669. *The Permanganate Oxidation of Nucleosides.*

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Oxidation of guanine-containing nucleosides with potassium permanganate at 37° at pH 9 gave guanidine and urea derivatives, which were probably glycosylureas. Thymidine uridine, cytidine, and deoxycytidine gave similar urea derivatives. Cytidine and deoxycytidine gave, in addition, derivatives of biuret.

THE permanganate oxidation of thymine and uracil and their 1-substituted derivatives gave urea and 1-substituted ureas;[1,2] cytosine and 1-substituted cytosines gave, in addition, derivatives of biuret.[2] The similar oxidation of the corresponding nucleosides and of guanosine and deoxyguanosine has now been studied.

Treatment of 2′,3′,5′-tri-*O*-acetylguanosine in unbuffered aqueous solution with potassium permanganate at 37° for 19 hours gave five products which were separated by paper chromatography in butanol–ethanol–water. The fastest-running component (A; R_F 0·72) was obtained crystalline and identified as a triacetylribosylurea. This follows from its analysis and conversion into ribose and urea on acid hydrolysis. Its infrared spectrum showed similarities to that of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylurea;[3] the latter showed stretching OH and NH frequencies at 3480, 3380, and 3240 cm.$^{-1}$, while those of substance A occurred at 3500, 3390, and 3250 cm.$^{-1}$. Absorption bands due to carbonyl groups of the acetate groups were shown by the glucose compound at 1750 and 1760 cm.$^{-1}$ and by substance A at 1743 and 1763 cm.$^{-1}$. Also the presence of an additional carbonyl band at 1687 cm.$^{-1}$ in spectra of both compounds suggested the presence of a urea group in substance A. In both cases the NH deformation band at 1620—1650 cm.$^{-1}$ was missing and was replaced by a band at 1575 cm.$^{-1}$.

The other major product from the oxidation of the triacetylguanosine was guanidine, which was identified chromatographically and by means of its crystalline picrate. The other three products were not isolated but they were shown to be deacetylated products of substance A.

[1] Benn, Chatamara, and Jones, *J.*, 1960, 1014.
[2] Chatamra and Jones, *J.*, 1963, 811.
[3] Helferich and Kosche, *Ber.*, 1926, **59**, 69.

When guanosine was oxidised with permanganate at pH 9, urea, ribose, and a compound (B; R_F 0·12), which was easily hydrolysed in aqueous solution to urea and ribose, were produced. Owing to its instability, attempts to isolate compound B were unsuccessful, but it was probably a ribosylurea.

The oxidation of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine gave a mixture whose components were not easily separated: but deoxyguanosine gave urea, 2-deoxyribose, and a product (C; R_F 0·25) which was easily hydrolysed to urea and 2-deoxyribose and was therefore probably a 2-deoxyribosylurea.

Thus the guanine residues in these nucleosides are oxidised to ureido-residues with the liberation of guanidine and possibly other products. These results are similar to those of Heinrich and Wilson [4] who obtained guanidine and urea from guanine by oxidation with permanganate at pH 1—2 at 100°.

Permanganate oxidation of uridine gave ribose, urea, and a product which had the properties of a ribosylurea and had the same R_F value as compound B. Similarly, thymidine gave 2-deoxyribose, urea, and a product which had the same properties as the deoxyribosylurea, C. Cytidine and deoxycytidine gave, in addition to urea derivatives, biuret and, probably, ribosylbiuret and deoxyribosylbiuret. The molar proportion of urea derivatives to buiret derivatives in the oxidation products was 4 : 1 in the case of cytidine and 3 : 1 in the case of deoxycytidine. Thus there is a much higher proportion of the urea derivative formed from these nucleosides than from cytosine or 1-methylcytosine.[2]

Owing to their instability, and the absence of pure synthetic compounds (the compound tentatively identified [5] as a D-ribofuranosylurea now appears to be a mixture), it has not yet been possible to establish the structure of the urea derivatives formed during the oxidations.

Experimental

Paper Chromatography.—Whatman No. 1 and 4 papers were used with the organic phase of the solvent system, butan-1-ol–ethanol–water (4 : 1 : 5). The components were detected by means of the silver nitrate spray: [6] carbohydrates gave brown spots; urea, biuret, and guanidine gave white spots; and glycosylureas and glycosylbiurets gave white spots which became brown on storage. Urea derivatives were first detected by means of the fructose–hydrochloric acid spray [1] and later by the more sensitive Ehrlich reagent.[7] Reducing sugars were detected by means of aniline hydrogen phthalate; [8] glycosylureas could also be detected with this reagent. Guanidine was detected by spraying with ninhydrin and then with ethanolic sodium hydroxide.[9]

Permanganate Oxidation of 2′,3′,5′-Tri-O-acetylguanosine.—A solution of 2′,3′,5′-tri-*O*-acetylguanosine (1 g.) and potassium permanganate (1·5 g.) in water (300 ml.) was set aside at 37° for 19 hr. (the pH rose to 9). A neutral solution of a mixture of sodium sulphite and sodium metabisulphite was added until no more permanganate remained. The precipitate was filtered off, and the filtrate concentrated to 30 ml. Paper chromatography showed the presence of at least six components. They had R_F values and gave colours with the silver nitrate spray as follows: (1) 0·093 (white); (2) 0·12 (brown); (3) 0·30 (white); (4) 0·32 (brown); (5) 0·51 (brown); (6) 0·70 (brown). None of the products had appreciable absorption of ultraviolet light.

The alkaline ninhydrin spray detected component 3 and showed that it was a guanidine derivative. It had the same R_F value as guanidine in the butan-1-ol–ethanol–water solvent, and also in the organic phase of the solvent system, butan-1-ol–acetic acid–water (4 : 1 : 5). Addition of picric acid to a portion of the solution of oxidation products gave a precipitate of the picrate which recrystallised from water as yellow needles, m. p. 308—310° (decomp.) [guanidine picrate, m. p. 310° (decomp.)] (Found: C, 29·5; H, 2·75; N, 28·9. Calc. for $C_7H_8N_6O_7$: C, 29·2; H, 2·8; N, 29·2%).

[4] Heinrich and Wilson, *J. Biol. Chem.*, 1950, **186**, 447.
[5] Benn and Jones, *J.*, 1960, 3837.
[6] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[7] Hubener, Bode, Mollat, and Wehner, *Z. physiol. Chem.*, 1952, **290**, 136.
[8] Partridge, *Nature*, 1949, **164**, 443.
[9] Jones and Thompson, *J. Chromatog.*, 1963, **10**, 248.

Component 1 was inorganic salts and was present in all subsequent oxidations, but it will not be recorded. Components 2, 4, 5, and 6 gave positive reactions with aniline hydrogen phthalate.

The concentrated solution of oxidation products was extracted with one volume of chloroform; this extracted component 6. The chloroform solution was evaporated to dryness and water added to the residue, which then crystallised slowly. Recrystallisation from water gave colourless cubes (A), m. p. 177—179°, R_F 0·72, of a triacetylribosylurea (Found: C, 45·7; H, 5·65; N, 8·8. Calc. for $C_{12}H_{18}N_2O_8$: C, 45·3; H, 5·6; N, 8·8%). Hydrolysis of this compound with N-hydrochloric acid at 100° for 1 hr. gave ribose (detected on paper chromatograms with the aniline hydrogen phthalate spray) and urea (detected with the fructose–hydrochloric acid spray).

The aqueous solution remaining after extraction with chloroform was evaporated and the residue extracted with methanol. The insoluble residue of inorganic material was filtered off, and the filtrate evaporated to dryness and dissolved in water, and the pH adjusted to 9. The solution was passed down a column of Zeocarb 225 (Na^+) to remove guanidine. The eluate contained the carbohydrate components 2, 4, and 5, and a trace of 6. Attempts were made to isolate components 2, 4, and 5 by partition chromatography on cellulose powder with the butan-1-ol–ethanol–water solvent, but owing to the instability of the compounds no pure product was obtained. Component 2 was obtained free from 4, 5, and 6, but contaminated with traces of urea and ribose. This component had the same R_F value as a component (B) formed during the oxidation of guanosine. It afforded ribose and urea, and was therefore probably a ribosylurea. Acetylation of component 2 by a mild procedure [3] and separation of the products by paper chromatography showed the presence of materials 2, 4, 5, and 6

Oxidation of Guanosine.—Guanosine (1 g.) was suspended in water (300 ml.), potassium permanganate (1·75 g.) was added, and the mixture was kept for 19 hr. at 37°. Manganese dioxide was then filtered off and the colourless filtrate concentrated to 30 ml. under reduced pressure. Guanidine was removed by adsorption on Zeocarb 225. Paper chromatography of the eluate from the ion-exchange resin showed the presence of urea, ribose, and a produce (B) of R_F 0·12. This could be detected with both the aniline hydrogen phthalate and the Ehrlich spray. A large amount of this material was separated by paper chromatography. Elution of the papers with water gave a product which was mainly substance B, but some degradation to ribose and urea had occurred. Hydrolysis of this impure product with N-hydrochloric acid at 37° for 24 hr. gave urea and ribose.

Oxidation of Deoxyguanosine.—Deoxyguanosine was oxidised in a similar manner to guanosine. Chromatography of the products disclosed guanidine, small amounts of 2-deoxyribose and urea, and a product (C) of R_F 0·25 which gave positive reactions for 2-deoxyribose and urea. The guanidine was removed with Zeocarb 225, the eluate from the resin evaporated to dryness, and the residue extracted with methanol. The methanol extract contained component C and traces of 2-deoxyribose and urea. Hydrolysis of this mixture with N-hydrochloric acid at 37° for 24 hr. gave 2-deoxyribose and urea. Pure synthetic 2-deoxyribosylureas were not available, but a component corresponding in chromatographic properties to C was found as a product of the acid-catalysed reaction of 2-deoxy-D-ribose with urea.[10]

Oxidation of Pyrimidine Nucleosides.—These were carried out as for the guanine derivatives. Oxidation was, however, faster, being complete in 4 hr. The products, which were identified chromatographically, were as follows:

(*a*) *Uridine:* urea, ribose, and a product, which corresponded in chromatographic properties and on treatment with acid or with alkali, to the ribosylurea, B.

(*b*) *Thymidine:* urea, 2-deoxyribose, and a product which corresponded to the deoxyribosylurea C.

(*c*) *Cytidine:* urea, biuret, ribose, the ribosylurea B, and a product (R_F 0·21) which on acid or alkaline hydrolysis gave biuret and ribose. The proportion of urea to biuret was measured as follows: The solution of the oxidation products was evaporated to dryness and extracted with methanol, and the methanol extract evaporated to dryness. A sample (20 mg.) of the residue was hydrolysed with N-hydrochloric acid (1 ml.) at 100° for 1 hr. The hydrolysate was neutralised and subjected to paper chromatography. The spots of urea and biuret were located by spraying marker strips and were eluted and their nitrogen contents were determined.

[10] Ross, Ph.D. Thesis, University of Birmingham, 1962.

Suitable blank determinations were also made. A control experiment showed that treatment of biuret with acid under these conditions gave no detectable urea. The molecular proportion of urea to biuret in the acid hydrolysate of the oxidation products was 79 : 21.

(*d*) *Deoxycytidine:* urea, biuret, 2-deoxyribose, the deoxyribosylurea C, and a product of R_F 0·32 which on acid hydrolysis gave 2-deoxyribose and biuret. This deoxyribosylbiuret on treatment with N-sodium hydroxide at 37° for 24 hr. gave a product of R_F 0·23 which also gave 2-deoxyribose and biuret on acid hydrolysis. The molecular proportion of urea to biuret derivatives in the oxidation products was measured as described above and found to be 74 : 26.

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