Nucleosides. Part VII.[1] The Reaction of Metal Salts of Thymine and Uracil with Tetra-acetyl-α-glucopyranosyl Bromide

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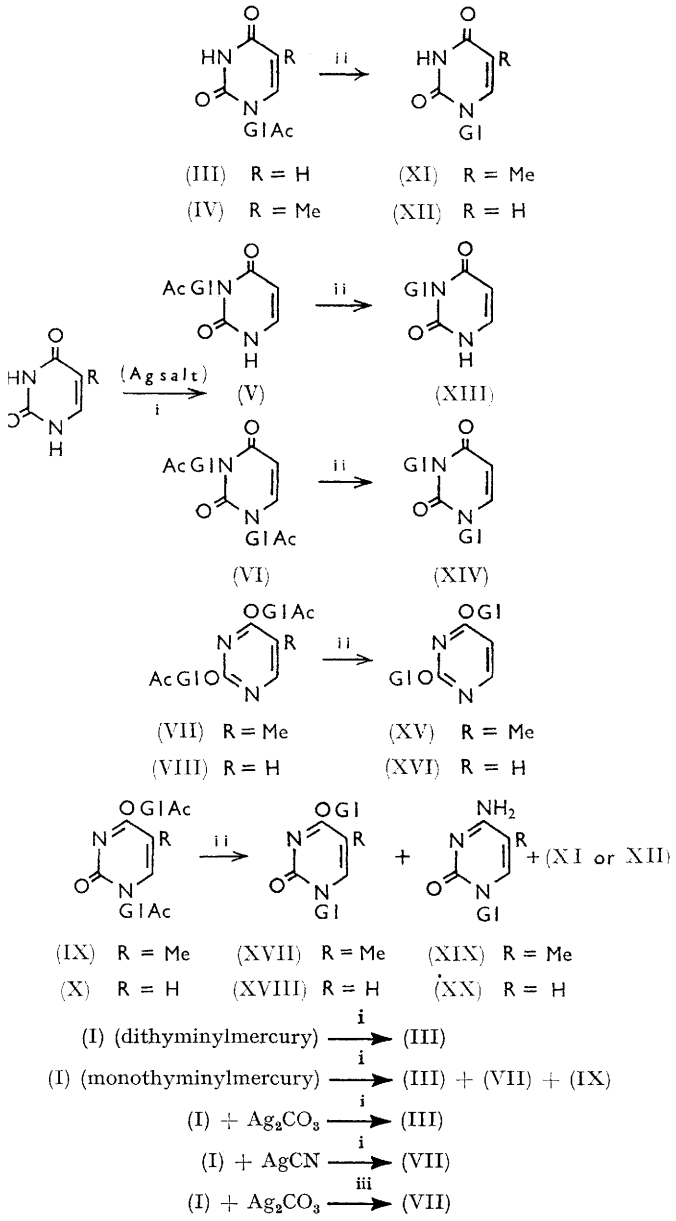
The formation of glucosides from the reaction of the silver salts of uracil and thymine with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide has been studied. Both thymine and uracil yield the acetylated *N*(3)-glucoside and *N*(3)*O*(6)-bisglucoside, and the *O*(2)*O*(6)-bisglucoside. In addition, uracil yields the acetylated *N*(1)-glucoside and *N*(1)*N*(3)-bisglucoside. Comparison with the products obtained from dithyminylmercury and monothyminylmercury is made.

EARLY workers attempting to synthesise pyrimidine nucleosides from metal salts of tautomeric bases containing the NH·CO group obtained products which were unstable in alkali and appeared to be *O*-glycosides.[2,3] The first successful synthesis of pyrimidine nucleosides, including the glucosides of thymine (XI) and uracil (XII), was accomplished by a different route.[4]

Later it was shown that thymine *N*-ribosides and

[1] Part VI, G. T. Rogers and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1968, 1929.
[2] E. Fischer and B. Helferich, *Ber.*, 1914, **47**, 1377.
[3] P. A. Levene and H. Sabotka, *J. Biol. Chem.*, 1925, **65**, 469.
[4] G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 4459.

-glucosides could be prepared by treating dithyminylmercury with the appropriate glycosyl halide.[5] However, when deoxyribosyl halides were used in this reaction



(I) (dithyminylmercury) —i→ (III)

(I) (monothyminylmercury) —i→ (III) + (VII) + (IX)

(I) + Ag_2CO_3 —i→ (III)

(I) + AgCN —i→ (VII)

(I) + Ag_2CO_3 —iii→ (VII)

Reagents: i, ABG–toluene at 110°; ii, NH_3–MeOH; iii, ABG–1,2-dimethoxyethane–molecular sieve.

AcGl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl; Gl = β-D-glucopyranosyl.

the products appeared to be *O*-deoxyribosides;[6,7] to obtain *N*-deoxyribosides it is necessary to use monothyminylmercury,[8] or, in the case of an amino-sugar, the silyl derivative of thymine.[7]

It was suggested[9] that in some cases pyrimidine nucleosides were obtained from pyrimidine mercury salts by prior formation of an *O*-glycoside, followed by a rearrangement catalysed by the mercuric halides formed in the reaction. This has been confirmed experimentally;[10–13] the failure of deoxyribosyl halides to yield *N*-glycosides with certain pyrimidine salts appears to be related to the instability of *O*-deoxyribosides, which are cleaved under rearrangement conditions.[13] The synthesis of nucleosides and related compounds, and *O*→*N* glycosyl rearrangements in derivatives of tautomeric heterocycles, have been extensively studied.[14,15]

Recently, the silver salt of uracil (II) has been reported to yield *N*(3)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil (IV) when treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (ABG) in xylene,[16] and treatment of thymine (I) with ABG in the presence of silver carbonate and a molecular sieve has been found to give *O*(2)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-*N*-glucopyranosyl)thymine[17] (VII). Since the silver salt of *N*-acetylcytosine gave a mixture of *O*- and *N*-glucosides with ABG in toluene,[12,18] the reaction of thymine (I) and uracil (II) silver salts under similar conditions seemed to merit further investigation.[19]

When a solution of silver nitrate was added to a solution of thymine (I) in ammonia or in sodium hydroxide, a solid was obtained containing *ca.* one and a half atoms of silver per molecule of thymine. This silver derivative, on treatment with ABG in toluene, gave a mixture of three compounds which were separated by preparative t.l.c. The first product was *N*(3)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thymine (III), which has previously been prepared from dithyminylmercury[5] and from the silyl derivative of thymine and ABG.[20,21] Its ^{1}H n.m.r. spectrum confirmed that it has the β-configuration;[22] the anomeric proton gave rise to a doublet at τ 4·10 ($J_{1,2}$ 9·0 c./sec.).[23] The product of deacetylation was stable to further treatment with alkali.

[5] J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Amer. Chem. Soc.*, 1956, **78**, 2117.
[6] M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777.
[7] M. L. Wolfrom and H. B. Bhat, *J. Org. Chem.*, 1967, **32**, 2757.
[8] M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Amer. Chem. Soc.*, 1959, **81**, 4112.
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[12] T. L. V. Ulbricht and G. T. Rogers, *J. Chem. Soc.*, 1965, 6125.
[13] T. L. V. Ulbricht and G. T. Rogers, *J. Chem. Soc.*, 1965, 1930.
[14] G. Wagner, *Z. Chem.*, 1966, **6**, 367.
[15] K. S. Kirby and T. L. V. Ulbricht, *Ann. Reports*, 1966, **63**, 537.
[16] I. A. Mikhailopulo, V. I. Gunar, and S. I. Zav'yabov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 470.
[17] G. Schmidt and J. Farkas, *Coll. Czech. Chem. Comm.*, 1966, **31**, 4442.
[18] David Thacker and T. L. V. Ulbricht, *Chem. Comm.*, 1967, 122; *J. Chem. Soc.* (*C*), 1968, 333.
[19] Preliminary communication, G. T. Rogers, R. S. Shadbolt, and T. L. V. Ulbricht, *Chem. Comm.*, 1968, 315.
[20] T. Nishimura and I. Iwai, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 357.
[21] E. Wittenburg, *Z. Chem.*, 1964, **4**, 303; *Chem. Ber.*, 1968, **101**, 1095.
[22] L. D. Hall, *Adv. Carbohydrate Chem.*, 1964, **19**, 51.
[23] T. Nishimura and B. Shimizu, *Agric. and Biol. Chem.* (*Japan*), 1964, **28**, 224.

The second product was the previously mentioned *O*(2)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine [17] (VII). Its ^{1}H n.m.r. spectrum showed the anomeric proton signals as a symmetrical quartet, two overlapping doublets centred at τ 3·89 and 3·96 ($J_{1,2}$ in each case 7·5 c./sec.), indicating the product to have the ββ-configuration.[22]

The third product had the correct analysis for a bisglucoside and had an i.r. spectrum which, like that of the *N*(3)-glucoside (III), showed carbonyl absorptions characteristic of an amide and an ester, but which did not contain an NH band, and reduced Fehling's solution even more readily than did the *O*(2)*O*(6)-bisglucoside (VII). When this compound was degraded with 0·2N-sodium hydroxide, *N*(3)-β-D-glucopyranosylthymine (XI) was the sole product, indicating that one sugar residue was attached at N-3. Reaction with methanolic ammonia gave three products; two of these had u.v. spectra corresponding to the *N*(3)-glucoside (XI) and a 5-methylcytosine *N*(3)-glycoside.[24] These results are consistent with the third product having the structure *N*(3)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine (IX), which would be expected to yield N^3-β-D-glucopyranosyl-5-methylcytosine (XIX) when treated with ammonia, since *N*(3)-β-D-glucopyranosylcytosine has been similarly prepared from 6-ethoxy-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)pyrimidin-2(3*H*)-one in the Hilbert-Johnson synthesis.[25]

The reaction of the *N*(3)*O*(6)-bisglucoside (IX) with ammonia also gave a third substance, with u.v. spectra resembling those of 6-ethoxy-3-methylpyrimidin-2(1*H*)-one; [26] after treatment with aqueous alkali (pH 13·0) for 2 hr. the solution gave a spectrum resembling that of *N*(3)-β-D-glucopyranosylthymine [24] (XI), indicating that the initial product was the deacetylated derivative, *N*(3)*O*(6)-bis-(β-D-glucopyranosyl)thymine (XVII). In the ^{1}H n.m.r. spectrum of the acetylated *N*(3)*O*(6)-bisglucoside (IX) the anomeric protons gave rise to a pair of overlapping doublets at τ 3·82 and 3·9 ($J_{1,2}$ 8·0 and 9·0 c./sec., respectively), confirming that the compound has the ββ-configuration.[22]

Uracil (II) also gave a silver derivative with an indefinite composition; this with ABG in toluene yielded five products (separated by preparative t.l.c.), of which four are new compounds. Three products corresponded to those formed in the case of thymine (I), that is, *N*(3)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-uracil (IV), *O*(2)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil (VIII), and *N*(3)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil (X). The *N*(3)-glucoside (IV) had previously been obtained as the sole product from the reaction of the silver salt [16] or the silyl derivative of uracil and ABG,[20,21] and by acetylation of *N*(3)-β-D-glucopyranosyluracil (XII) (from the Hilbert-Johnson reaction).[4] On deacetylation it gave a single product with an unchanged u.v. spectrum, which was stable to further treatment with dilute alkali. The ^{1}H n.m.r. spectrum of the *N*(3)-glucoside (IV) showed the anomeric proton signal as a doublet at τ 4·04 ($J_{1,2}$ 8·0 c./sec.), confirming the β-configuration. The i.r. spectrum of the *O*(2)*O*(6)-bisglucoside (VIII) showed carbonyl absorptions characteristic of an ester but it did not contain an NH or CO amide band. Its ^{1}H n.m.r. spectrum revealed the anomeric protons signals as a pair of doublets with centres at τ 4·0 and 4·3 ($J_{1,2}$ 7·5 c./sec. in both cases), confirming the ββ-configuration. The structure of the *N*(3)*O*(6)-bisglucoside (X) was proved in a manner similar to that of the corresponding thymine compound; on treatment with ammonia two products were obtained with u.v. spectra corresponding to *N*(3)-β-D-glucopyranosyluracil and *N*(3)-β-D-glucopyranosylcytosine; a third product had properties expected for *N*(3)*O*(6)-bis-(β-D-glucopyranosyl)uracil (XVIII). The anomeric protons gave rise to a pair of overlapping doublets with centres at τ 3·92 and 3·94 ($J_{1,2}$ 7·5 and 8·0 c./sec. respectively).

The fourth product obtained from the silver salt of uracil was a monoglucoside with u.v. spectrum resembling that of 1-methyluracil,[26] unchanged after deacetylation and heating with dilute alkali. The product was therefore *N*(1)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil (V), assigned the β-configuration on the basis of the ^{1}H n.m.r. spectrum, which showed the anomeric proton signal as a doublet with centre at τ 4·29 ($J_{1,2}$ 7·5 c./sec.). Previously the only method of synthesis of (V) involved cyclisation of the corresponding glycosylurea.[27] The fifth product had the correct analysis for a bisglucoside and showed i.r. carbonyl absorptions corresponding to an amide and an ester but no NH band. Its u.v. spectrum resembled that of 1,3-dimethyluracil,[26] and was unchanged by deacetylation with methanolic ammonia. However, the deacetylated product was degraded when heated with 0·2N-sodium hydroxide at 100° for 10 min. 1,3-Dimethyluracil is also unstable in alkali [26] and it was concluded that the product was *N*(1)*N*(3)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil (VI). The ^{1}H n.m.r. spectrum revealed the anomeric protons as a pair of overlapping doublets with centres at τ 4·06 and 4·23 ($J_{1,2}$ 9·0 and 8·0 c./sec. respectively).

The bis-*O*-glucosides (VII) and (VIII) on treatment with ammonia gave single products with u.v. spectra resembling those of 2,6-dimethoxy-5-methylpyrimidine [17] and 2,6-diethoxypyrimidine,[26] indicating that they were *O*(2)*O*(6)-bis-(β-D-glucopyranosyl)thymine (XV) and *O*(2)*O*(6)-bis-(β-D-glucopyranosyl)uracil (XVI); they were hydrolysed completely when heated with 0·2N-sodium hydroxide at 100° for 10 min. to give thymine and uracil respectively.

The formation of thymine glucosides from mercury

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[25] G. E. Hilbert and E. F. Jansen, *J. Amer. Chem. Soc.*, 1936, **58**, 60.

[26] D. Shugar and J. J. Fox, *Biochem. Biophys. Acta*, 1952, **9**, 199.

[27] Japan. Pat. 2878 (*Chem. Abs.*, 1964, **60**, 15,976).

salts was also studied. The reaction of dithyminylmercury with ABG in toluene [5] gave (88%) the *N*(3)-glucoside (III), with only traces of other products. In contrast, monothyminylmercury [8] under similar conditions gave a mixture of the *N*(3)-glucoside (III), the *O*(2)*O*(6)-bisglucoside (VII), and the *N*(3)*O*(6)-bisglucoside (IX). The *O*(2)*O*(6)-bisglucoside (VII) is known to be the major product when thymine (I) is treated with ABG in ethylene glycol ethyl methyl ether in the presence of silver carbonate and a molecular sieve.[17] When this reaction was carried out in toluene and the molecular sieve omitted, the sole product was the *N*(3)-glucoside (III), and the yield (46%) was the same whether one or two equivalents of ABG were used. Replacement of silver carbonate with silver cyanide resulted in the formation of the *O*(2)*O*(6)-bisglucoside (VII) (9·0%).

It appears, therefore, that in reactions involving displacement of hydrogen atoms in thymine by glycosyl residues in the presence of acid acceptors, it is the *O*(2)*O*(6)-bisglucoside which is formed. When molecular sieve is omitted, *i.e.*, when traces of water and acid are not rigorously excluded, the *N*(3)-glucoside is obtained instead, probably by an acid-catalysed rearrangement of the bis-*O*-glucoside.[28]

The formation of a mixture of products from monothyminylmercury may be due to the fact that insufficient bromide ions are liberated in this reaction to convert all the mercury into mercuric bromide, since the mercuric bromide-catalysed *O*→*N*-glycosyl rearrangement is known to be concentration dependent.[12,13]

Experiments on the rearrangement of uracil and thymine *O*-glucosides are presented in the following paper, and the mode of formation of the products obtained in the present work is discussed more fully. The striking difference between the reactions of uracil and of thymine is noteworthy. It does not depend on the mode of preparation of the silver salts, since thymine yields the same three products, in very similar yields, whether the salt is prepared in ammonia or in sodium hydroxide. In the absence of a 5-methyl group, the reactivities of the two nitrogen atoms are apparently, therefore almost the same.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was carried out on silica gel (Merck GF_{254}); preparative t.l.c. was performed with plates (40 × 20 cm.) carrying silica layers 2 mm. thick, with a loading of *ca.* 400 mg. per plate, in the following solvent systems: 1% ethanol–chloroform (A), ether (B), ethyl acetate (C), n-butanol saturated with water (D), and n-butanol–ethanol–water in an atmosphere of ammonia (9 : 1 : 2) (E). Preparative thin-layer chromatograms were subjected to multiple developments (generally 4—6) until the maximum separation of bands was obtained. Paper chromatography (descending) was carried out with Whatman no. 1 paper in n-butanol saturated with water (F) or n-butanol–ethanol–water in an atmosphere of ammonia (9 : 1 : 2) (G). Compounds were detected by irradiation with a short-wave u.v. lamp. When compounds were extracted from analytical chromatograms $\lambda_{max.}$ and $\lambda_{min.}$ values were determined for solutions in 50% ethanol with a Hilger and Watts Ultrascan. Values of $\lambda_{max.}$ and $\lambda_{min.}$ under acid or alkaline conditions vary with time for the *O*-glucosides, owing to hydrolysis; small variations in $\lambda_{max.}$ and $\lambda_{min.}$ for acetylated glucosides occur under acid or alkaline conditions for the same reason. Molar extinction coefficients (given in parentheses after $\lambda_{max.}$ or $\lambda_{min.}$) were determined at *ca.* 6 mg./100 ml. for solutions in 95% ethanol with a Hilger and Watts Uvispek. I.r. spectra were determined for solutions in methylene chloride with a Perkin-Elmer 237 instrument, and ^{1}H n.m.r. spectra were determined with a Varian A60 or HA 100 for solutions in deuteriochloroform with tetramethylsilane as internal standard. Dithyminyl mercury [5] and monothyminyl mercury [8] were prepared by literature methods. Yields of glucosides obtained from uracil or thymine metal derivatives are overall yields based on uracil or thymine, and take no account of the amount of ABG used.

Reaction of the Silver Derivative of Thymine with ABG.—Thymine (2·52 g.) was suspended in water (40 ml.) at 60° and concentrated ammonia (50 ml.) was added until a clear solution was obtained. A solution of silver nitrate (3·4 g.) in water (40 ml.) was added, and the solution was heated to boiling; air was bubbled through until the excess of ammonia had been removed. The solution was cooled and the solid was filtered off, washed with water, ethanol, and ether, and dried at 70°/20 mm. (3·56 g.) (Found: Ag, 53·6; N, 8·9. $C_5H_{4.5}Ag_{1.5}N_2O_2$ requires Ag, 54·8; N, 10·1%). A similar product was obtained when the reaction was carried out in sodium hydroxide (Found: Ag, 54·8; N, 8·7%).

The silver salt (3·56 g.) and toluene (60 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (6·28 g.) was added to the hot suspension, which was stirred and heated under reflux for 30 min. The mixture was filtered. The residue was washed with ethyl acetate, and the combined filtrates were evaporated to give a syrup (6·34 g., 70%). A portion (1·93 g.) was then subjected to preparative t.l.c. in solvent (B) to give two major bands. The slower-running band was extracted to give a syrup (433 mg.), which was purified by preparative t.l.c. in solvent (A), and then again in solvent (B) to give, after extraction, a syrup (328 mg., 7%), which yielded N(3)O(6)-*bis*-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*thymine* (IX) (242 mg.), m.p. 128—131° (from methylene dichloride–di-isopropyl ether) (Found: C, 50·0; H, 5·4; N, 3·4. $C_{33}H_{42}N_2O_{20}$ requires C, 50·4; H, 5·4; N, 3·55%), $\nu_{max.}$ 1760 (CO ester) and 1685 (CO amide) cm.$^{-1}$, $\lambda_{max.}$ 290 mμ (6510), $\lambda_{min.}$ 243 mμ (1530), $\lambda_{max.}$ (pH 13) 280 mμ, $\lambda_{min.}$ 247 mμ.

The faster-running band was extracted to give a syrup (1·313 g.) which was subjected to preparative t.l.c. in solvent (A) to give two major bands. The slower-running of these was extracted to give a syrup (385 mg., 14%) which gave *N*(3)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thymine hemihydrate (III) (163 mg.), m.p. 154—155° (from methanol) (lit.,[21] 156—158°) (Found: C, 48·8; H, 5·2; N, 5·9. Calc. for $C_{19}H_{24}N_2O_{11},0{\cdot}5H_2O$: C, 49·0; H, 5·4; N, 6·0%), $\nu_{max.}$ 3380 (NH), 1765 (CO of ester), and 1695 (CO of amide) cm.$^{-1}$, $\lambda_{max.}$ 261·5 mμ (10,290), $\lambda_{min.}$ 232 mμ (3500), $\lambda_{max.}$ (pH 13) 265 mμ, $\lambda_{min.}$ 245 mμ [lit.,[5,20] $\lambda_{max.}$ 262·5 mμ, $\lambda_{min.}$ 232·5 mμ, $\lambda_{max.}$ 261 mμ (10,050)].

The faster-running band was extracted to give a syrup

[28] G. Schmidt and J. Farkas, *Tetrahedron Letters*, 1967, 4251.

(894 mg., 19%) which was crystallized twice from ethyl acetate–light petroleum (b.p. 60—80°) to give *O*(2)*O*(6)-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thymine (VII) (415 mg.), m.p. 166—167° (lit.,[17] 171·5°) (Found: C, 50·5; H, 5·3; N, 3·6. Calc. for $C_{33}H_{42}N_2O_{20}$; C, 50·4; H, 5·4; N, 3·55%), $\nu_{max.}$ 1760 cm.$^{-1}$ (ester), $\lambda_{max.}$ 263·5 mμ (6790), $\lambda_{min.}$ 235·5 mμ (1550), $\lambda_{max.}$ (pH 13·0) 265 mμ, $\lambda_{min.}$ 238 mμ (lit.[17] $\lambda_{max.}$ 261 mμ, $\lambda_{min.}$ 235 mμ).

Compounds (IX), (III), and (VII) had R_F values [t.l.c.] 0·12, 0·14, and 0·31 [solvent (A)], 0·10, 0·47, and 0·47 [solvent (B)], and 0·80, 0·78, and 0·88 [solvent (C)].

The same three products were obtained, in similar yields, when the thymine silver salt was prepared in sodium hydroxide.

Reaction of the Silver Derivative of Uracil with ABG.—A solution of silver nitrate (7·59 g.) in water was added to a solution of uracil (5 g.) in water (400 ml) containing N-sodium hydroxide (44·65 ml.). Celite filter aid (1 g.) was added, and the gelatinous suspension was stirred at 60° for 1 hr in the absence of light The precipitate was filtered off, washed with water, ethanol, and ether, and dried at 70°/20 mm (6·75 g.).

The silver salt (6·75 g.) and toluene (70 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (12·68 g.) was added to the hot suspension which was stirred and heated under reflux for 20 min. The mixture was filtered, the residue was washed with chloroform, and the combined filtrates were added to light petroleum to give a white solid (11·15 g., 56%). A portion of this product (5·9 g.) was then subjected to preparative t.l.c. in solvent (B) to give three major bands.

The slowest-running band was extracted, rechromatographed in solvent (A), and extracted to give a solid (427 mg., 40%) which gave N(1)-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*uracil hemihydrate* (V) (250 mg.), m.p. 110—111° (from di-isopropyl ether) (Found: C, 48·1; H, 5·0; N, 5·9. $C_{18}H_{22}N_2O_{11},0{\cdot}5H_2O$ requires C, 47·9; H, 5·1; N, 6·2%), $\nu_{max.}$ 3420 (NH), 1760 (CO of ester), and 1680 (CO of amide) cm.$^{-1}$, $\lambda_{max.}$ 264 mμ (9400), $\lambda_{min.}$ 231 mμ (1760), $\lambda_{max.}$ (pH 1·0) 264 mμ, $\lambda_{min.}$ 231 mμ, $\lambda_{max.}$ (pH 13·0) 293 mμ, $\lambda_{min.}$ 246 mμ.

The middle band was extracted to give a syrup (1·32 g.), purified by preparative t.l.c. in solvent (A); the major band was extracted to give a solid (1·22 g., 11·0%) which gave *N*(3)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)uracil hemihydrate (IV) (970 mg., 7·0%), m.p. 152—153° (from 95% ethanol) (lit.,[4] 154—155°) (Found: C, 47·9; H, 4·9; N, 6·2. Calc. for $C_{18}H_{22}N_2O_{11},0{\cdot}5H_2O$: C, 47·9; H, 5·1; N, 6·2%), $\nu_{max.}$ 3380 (NH), 1760 (CO of ester), and 1700 (CO of amide) cm.$^{-1}$, $\lambda_{max.}$ 260 mμ (9350), $\lambda_{min.}$ 230 mμ (2510), $\lambda_{max.}$ (pH 1) 260 mμ, $\lambda_{min.}$ 230 mμ, $\lambda_{max.}$ (pH 13) 260 mμ, $\lambda_{min.}$ 245 mμ.

The fastest-running band was extracted to give a solid (3·45 g.) which was extracted with boiling di-isopropyl ether (17 × 100 ml.). The insoluble material (900 mg., 4·9%) gave N(3)O(6)-*bis*-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*uracil* (X) (600 mg.), m.p. 267—268·5° [from methanol (450 ml.)] (Found: C, 49·8; H, 5·2; N, 3·6. $C_{32}H_{40}N_2O_{20}$ requires C, 49·7; H, 5·2; N, 3·6%), $\nu_{max.}$ 1760 (CO of ester) and 1685 (CO of amide) cm.$^{-1}$, $\lambda_{max.}$ 280 mμ (6560), $\lambda_{min.}$ 235 mμ (2070), $\lambda_{max.}$ (pH 1) 275 mμ, $\lambda_{min.}$ 241 mμ, $\lambda_{max.}$ (pH 13) 276 mμ, $\lambda_{min.}$ 241 mμ.

The material soluble in di-isopropyl ether (2·6 g.) was separated into three components by preparative t.l.c. in solvent (B). The slowest-running band on rechromatography consistently gave several bands, indicating that the product was decomposing on the plates. This fraction was not investigated further.

The middle band (878 mg., 4·8%) was purified by preparative t.l.c. in solvent (A) and the extracted material gave N(1)N(3)-*bis*-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-*uracil* (VI) (800 mg.), m.p. 122—123° (from aqueous ethanol) (Found: C, 49·9; H, 5·2; N, 3·7. $C_{32}H_{40}N_2O_{20}$ requires C, 49·7; H, 5·2; N, 3·6%), $\nu_{max.}$ 1760 (CO of ester) and 1690 (CO of amide) cm.$^{-1}$, $\lambda_{max.}$ 260 mμ (9620), $\lambda_{min.}$ 231 mμ (4210), $\lambda_{max.}$ (pH 1) 263 mμ, $\lambda_{min.}$ 231 mμ, $\lambda_{max.}$ (pH 13·0) 264 mμ, $\lambda_{min.}$ 237 mμ.

The fastest-running band (183 mg., 1·0%) was extracted and crystallised twice from ethyl acetate–light petroleum to give O(2)O(6)-*bis*-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*uracil* (VIII) (160 mg.), m.p. 222—223° (Found: C, 49·5; H, 5·4; N, 3·45. $C_{32}H_{40}N_2O_{20}$ requires C, 49·7; H, 5·2; N, 3·6%), $\nu_{max.}$ 1760 cm.$^{-1}$ (CO of ester), $\lambda_{max.}$ 260 mμ (7840), $\lambda_{min.}$ 233 mμ (2280), $\lambda_{max.}$ (pH 1) 260 mμ, $\lambda_{min.}$ 233 mμ, $\lambda_{max.}$ (pH 13) 260 mμ, $\lambda_{min.}$ 233 mμ.

Compounds (V), (IV), (X), (VI), and (VIII) had R_F values [t.l.c.] 0·04, 0·06, 0·06, 0·10, and 0·08 [solvent (A)], 0·10, 0·31, 0·01, 0·26, and 0·43 [solvent (B)], and 0·56, 0·83, 0·87, 0·91, and 0·89 [solvent (C)], respectively.

Reaction of the Acetylated Glucosides with Methanolic Ammonia.—The acetylated glucoside (20 mg.) was set aside in saturated methanolic ammonia at 0° for 16 hr. The solution was then chromatographed, the spots were extracted, and the u.v. spectra were measured.

(*a*) Thymine *N*(3)-glucoside (III) gave a single product with u.v. spectra corresponding to *N*(3)-β-D-glucopyranosylthymine [24] (XI), R_F 0·21 (D), 0·15 (E), 0·17 (F), and 0·14 (G), $\lambda_{max.}$ 265 mμ, $\lambda_{min.}$ 234 mμ, $\lambda_{max.}$ (pH 13) 265 mμ, $\lambda_{min.}$ 245 mμ.

(*b*) Uracil *N*(3)-glucoside (IV) gave a single product with u.v. spectra corresponding to *N*(3)-β-D-glucopyranosyluracil [24] (XII), R_F 0·11 (D) and 0·05 (E), $\lambda_{max.}$ 259·5 mμ, $\lambda_{min.}$ 229 mμ, $\lambda_{max.}$ (pH 1) 260 mμ, $\lambda_{min.}$ 229 mμ, $\lambda_{max.}$ (pH 13) 259·5 mμ, $\lambda_{min.}$ 243 mμ.

(*c*) Uracil *N*(1)-glucoside (V) gave a single product with u.v. spectra corresponding to *N*(1)-β-D-glucopyranosyluracil [29] (XIII), R_F 0·20 (D), 0·13 (E), 0·09 (F), and 0·13 (G), $\lambda_{max.}$ 264 mμ, $\lambda_{min.}$ 231 mμ, $\lambda_{max.}$ (pH 1) 267 mμ, $\lambda_{min.}$ 231 mμ, $\lambda_{max.}$ (pH 13) 293 mμ, $\lambda_{min.}$ 248 mμ.

(*d*) Thymine *O*(2)*O*(6)-bisglucoside (VII) gave a single product with u.v. spectra resembling those of 2,6-dimethoxy-5-methylpyrimidine; [17] R_F 0·07 (D) and 0·09 (E), $\lambda_{max.}$ 263 mμ, $\lambda_{min.}$ 236 mμ, $\lambda_{max.}$ (pH 13) 264 mμ, $\lambda_{min.}$ 238 mμ.

(*e*) Uracil *O*(2)*O*(6)-bisglucoside (VIII) gave a single product with u.v. violet spectra resembling those of 2,6-diethoxypyrimidine; [20] R_F 0·05 (D), 0·06 (E), 0·04 (F) and 0·11 (G), $\lambda_{max.}$ 259 mμ, $\lambda_{min.}$ 233 mμ, $\lambda_{max.}$ (pH 1) 262 mμ, $\lambda_{min.}$ 233 mμ, $\lambda_{max.}$ (pH 13) 262 mμ, $\lambda_{min.}$ 233 mμ.

(*f*) Uracil *N*(1)*N*(3)-bisglucoside (VI) gave a single product with u.v. spectra resembling those of *N*(1)*N*(3)-dimethyluracil; [26] R_F 0·03 (D), 0·02 (F), and 0·06 (G), $\lambda_{max.}$ 259 mμ, $\lambda_{min.}$ 231 mμ, $\lambda_{max.}$ (pH 13) 263 mμ, $\lambda_{min.}$ 237 mμ.

(*g*) Thymine *N*(3)*O*(6)-bisglucoside (IX) gave three products. The first [R_F 0·17 (E) and 0·16 (F)] had u.v. spectra corresponding to *N*(3)-β-D-glucopyranosylthymine (XI). The second [R_F 0·07 (E) and 0·06 (F)] had u.v. spectra corresponding to a 5-methylcytosine pyranoside [$\lambda_{max.}$ 241 and 276 mμ, $\lambda_{min.}$ 260 mμ, $\lambda_{max.}$ (pH 1) 286 mμ,

[29] M. Sano, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 320.

$\lambda_{min.}$ 246 mμ, $\lambda_{max.}$ (pH 14) 278 mμ, $\lambda_{min.}$ 258 mμ] [lit. [24] $\lambda_{max.}$ 237 and 275 mμ, $\lambda_{min.}$ 257 mμ, $\lambda_{max.}$ (pH 1) 285 mμ, $\lambda_{min.}$ 244 mμ, $\lambda_{max.}$ (pH 14) 277 mμ, $\lambda_{min.}$ 257 mμ]. A third product [R_F 0·23 (E) and 0·37 (F)] had a u.v. spectrum with $\lambda_{max.}$ 281 mμ, $\lambda_{min.}$ 242 mμ; the spectra showed little change on addition of acid or alkali, but after the alkaline solution had been left for 2 hr., the spectrum resembled that of *N*(3)-β-D-glucopyranosylthymine (XI) [6-ethoxy-3-methylpyrimidine-2(3*H*)-one [26] has $\lambda_{max.}$ 274·5 mμ].

(*h*) Uracil *N*(3)*O*(6)-bisglucoside (X) also gave three products. The first [R_F 0·09 (F)] had u.v. spectra corresponding to *N*(3)-β-D-glucopyranosyluracil (XII). The second [R_F 0·05 (F)] had $\lambda_{max.}$ 270 mμ, $\lambda_{min.}$ 230 mμ, $\lambda_{max.}$ (pH 1) 276 mμ, $\lambda_{min.}$ 239 mμ, $\lambda_{max.}$ (pH 13) 240 and 267 mμ, $\lambda_{min.}$ 250 mμ. This agrees with the published data for *N*(3)-β-D-glucopyranosyl cytosine (XX). A third product [R_F 0·01 (F)] had $\lambda_{max.}$ 280 mμ, $\lambda_{min.}$ 235 mμ, but when the compound was left in alkali for 2 hr. the spectrum changed to that of *N*(3)-β-D-glucopyranosyluracil.

Reactions of the Acetylated Glucosides with Sodium Hydroxide.—The acetylated glucoside (20 mg.) and 0·2N-sodium hydroxide (1 ml.) were heated at 100° for 10 min. The solutions were chromatographed, the spots extracted, and the u.v. spectra determined.

(*a*) The *N*-glucoside (III), (IV), or (V) gave the corresponding de-acetylated glucoside under these conditions.

(*b*) The *O*(2)*O*(6)-bisglucosides (VII) and (VIII) were hydrolysed completely under these conditions and gave products with u.v. spectra corresponding to thymine [R_F 0·6 (D), and 0·57 (E)] and uracil [R_F 0·35 (F) and 0·45 (E)].

(*c*) Uracil *N*(1)*N*(3)-bisglucoside (VI) decomposed when treated with sodium hydroxide. The nature of the product was not investigated.

(*d*) Thymine *N*(3)*O*(6)-bisglucoside (IX) gave a single product with u.v. spectra and R_F values in solvents (D) and (E) corresponding to *N*(3)-β-D-glucopyranosylthymine (XI).

(*e*) Uracil *N*(3)*O*(6)-bisglucoside (X) gave a single product with u.v. spectra and R_F values in solvents (D) and (E) corresponding to *N*(3)-β-D-glucopyranosyluracil (XII).

Reaction of Monothyminylmercury with ABG.—Monothyminyl mercury (162 mg.) and toluene (20 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (206 mg.) was added to the hot solution and the mixture was stirred and heated under reflux for 30 min. The mixture was filtered, the residue was washed with chloroform, and the combined filtrates were washed with 30% potassium iodide solution and water and then dried and evaporated. The residue was purified by preparative t.l.c. in solvent (B) to give two bands.

The slower-running band was extracted and the product rechromatographed in solvent (B) to give a single band (36 mg., 6%), shown to contain the *N*(3)*O*(6)-bisglucoside (IX) (by t.l.c. and u.v. violet spectra, and deacetylation, t.l.c., and u.v. spectra).

The faster-running band was extracted and the product rechromatographed in solvent (A) to give two bands. The faster-running was the *O*(2)*O*(6)-bisglucoside (VII) (53 mg., 9%) and the slower-running was the *N*(3)-glucoside (37 mg., 11%). These products were identified in a similar fashion to the *N*(3)*O*(6)-bisglucoside (IX).

Reaction of Dithyminylmercury with ABG.—Dithyminylmercury (450 mg.) and toluene (25 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (820 mg.) was added to the hot solution and the mixture was stirred and heated under reflux for 30 min. The mixture was worked up as described for the reaction with monothyminylmercury, and then subjected to preparative t.l.c. in solvent (A) to give a single band, which was extracted (800 mg., 88%), and the product was crystallised from methanol to give the *N*(3)-glucoside (III) (540 mg.), m.p. 154—155°.

Reaction of Thymine with ABG in the Presence of Silver Carbonate.—(*a*) Thymine (I) (126 mg.), silver carbonate (276 mg.), and toluene (20 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (822 mg.) was added to the hot solution and the mixture was stirred and heated under reflux for 1 hr. The solution was filtered, the residue was washed with chloroform, and the combined filtrates were evaporated. The residue was subjected to preparative t.l.c. in solvent (A) to give a single band, which was extracted (209 mg., 46%), and the product was crystallized from the methanol to give the *N*(3)-glucoside (III) (117 mg.), m.p. 149—150°. The yield of crystalline *N*(3)-glucoside (III) was the same when the reaction was repeated with ABG (1 equiv.).

(*b*) Thymine (126 mg.), silver carbonate (552 mg.), ABG (822 mg.), molecular sieve type 3A (Union Carbide and Carbon Corporation; 1·0 g.), and 1,2-dimethoxyethane (20 ml.) were stirred and heated under reflux for 4 hr. The mixture was filtered, the residue was washed with chloroform, and the combined filtrates were evaporated. The residue was purified by preparative t.l.c. first in solvent (B) and then in (A) to give a gum (277 mg., 35%), which gave the *O*(2)*O*(6)-bisglucoside (VII) (130 mg.), m.p. 163—165° [from ethyl acetate–light petroleum (b.p. 60—80°)].

Reaction of Thymine with ABG in the Presence of Silver Cyanide.—Thymine (I) (252 mg.), silver cyanide (534 mg.), and toluene (20 ml.) were heated to boiling and toluene (10 ml.) was distilled off. ABG (822 mg.) was added to the hot solution which was stirred and heated under reflux for 1 hr. The mixture was worked up as described previously to give the *O*(2)*O*(6)-bisglucoside (VII) (73 mg., 9%) as a gum.

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