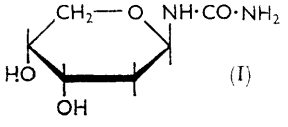
451. *Glycosylureas. Part II.*[1] *The Synthesis and Properties of 2-Deoxy-D-ribosylureas*

By W. E. Jensen, A. S. Jones, and G. W. Ross

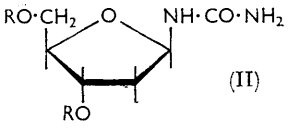
The acid-catalysed condensation of 2-deoxy-D-ribose with urea gave a mixture of 2-deoxyribosylureas. The major product, 2-deoxy-*N*-β-D-ribopyranosylurea (I), was isolated in crystalline form and characterised. Attempts to obtain 2-deoxy-*N*-β-D-ribofuranosylurea (II; R = H) by base-catalysed methanolysis of 3,5-bis-*p*-nitrobenzoyl-2-deoxy-*N*-β-D-ribofuranosylurea were unsuccessful owing to the instability of the former. Permanganate oxidation of 3′,5′-bis-*p*-nitrobenzoylthymidine gave 3,5-bis-*p*-nitrobenzoyl-2-deoxy-*N*-β-D-ribofuranosylurea.

GLYCOSYLUREAS have been synthesised by the acid-catalysed condensation of an aldose with urea, *e.g.*, D-glucose gave *N*-β-D-glucopyranosylurea and *NN*′-di-β-D-glucopyranosylurea,[1,2] whereas D-ribose gave a mixture of products including an *N*-D-ribopyranosylurea and a compound which was tentatively identified as an *N*-D-ribofuranosylurea. It now appears that both compounds were ribopyranosylureas.[3] This Paper reports attempts to synthesise 2-deoxy-*N*-β-D-ribofuranosylurea, which is important in connection with the structure and properties of permanganate-oxidised deoxyribonucleic acid,[4] and the first attempts were made by using this type of condensation.

2-Deoxy-D-ribose reacted with urea in the presence of acid to give at least four products. Two of these (components C and D) appeared to be mono-2-deoxy-D-ribosylureas in view of their mobility on paper chromatograms and reactions with the sprays specific for



(I)



(II)

2-deoxyribose and the urea residues. Component C was isolated in pure crystalline form and shown to be 2-deoxy-β-D-ribopyranosylurea (I) on the basis of the following evidence.

Component C reacted rapidly with 1·3 moles of periodate, thus indicating that it was a pyranose derivative (over-oxidation is common with glycosylureas [1]). The infrared spectrum showed that there was only very weak type 1 absorption at 920 cm.$^{-1}$ (shoulder),

[1] Part I, M. H. Benn and A. S. Jones, *J.*, 1960, 3837.
[2] A. S. Jones and G. W. Ross, *Tetrahedron*, 1962, **18**, 189.
[3] T. Naito, T. Kawakami, M. Sano, and M. Hirata, *Chem. and Pharm. Bull.* (*Japan*), 1961, **9**, 249.
[4] A. S. Jones, G. W. Ross, S. Takemura, T. W. Thompson, and R. T. Walker, *J.*, 1964, 373; A. S. Jones and R. T. Walker, *Nature*, 1964, **202**, 24, 1108.

and type 3 absorption (760—780 cm.$^{-1}$) was very weak or absent. There was a strong band at 856 cm.$^{-1}$ (CH_2 rocking), but none at 840—850 cm.$^{-1}$. Therefore, the absence of type 2a absorption (844 ± 8 cm.$^{-1}$), although not certain, was probable. This spectrum indicated, therefore, that the glycosidic linkage was β.[5] The usual urea double peak was present at 1585 and 1685 cm.$^{-1}$.

Component D was not identified, but it was probably the α-pyranose isomer because a mixture of C and D consumed 1·4 moles of periodate. When a mixture of C and D had been allowed to stand in water at +4° for several weeks a small amount of an additional component (E) of higher R_F value appeared. This corresponded in R_F to a product obtained from the permanganate oxidation of deoxyribonucleosides,[6] and gave reactions typical of a deoxyribosylurea. It was probable that E was 2-deoxy-β-D-ribofuranosylurea (II; R = H). In order to confirm this, and also to establish the nature of the products obtained from the permanganate oxidation of deoxyribonucleosides, an alternative synthesis of (II; R = H) was attempted.

3,5-Bis-*p*-nitrobenzoyl-2-deoxy-β-D-ribofuranosylurea (II; R = *p*-NO_2·C_6H_4·CO) was synthesised as described by Hoffer.[7] The *p*-nitrobenzoyl groups were removed by sodium methoxide in dry methanol. The solution contained a deoxyribosylurea which was chromatographically identical with E. Attempts to isolate the compound were unsuccessful owing to its ready partial decomposition to C. The oxidation of 3′,5′-bis-*p*-nitrobenzoylthymidine with potassium permanganate in aqueous acetone gave, in low yield, a product which was identical with (II; R = *p*-NO_2·C_6H_4·CO) synthesised by Hoffer's method.[7] It appeared that the low yield was due to the removal of some of the *p*-nitrobenzoyl groups because urea and *p*-nitrobenzoic acid were products of the oxidation. The formation of (II; R = *p*-NO_2·C_6H_4·CO) during the oxidation of the thymidine derivative was, however, additional evidence that, in the oxidation of deoxyribonucleic acids by permanganate, the thymine residues are converted into ureido residues.[4,8]

Experimental

2-Deoxy-β-D-ribopyranosylurea.—A solution of 2-deoxy-D-ribose (1·14 g.) and urea (1·4 g.) in 5% aqueous sulphuric acid (1 ml.) was kept at 30° for 44 hr. The solution was neutralised with barium carbonate, centrifuged, and the supernatant liquid freeze-dried (2·15 g.). Paper chromatography on Whatman No. 4 paper in butan-1-ol–ethanol–water (4 : 1 : 5) showed the presence of four components, A—D (R_{urea} 0·27, 0·32, 0·57, 0·68), in addition to urea and 2-deoxyribose. The four components gave positive reactions for urea and for 2-deoxyribose with the *p*-dimethylaminobenzaldehyde [9] and thiobarbituric acid [10] sprays, respectively. A total of 1·28 g. of the reaction mixture was applied as a streak to 9 sheets of Whatman No. 3 paper (previously washed with butan-1-ol–ethanol–water), and the chromatograms were run for 2 days in butan-1-ol–ethanol–water. The areas corresponding to the components C and D (these were not completely separated on this paper) were eluted with water and freeze-dried, to give a product which was a mixture of C and D (275 mg.) (Found: N, 15·7. Calc. for $C_6H_{12}N_2O_4$: N, 15·9%). This was dissolved in a minimum of water and the solution allowed to stand for several days at 4°, when crystals slowly formed. Several crops of these were collected, to give *2-deoxy-β-D-ribopyranosylurea* (65 mg.), m. p. 100—102°, $[\alpha]_D^{20}$ −11·5° (*c* 1·0 in water) (Found: C, 37·1; H, 7·3; N, 14·4; H_2O, 9·8. $C_6H_{12}N_2O_4$,H_2O requires C, 37·1; H, 7·2; N, 14·5; H_2O, 9·3%). Paper chromatography showed that the crystals contained only component C.

Periodate Oxidation.—The substance (34 mg.) was dissolved in 0·002M-sodium metaperiodate (100 ml.) and allowed to stand at 3° in the absence of light. At intervals samples (10 ml.) were withdrawn and the amount of periodate consumed determined by Fleury and Lange's method.[11]

[5] S. A. Barker, E. J. Bourne, and D. H. Whiffen, "Methods of Biochemical Analysis III," Interscience, New York, 1956, p. 273.
[6] A. S. Jones and R. T. Walker, *J.*, 1963, 3554.
[7] M. Hoffer, U.S. Pat. 2,949,449/1960, (*Chem. Abs.*, 1961, **55**, 586).
[8] M. H. Benn, B. Chatamra, and A. S. Jones, *J.*, 1960, 1014.
[9] H. J. Hübener, F. Bode, H. J. Mollat, and M. Wehner, *Z. physiol. Chem.*, 1952, **290**, 136.
[10] L. D. Saslaw and V. S. Warawdekar, *Arch. Biochem. Biophys.*, 1960, **90**, 245.
[11] P. Fleury and J. Lange, *J. Pharm. Chim.*, 1933, **17**, 107.

The number of mol. of periodate consumed were 1·16, 1·3, 1·4, and 1·5, after 3, 19·5, 51, and 403 hr., respectively. The mixture of C and D was treated with periodate in the same way; 1·11 and 1·4 mol. were consumed after 20 and 146 hr., respectively.

Attempts to crystallise component D from the mother-liquors obtained from the crystallisation of C failed. On prolonged standing of the solution an additional component (E) (R_{urea} 0·83 in butan-1-ol–ethanol–water) appeared. This gave positive reactions for urea and 2-deoxyribose residues.

*3,5-Bis-*p*-nitrobenzoyl-2-deoxy-*D*-ribofuranosylurea.*—This was synthesised as described by Hoffer,[7] m. p. 196°, $[\alpha]_D^{25}$ +54° (*c* 1·0 in acetic acid) (lit.,[7] m. p. 196°, $[\alpha]_D^{22}$ +54°) (Found: C, 50·2; H, 4·47; N, 12·0. Calc. for $C_{20}H_{18}N_4O_{10}$: C, 50·7; H, 3·83; N, 11·8%).

*3′,5′-Bis-*p*-nitrobenzoylthymidine.*—Thymidine (0·5 g.) in dry pyridine (15 ml.) was mixed, with cooling, with *p*-nitrobenzoyl chloride (1·5 g.) in dry pyridine (10 ml.). The mixture was left at room temperature for 18 hr. and poured, with stirring, into ice–water (250 ml.). The resulting precipitate was filtered off, washed with water, and dried, to give the crude product (1·14 g.), m. p. 110—135°. This material was crystallised with some difficulty from acetone–ethanol, to give 3′,5′-bis-*p*-nitrobenzoylthymidine (0·29 g.), m. p. 166—168° (lit.,[7] 166—167) (Found: N, 10·6. Calc. for $C_{24}H_{20}N_4O_{11}$: N, 10·4%).

*Permanganate Oxidation of 3′,5′-Bis-*p*-nitrobenzoylthymidine.*—A solution of 3′,5′-bis-*p*-nitrobenzoylthymidine (53 mg.) in freshly distilled acetone (5 ml.) and 0·2M-acetate buffer (pH 5·2) (0·1 ml.) was heated to 37°, mixed with a solution of potassium permanganate (53 mg.) in the same solvent (5·1 ml.) at 37°, and the mixture maintained at 37° for 12 hr. The precipitate which formed was centrifuged off (this contained only inorganic material) and the supernatant liquid was decolourised with an aqueous solution of sodium pyrosulphite (adjusted to pH 7). The precipitate which formed was centrifuged off and crystallised from pyridine to give 3,5-bis-*p*-nitrobenzoyl-2-deoxy-D-ribofuranosylurea (2·5 mg.), m. p. and mixed m. p. with material synthesised by Hoffer's method [7] (after drying at 110° for 3 hr. *in vacuo*) 196°. Material from a number of experiments was combined and the optical rotation was $[\alpha]_D^{25}$ +54°. The infrared spectra of the compounds prepared by the two methods were identical over the range 750—4000 cm.$^{-1}$, and the two products had identical R_F values (0·82) in butan-1-ol–ethanol–water (4 : 1 : 5). The pyridine mother-liquors were concentrated *in vacuo*, and the product which separated on standing (17 mg.) was filtered off. This had m. p. 120° and was probably impure starting material. The filtrate was chromatographed on Whatman No. 1 paper in butan-1-ol–ethanol–water (4 : 1 : 5) and sprayed with the *p*-dimethylaminobenzaldehyde spray.[9] Yellow spots corresponding to urea and *p*-nitrobenzoic acid were detected.

*Attempted Synthesis of 2-Deoxy-β-*D*-ribofuranosylurea.*—3,5-Bis-*p*-nitrobenzoyl-2-deoxy-β-D-ribofuranosylurea (100 mg. dried at 110° *in vacuo* for 2 hr.) was suspended in dry methanol (50 ml.), dry sodium methoxide (10 mg.) added, and the mixture shaken for 24 hr. at room temperature. A chromatogram run in butan-1-ol–ethanol–water (4 : 1 : 5) on Whatman No. 1 paper showed the presence of *p*-nitrobenzoic acid (R_{urea} 1·28) and another component (R_{urea} 0·85) which gave a positive reaction for urea [9] and corresponded to the component E which was formed in the reaction product of 2-deoxyribose with urea. Zeo-Karb 225 (H^+ form dried *in vacuo* at 110°) was added to the methanol solution, and after shaking for 2 hr. the suspension was filtered. Chromatography of the filtrate showed the presence of an additional substance which corresponded to component C which was formed by the reaction of 2-deoxyribose with urea. A similar result was obtained when the pyridinium form of the resin was used.

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CHEMISTRY DEPARTMENT, THE UNIVERSITY, BIRMINGHAM 15. [*Received, October 21st,* 1964.]