Tetrahedron Vol 27. pp 65 to 69. Pergamon Press 1971. Printed in Great Britain

THE PERMANGANATE OXIDATION OF CYTOSINE DERIVATIVES

R. S. GOODY, A. S. JONES and R. T. WALKER
Chemistry Department, The University of Birmingham, Birmingham 15

(*Received in the UK* 18 *September* 1970; *Accepted for publication* 22 *September* 1970)

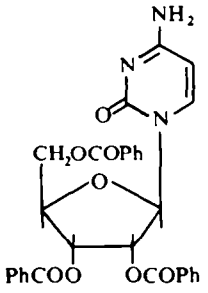
Abstract—The oxidation by potassium permanganate of cytosine and cytidine to derivatives of urea and of biuret has been confirmed. N-Formylurea has also been identified as one of the oxidation products of cytosine. Derivatives of urea, but not those of biuret were formed when 2′, 3′, 5′-tri-O-benzoylcytidine (I) was similarly oxidised. The major product was N-(2,3,5-tri-O-benzoyl-D-ribofuranosyl) N′-oxalylurea. This was unstable and decomposed readily to another oxidation product, namely N-(2,3,5-tri-O-benzoyl-D-ribofuranosyl) urea. These two compounds were also obtained from the oxidation of 2′,3′,5′-tri-O-benzoyluridine. The reasons for the absence of biuret derivatives in the permanganate oxidation products of I and of DNA and RNA are discussed.

IT HAS been shown previously that cytosine is oxidised by potassium permanganate in bicarbonate buffer (pH9) at 37° to give *inter alia*, urea and biuret,[1] and that cytidine and deoxycytidine gave derivatives of both urea and biuret.[2] However, when DNA and RNA were oxidised under similar conditions, although all of the cytosine residues were oxidised, no biuret residues were produced.[3,4] In order to discover the reason for this difference, the permanganate oxidation of cytosine and some derivatives has been studied in greater detail.

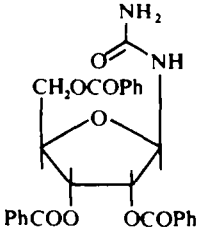
In the earlier work on the oxidation of cytosine[1] no attempt was made to obtain complete oxidation but in the present case an excess of potassium permanganate was used (i.e. 3·5 mole *c/f* 3·33 moles needed for complete oxidation to urea and carbon dioxide) and complete oxidation of the cytosine was achieved. The major N-containing products were urea and buiret. There was produced, also, a small amount of N-formylurea (identified by comparison with an authentic specimen). These products were present in molar ratio, urea: N-formylurea: biuret of 1·8: 0·26 : 1. This result is appreciably different from that previously obtained, (namely a molar ratio urea: biuret of 1 : 1·5) but this could be due to the higher concentration of permanganate used and the absence of bicarbonate in solution.

In the previous work on the permanganate oxidation of cytidine,[2] urea and biuret derivatives were formed in the molar ratio of 4 :1. Because the mixture of oxidation products was complex and because this may have been due to oxidation of sugar OH groups, in the present work these OH groups were protected. The cytosine derivative chosen was 2′,3′,5′-tri-O-benzoylcytidine (I) which was synthesized by the selective de-N-acylation of 4-N-acetyl-2′,3′,5′-tri-O-benzoylcytidene under acidic conditions to give 2′, 3′, 5′-tri-O-benzoylcytidine. The oxidation was carried out in t-butanol-water (1 : 1) because of the insolubility of the compound in water. The oxidation products were isolated in good yield. Silica gel TLC showed the presence of a major, a minor and a trace component. The minor component (about 20% of the products) was shown to be

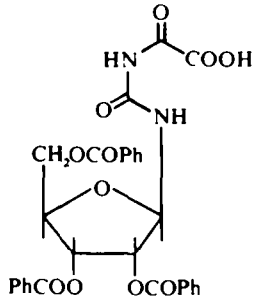
N-2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) urea (II) by comparison with an authentic specimen. This was obtained by treatment of 2,3,5-tri-O-benzoylribofuranosyl chloride with silver cyanate to give the corresponding isocyanate, which on treatment with methanolic ammonia gave II.[5] The major component (about 65% of the products) decomposed to II on repeated development on the TLC plate. It showed acidic properties and gave oxalic acid upon alkaline hydrolysis. This, and its elemental analysis indicated that the compound was an N-oxalyl derivative of II. The fact that the compound reacted very slowly with the Ehrlich reagent showed that both N atoms of the urea residue were substituted. It was concluded, therefore, that the compound was N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) N′-oxaly N′-oxalylurea (III).



I



II

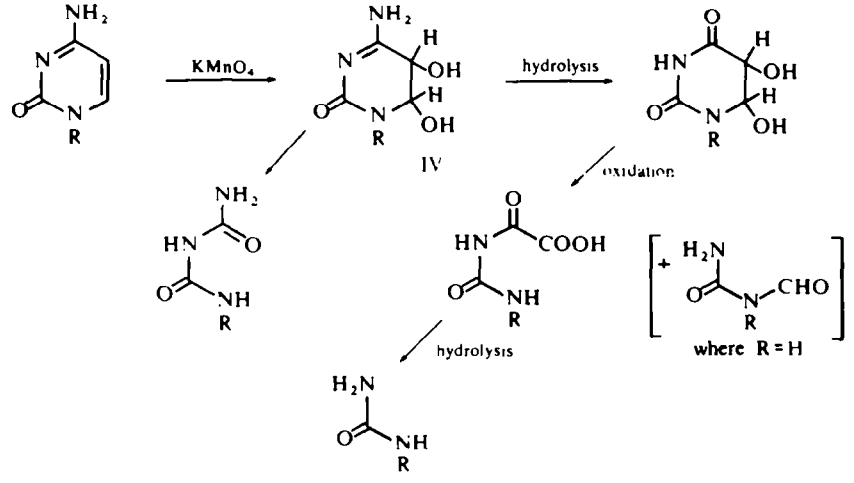


III

Compound III was also obtained as the major product and II as the minor product when 2′, 3′, 5′,-tri-O-benzoyluridine was oxidised under similar conditions.

Acidic hydrolysis of the total oxidation products of I gave urea but no biuret. Under the conditions used, biuret was not hydrolysed to urea, so it was concluded that no biuret derivatives had been formed in the oxidation of I. This reaction is similar in this respect, therefore, to that occurring with DNA and RNA and different from that taking place with cytosine, cytidine and deoxycytidine.

The probable first stage in the oxidation of cytosine and its derivatives is, by analogy



with the corresponding oxidation of thymine derivatives,[6] the formation of the 5,6-diol, IV. Because biuret derivatives are not hydrolysed or oxidised to urea derivatives under the conditions used, the two types of derivative must be produced by independent pathways. It seems probable that this could occur by the intermediate, IV, undergoing two types of reaction, namely (I) oxidation to give a biuret derivative and (2) deamination to a uracil derivative followed by oxidation to give a urea derivative as follows:

The ratio of urea to biuret derivatives produced would be dependent, therefore, on the relative ease of deamination or oxidation of IV. The ease of deamination of 5,6-dihydrocytosine derivatives appears to be relevant in this connection and it has been shown that it is dependent upon the nature of the substituent on N^1 and that it is in the order: 5,6-dihydrocytidine > 5,6 dihydrodeoxycytidylic acid > 5,6-dihydro-2′-deoxycytidine > 5,6-dihydrocytosine.[7] 5,6-Dihydrocytidylic acid is readily deaminated; deamination to 5,6-dihydrouridylic acid occurs during the hydrogenation of cytidylic acid under mild conditions.[8] As a rough approximation, therefore, it appears that the more bulky the group substituted at N^1, the easier the deamination. If a similar effect operates in the deamination of the proposed intermediate, IV and also a possible retardation of the oxidation of IV by the bulky substituent on N^1, then the present results can be explained.

EXPERIMENTAL

Chromatography. The following solvents were used: (1) butan-1-ol: ethanol: water (4:1:5) (organic phase); (2) butan-2-ol: water (7:5); (3) propan-2-ol: ammonia (*d*, 0·88): water (35:3:15). Urea and biuret derivatives were detected by means of Ehrlich's *p*-dimethylaminobenzaldehyde reagent.[9] Urea and glycosylureas gave a yellow colour upon drying the chromatograms. Other substituted ureas gave the yellow colour more slowly; biuret derivatives gave the colour slowly and with significantly lower intensity.

The oxidation of cytosine with potassium permanganate. A soln of cytosine (50 mg) and $KMnO_4$ (249 mg) in water (15 ml) was kept at 37° for 19 hr. (The soln rapidly reached pH9 and stayed almost constant at this pH). A drop of neutral Na_2SO_3aq was then added to convert the excess of permanganate into MnO_2. The suspension was centrifuged, the deposit well washed with water and the combined supernatant and washings evaporated to dryness. A portion of the residue was examined by paper chromatography in solvent 2. Urea (R_f 0·36) and biuret (R_f 0·49) were present. A component (R_f 0·58) which slowly gave a yellow colour with the Ehrlich reagent was also present. This was identified as N-formylurea by comparison by paper chromatography in solvents 1 and 2 with an authentic sample. In the latter solvent parabanic acid had an R_f of 0·72 and oxaluric acid had an R_f of 0·16. The component also corresponded to N-formylurea in solvent 3 but in this case some decomposition to urea occurred and it behaved similarly to N-formylurea upon TLC on silica gel with acetone: chloroform (3:7) as the solvent. Acidic or alkaline hydrolysis of the compound gave urea. The molar ratios of the products formed during the oxidation were determined by measuring the N contents of the components obtained by running chromatograms on Whatman No. 3 paper in solvent 2. They were urea: N-formylurea: biuret, 1·8:0·26:1·0. The total recovery of N-containing compounds from the chromatogram was 77% (assuming that a molecule of ammonia was lost for each molecule of urea derivative formed). A control experiment was carried out in which a known mixture of urea and biuret was treated with permanganate under the same conditions as the cytosine. The recoveries of urea and biuret were found to be 78% and 74% respectively.

The preparation of 2′,3′,5′-tri-O-benzoylcytidine. 4-N-Acetylcytosine mercury (2·3 g) was condensed with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (5·04 g) in a mixture of xylene and benzene as described.[10] The product, 4-N-acetyl-2′,3′,5′-tri-O-benzoyl-cytidine, on recrystallization from EtOH was obtained in 32% overall yield from cytosine m.p. 191°–192° λ_{max} in EtOH 231 nm (ε.40,700) and 284 nm ε,7,490), shoulder at 295nm. (Found: C,64·64; H, 4·42; N, 6·50. Calc. for $C_{32}H_{27}N_3O_9$; C, 64·30; H, 4·56; N, 7·03%).

This compound (1·3 g) was dissolved in chloroform/EtOH (3:1; 100 ml) and 1N HCl (11 ml) added. The soln was allowed to stand at room temp for 3 days.at the end of which time the UV absorption of the soln

at 300 nm had dropped to zero. The solvent was removed under reduced pressure and EtOH added to the residue which was evaporated to dryness several times to remove the HCl. TLC on silica (10% EtOH in benzene), showed the presence of a single UV absorbing compound which was recrystallised from boiling chloroform/EtOH (1 : 4) to which light petroleum (60°–80° fraction) was added to incipient cloudiness and the soln allowed to cool to give pure 2′,3′,5′-tri-O-benzoylcytidine hydrochloride (1·1 g; 40% overall yield from cytidine), m.p. 226–227° (d). λ_{max} in EtOH 230 nm (ε,29,700) and 280 nm (ε,9,650); λ_{min} in EtOH 253 nm. (Found: C, 60·54; H, 4·41; N, 7·10. $C_{30}H_{25}N_3O_8$. HCl requires: C, 60·87; H, 4·44; N, 7·10%).

The oxidation of 2′,3,5′-*tri-O-benzoylcytidine with potassium permanganate.* 2′,3′,5′-tri-O-benzoylcytidine hydrochloride (0·5 g) was dissolved in t-BuOH (100 ml; previously distilled from $KMnO_4$) and water (100 ml) added. The pH of the soln was adjusted to 7, $KMnO_4$ (0·5 g) added and the soln kept at 37° for 19 hr. The excess of $KMnO_4$ was then destroyed with neutral Na_2SO_3 and the solvent removed by evaporation under reduced pressure. Water (50 ml) was added to the residue, which was a mixture of MnO_2 and oxidation products and the MnO_2 dissolved by the addition of neutral Na_2SO_3. The resulting white suspension was extracted several times with chloroform, the chloroform extract dried over $MgSO_4$ and evaporated to dryness to give a white powder.

A sample of the product was examined by TLC on silica gel using 5% EtOH/chloroform as solvent. Six UV-absorbing components (1–6) were obtained. Component 1 (slowest running component) comprised 66% of the UV absorption of the products (at 231 nm) and component 2 about 20% (component 3 ran very close and made an exact determination impossible; total of 2 + 3 was 24%). Components 2 and 3 gave an immediate yellow colour with the Ehrlich spray, component 1 did so after standing overnight. Component 2 was found to be identical chromatographically with the sample of N-(2,3,5-tri-O benzoyl-β-D-ribofuranosyl) urea synthesized as described below. It was noticed that upon repeated running of the chromatoplate that component 1 changed into component 2, conversion being complete after 4 runs. A crystalline sample of component 2 was obtained from a preparative scale. TLC plate which had been repeatedly run in 5% EtOH in chloroform. This had the same m.p. as the authentic material.

The oxidation of 2′,3′,5′-*tri-O-benzoyluridine with potassium permanganate.* The oxidation was carried out as described above except that a larger volume of solvent was used (500 ml of 1 : 1 t-BuOH-water). Silica gel TLC of the products showed the presence of components which corresponded to 1,2 and 3 from the oxidation of 2′,3′,5′-tri-O-benzoylcytidine. Component 1 accounted for 62% of the absorption at 231 nm of the products and 2 and 3 combined to 38%. Component 2 had an IR spectrum and R_F values in several solvent systems identical N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) urea.

Isolation and identification of component 1. The mixture of oxidation products (100 mg) (from either tri-O-benzoylcytidine or tri-O-benzoyluridine) was dissolved in a minimum volume of chloroform and ethanol added until precipitation was complete. The ppt was filtered off, dried (yield, 50 mg) and then dissolved in a minimum volume of acetone and one drop of 4N HCl added. After standing at room temp for 30 min, crystallisation occurred, the crystals were filtered off, washed and dried (yield, 20 mg). The products from the oxidation of both compounds had identical m.ps (172°–175°d.) and IR spectra. Silica gel TLC showed the presence of component 1 and a small amount of 2 (presumably arising from the slow decomposition of 1). From the evidence given below it was concluded to be N-(2,3,5-*tri-benzoyl*-β-D-*ribofuranosyl*)-N′-*oxalylurea*. Found: C, 60·2; H, 4·33; N, 5·10. $C_{29}H_{24}N_2O_{11}$ requires: C, 60·4; H, 4·16; N, 4·86%). The compound was acidic and after hydrolysis with boiling 1N alkali for 30 min it gave oxalic acid which was identified by precipitation of its calcium salt and decolourisation of acid permanganate. Cellulose TLC in solvent 1 of the alkaline hydrolysate showed the presence of 3 components which gave a yellow colour with the Ehrlich reagent. One of these was urea (R_F 0·50) and the other two (R_F 0·29 and 0·22) corresponded to components identified in the reaction mixture of D-ribose and urea in the presence of acid and shown to be D ribosylureas.[11]

Absence of biuret residues in the oxidation products of 2′,3′,5′-*tri-O-benzoylcytidine.* A sample (5 mg) of the total oxidation products of the 2′,3′,5′-tri-O-benzoylcytidine was added to N HCl (2 ml) and the mixture kept at 37° for 20 hr. The soln was neutralised, evaporated to dryness and the residue extracted exhaustively with hot EtOH. The extract was examined by cellulose TLC in solvents 1, 2 and 3. In each case a component identical to urea was detected but there was no component corresponding to biuret. When biuret was treated with acid and the products examined in a similar way no urea was detected.

2,3,5-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl isocyanate.* 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose[12] (20 g) was suspended in dry ether (250 ml) containing dry HCl (33·6 g) and the suspension kept at 2° with shaking for 16 days. A small insoluble residue was filtered off, the filtrate evaporated to dryness and the residue co-evaporated with dry benzene (twice) and dry toluene. The gum so obtained (2,3,5-tri-O-benzoyl-

D-ribofuranosyl chloride) was dissolved in dry toluene (114 ml), freshly-prepared AgCNO (13 g) added and the mixture boiled under reflux in the absence of light for 2·5 hr. The solid material was then filtered off, washed with toluene and the combined filtrate and washings evaporated *in vacuo* at 40° to give a yellow gum which crystallised on standing to give needles (21 g). This product gave a peak in the IR spectrum typical of an isocyanate (2265 cm^{-1}) and a negative reaction for a urea derivative with the Ehrlich reagent. A small sample was crystallised from toluene-ether to give a *product*, m.p. 212–216° (d), $[\alpha]_D^{30}$-28·3° (*c*, 0·6 in C_5H_5N). (Found: C, 66·2; H, 4·67; N, 2·76. $C_{27}H_{21}O_8N$ requires: C, 66·5; H, 4·34; N, 2·87%).

N-(2,3,5-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl*) *urea*. The crude isocyanate prepared as described above was dissolved in MeOH (12 ml) saturated at 0° with ammonia and the soln shaken at 0° for 1 hr. The isocyanate did not completely dissolve, so chloroform (40 ml) was added and the shaking continued for 30 min. The soln was then concentrated to dryness at 5° and the residue co-evaporated with MeOH (twice). The residue was triturated with ether to give an amorphous, hygroscopic solid (10 g). This material was crystallised with difficulty from MeOH to give a crude product (6g). After two recrystallisations from the same solvent, N-(2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*) *urea* was obtained which sintered at 176° and melted at 180–181°, $[\alpha]_D^{28}$-30° (*c*, 1·4 in C_5H_5N). (Found: C, 64·9; H, 4·64; N, 5·0 $C_{27}H_{24}O_8N_2$ requires: C, 64·3; H, 4·8; N, 5·5%). The compound was homogeneous by paper chromatography in 3 solvent systems, gave a positive reaction with the Ehrlich reagent and gave a white spot with a brown halo with a $AgNo_3$ spray. This is characteristic of ureido sugars.[11] The IR spectrum showed a peak at 1647 cm^{-1} typical of the —CONH group in ureido sugars.

The authors thank Dr. E. M. Badawi for synthesising N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) urea and the Science Research Council for a Research Studentship (to R. S. G.).

REFERENCES

[1] B. Chatamra and A. S. Jones; *J. Chem. Soc.* 811 (1963)
[2] A. S. Jones and R. T. Walker, *Ibid.* 3554 (1963)
[3] A. S. Jones, G. W. Ross, S. Takemura, T. W. Thompson and R. T. Walker, *Ibid.* 373 (1964)
[4] J. J. Holbrook, A. S. Jones and M. J. Welch, *Ibid.* 3998 (1965)
[5] E. M. Badawi, PhD. Thesis University of Birmingham (1964)
[6] M. H. Benn, B. Chatamra and A. S. Jones, *J. Chem. Soc.* 1014 (1960)
[7] M. Green and S. S. Cohen, *J. Biol. Chem.* **228**, 601 (1957)
[8] W. E. Cohn and D. G. Doherty, *J. Am. Chem. Soc.* **78**, 2863 (1956)
[9] H. J. Hubener, F. Bode, H. J. Mollat and M. Wehner, *Z. Physiol. Chem.* **290**, 136 (1952)
[10] J. J. Fox, N. Yung, I. Wempen and I. L. Doerr, *J. Am. Chem. Soc.* **79**, 5060 (1957)
[11] M. H. Benn and A. S. Jones, *J. Chem. Soc.* 3837 (1960)
[12] H. M. Kissiman, C. Pidacks and B. R. Baker, *J. Am. Chem. Soc.* **77**, 18 (1955)