1141. *Nucleosides. Part I.* O-*Glucosides of Cytosine and their Rearrangement to* N-*Glucosides*

By T. L. V. Ulbricht and G. T. Rogers

The reaction of metal salts of pyrimidines and purines with glycosyl halides is discussed. The silver salts of cytosine and *N*-acetylcytosine react with acetobromoglucose to give acetylated *O*-glucosides, which rearrange to *N*-glucosides in the presence of mercuric bromide.

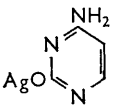
The first nucleoside synthesis is that due to Fischer and Helferich,[1] who investigated the reaction of the silver salts of purines and pyrimidines with glycosyl halides and were successful in obtaining several purine N^9-glycosides. Two observations[1,2] of particular interest in connection with the present investigation are: (1) the silver salt of adenine is sufficiently basic to dehydrohalogenate glycosyl halides, so that the less basic 2,8-dichloroadenine had to be used (this observation was later confirmed,[3] and the use of *N*-acetyladenine introduced); (2) silver salts of purines and pyrimidines containing the tautomeric group $-NH-CO- \rightleftharpoons -N=C(OH)-$, such as 4-methyluracil, cytosine, and theobromine, react with glycosyl halides to give products which reduce Fehling's solution and are labile to both acid and alkali, quite unlike the natural nucleosides. Fischer concluded that

[1] E. Fischer and B. Helferich, *Ber.*, 1914, **47**, 210.
[2] E. Fischer, *Ber.*, 1914, **47**, 1377.
[3] J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 1650.

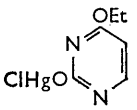
these derivatives are probably *O*-glycosides. Levene and Sobotka [4] came to the same conclusion from experiments in which silver and potassium salts of pyrimidines (cytosine and a number of substituted uracils) were condensed with glycosyl halides; unstable products were invariably obtained.

As a consequence of these unpromising results, and the fact that the Hilbert–Johnson reaction [5,6] made possible the synthesis of natural pyrimidine nucleosides,[7] the reaction of metal salts of pyrimidines with glycosyl halides was not investigated for thirty years, until the successful synthesis of purine nucleosides by use of mercury salts of purines [3] prompted the investigation of such derivatives of pyrimidines, and it was found [8,9] that *N*-glycosides could be obtained from them in good yield; for example, thymine riboside can be prepared from dithyminylmercury.[8] However, reaction of the same mercury derivative with a deoxyribosyl halide gives not an *N*-deoxyriboside but, apparently, an *O*-deoxyriboside; [10] to obtain *N*-deoxyribosides it is necessary to use a different kind of pyrimidine mercury salt, containing the pyrimidine base and mercury in a 1 : 1 ratio.[11,12]

A number of hypotheses were put forward to account for these results, and to explain why silver and mercury salts yield different products.[13] It was suggested that chloromercuripyrimidines, dipyrimidinylmercury, and pyrimidine silver salts are oxygen deriv-



(I)



(II)

atives (*e.g.*, I and II), that these derivatives react with glycosyl halides to give, initially, *O*-glycosides, many of which, under the conditions of the reaction, rearrange to *N*-glycosides (the possibility of rearranging *O*-glycosides to *N*-glycosides had been suggested in a Paper on *O* ⟶ *N*-alkyl rearrangements in alkoxypyrimidines [14]), and that monopyrimidinemercury compounds probably have a structure in which mercury is attached to nitrogen and give *N*-glycosides directly.

This Paper is the first of a series in which the validity of these hypotheses is examined and an attempt made to answer the questions raised above.

Fischer's procedure for the preparation of the silver salt of cytosine (I) and its condensation with acetobromoglucose in refluxing xylene were modified, but his results essentially confirmed. The acetylated glycoside obtained reduces Fehling's solution, and its ultraviolet spectrum ($\lambda_{max.}$ 271 mμ) is very similar to that of 6-amino-2-methoxypyrimidine ($\lambda_{max.}$ 270·5) [15] and is unchanged in cold alkali, but on heating, the spectrum of cytosine is obtained. In acid, the spectrum of cytosine is obtained immediately. Fischer's conclusion that the condensation product is an *O*-glycoside (IV; R = H) can therefore be accepted without reservation.

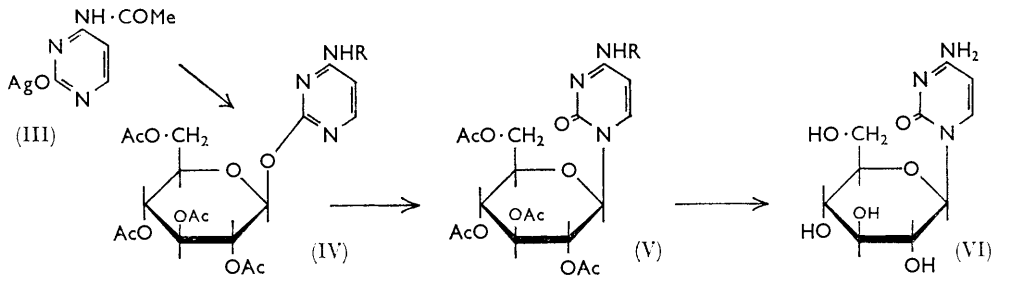
When the acetylated *O*-glycoside (IV; R = H) was heated with mercuric bromide in xylene, considerable decomposition occurred. To some extent this decomposition could be reduced by using a mixture of dimethylformamide and toluene as solvent. The product of this reaction was treated with methanolic ammonia and the deacetylated material fractionated by column chromatography on an acidic ion-exchange resin. In addition to

[4] P. A. Levene and H. Sobotka, *J. Biol. Chem.*, 1925, **65**, 469.
[5] G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 4489.
[6] G. E. Hilbert and E. F. Jansen, *J. Amer. Chem. Soc.*, 1936, **58**, 60.
[7] G. A. Howard, B. Lythgoe, and A. R. Todd, *J.*, 1947, 1052.
[8] J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Amer. Chem. Soc.*, 1956, **78**, 2117.
[9] J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Amer. Chem. Soc.*, 1957, **79**, 5060.
[10] M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777.
[11] M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Amer. Chem. Soc.*, 1959, **81**, 4112.
[12] J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *J. Amer. Chem. Soc.*, 1961, **83**, 4066.
[13] T. L. V. Ulbricht, *Angew. Chem.* (*Internat. Edn.*), 1962, **1**, 476.
[14] T. L. V. Ulbricht, *J.*, 1961, 3345.
[15] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, 1952, **9**, 199.

cytosine, a small quantity of a nucleoside stable to acid and to alkali was obtained, which was identified as N^3-β-D-glucopyranosylcytosine (VI) from its known u.v. spectrum [16] and by comparison with an authentic sample. The anomeric configuration of this product was determined by measurement of its optical rotatory dispersion. It had a positive Cotton effect, which confirmed that it was the β-anomer.[17–19]

In view of the interference of the free amino-group of adenine in nucleoside synthesis [1,3] (see above) and the fact that the amino-group of cytosine is protected in the synthesis of cytidine and of 2′-deoxycytidine,[12] it appeared possible that the low yield of *N*-glucoside in the above rearrangement might be due to the presence of the unprotected amino-group, if the rearrangement reaction involved regeneration of the glycosyl halide. This was shown to be so.

The silver salt (III) of *N*-acetylcytosine was prepared and condensed with acetobromoglucose in refluxing xylene. The product, which reduces Fehling's solution, has a characteristic u.v. spectrum ($\lambda_{max.}$ 230 and 272 mμ) unchanged in the cold in both acid and alkali, though in acid the spectrum of *N*-acetylcytosine is soon obtained; it is therefore



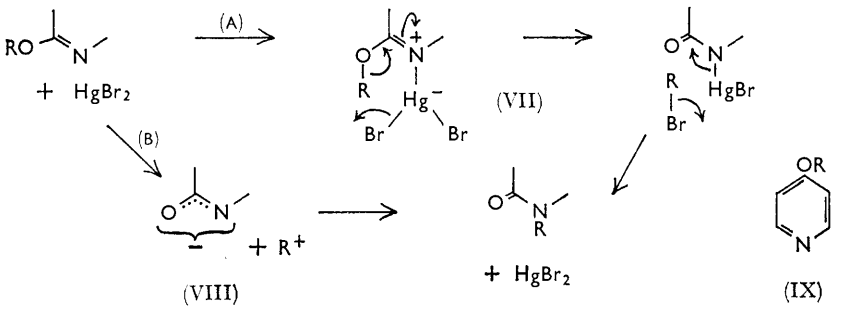
the *O*-glucoside (IV; R = COMe). In accordance with the known stereochemistry of nucleoside synthesis, this *O*-glucoside should have the β-configuration, since it is the product of a reaction between a metal salt and a glycosyl halide containing a 2-acyloxy-group,[20,21] and this was confirmed by the optical rotatory dispersion, which showed a positive Cotton effect.

The rearrangement of this *O*-glucoside with mercuric bromide in toluene could be conveniently followed by ultraviolet spectroscopy, as the spectrum of the product (V) ($\lambda_{max.}$ 250 and 299) is quite different. The reaction, using one equivalent of mercuric bromide, was complete in 90 minutes, and crystallisation of the product from ethanol gave a 55% yield of N^3-β-D-tetra-*O*-acetylglucopyranosylcytosine (V), identical with an authentic sample. This compound is already known to have the β-configuration and, as expected, its optical rotatory dispersion curve showed a positive Cotton effect.

Similar rearrangements have recently been reported for *O*-glycosides of α-pyridone,[22,23] and of 3-hydroxypyrazines.[24] Of particular interest is the reinvestigation [25] of the reaction of the chloromercuriuracil derivative (II) with acetobromoglucose. The product is an *O*-glucoside, in agreement with the suggestion that salts like (II) are *O*-derivatives; only after rearrangement is the *N*-glucoside obtained. All these rearrangements require catalysis by mercuric salts.

[16] J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, 1952, **9**, 369.
[17] T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Letters*, 1964, 695.
[18] T. R. Emerson and T. L. V. Ulbricht, *Chem. and Ind.*, 1964, 2129.
[19] T. R. Emerson, W. Klyne, R. J. Swan, and T. L. V. Ulbricht, unpublished results.
[20] T. L. V. Ulbricht, in "Comprehensive Biochemistry," vol. 8, ed. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1963.
[21] T. L. V. Ulbricht, "Purines, Pyrimidines and Nucleotides," Pergamon Press, London, 1964.
[22] G. Wagner and H. Pischel, *Arch. Pharm.*, 1962, **295**, 373.
[23] T. Ukita, R. Funakoshi, and Y. Hirose, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 828.
[24] G. Wagner and D. Heller, *Naturwiss.*, 1963, **50**, 497.
[25] T. Ukita, H. Hayatsu, and Y. Tomita, *Chem. and Pharm. Bull.* (*Japan*), 1963, **11**, 1068.

In a preliminary report of the present work,[26] a mechanism (mechanism A) for the rearrangement was suggested involving a mercuric complex (VII). An alternative mechanism (mechanism B) has been proposed by Wagner and Pischel,[22] who suggested that, under the influence of mercuric salts, the *O*-glucoside ionises into an anion (VIII) and a



R = 2,3,4,6-Tetra-*O*-acetylglucopyranosyl

glucosyl cation; since the anion is mesomeric, recombination of the ions can yield the *N*-glucoside.

The rearrangement of γ-pyridone *O*-glucoside (IX) constitutes a test case, since rearrangement by mechanism (A) is excluded, whereas, by mechanism (B), there is no reason why (IX) should not rearrange as readily as the α-isomer. In fact, the γ-isomer does not rearrange.[22] Other evidence regarding the mechanism of this reaction is discussed in Part II (following Paper).[27]

Experimental

Ultraviolet absorption spectra were measured on a Perkin-Elmer recording spectrophotometer, model 137 U.V. Paper chromatography (descending) was carried out with Whatman No. 1 paper in the following solvent systems: (A) ethyl acetate saturated with 0·1M-phosphate buffer (pH 6·0); (B) butan-1-ol saturated with water; (C) butan-1-ol–acetic acid–water (4 : 1 : 5); (D) saturated aqueous ammonium sulphate–propan-2-ol–0·1M-phosphate buffer (pH 7·2) (79 : 2 : 19). All experiments with silver salts were carried out in subdued light. Light petroleum refers to the fraction of b. p. 60—80°.

Cytosine Silver Salt.—A solution of silver nitrate (1·317 g.) in water (10 ml.) was added to a solution of cytosine (1·0 g.) in hot dilute aqueous ammonia (1 : 10; 100 ml.) in a crystallising dish, left on a steam-bath for 2 hr., and cooled overnight, to yield white crystals (1·7 g.).

Cytosine-O-glucoside Tetra-acetate (IV; R = H).—A suspension of cytosine silver salt (1·5 g.) in anhydrous xylene (40 ml.) was stirred, and a little of the solvent distilled to remove traces of water. Acetobromoglucose (2·7 g.) was added, and the mixture stirred and refluxed for 20 min. The hot solution was filtered and the residue washed with a little chloroform. The filtrate was poured slowly into light petroleum (300 ml.), with stirring, and the precipitate filtered off, washed thoroughly with the same solvent, and dried *in vacuo* at room temperature, giving a pale buff powder (1·8 g.), m. p. 75—85°. (When toluene was used in place of xylene as solvent, the yield was 0·75 g.) A colourless product, m. p. 85—90°, was obtained by dissolution in carbon tetrachloride (charcoal) and reprecipitation with light petroleum (Found: C, 49·3; H, 5·1; N, 9·2. Calc. for $C_{18}H_{23}N_3O_{10}$: C, 49·0; H, 5·2; N, 9·5%). The product reduces Fehling's solution. $\lambda_{max.}$ (95% EtOH) 271 (ε 6900), $\lambda_{min.}$ 248. At pH 13 in the cold: $\lambda_{max.}$ 271, $\lambda_{min.}$ 247; after heating to boiling point, $\lambda_{max.}$ 281, $\lambda_{min.}$ 249. At pH 1, the spectrum of cytosine is immediately obtained, $\lambda_{max.}$ 276, $\lambda_{min.}$ 239 mμ.

N^3-β-D-*Glucopyranosylcytosine* (VI).—The above product (1·4 g.) was dissolved in a mixture of anhydrous dimethylformamide (20 ml.) and toluene (10 ml.), and a little solvent distilled to remove traces of water. A solution of mercuric bromide (2·29 g., 2 mol.) in toluene (60 ml.)

[26] T. L. V. Ulbricht, *Proc. Chem. Soc.*, 1962, 298.
[27] T. L. V. Ulbricht and G. T. Rogers, following Paper.

was added, and the clear solution refluxed for 1½ hr. Removal of the solvents *in vacuo* left a brown gum, which was dissolved in chloroform, washed with 30% potassium iodide and with water, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in anhydrous methanol, saturated with ammonia at 0°, and left for 2 days at 0°. After evaporation to dryness, acetamide was removed at 40° *in vacuo*, and the residue dissolved in water and chromatographed on a column of Amberlite CG-50 (15 × 2 cm.) which was eluted with water (to tube 185) and then with 0·1N-ammonia, approx. 10 ml. being collected in each tube. The optical density of the eluate was measured at 270 mμ. Fraction 1 (tubes 10—35) contained cytosine, $\lambda_{max.}$ (pH 1) 276, (pH 7) 267, (pH 13) 281 mμ; R_F values in solvent A 0·09, B 0·30, C 0·36, D 0·63. Fraction 2 (tubes 45—123, 1300 o.d. units ≡ 39 mg.) contained N^3-β-D-glucopyranosylcytosine, $\lambda_{max.}$ (pH 1) 276, (pH 7—13) 268 mμ. R_F values in solvent B 0·05, C 0·20; identical with product obtained by deacetylation of (V).

N-*Acetylcytosine Silver Salt* (III).—*N*-acetylcytosine (1·53 g.) was dissolved in water (750 ml.) and sodium hydroxide solution (1N; 10 ml.) by warming to 45°, and an aqueous solution of silver nitrate (1·7 g.) added. After heating to 60°, the white *precipitate* was filtered off and washed with water, ethanol, and ether (yield, 2·2 g.) (Found: C, 27·3; H, 2·7; Ag, 41·9; N, 16·3. $C_6H_6AgN_3O_2$ requires C, 27·7; H, 2·3; Ag, 41·5; N, 16·2%).

O-(β-D-*Tetra*-O-*acetylglucopyranosyl*)-N-*acetylcytosine*.—The condensation was carried out as for (IV; R = H), using the above silver salt (3·0 g.), acetobromoglucose (4·74 g.), and xylene (100 ml.). Stirring and heating under reflux for 20 min. gave 3·6 g. (64%) of product, m. p. 80—90°. This was purified by slowly pouring a dilute benzene solution into light petroleum and recrystallising the precipitate from di-isopropyl ether, giving colourless crystals of O-(β-D-*tetra*-O-*acetylglucopyranosyl*)-N-*acetylcytosine*, m. p. 90—95° (Found: C, 49·7; H, 5·4; N, 8·2. $C_{20}H_{25}N_3O_{11}$ requires C, 49·7; H, 5·2; N, 8·6%). The product reduces Fehling's solution. $\lambda_{max.}$ (95% EtOH) 272 (ε 7,500), $\tau_{min.}$ 243, $\lambda_{max.}$ 230, $\lambda_{min.}$ 220 mμ. The u.v. spectrum is unchanged in acid and alkali, but after heating at pH 1, $\lambda_{max.}$ = 306 mμ (*N*-acetylcytosine).

When, after heating the above reaction mixture under reflux for 20 min., a solution of mercuric bromide in toluene was added and heating continued, no further reaction occurred and the *O*-glucoside was isolated.

N^3-(β-D-*Tetra*-O-*acetylglucopyranosyl*)-N-*acetylcytosine* (V).—The above *O*-glucoside (IV; R = COMe) (1·5 g.) was dissolved in toluene (20 ml.), and a little solvent distilled to remove traces of water. After addition of a solution of mercuric bromide (1·23 g., 1·1 mol.) in toluene (35 ml.), the clear, pale yellow solution was stirred and heated under reflux for 1½ hr., during which time it became cloudy. It was then poured into light petroleum (250 ml.), the precipitate dissolved in chloroform, washed with 30% potassium iodide solution and with water, and the chloroform solution dried ($MgSO_4$) and evaporated to dryness. The residual gum, dissolved in the minimum of hot ethanol, slowly crystallised in the cold to give 0·82 g. (55%) of product, m. p. 216—218° (from ethanol) (partial melting at 130—150° and resolidification), characteristic of (V). Mixed m. p. with an authentic sample [kindly supplied by Dr. I. Wempen (ref. 9)] was undepressed. $\lambda_{max.}$ (95% EtOH) 250, 299 mμ.

When the above rearrangement was repeated in the presence of silver bromide, no reaction occurred, and unchanged *O*-glucoside was isolated.

N^3-β-D-*Glucopyranosylcytosine Hydrochloride* (*from* V).—The above acetylated *N*-glucoside (V) was dissolved in anhydrous methanol, and the solution saturated with ammonia at 0°, and left for 2 days at 0°. After evaporation to dryness and removal of acetamide at 50° *in vacuo*, the residue was dissolved in 95% ethanol and conc. hydrochloric acid added until the solution was strongly acid. After cooling overnight, colourless crystals of the hydrochloride were obtained, m. p. and mixed m. p. 199—200° (decomp.).

Optical Rotatory Dispersion Measurements.—These were carried out at Westfield College and it is hoped to present full details in a later publication.[19]

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