761. *Glycosylureas. Part I. Preparation and Some Reactions of* D-*Glucosylureas and* D-*Ribosylureas.*

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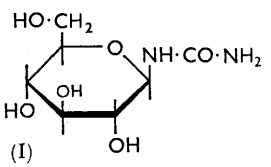
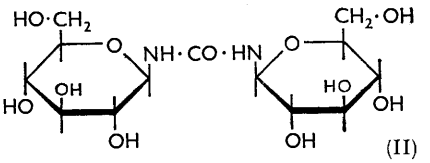
N-β-D-Glucopyranosyl- and *NN'*-di-β-D-glucopyranosyl-urea were prepared by the acid-catalysed condensation of D-glucose with urea; D-ribose gave *N*-D-ribopyranosyl-, *N*-D-ribofuranosyl-, and various *NN'*-di-D-ribosyl-ureas. The monoglycosylureas are rapidly degraded to the sugar by nitrous acid in N-sulphuric acid at 0°, but hydrolysis by N-sulphuric acid alone is very slow. Alkaline degradation of the glycosylureas to sugar is effected but is complex. No evidence of Amadori or Lobry de Bruyn–van Eckenstein rearrangements was found.

Glycosylureas (ureido-sugars) are of interest as potential intermediates in the synthesis [1] and degradation [2,3] of nucleosides, nucleotides, and nucleic acids. D-Glucosylurea was first obtained by Schoorl [4] on acid-catalysed condensation of D-glucose with urea; his poor yield was later improved by others.[5] It has also been produced by the action of aqueous ammonia on 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl isocyanate.[6]

The present investigation on the condensation of D-glucose with urea under a variety of conditions has shown that *N*-β-D-glucopyranosyl- (I) and *NN'*-di-β-D-glucopyranosyl-urea (II) are formed, the former being the major product when >1 mol. of glucose was used in 5% aqueous sulphuric acid, and the diglucosylurea (II) when 2 mol. were used. It has already been shown [7] that the diglucosylurea (II) is the *NN'*- and not the *NN*-compound.



(I)



(II)

N-D-Glucosyl- (I) and *NN'*-di-D-glucosyl-urea (II), when oxidised with sodium metaperiodate in aqueous unbuffered solution at 4° in the dark, consumed 2 and 4 mols. of periodate respectively. Neither *N*-formyl- nor *N*-2-hydroxyethyl-urea consumed periodate under these conditions. These results are in accord with the reported stability to periodate oxidation of the $-CH(OH)\cdot CH_2\cdot NH\cdot COR$ system in acylserine derivatives [8] and the consumption of 2 mol. of periodate by *N*-acetyl-D-glucopyranosylamine,[9] and they indicate that the glucosylureas are not in the acyclic form proposed by Schoorl.[4] Evidence from synthetic studies [1] strongly suggests that the ring in *N*-D-glucosylurea is pyranose and that the glycosidic linkage is β. In *NN'*-di-D-glucosylurea the structure appears to be similar.[7]

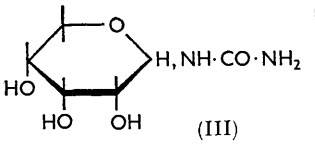
N-β-D-Glucosylurea was slowly hydrolysed by dilute sulphuric acid at 0° to D-glucose. Dilute nitrous acid at 0° rapidly converted the glucosylurea into D-glucose and similarly converted 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosylurea to 2,3,4,6-tetra-*O*-acetyl-glucopyranose. No evidence of an Amadori rearrangement to a fructosylurea was found, although *N*-D-glucosylglycine undergoes acid-catalysed irreversible rearrangement to

[1] Goodman, *Adv. Carbohydrate Chem.*, 1958, **13**, 215.
[2] Cohn and Doherty, *J. Amer. Chem. Soc.*, 1956, **78**, 2863.
[3] Bayley and Jones, *Trans. Faraday Soc.*, 1959, **55**, 492.
[4] Schoorl, *Rec. Trav. chim.*, 1903, **22**, 31.
[5] Helferich and Kosche, *Ber.*, 1926, **59**, 69; Hynd, *Biochem. J.*, 1926, 205; B.P. 653,775/1951.
[6] Fischer, *Ber.*, 1914, **47**, 1389.
[7] Johnson and Bergmann, *J. Amer. Chem. Soc.*, 1932, **54**, 3360.
[8] Posternak and Pollaczek, *Helv. Chim. Acta*, 1941, **24**, 1190.
[9] Nieman and Hays, *J. Amer. Chem. Soc.*, 1940, **62**, 2960.

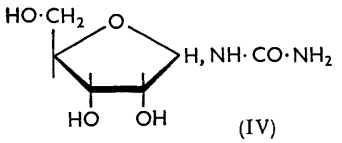
N-D-fructosylglycine.[10] The failure of the glucosylurea to undergo an Amadori rearrangement may be due to the low basicity of the substituted nitrogen atom, to which, in the first step of the accepted mechanism for the rearrangement,[11] a proton becomes attached—the very weakly basic *N*-acetyl-D-glucosylamine also does not undergo this rearrangement.[12]

Schoorl [4] observed that boiling barium hydroxide solution decomposed *N*-β-D-glucopyranosylurea with the evolution of ammonia. At lower temperatures, however, a more subtle change took place in which the optical rotation of the reaction mixture fell slowly to a constant value; unchanged *N*-β-D-glucopyranosylurea, and a syrup which Schoorl suggested was a mixture of hexosylureas formed by a Lobry de Bruyn–van Eckenstein rearrangement were then recovered. We found that at 37° the optical rotation was unchanged at pH 9, and only very slightly changed at pH 11, but was markedly changed after 3 days in N-sodium hydroxide. In the last case paper chromatography showed that most of the glucosylurea was unaltered but that small amounts of D-glucose, urea, and unidentified products were present. Treatment of the reaction mixture with nitrous acid gave D-glucose and a small amount of an unidentified component, but no D-mannose or D-fructose. There was, therefore, no evidence of the occurrence of a Lobry de Bruyn–van Eckenstein rearrangement. The change in optical rotation may have been due to the production of D-glucose which is then further degraded by the alkali to acidic products.[13] After 3 weeks' treatment with N-sodium hydroxide at 37° much unchanged glucosylurea was still present, but an appreciable amount of glucose, some urea, and a number of unidentified products were also formed.

Condensation of D-ribose with urea under the conditions used for the preparation of *N*-β-D-glucopyranosylurea gave a product which was shown by paper chromatography to contain, in addition to urea and D-ribose, three slow-moving (A, B, and C) and two fast-moving (D and E) compounds. Column chromatography on charcoal–Celite afforded chromatographically pure (D) and almost pure (E), but compounds (A), (B), and (C) were not separated from each other. Compounds (D) and (E) gave correct analyses for D-ribosylurea, were rapidly degraded to D-ribose by nitrous acid, and on 48 hours' treatment at 4° in the dark consumed 2·2 and 1·4 mol. respectively of periodate. This indicated that compound (D) was *N*-D-ribopyranosylurea (III), and that compound (E) was probably *N*-D-ribofuranosylurea (IV). The rather high periodate uptake of the latter could be ascribed to over-oxidation, as observed [1] with glucosylureas at 25°, or to isomerisation to the pyranosyl form under the acidic conditions of the oxidation. The latter explanation seemed probable since in dilute acid at 0° hydrolysis of these ureas to D-ribose was slow but some of compound (E) was converted into (D). The structural assignments are



(III)



(IV)

supported by the relative optical rotations and mobility on paper chromatograms, because in the D-ribose series furanosyl compounds have the higher rotations [14] and travel the faster in certain solvent systems.[15] It appeared that compounds (A), (B), and (C) were *NN'*-di-D-ribosylureas because the reaction of 2 mols. of D-ribose with urea gave a crystalline product, giving correct analyses for diribosylurea and shown by paper chromatography to contain these three compounds. This mixture consumed 3·8 mol. of

[10] Anet, *Austral. J. Chem.*, 1957, **10**, 193.
[11] Isbell, *Ann. Rev. Biochem.*, 1948, **12**, 205.
[12] Mitts and Hixon, *J. Amer. Chem. Soc.*, 1944, **66**, 483.
[13] Nef, *Annalen*, 1910, **376**, 1.
[14] Chargaff and Davidson, "Nucleic Acids," Vol. I, Academic Press, New York, 1955, p. 65.
[15] Isherwood and Jermyn, *Biochem. J.*, 1951, **48**, 515.

periodate in 48 hr. at 4° in the dark, so that when over-oxidation is taken into account the presence of both pyranose and furanose isomers can be assumed.

Both *N*-D-ribopyranosyl- and *N*-D-ribofuranosyl-urea were stable at pH 9 at 37°, but in 0·2N-barium hydroxide at 37° slow hydrolysis to D-ribose took place.

Experimental

Paper Chromatography.—Descending chromatograms were run on Whatman No. 1 paper in butan-1-ol–ethanol–water (4 : 1 : 5) for 48 hr. (system I) or in butan-1-ol–acetic acid–water (4 : 1 : 5) for 60 hr. for glucose derivatives or 36 hr. for ribose derivatives (system II). The spots were located with silver nitrate [16] or aniline hydrogen phthalate,[17] and their motion relative to glucose (R_G) measured. With silver nitrate the glycosylureas appeared first as a white spot which quickly developed a brown halo, and with aniline hydrogen phthalate glucosylurea gave a brown spot and ribosylurea a reddish spot.

N-β-D-*Glucopyranosylurea.*—This compound was prepared by the four published procedures.[4,5] In all cases the product had m. p. 206—208° (decomp.), $[\alpha]_D^{23}$ −22° (*c* 2 in H_2O) and on paper chromatography (system II) gave two spots of R_G 0·14 and 0·63, the latter being the more intense. Fractional crystallisation failed to remove the slower component (later identified as a diglucosylurea). Hynd's procedure [5] gave the best yield of almost pure *N*-β-D-glucopyranosylurea, and chromatographically pure material was obtained from this product by chromatography on a charcoal–Celite column.[18] The following procedure, however, has the advantage of requiring a shorter reaction time.

D-Glucose hydrate (10 g.) and urea (10 g.) in 5% aqueous sulphuric acid (5 ml.) were stirred at 70° for 18 hr. More 5% aqueous sulphuric acid (5 ml.) was added and reaction continued for a further 24 hr. The mixture was cooled and the crystals which were formed were triturated with methanol, filtered off, and air-dried, to give crude *N*-β-D-glucopyranosylurea–urea complex (10·2 g.), m. p. 160—170° (lit.,[5] m. p. 171—172°). The ground complex (5 g.) was extracted six times with boiling methanol (15 ml.), to give *N*-β-D-glucopyranosylurea (3·5 g.), m. p. 204—205°. An aqueous solution (15 ml.) of this product (1·6 g.) was placed on charcoal–Celite (30 cm. × 15·9 cm.^{2}) and the column eluted with water. Fractions of 25 ml. were collected and small portions (0·75 ml.) tested with Dreywood's reagent.[19] Positive tests were given by fractions 19—40, which were combined and freeze-dried to give chromatographically pure *N*-β-D-glucopyranosylurea (1·03 g.), R_G 0·63 (system II); when crystallised from aqueous ethanol this had m. p. 208—209°, $[\alpha]_D^{18\cdot5}$ −22° (*c* 2 in H_2O). Further elution of the column with 6% aqueous ethanol gave a second group of fractions which were combined and freeze-dried to give chromatographically pure *NN'*-di-β-D-glucopyranosylurea (0·09 g.), R_G 0·14 (system II), which when crystallised from aqueous methanol had m. p. >300° (decomp.), $[\alpha]_D^{19}$ −33° (*c* 2 in H_2O).

NN'-*Di*-β-D-*glucopyranosylurea.*—D-Glucose hydrate (35 g.) and urea (5 g.) in 5% aqueous sulphuric acid (20 ml.) were stirred at 70° for 18 hr. The mixture was cooled, diluted with water (20 ml.), neutralised with barium carbonate, decolorised with charcoal, and concentrated under reduced pressure to an oil which crystallised when extracted with boiling methanol. The crystals (16 g.) were recrystallised twice by dissolving them in hot water and adding two volumes of hot methanol. On cooling, chromatographically pure *NN'*-di-β-D-glucopyranosylurea, m. p. 345° (decomp.), $[\alpha]_D^{18\cdot5}$ −34° (*c* 2 in H_2O), R_G 0·15 (system II), was obtained (Found: C, 40·7; H, 6·5; N, 7·2. Calc. for $C_{13}H_{24}N_2O_{11}$: C, 40·6; H, 6·3; N, 7·3%).

D-*Ribosylureas.*—D-Ribose (3 g.) and urea (3 g.) in 5% aqueous sulphuric acid (2 ml.) were stirred at 60° for 18 hr. The cooled mixture was diluted with water (3 ml.), neutralised with barium carbonate, decolorised with charcoal, and concentrated under reduced pressure at <45° to a syrup (4·2 g.) which did not crystallise. Paper chromatography of this product (system I) showed the presence of five compounds in addition to ribose and urea, with the following R_G values and colours with the silver nitrate spray reagent: A, 0·25, brown; B, 0·34, brown; C, 0·46, brown; D, 0·91, white with brown halo; E, 1·26, white with brown halo. The syrup

[16] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[17] Partridge, *Nature*, 1949, **164**, 443.
[18] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677; Barker, Bourne, and Theander, *J.*, 1955, 4276.
[19] Morris, *Science*, 1948, **107**, 254; Dreywood, *Ind. Eng. Chem. Analyt.*, 1946, **18**, 499.

(2·5 g.) was fractionated by column chromatography on charcoal–Celite as described above. 25 ml. fractions were collected, the first 100 being of water, and the next 50 of water–ethanol (92 : 8). Fractions 41—50 contained compound D (0·351 g.), fractions 51—60 contained compound D and a little compound E (0·270 g.), fractions 61—70 contained almost pure E (0·185 g.), and fractions 99—150 contained A, B, and C (0·637 g.) with traces of D and E. Compound D was isolated as an amorphous solid (0·351 g.) which was identified as N-D-*ribopyranosylurea* (Found: C, 37·4; H, 6·3; N, 14·75. $C_6H_{12}N_2O_5$ requires C, 37·5; H, 6·3; N, 14·6%), $[\alpha]_D^{18}$ −27° (*c* 2 in H_2O). The almost pure compound E was isolated similarly and identified as *N*-D-ribofuranosylurea (Found: N, 14·2%), $[\alpha]_D^{23}$ +24° (*c* 2 in H_2O).

*Di-*N*-ribosylureas.*—D-Ribose (0·85 g.) and urea (0·165 g.) in 8% aqueous sulphuric acid (0·5 ml.) were heated at 50° for 24 hr. The cooled mixture was diluted with water (5 ml.), neutralised with barium carbonate, decolorised with charcoal, and freeze-dried. The residue was dissolved in hot water (2 ml.), and hot methanol (20 ml.) was added. The crystals which separated (0·29 g.) were recrystallised from aqueous methanol to give prisms of di-*N*-ribosylurea, m. p. 249° (decomp. from 235°), $[\alpha]_D^{25}$ −35° (*c* 2 in H_2O) (Found: C, 40·5; H, 6·25; N, 8·35. Calc. for $C_{11}H_{20}N_2O_9$: C, 40·75; H, 6·2; N, 8·6%). Paper chromatography (systems I and II) showed the presence of compound A, B, and C in this mixture.

Periodate Oxidations.—These were carried out in the dark at 4° by Fleury and Lange's method.[20] After 72 hr., *N*-β-D-glucopyranosyl- and *NN'*-di-β-D-glucopyranosyl-urea consumed 1·98 and 4·03 mols. of periodate respectively. At 20—25° there was considerable over-oxidation. At 4° *N*-formyl-[21] and *N*-2-hydroxyethyl-urea[22] did not react with periodate. *N*-Ribopyranosylurea (D), *N*-ribofuranosylurea (E), and diribosylureas (A, B, and C) took up 2·2, 1·4, and 3·8 mols. of periodate respectively in 48 hr.

Reactions with Nitrous Acid.—(*a*) A solution of *N*-β-D-glucopyranosylurea (200 mg.) in ice-cold N-sulphuric acid (5 ml.) was divided into two equal portions. To one was added sodium nitrite (100 mg.). Both portions were left at 0° and at intervals samples were removed and examined by paper chromatography (system II). In the solution containing sodium nitrite all the glucosylurea had been converted into glucose in 4 hr. In the other, hydrolysis was very slow and only became apparent after 24 hr. In neither case was fructose detected. Similar treatment of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)urea with nitrous acid gave 2,3,4,6-tetra-*O*-acetylglucopyranose; treatment with N-sulphuric acid at 0° for 24 hr. resulted in only slight hydrolysis.

(*b*) A solution of *N*-ribopyranosylurea (compound D; 50 mg.) in ice-cold 0·1N-hydrochloric acid (6·25 ml.) containing potassium nitrite (150 mg.) was set aside at 3°. At intervals, samples (1 ml.) were withdrawn, neutralised with barium carbonate, and examined by paper chromatography (system II). After 4 hr. the ribosylurea had been completely converted into ribose. In absence of the potassium nitrite hydrolysis was incomplete after 48 hr. Similar results were obtained when compound E (*N*-ribofuranosylurea) was examined by the same procedure, but a spot due to compound D also appeared on the chromatograms of both nitrite and non-nitrite treated solutions.

Effect of Alkalis.—(*a*) Aqueous 2% solutions of *N*-β-D-glucopyranosylurea in (i) sodium hydrogen carbonate (pH 9), (ii) sodium carbonate (pH 11), and (iii) N-sodium hydroxide were left at 37° and at intervals examined in the polarimeter. The results are tabulated.

Hours	0	1	2	4	7	12	24	48	72
(i), $[\alpha]_D^{22}$	−22°	−22°	—	—	−23°	—	−22°	−22°	−22°
(ii), $[\alpha]_D^{22}$	−22°	−22°	−22°	—	—	—	−20°	−20°	−19°
(iii), $[\alpha]_D^{22}$	−23°	−16°	—	−12°	−11°	−10°	−9·7°	−9·2°	−9·0°

After 72 hr., the mixtures were neutralised and examined by paper chromatography (system II). Mixtures (i) and (ii) contained only glucosylurea, and mixture (iii) contained in addition a little glucose, urea, and unidentified products. After 3 weeks at 37°, mixture (iii) still contained unchanged glucosylurea and some glucose, urea, and unidentified products. After 72 hr. at 37° a portion of mixture (iii) was acidified, cooled to 0°, and treated with sodium nitrite for 4 hr. On neutralisation and examination by paper chromatography (system II),

[20] Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 107.
[21] Maynert and Washburn, *J. Org. Chem.*, 1950, **15**, 259.
[22] Charlton and Day, *J. Org. Chem.*, 1937, **1**, 552.

glucose and a small amount of an unidentified component (R_G 1·32; white spot with the silver nitrate spray) were detected.

(*b*) Aqueous 2% solutions of *N*-D-ribopyranosyl- (compound D) and *N*-D-ribofuranosyl-urea (compound E) in (i) sodium hydrogen carbonate (pH 9) and (ii) 0·2N-barium hydroxide were left at 37°. At intervals up to 72 hr., portions were withdrawn, cooled, made faintly acidic with dilute sulphuric acid, neutralised with barium carbonate, and examined by paper chromatography (system I). At pH 9 there was no apparent degradation or isomerisation, but in 0·2N-barium hydroxide slow hydrolysis to ribose took place. Some isomerisation of compound (E) to (D) was also apparent.

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