Transfer RNA Components. Part I.† Direct Synthesis of Nucleosides of Cytosine and 2-Thiocytosine

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The direct synthesis of cytosine nucleosides results from the reaction of cytosine or *N*-acetylcytosine with a glycosyl halide in the presence of mercury(II) cyanide. The nature of the products varies with the solvent and the presence or absence of molecular sieve. With acetobromoglucose in toluene, cytosine gives a mixture of the *N*(1),*N*(3),*N*(6)-triglucoside (VI) and the *O*(2)-glucoside (V). In nitromethane, cytosine, *N*-acetylcytosine, and 2-thiocytosine give satisfactory yields of the corresponding *N*(3)-glycosides. A convenient synthesis and purification of 2',3',5'-tri-*O*-acetyl-2-thiocytidine (XI) is described.

We have shown previously that uracil reacts directly with acetobromoglucose (ABG) in the presence of mercury(II) cyanide to give the *N*(3)-glucoside and the *N*(1),*N*(3)-diglucoside, the latter compound being formed from the *N*(3)-glucoside *via* an *N*(3),*O*(6)-diglucoside intermediate.[1,2] Evidence was presented for the existence of two alternative mechanisms—an acid-catalysed reaction which is favoured by the use of a polar solvent

† This paper is to be considered as Part X of the series 'Nucleosides'; for Part IX, see ref. 1.

[1] G. T. Rogers and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1969, 2450.

[2] G. T. Rogers and T. L. V. Ulbricht, *Chem. Comm.*, 1965, 508.

(acetonitrile) and a mercury(II) bromide-catalysed reaction which takes place readily in a non-polar solvent (toluene). The method can be used to synthesise tri-*O*-acetyluridine directly from uracil in a yield of 54%.[1,2] Recently, Watanabe and Fox have briefly reported the direct synthesis of uracil nucleosides by use of mercury(II) cyanide,[3] without, however, establishing the mechanism of the reaction or isolating any products other than the *N*(3)-nucleosides.

In the present work the direct reaction of cytosine with ABG in various solvents, with mercury(II) cyanide as an acid acceptor has been investigated. Earlier workers [4] treated *N*(6)-benzoylcytosine with 2,3,4,6-tetra-*O*-acetylglucopyranosyl chloride in boiling nitromethane containing mercury(II) cyanide and obtained a good yield (46%) of the *N*(3)-β-glucoside; no other product was isolated. We have now shown that an essentially similar reaction takes place when *N*(6)-acetylcytosine (I) reacts with ABG in nitromethane containing an equivalent of mercury(II) cyanide; the *N*(3)-β-glucoside (58%) from *N*-acetylcytosine and ABG was obtained when the reaction was carried out in acetonitrile in the presence of molecular sieve, conditions which favour the stability of the labile product. The *O*(2)- and *N*(3)-glucosides produced in these reactions were identical to the *O*- and *N*-glucosides previously synthesised from the silver salt of *N*-acetylcytosine.[5]

On the basis of our earlier work on the rearrangement of *O*(2)-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-acetylcytosine [5] we concluded that it should be possible to rearrange the *O*(2)-glucoside formed from *N*-acetylcytosine in nitromethane prior to isolation, to afford an improved yield of the *N*(3)-glucoside. This was achieved by filtering the reaction mixture, adding toluene to the filtrate, boiling off the nitromethane, and treating with mercury(II) bromide; the *N*(3)-isomer was obtained as the major product (55%).

Nucleoside synthesis involving the use of free cytosine has not been previously studied in detail because of the poor yields and because unidentified products were obtained.[6] We have found that when cytosine (IV) is heated in toluene with mercury(II) cyanide and ABG two

TABLE 1

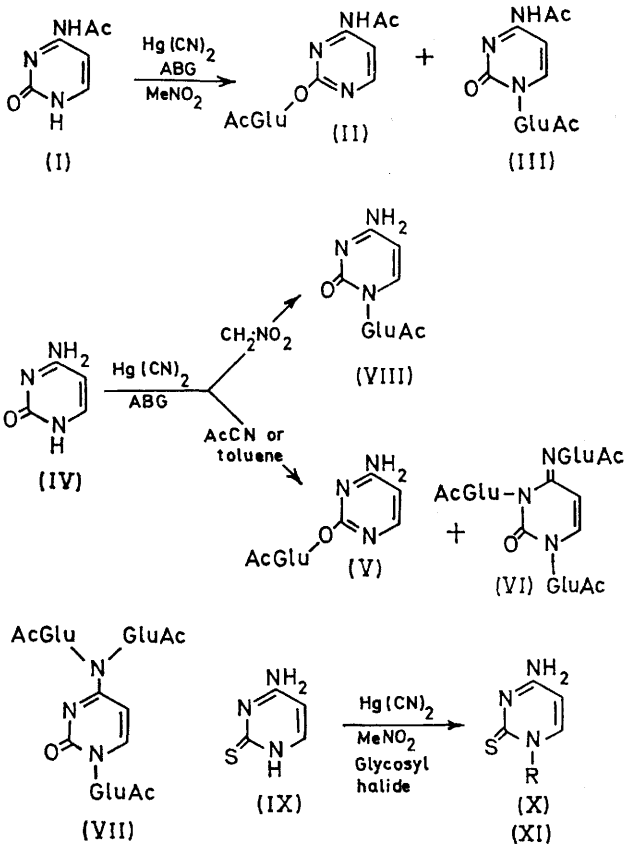
Reaction of *N*-acetylcytosine with acetobromoglucose

Solvent	Sieve	Time (min.)	Products (%) *O*(2)-Glucoside (II)	*N*(3)-Glucoside (III)
MeCN	+	60	58	5·5
MeCN	—	60	21	Trace
$MeNO_2$	—	150		34
$MeNO_2$	+	150	39	35
MeCN	—	150	2	Trace

TABLE 2

Reaction of cytosine with acetobromoglucose

Solvent	Sieve	Time (min.)	Equiv. ABG	Products (%) *O*(2)-Glucoside (V)	*N*(3)-Glucoside (VIII)	Triglucoside (VI)
MeCN	+	50	1·0	9·0		17
PhMe	+	50	1·0	13·5		15
PhMe	+	50	3·0	8·0		22
PhMe	—	50	1·0	7·5		14
$MeNO_2$	+	60	3·0	Trace	50	3
$MeNO_2$	+	60	1·0		12	



(X) R = 2,3,4,6-Tetra-*O*-acetylglucopyranosyl
(XI) R = 2,3,5-Tri-*O*-acetylribofuranosyl
GluAc = 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl

tetra-acetate (III, 34%), was the only product detected. When this reaction was repeated in the presence of molecular sieve, the *N*(3)-glucoside (III) was again formed in similar yield, and in addition the labile *O*(2)-glucoside (II) (39%) could be isolated. The latter product presumably survives in these conditions because of the rigorous exclusion of traces of acid and water. When the reaction is carried out under conditions known to favour acid-catalysed rearrangement, *i.e.* in acetonitrile in the absence of molecular sieve,[1] only a trace of the *N*(3)-glucoside is obtained, indicating that the *N*(3)-glucoside of *N*-acetylcytosine is unlikely to be formed by such a mechanism. The maximum yield of the *O*(2)-glucoside

[3] K. A. Watanabe and J. J. Fox, *J. Heterocyclic Chem.*, 1969, **6**, 109.
[4] N. Yamaoka, J. Aso, and K. Matsuda, *J. Org. Chem.*, 1965, **30**, 149.
[5] T. L. V. Ulbricht and G. T. Rogers, *J. Chem. Soc.*, 1965, 6125.
[6] J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Amer. Chem. Soc.*, 1957, **79**, 5060.

products are formed, which can be separated by preparative t.l.c. in ethyl acetate. The component of lower R_F value (7·5%) showed $\lambda_{max.}$ 270 mμ, reduced Fehling's solution, and yielded cytosine when heated with alkali; it was shown to be the *O*(2)-glucoside (V).[5]

The component of higher R_F value (14%), purified by t.l.c. in ether, did not reduce Fehling's solution, and microanalysis indicated that it was a triglucosylcytosine. This was confirmed by the i.r. spectrum (absence of an NH band and presence of carbonyl absorptions characteristic of an ester and a tertiary amide). Of the two possible structures, (VI) and (VII), the former was more likely on the basis of the u.v. spectrum [$\lambda_{max.}$ (pH 7·0) 223 and 285 mμ, $\lambda_{min.}$ 246 mμ; $\lambda_{max.}$ (pH 1·0) 288 mμ, $\lambda_{min.}$ 247 mμ]. These values are in close agreement with those quoted for *N*(1),*N*(3),*N*(6)-trimethylcytosine [7] [$\lambda_{max.}$ (EtOH) 222 and 285—286 mμ, $\lambda_{min.}$ 248; $\lambda_{max.}$ (0·1N-HCl) 287, $\lambda_{min.}$ 248 mμ]. On the other hand the trimethyl compound corresponding to the triglucoside (VII) has a u.v. spectrum which shows a characteristic shift of 8 mμ when the pH is lowered from 7·0 to 1·0; $\lambda_{max.}$ (EtOH) 282, $\lambda_{min.}$ 240; $\lambda_{max.}$ (pH 1·0) 221 and 290, $\lambda_{min.}$ 247 mμ; a similar shift is given by *N*(6)-dimethylcytidine.[8]

The structure of (VI) was confirmed by degradation with boiling aqueous formamide, conditions known to cause extensive deamination of cytidine.[9] Compounds (VI) and (VII) would be expected to give the deaminated products *N*(1),*N*(3)-diglucopyranosyluracil and *N*(3)-glucopyranosyluracil respectively. The degradation was followed by t.l.c. and shown to be complete in 3 hr., giving a product with the chromatographic properties of and a similar u.v. spectrum to *N*(1),*N*(3)-diglucopyranosyl uracil,[10] showing that the triglucoside was (VI).

When the reaction of cytosine with ABG and mercury(II) cyanide was carried out in the presence of molecular sieve, the same two products, (V) and (VI), were obtained in either toluene or acetonitrile. No *N*(3)-glucoside was detected, and in a separate experiment it was shown that it does not undergo reaction with ABG to give the triglucoside and, therefore, (VIII) is not an intermediate in the formation of (VI). Probably reaction initially occurs at the exocyclic amino-group; it is known that free amino-groups interfere in nucleoside synthesis.[5,11]

As expected, the yield of the triglucoside could be improved (22%) by using three equivalents of ABG. Attempts to isolate possible intermediates in the formation of the triglucoside by reducing either the temperature or the time of the reaction were unsuccessful; only traces of the *O*(2)- and tri-glucosides could be isolated.

As in the case of *N*-acetylcytosine, nitromethane proved to be the best solvent for preparing the *N*(3)-glucoside in good yield directly from cytosine. When the reaction was carried out with three equivalents of ABG in the presence of molecular sieve, *N*(3)-(tetra-*O*-acetylglucopyranosyl)cytosine (VIII) was isolated as the major product (50%) together with traces of the triglucoside and the *O*(2)-glucoside. When one equivalent of ABG was used the *N*(3)-glucoside was the only product that could be isolated, and the yield was reduced to 12%.

As an extension of this work we have successfully synthesised nucleosides of 2-thiocytosine (reported in a preliminary communication [2]). Nucleosides of thiopyrimidines have been shown to occur in the transfer RNA of certain bacteria [12,13] and studies of the synthesis of such thionucleosides have been prompted in order to make such compounds more readily available for biochemical and crystallographic studies.

Whereas 6-thiopyrimidine nucleosides are readily accessible from the corresponding 6-oxopyrimidine nucleosides by thiation with phosphorus pentasulphide,[8] the synthesis of 2-thiopyrimidine nucleosides has necessitated the use of elaborate routes [14,15] from uridine, or the mercury salt condensation.[16] In the latter method the thio-base must first be acetylated in order to yield a suitable mercury derivative, and the overall yield is low.

The results of our work with cytosine in nitromethane indicated that it should be possible to synthesise nucleosides of 2-thiocytosine directly, and we have prepared the glucosyl and ribosyl derivatives in this way. Treatment of 2-thiocytosine with ABG in nitromethane containing mercury(II) cyanide and molecular sieve gave a product which was shown to consist of two components by t.l.c. The main band was purified by preparative t.l.c. and shown to be *N*(3)-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thiocytosine (X) (40% yield), $\lambda_{max.}$ (pH 7·0) 252 and 274sh mμ, $\lambda_{max.}$ (pH 1·0) 233, 280, and 310sh mμ, characteristic for 2-thiocytosine *N*(3)-glycosides.[14,15] The structure of (X) was confirmed by methylation and subsequent alkaline hydrolysis [14] to *N*(3)-β-D-glucopyranosylcytosine, identical with the product obtained by deacetylation of compound (III) or (VIII). The minor component was not characterised.

A similar reaction between 2-thiocytosine and 2,3,5-triacetylribofuranosyl bromide [17] afforded 2′,3′,5′-tri-*O*-acetyl-2-thiocytidine in 29% yield after purification by t.l.c. The product had the expected u.v. spectrum, and methylation followed by alkaline hydrolysis gave cytidine, identical with authentic material. As an alternative to preparative t.l.c. (which is confined to small-scale experiments) the crude triacetyl-2-thiocytidine can be purified on a silica column (see Experimental section).

[7] G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 1955, 855.
[8] I. Wempen, R. Duschinsky, L. Kaplan, and J. L. Fox, *J. Amer. Chem. Soc.*, 1961, **83**, 4755.
[9] D. H. Hayes, *J. Chem. Soc.*, 1960, 1184.
[10] G. T. Rogers, R. S. Shadbolt, and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1969, 203.
[11] J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 1650.
[12] M. N. Lipsett, *J. Biol. Chem.*, 1965, **240**, 3975.
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[14] T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. and Pharm. Bull* (*Japan*), 1968, **16**, 1788.
[15] W. V. Ruyle and T. Y. Shen, *J. Medicin. Chem.*, 1967, **10**, 331.
[16] H. J. Lee and P. W. Wigler, *Biochemistry*, 1968, **7**, 1427.
[17] M. Winkley and R. K. Robins, *J. Org. Chem.*, 1968, **33**, 2822.

The synthesis and purification of 2-thiocytidine by the method described here should be suitable for large-scale preparations.

There are some marked differences between reactions in acetonitrile and in nitromethane. Although these two solvents have essentially similar dielectric constants the differences in their b.p.s is not negligible (20°). Moreover, nitromethane favours S_N1 reactions more than does acetonitrile, and also with nitromethane one may expect more nucleophilic solvent intervention.[18] It would require, however, a detailed investigation of the mechanism of the reactions described in the present paper to explain the observed differences more precisely.

EXPERIMENTAL

U.v. spectra, for solutions in 95% ethanol, were recorded with a Hilger-Watts Ultrascan and a Unicam SP 800 spectrophotometer. I.r. spectra, for solutions in methylene chloride, were obtained with a Perkin-Elmer 237 grating spectrophotometer. Preparative t.l.c. was carried out on Merck silica gel 254 as described earlier.[10] All m.p.s were determined with a Büchi apparatus. All solvents were redistilled before use. Molecular sieve refers to BDH powder type 3A. Column chromatography was carried out with Merck silica gel 0·2—0·5 mm.

Reaction of N(6)-*Acetylcytosine with Acetobromoglucose in Nitromethane.*—(a) *Without molecular sieve.* A mixture of *N*-acetylcytosine (306 mg.), mercury(II) cyanide (252 mg., 1 equiv.) and nitromethane (17 ml.) was heated to boiling, with stirring, and a little solvent was distilled off. Acetobromoglucose (ABG) (822 mg.) was then added and the heating was continued for 2·5 hr. The mixture was evaporated *in vacuo* and the residue was dissolved in chloroform (30 ml.), washed with potassium iodide solution (30%; 2 × 20 ml.), and water. The organic layer was separated, dried, and evaporated to a pale yellow foam, which was subjected to preparative t.l.c. in ethyl acetate to give one main band (R_F 0·2) corresponding to *N*(3)-tetra-*O*-acetyl-β-D-glucopyranosyl-*N*(6)-acetylcytosine (III). The product did not reduce Fehling's solution. Extraction and evaporation gave a pale yellow solid (330 mg., 34%) [m.p. 226—227° (from ethanol)], identical with the previously reported *N*-acetylcytosine *N*(3)-glucoside tetra-acetate [5,8] (Found: C, 49·4; H, 5·2; N, 8·5. Calc. for $C_{20}H_{25}N_3O_{11}$: C 49·7; H, 5·2; N, 8·6%), $\nu_{max.}$ 3375 and 3460 (NH), 1760 (ester Cu.c.), and 1720sh (CO amide) cm.$^{-1}$; $\lambda_{max.}$ (pH 7·0) 250 (ε 15,800) and 300 (4,800) mμ, $\lambda_{min.}$ 227 and 278 mμ.

(b) *With molecular sieve.* When the above experiment was repeated with the addition of molecular sieve (1·5 g.), two products were formed and separated by t.l.c. in ethyl acetate. The component with R_F 0·2 was shown to be the *N*(3)-glucoside (338 mg., 35%), identical with the product already described. The component R_F 0·8 was extracted and crystallised from methylene chloride–di-isopropyl ether, and shown to be *O*(2)-tetra-*O*-acetyl-β-D-glucopyranosyl-*N*(6)-acetylcytosine (II), m.p. 133° (378 mg., 39%). The product reduced Fehling's solution and was identical to the *O*-glucoside previously obtained in this laboratory (Found: C, 49·4; H, 5·3; N, 8·65. Calc. for $C_{20}H_{25}N_3O_{11}$: C, 49·7; H, 5·2; N, 8·6%), $\nu_{max.}$ 3400 (NH), 1760 (ester CO), and 1723 (amide CO) cm.$^{-1}$, $\lambda_{max.}$ (pH 7·0) 230 (ε 10,300) and 272 (12,400) mμ, $\lambda_{min.}$ 243 mμ.

(c) N(3)-(2′,3′,4′,6′-*Tetra-O-acetyl*-β-D-*glucopyranosyl*)-N(6)-*acetylcytosine* (III). *N*-Acetylcytosine (153 mg.) was heated under reflux for 2½ hr. with mercury(II) cyanide (126 mg., 1 equiv.) and molecular sieve (1 g.) in nitromethane (10 ml.) containing ABG (411 mg., 1 equiv.). The mixture was filtered and the solid was washed with hot nitromethane. Toluene (12 ml.) was added to the solution and the nitromethane was distilled off. Mercury(II) bromide (722 mg.) was added to the remaining toluene solution (9 ml.), and the mixture was heated under reflux for 20 min., dissolved in chloroform (40 ml.), washed with potassium iodide solution and water, and dried. On evaporation a white foam of the *N*(3)-glucoside (III) was obtained (265 mg., 55%). The product showed a single spot on t.l.c. in ethyl acetate, with $\lambda_{max.}$ 250 and 299 mμ. After crystallisation from ethanol it was identical with that already described.

Reaction of N(6)-*Acetylcytosine with Acetobromoglucose in Acetonitrile.*—(a) *Without molecular sieve.* *N*-Acetylcytosine (306 mg.) and mercury(II) cyanide (252 mg.) were dissolved in boiling acetonitrile (17 ml.). ABG (823 mg.) was added and the mixture was heated under reflux for 1 hr. The hot mixture was filtered, and worked up as in the previous experiment (a). Preparative t.l.c. in ethyl acetate showed a single band which was extracted to give a sticky solid (429 mg.). The product was purified by rechromatography in ethyl acetate (R_F 0·8) and again in ether to give pure *O*(2)-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*(6)-acetylcytosine (II) (201 mg., 21%). When this experiment was repeated with a longer reaction time (150 min.) only a trace of the *O*(2)-glucoside (II) (2·0%) could be isolated.

(b) *With molecular sieve.* When experiment (a) was repeated in the presence of molecular sieve (1·5 g.), the *O*(2)-glucoside (II) was formed as the major product (557 mg., 58%), accompanied by some of the *N*(3)-glucoside (III) (53 mg., 5%).

Reaction of Cytosine with ABG.—(a) *In toluene, without molecular sieve.* Cytosine (222 mg.) and mercury(II) cyanide (252 mg.) were suspended in toluene (17 ml.) and the mixture heated to boiling. ABG (822 mg.) was added and the heating was continued for 50 min. The mixture was filtered and worked up as already described. Preparative t.l.c. in ethyl acetate showed two bands, R_F 0·85 and 0·75. Each band was further purified by rechromatography in ether. The slower-running band was extracted and evaporated to give a pale yellow foam (66 mg.). The product reduced Fehling's solution and had $\lambda_{max.}$ 270 mμ, $\lambda_{min.}$ 248 mμ, identical to *O*(2)-(2′,3′,4′,6′-tetra-*O*-acetylglucopyranosyl)cytosine (V), previously prepared.[5] The faster-running band was similarly extracted and evaporated to leave a pale yellow solid (317 mg., 14%), and gave a *solid*, m.p. 157—158° (from 95% ethanol), $\nu_{max.}$ 1760 (ester CO) and 1730sh (tert. amide) cm.$^{-1}$ (no NH band) (Found: C, 49·2; H, 5·3; N, 4·0. $C_{46}H_{59}N_3O_{28}$,H_2O require sC, 49·3; H, 5·5; N, 3·8), $\lambda_{max.}$ (pH 1·0) 288 mμ, $\lambda_{max.}$ (pH 7·0) 223 and 285 mμ (ε 10,300), $\lambda_{min.}$ (pH 1·0) 247 mμ, $\lambda_{min.}$ (pH 7·0) 246 mμ, in close agreement with the spectrum of *N*(1),*N*(3),*N*(6)-trimethylcytosine,[7] indicating the product to be *N*(1),*N*(3)-bis-(2′,3′,4′,6′-tetra-*O*-acetylglucopyranosyl)6-(2′,3′,4′,6′-tetra-*O*-acetylglucopyranosylimino)-3,6-dihydro-2(1*H*)-pyrimidone (VI).

(b) *In the presence of molecular sieve.* When experiment (a) was repeated in the presence of molecular sieve (1 g.) the

[18] S. Winstein, B. Appel, R. Baker, and A. Diaz, *Chem. Soc. Special Pub.*, 1965, No. 19, p. 109.

crude product showed two bands on t.l.c. in ethyl acetate. The slower-running component was identical with the previously described *O*(2)-glucoside (V) (119 mg., 13·5%), crystallised from chloroform–di-isopropyl ether (90 mg.; m.p. 129—130°). The faster-running band (330 mg.) was the triglucoside (VI) (15·0%).

(c) *With acetonitrile as solvent.* When experiment (b) was repeated with acetonitrile (17 ml.), the *O*(2)-glucoside (V) and the glucoside (VI) were obtained (9·0 and 17%, respectively).

(d) *Repeat of Experiment* (*b*) *with ABG* (3 *equiv.*).—Cytosine (222 mg.) was condensed with acetobromoglucose (2·46 g.) as described in experiment (b). The crude foam was then dissolved in methylene chloride (10 ml.) and treated with di-isopropyl ether until complete precipitation had occurred. The solid (720 mg.) was filtered off, washed with di-isopropyl ether, and then subjected to multiple-elution preparative t.l.c. in chloroform. The major band was extracted (476 mg.) and shown to be the triglucoside (VI) (22%). It was crystallised from ethanol to give a product identical with that already described. The filtrate was evaporated to give a foam (1·1 g.), which when subjected to repeated preparative t.l.c. in chloroform gave a main band of the *O*(2)-glucoside (V) (76 mg., 8·6%). This fraction also contained some unidentified products.

Deamination of the Triglucoside (VI).—The triglucoside (VI) (20 mg.) and 50% aqueous formamide (10 ml.) were heated to boiling; the reaction was followed at 15 minute intervals by t.l.c. in water. It was complete in 3 hr. The u.v. spectrum of the product showed $\lambda_{max.}$ (pH 1·0) 264 mμ, $\lambda_{max.}$ (pH 13·0) 265 mμ, similar to the spectrum of *N*(1),*N*(3)-diglucopyranosyluracil.[10] After removal of formamide the product had R_F 0·75 (t.l.c. in water), identical with *N*(1), *N*(3)-diglucopyranosyluracil run concurrently.

Reaction of Cytosine with ABG in Nitromethane.—(a) *With ABG* (3 *equiv.*). Cytosine (222 mg.), mercury(II) cyanide (756 mg.), molecular sieve (2 g.), and nitromethane (17 ml.) were heated to boiling and about 1 ml. of solvent was distilled off. ABG (2·46 g.) was added and the heating was continued for 1 hr. The yellow suspension was filtered and the solid was washed with nitromethane. The filtrate was evaporated, chloroform (20 ml.) was added, and the mixture was washed with potassium iodide solution and water, and dried. The solution was evaporated and left *in vacuo* to give a yellow solid (2·5 g.). Methylene chloride (10 ml.) was added and an excess of di-isopropyl ether until precipitation was complete. The solid (1 g.) was subjected to multiple preparative t.l.c. in chloroform. Three major bands were obtained. A band at the origin (106 mg.) was extracted and showed $\lambda_{max.}$ 259 and 306 mμ—this was not investigated further. The middle band (72 mg.) was extracted and shown to be the triglucoside (VI) (3·0%). The third band (440 mg.) was shown to be *N*(3)-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)cytosine (VIII) (50%), m.p. 134—135° (from aqueous ethanol), $\lambda_{max.}$ 3310 and 3460 (NH_2), 1760 (ester CO), and 1720 (amide CO) cm.$^{-1}$, $\lambda_{max.}$ (pH 1·0) 276 mμ, $\lambda_{max.}$ (pH 7·0) 269 mμ (ε 9200), $\lambda_{min.}$ 244 mμ (Found: C, 49·2; H, 5·1; N, 9·2%. Calc. for $C_{18}H_{23}N_3O_{10}$: C, 49·0; H, 5·2; N, 9·5%). No other products were obtained from the filtrate.

(b) *With ABG* (1 *equiv.*). When experiment (a) was repeated with ABG (1 equiv.), only the *N*(3)-glucoside (VIII) could be isolated (150 mg., 12%).

N(3)-(2′,3′,4′,6′-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*thiocytosine* (X).—2-Thiocytosine (IX) (254 mg.), mercury(II) cyanide (504 mg.), molecular sieve (1 g.), and nitromethane (17 ml.) were heated to boiling with stirring. ABG (1·64 g., 2 equiv.) was then added and the heating was continued for 1 hr. The mixture was filtered and evaporated under reduced pressure. Chloroform (20 ml.) was added to the residue, which was then washed with potassium iodide solution (30%; 2 × 20 ml.) and water, dried ($MgSO_4$), and evaporated (1·35 g.). A portion of the product (500 mg.) was purified by preparative t.l.c. in ethyl acetate to give a major band (136 mg., 40%) of the *N*(3)-glucoside (X), which crystallised as needles from aqueous ethanol, m.p. 154—155° (Found: C, 45·6; H, 5·0; N, 8·6. $C_{18}H_{23}N_3O_9S,H_2O$ requires C, 45·5; H, 5·3; N, 8·8%), $\nu_{max.}$ 3400 and 3470 (NH_2) and 1765 (ester CO) cm.$^{-1}$, $\lambda_{max.}$ (pH 1·0) 233, 280, and 310sh mμ, $\lambda_{max.}$ (pH 7·0) 252 (ε 17,400) and 274 mμ, $\lambda_{min.}$ (pH 1·0) 2·57 mμ, $\lambda_{min.}$ (pH 7·0) 223 mμ, which is in close agreement with the published u.v. spectra for 2-thiocytidine.[14,15]

N(3)-(2′,3′,5′-*Tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2-*thiocytosine* (XI).—2′,3′,5′-Tri-*O*-acetylribofuranosyl bromide, prepared from tetra-*O*-acetylribose (5 g.),[17] was added to a mixture of 2-thiocytosine (1·0 g.), mercury(II) cyanide (1·98 g.), molecular sieve (4 g.), and nitromethane (45 ml.), and the suspension was heated under reflux for 1 hr. The mixture was filtered, evaporated under reduced pressure, dissolved in chloroform (40 ml.), and washed with potassium iodide solution (30%; 2 × 20 ml.) and water. The dried organic phase was evaporated to leave a pale yellow viscous foam (5·6 g.). This product can be purified (*a*) by preparative t.l.c. in ethyl acetate or (*b*) by column chromatography on silica gel.

(*a*) The product (2·8 g.) gave a single band (R_F 0·4) on preparative t.l.c. and was extracted with ethyl acetate to give pure 2′,3′,5′-tri-*O*-acetyl-2-thiocytidine (XI) (420 mg., 28%). The product crystallised from 20% ethanol–water, as a hemihydrate, m.p. 139—140° (Found: C, 45·9; H, 4·9; N, 10·4. $C_{15}H_{18}N_3O_7S,0{\cdot}5H_2O$ requires C, 45·7; H, 5·1; N, 10·7%), $\nu_{max.}$ 3380 and 3450 (NH_2) and 1750 (ester CO) cm.$^{-1}$, $\lambda_{max.}$ (pH 1·0) 232, 279, and 310sh mμ, $\lambda_{max.}$ (pH 7·0) 250 (ε 18,000) and 270sh mμ, $\lambda_{min.}$ (pH 1·0) 253 mμ, $\lambda_{min.}$ (pH 7·0) 223 mμ.

(*b*) The crude product (2·8 g.) was dissolved in chloroform and put on a column of silica gel (20 g.) previously washed with chloroform. Elution with ether–benzene (1 : 1) (300 ml.) removed unchanged sugar. 2-Thiocytidine was recovered from the column with ethyl acetate (250 ml.); evaporation gave pure (XI) (426 mg., 29%).

Methylation and Hydrolysis of 2-*Thiocytosine Glycosides* (X) *and* (XI).—The glycoside (*ca.* 10 mg.) and an excess of methyl iodide in absolute methanol (3 ml.) were heated at 60° for 3 hr. The solvent was removed and the residue was dissolved in 2N-sodium hydroxide (2 ml.) and neutralised with BioRad cation exchange resin (AG 50W — X2). The 2-thiocytosine glucoside (X) gave a product which showed $\lambda_{max.}$ (pH 7·0 and 13·0) 270 mμ, $\lambda_{max.}$ (pH 1·0) 278 mμ, characteristic of *N*(3)-glucopyranosylcytosine, and which ran concurrently with a sample of deacetylated *N*(3)-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)cytosine (VIII), on t.l.c. in water (R_F 0·85). The riboside (XI) gave a product identical with cytidine.

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