08 g; 50% dispersion in oil pre-washed with petroleum) in dimethylformamide (25 ml). The solution was stirred at room temperature for 2 h and then *S*-3-bromopropyl thioacetate (3·94 g) was added. The solution was heated to 80° for 14 h, then the temperature was raised to 150°, more 3-bromopropyl thioacetate (1 g) was added, and the solution was stirred for 30 min. The solution was cooled and evaporated, and the residue was extracted with chloroform. The extract afforded 1-(3-acetylthiopropyl)-uracil (see before).

Reaction of 3-(3-*Acetylthiopropyl*)*uracil with Methanolic Ammonia.*—The N-3 isomer (50 mg) was treated with methanolic ammonia (20 ml; pre-saturated at 0°) at room temperature for 6 h. Evaporation left a white solid which was recrystallized from aqueous methanol; m.p. 242—243° (Found: M^+, 370), $\lambda_{max.}$ (H_2O) 262 and 203, $\lambda_{min.}$ 235, $\lambda_{max.}$ (0·1N-NaOH) 285, $\lambda_{min.}$ 244 nm. The compound gave a positive disulphide test and was identified as the 3,3-linked disulphide (XVII).

1-(3-*Acetylthiopropyl*)-4-*methylthiopyrimidin*-2(1H)-*one* (XVIII).—*S*-Methyl-4-thiouracil (500 mg), potassium carbonate (486 mg), and *S*-3-bromopropyl thioacetate (1·38 g) were heated at 70° for 5 h in dimethylformamide (20 ml). The solvent was evaporated off and the residual oil extracted with chloroform. The extracts, after drying and evaporation, gave an oil which crystallized during 12 h at −15°. Recrystallization from benzene gave pale yellow *crystals*, m.p. 60° (Found: C, 46·7; H, 5·45; N, 10·9; S, 25·0%; M^+, 258. $C_{10}H_{14}N_2O_2S$ requires C, 46·5; H, 5·4; N, 10·9; S, 24·8%; M, 258), τ ($CDCl_3$) 7·71 and 7·50 (s, CH_3), 7·96 (q, CH_2·CH_2·CH_2), 7·12 (t, CH_2·S), 6·14 (t, CH_2·N), and 3·82 and 2·67 (both d, 5- and 6-H, J 7 Hz), $\lambda_{max.}$ (EtOH) 305 (ε 11,810), $\lambda_{min.}$ 248 (3850), $\lambda_{infl.}$ 226 (9627) and 280 nm (8792).

Reactions of 1-(3-*Acetylthiopropyl*)*uracil with Methanolic Ammonia and with Hydrochloric Acid.*—The thioacetate (50 mg) was treated with methanolic ammonia (5 ml; pre-saturated at 0°) for 2 h at room temperature. Four components were revealed by t.l.c. but repeated crystallization from water gave one product. *Perhydropyrimido*-[4,3-b][1,3]*thiazine*-6,8-*dione* (XIX) had m.p. 230—234° (Found: C, 45·0; H, 5·3; N, 15·0; S, 17·1%; M^+, 186.

$C_7H_{10}N_2O_2S$ requires C, 45·1; H, 5·4; N, 15·1; S, 17·2%; *M*, 186), τ (CF_3·CO_2D) 8·06 (q, CH_2·CH_2·CH_2), 7·19 (d, *J* 13 Hz, one of N·CH_2·CH_2), 6·70 and 6·50 (both d, *J* 8 Hz, J_{gem} 18 Hz), 5·22 (d, *J* 13 Hz, other N·CH_2·CH_2), and 4·94 (d, *J* 8 Hz).

The same product was obtained when the thioacetate (100 mg) was refluxed for 2 h with N-hydrochloric acid. It separated needles on cooling; more was obtained on removal of solvent (yield 75%).

Bis-3-(1,2,3,4-tetrahydro-2,4-dioxopyrimidin-1-yl)propyl Disulphide (X).—The cyclic sulphide (XIX) (50 mg) was dissolved in 0·1N-sodium hydroxide (10 ml) and left at room temperature for 24 h. Addition of N-hydrochloric acid precipitated the *disulphide*, m.p. 185° (decomp.) (from water), which gave a positive test for a disulphide (Found: C, 44·6; H, 4·7; N, 15·2; S, 17·0%; M^+, 370. $C_{14}H_{18}N_4O_4S_2$ requires C, 45·4; H, 4·9; N, 15·2; S, 17·3%; *M*, 370), τ (CF_3·CO_2D) 7·92 (q, CH_2·CH_2·CH_2), 7·40 (t, CH_2·S), 6·01 (t, CH_2·N), and 3·67 and 2·15 (both d, 5- and 6-H, *J* 8 Hz), $\lambda_{max.}$ (EtOH) 266 nm (ε 20,989).

Reaction of N(4)-Acetylcytosine with 1,2-Dibromoethane.—*N*(4)-Acetylcytosine (200 mg), potassium carbonate (180 mg), and 1,2-dibromoethane (2 ml) were stirred for 20 h at room temperature in dry dimethyl sulphoxide (20 ml). The solvent was removed under high vacuum and the residue taken up in water (20 ml). Extraction with chloroform gave no identifiable product so the aqueous solution was evaporated and the residue crystallized from ethyl acetate–ethanol. The mass spectrum showed a molecular ion at *m/e* 155; u.v. and t.l.c. showed the product to be 1-(2-hydroxyethyl)cytosine (IIb) (comparison with an authentic specimen). The procedure was repeated but no attempt was made to isolate the product. The mass spectrum of the semi-solid produced by evaporation of the solvent had a molecular ion at *m/e* 179 and a fragmentation pattern consistent with the structure of 7-acetylimino-2,3-dihydro-oxazolo[2,3-*b*]pyrimidine (XIV).

N(4)-Acetyl-1-(2-acetylthioethyl)cytosine (XIa).—Sodium hydride (63 mg; 50% dispersion in oil, pre-washed with petroleum) was suspended in dimethylformamide (10 ml), and thioacetic acid (0·2 ml) was added. After evolution of hydrogen had ceased, the crude mixture from the previous experiment [from *N*(4)-acetylcytosine (200 mg)] dissolved in dimethylformamide (10 ml) was added, and the whole was stirred at room temperature for 1 h. The orange solution was evaporated ***in vacuo*** giving an oil which was taken up in water and extracted with chloroform. The extracts were dried (Na_2SO_4), boiled with charcoal, and evaporated to give an orange gel which could not be crystallized, although t.l.c. indicated it to be one compound (Found: M^+, 255. $C_{10}H_{13}N_3O_3S$ requires *M*, 255), τ ($CDCl_3$) 7·70 and 7·73 (both s, CH_3·CO), 6·76 (t, CH_2·S), 6·00 (t, CH_2·N), and 2·58 and 2·38 (both d, 5- and 6-H, *J* 7 Hz), $\lambda_{max.}$ (EtOH) 216, 245, and 301 nm.

Bis-2-(4-amino-1,2-dihydro-2-oxopyrimidin-1-yl)ethyl Disulphide (XIIa).—The gel obtained in the foregoing reaction was treated with methanolic ammonia (pre-saturated at 0°) for 20 h at room temperature. Evaporation of the solvent and crystallization from water (charcoal) gave colourless *crystals*, m.p. 244—245° (decomp.) (Found: C, 39·9; H, 5·0; N, 23·4. $C_{12}H_{16}N_6O_2S_2,H_2O$ requires C, 40·2; H, 5·05; N, 23·4%), $\lambda_{max.}$ (0·1N-HCl) 283 (ε 22,956) and 214 (17,760), $\lambda_{min.}$ 242 nm (3179).

We thank Newnham College, Cambridge, for a Research Scholarship and the S.R.C. for a Research Studentship (to C. M. T.), and the M.R.C. for a grant.

[2/763 *Received, 5th April,* 1972]