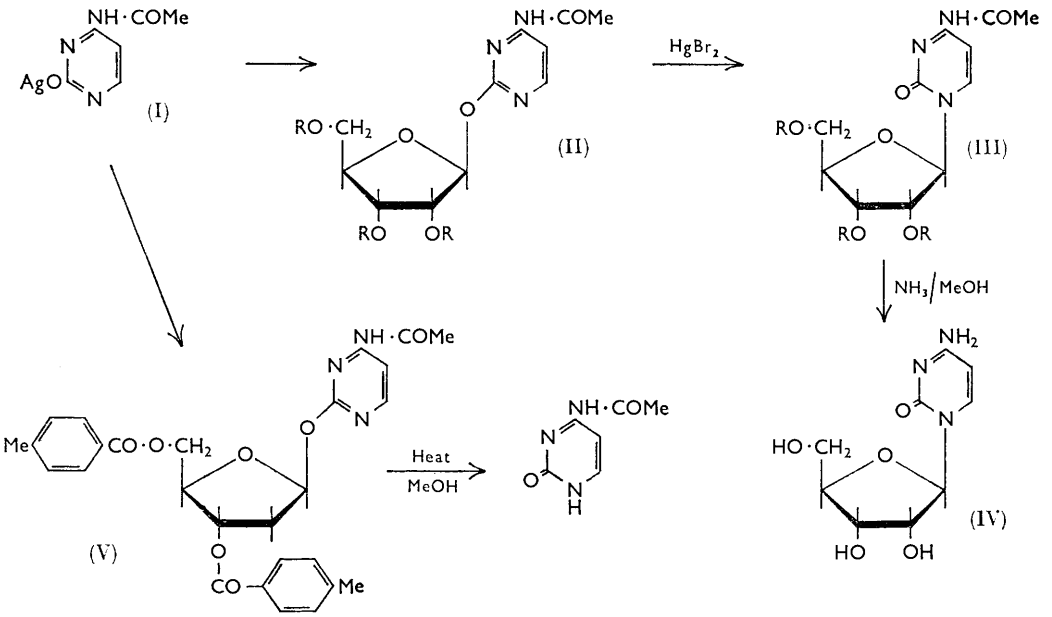
## 1142. *Nucleosides. Part II.*[1] *Further Studies with O-Glycosides of Cytosine*

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

The preparation of acylated *O*-ribosides and an *O*-deoxyriboside of *N*-acetylcytosine is described. The *O*-ribosides can be rearranged to the corresponding *N*-glycosides (cytidines) but the very labile *O*-deoxyriboside is cleaved and liberates the pyrimidine base. The conditions of the rearrangement (catalyst, solvent, concentration, temperature) have been examined, and are discussed in relation to the mechanism of the reaction.

In Part I [1] the synthesis of acetylated *O*-glucosides of cytosine and *N*-acetylcytosine and their rearrangement to the corresponding *N*-glucosides was described. As the silver salt of *N*-acetylcytosine reacted cleanly, and the rearrangement of the derived *O*-glucoside proceeded in high yield, subsequent studies were carried out on derivatives of *N*-acetylcytosine.

The silver salt of this pyrimidine reacted with tri-*O*-acetyl- and tri-*O*-benzoyl-ribofuranosyl chloride to give *O*-glycosides (II). In the case of the benzoyl derivative, the ultraviolet spectrum of the product did not give any information about its structure because of the absorption by the benzoyl groups, but it reduced Fehling's solution and could be hydrolysed to cytosine. Rearrangement with mercuric bromide and subsequent deacylation with sodium methoxide gave β-cytidine (IV), isolated as its hydrochloride.



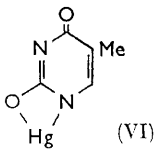

The acetylated *O*-riboside (II; R = COMe) had the expected [1] ultraviolet spectrum ($\lambda_{max.}$ 273 and 231 mμ). The optical rotatory dispersion showed a positive Cotton effect and the compound is therefore assigned the β-configuration.[2-4] The rearrangement with mercuric bromide was followed by the change in this spectrum to that of the product [1] ($\lambda_{max.}$ 250 and 300 mμ) and appeared to be complete in 30 minutes—considerably faster

[1] Part I, T. L. V. Ulbricht and G. T. Rogers, preceding Paper.
[2] T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Letters*, 1964, 695.
[3] T. R. Emerson and T. L. V. Ulbricht, *Chem. and Ind.*, 1964, 2129.
[4] T. R. Emerson, W. Klyne, R. J. Swan, and T. L. V. Ulbricht, unpublished results.

than the rearrangement of the *O*-glucoside. The product, *N*-acetyl-2′,3′,5′-tri-*O*-acetylcytidine (III; R = COMe), which has not previously been described, was isolated in 49% yield, and was identical with the substance obtained by the acetylation of cytidine. That the rearrangement product was the β-anomer was confirmed by its optical rotatory dispersion, and by deacetylation to β-cytidine. Formally this constitutes a new synthesis of cytidine.

No identifiable product could be isolated from the reaction of the silver salt of *N*-acetylcytosine with di-*O*-*p*-toluoyldeoxyribofuranosyl chloride under the usual conditions, *i.e.*, in refluxing toluene. In view of the high reactivity of this glycosyl halide, the reaction was carried out at room temperature in dimethylformamide–toluene. The product, obtained as a gum, reduced Fehling's solution and appeared to be an *O*-deoxyriboside. Extensive decomposition occurred when rearrangement was attempted, and, when a methanolic solution was concentrated by boiling, a crystalline *N*-acetylcytosine separated. The product from the sugar moiety (presumably a glycal) was not isolated. This is not unexpected, as the first glycal with a furanose structure to be described [the phenyl analogue of the *p*-toluoyl glycal which would be formed from (V)] is very labile.[5] It is of interest that both thymine and a glycal have been obtained from the reaction of dithyminylmercury with a deoxyglucopyranosyl halide,[6] but from the reaction of the same mercury derivative with di-*O*-*p*-toluoyldeoxyribofuranosyl chloride, which also appears to give an *O*-glycoside initially,[7] only thymine could be isolated.[8]

The conclusion to be drawn from these results is that *O*-deoxyribosides are too unstable to undergo rearrangement; the elimination reaction supervenes. This explains the necessity, discussed in Part I,[1] of using a different kind of mercury derivative, in which the pyrimidine : mercury ratio is 1 : 1, for the synthesis of deoxyribonucleosides. The only structure so far proposed for such a derivative is (VI) for thyminylmercury.[7] As the valency angle of bivalent mercury is believed to be 180°, both on theoretical grounds and on the basis of combined *X*-ray and neutron-diffraction studies,[9] this structure is inherently unlikely and a polymeric structure is more probable. It is certainly reasonable to conclude, however, that one feature of the structure of thyminylmercury, and similar derivatives used in the synthesis of pyrimidine deoxyribonucleosides, is that the metal atom is directly attached to nitrogen, so that reaction with glycosyl halides leads to *N*-glycosides directly.



(VI)

The rearrangement of the acetylated *O*-glucoside of *N*-acetylcytosine has been studied under a variety of conditions (see Table). Neither sodium iodide, which effects the

Rearrangement of *N*-acetylcytosine tetra-*O*-acetyl-*O*-glucoside

| Catalyst | Molar ratio catalyst : nucleoside | Solvent | Temp. | Time * | Rearrangement |
|---|---|---|---|---|---|
| $HgCl_2$ ..... | 1 : 1 | Toluene | 110° | 3 hr. | None † |
| $HgCl_2$ ..... | 1 : 1 | Xylene | 140 | 3 hr. | Complete ‡ |
| NaI ..... | 2 : 1 | DMF | 100 | 1½ hr. | None † |
| LiBr ..... | 1·3 : 1 | Toluene–DMF | 110—120 | 1½ hr. | None † |
| $HgBr_2$ ..... | 1 : 1 | DMF–benzene | Up to 100 | 2 hr. | None † |
| $HgBr_2$ ..... | 1 : 1 | ,, | 115 | 1½ hr. | Complete ‡ |
| $HgBr_2$ ..... | 1 : 1 | Toluene | 110 | 1½ hr. | Complete ‡ |
| $HgBr_2$ ..... | | As above, but diluted 10-fold | | | None † |
| $HgBr_2$ ..... | 2 : 1 | Toluene | 110 | 20 min. | Complete ‡ |
| $LiClO_4$ ..... | 2 : 1 | Toluene | 110 | 1½ hr. | None † |

* Where reaction occurred, time stated is minimum time for complete reaction. † No change in u.v. spectrum. ‡ Completeness estimated by change in u.v. spectrum; product isolated and crystallised.

[5] R. K. Ness and H. G. Fletcher, jun., *J. Org. Chem.*, 1963, **28**, 435.
[6] W. W. Zorbach and J. J. Durr, jun., *J. Org. Chem.*, 1962, **27**, 1474.
[7] M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777.
[8] M. Hoffer, personal communication.
[9] J. Hvoslef, *Acta Chem. Scand.*, 1958, **12**, 1568.

*O* ⟶ *N* rearrangement of alkoxypyrimidines,[10] nor lithium bromide brought about any reaction. Mercuric chloride is effective, but requires a higher temperature than mercuric bromide; this is to be expected if the breaking of a mercury–halogen bond is a key step in the reaction.[1] The extremely marked effect of a 10-fold dilution (there is no detectable rearrangement) clearly indicates that the reaction is not intramolecular; this is also indicated by the effect of doubling the molar proportion of mercuric bromide.

Winstein, Friedrich, and Smith demonstrated that the salt effects on the rate of ionisation of organic substrates can become enormous in poorly ionising solvents.[11] Of a number of salts investigated,[11] lithium perchlorate proved particularly effective in promoting ionisation, by preventing ion-pair return ("covalent return"). If the O ⟶ *N*-glycoside rearrangement involved salt-promoted ionisation, one might expect that lithium perchlorate would be a catalyst for the reaction, but it was found to be ineffective. In agreement with this, the reaction also appears to be independent of the ionising power of the solvent, since changing the solvent from toluene to dimethylformamide (this contained a small proportion of benzene, to facilitate drying of the reaction mixture) did not accelerate the reaction or permit it to be carried out at a lower temperature. These results, which suggest that it is necessary to offer an explanation for the specific catalysis by mercuric salts, are all consistent with one of the mechanisms which has been proposed [12] (mechanism A)[1] but are less readily explained on the basis of a mechanism [13] involving ionisation (mechanism B).[1]

## Experimental

For details of spectral and chromatographic determinations, see Part I.[1] Experiments with silver salts were carried out in subdued light. Light petroleum had b. p. 60—80°.

N-*Acetyl*-O-(*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*cytosine* (II; R = COPh).—A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose (900 mg.) in anhydrous ether (25 ml.) was saturated with anhydrous hydrogen chloride at 0°, and the flask sealed and left at 0° for 5 days. The solution was evaporated under reduced pressure, the residue dissolved in anhydrous benzene, and the solution evaporated. This procedure was twice repeated and a benzene solution of the residue added to a stirred, dry suspension of *N*-acetylcytosine silver salt (511 mg., 1·1 mol.) in xylene (20 ml.). The benzene was distilled and the mixture stirred and refluxed for 1 hr., filtered, the filtrate poured into light petroleum, and the precipitate filtered and dried *in vacuo*, giving the colourless *product* (670 mg.), m. p. 70—80° (Found: C, 66·5; H, 4·8; N, 6·9. $C_{32}H_{27}N_3O_8$ requires C, 66·1; H, 4·7; N, 7·2%). The product reduces Fehling's solution; $\lambda_{max.}$ (95% EtOH) 230, 273 mμ.

*Hydrolysis of* (II; R = COPh).—The above product (20 mg.) was added to a solution of sodium (10 mg.) in methanol (5 ml.) and refluxed for 45 min. The solution was evaporated to dryness, the residue dissolved in aqueous ethanol, neutralised with dilute hydrochloric acid, and evaporated to dryness. The residue, after two extractions with hot benzene (discarded), was cytosine, $\lambda_{max.}$ (pH 1) 276, (pH 7) 627, (pH 13) 281 mμ; $R_F$ values in solvent A 0·09, B 0·30, C 0·36, D 0·63.

*Rearrangement of* (II; R = COPh).—A solution of mercuric bromide (560 mg., 1·1 mol.) in toluene (20 ml.) was added to a solution of the *O*-riboside (II; R = COPh) (820 mg.) in toluene (20 ml.), and the solution stirred and refluxed for 70 min. It was poured into light petroleum, the precipitate dissolved in chloroform, washed with 30% aqueous potassium iodide solution and with water, the chloroform solution dried ($MgSO_4$) and evaporated. The residue was dissolved in ethanol and refluxed with small portions of sodium until the solution remained alkaline. After acidification with hydrochloric acid and extraction with ether (discarded), the solution was adsorbed on a charcoal column, washed with water, and eluted with ethanol–water–conc. ammonia (2 : 2 : 1), giving a solution of cytidine, $\lambda_{max.}$ (pH 1) 279, (pH 7) 270,

[10] T. L. V. Ulbricht, *J.*, 1961, 3345.
[11] S. Winstein, E. C. Friedrich, and S. Smith, *J. Amer. Chem. Soc.*, 1964, **86**, 305.
[12] T. L. V. Ulbricht, *Proc. Chem. Soc.*, 1962, 298.
[13] G. Wagner and H. Pischel, *Arch. Pharm.*, 1962, **295**, 373.

(pH 13) 271; $R_F$ values in solvent B 0·17, C 0·27. A portion of this product was chromatographed on a column of Amberlite CG-50 and eluted with water, and, after a small preliminary fraction which was discarded, cytidine was obtained in the main fraction and converted into the hydrochloride in anhydrous methanol solution and recrystallised from the same solvent, m. p. and mixed m. p. 219° (lit.,[14] 218°).

N-*Acetyl*-O-(*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*cytosine* (II; R = COMe).—1,2,3,5-Tetra-*O*-acetylribofuranose (3·67 g.) was converted into the glycosyl chloride with hydrogen chloride in the usual way, and the condensation with *N*-acetylcytosine silver salt (3·0 g.) carried out in xylene (75 ml.) with glass beads (5 g.) at the reflux temperature for 20 min. The solution was filtered, and evaporated to dryness *in vacuo*, giving a pale yellow solid (3·9 g., 83%), purified by dissolution in chloroform and addition of the solution, drop by drop, to ice-cooled light petroleum with vigorous shaking. The precipitate was rapidly filtered off, washed with light petroleum, and dried *in vacuo*, giving the *product* (3·12 g.), m. p. 55—60° (Found: C, 49·0; H, 5·0; N, 9·9. $C_{17}H_{21}N_3O_9$ requires C, 49·6; H, 5·1; N, 10·2%). The product reduces Fehling's solution; $\lambda_{max.}$ (95% EtOH) 273 (ε 6800), 231 mμ. Treatment with dilute mineral acid gives cytosine.

N-*Acetyltri*-O-*acetylcytidine* (III; R = COMe).—(a) *From cytidine.* Cytidine (1·0 g.) was heated on a steam-bath with acetic anhydride (15 ml.) containing a few drops of pyridine, until dissolution occurred. After standing for 2 hr. at room temperature, the solution was evaporated to dryness *in vacuo*, and re-evaporated with benzene and methanol until the residue was obtained as a solid (1·6 g.). The *product* was obtained as colourless crystals from di-isobutyl ketone–light petroleum, m. p. 195—196° (decomp.) after partial melting at 60—70° and resolidification (Found: C, 49·7; H, 5·4; N, 9·8. $C_{17}H_{21}N_3O_9$ requires C, 49·6; H, 5·1; N, 10·2%), $\lambda_{max.}$ (95% EtOH) 250 (ε 16·5 × $10^3$), 300 (ε 6·95 × $10^3$). $R_F$ values in solvent C, 0·85, B (thin-layer chromatography on silica gel) 0·24.

(b) *From* (II; R = COMe). A solution of mercuric bromide (6·8 g.) in toluene was added to a solution of the *O*-riboside (II; R = COMe) (3·9 g. of crude product) in toluene (50 ml.) which had been dried by distilling some solvent, and refluxed for 30 min. The solution was filtered, evaporated to dryness *in vacuo*, the residue dissolved in chloroform and washed with 30% aqueous potassium iodide solution and water, and dried ($MgSO_4$), and the solvents were removed *in vacuo*. The residue was re-evaporated several times with benzene until the product was obtained as a dry powder (1·9 g., 49%), $R_F$ values (single spots) in solvent C 0·85, B (silica gel thin-layer chromatography) 0·24. Recrystallisation from di-isobutyl ketone–light petroleum gave *N*-acetyltri-*O*-acetylcytidine, m. p. 195—196° (decomp.) after partial melting at 60—70° and resolidification. Mixed m. p. with product obtained under (a) above was undepressed. A portion of the product was deacetylated with methanolic ammonia and converted into the hydrochloride in the usual way. Recrystallisation from anhydrous methanol gave cytidine hydrochloride, m. p. and mixed m. p. 219°.

N-*Acetyl*-O-(2-*deoxy*-3,5-*di*-O-p-*toluoyl*-β-D-*ribofuranosyl*)*cytosine* (V).—A stirred suspension of *N*-acetylcytosine silver salt (2·3 g.) in a mixture of dimethylformamide (60 ml.) and toluene (30 ml.), was dried by distilling some of the solvent. After cooling, 2-deoxy-3,5-di-*O*-*p*-toluoyl-ribofuranosyl chloride [7] (1·87 g., 0·5 mol.) was added, and the mixture shaken in the dark for 5 days. After filtering, the solution was evaporated, the residual gum re-evaporated with xylene, the residue dissolved in toluene (charcoal), filtered, poured into light petroleum, and cooled. The solvent was decanted and the gum freed from solvent *in vacuo*. This product reduced Fehling's solution, was completely soluble in benzene, and gave a single spot ($R_F$ 0·93) in solvent C. When a solution in anhydrous methanol was boiled, a colourless crystalline solid separated, identified as *N*-acetylcytosine, $\lambda_{max.}$ (pH 1) 237, 306, (pH 7) 243, 294, (pH 13) 299 mμ; no m. p. <300°.

*Optical Rotatory Dispersion measurements.*—These were carried out at Westfield College and it is hoped to present full details in a later publication.[4]

TWYFORD LABORATORIES, 309 ELVEDEN ROAD, LONDON N.W.10. [*Received, March 22nd,* 1965.]

[14] P. A. Levene and W. A. Jacobs, *Ber.*, 1910, **43**, 3155.