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GLYCOSYLUREAS—III

THE SYNTHESIS OF D-MANNOSYL- AND D-GALACTOSYLUREAS

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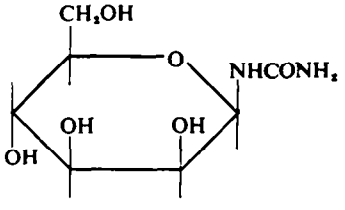
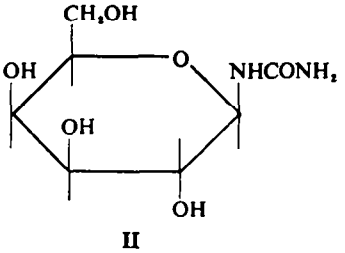
Abstract—The acid-catalysed condensation of D-mannose and D-galactose with urea gave N-β-D-mannopyranosylurea and N-β-D-galactopyranosylurea respectively as the main products. The pyranose structure of these compounds was established by the use of periodate oxidation. The β-configuration of the glycosidic linkage was indicated by the fact that the products of the reaction of periodate had the same optical rotation as the products of the reaction of periodate with N-β-D-glucopyranosylurea. This assignment of configuration was confirmed in the case of the mannose derivative by the unambiguous synthesis of the corresponding α anomer (as the tetra-O-acetate) and in the case of the galactose derivative by comparison of the optical activity of the glycosylureas with that of the appropriate methyl glycosides.

IN previous papers of this series,[1] the synthesis of urea derivatives of D-glucose, D-ribose and 2-deoxy-D-ribose, by the acid-catalysed condensation of the aldose with urea, has been reported. The present paper reports similar syntheses of urea derivatives of D-mannose and D-galactose. A galactosylurea was first synthesized by this method by Schoorl[2] but it was not obtained pure nor adequately characterized. The same worker also obtained a dimannosylurea but not a monomannosylurea.

In the present work D-mannose and D-galactose were treated with urea in the presence of aqueous acid under conditions similar to those used for the synthesis of N-β-D-glucopyranosylurea. As with the latter, the crude products were mixtures which were purified by chromatography on charcoal–celite columns to give the required monoglycosylureas (I and II). A tetra-O-acetate was obtained in each case from the



I



II

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[1] M. H. Benn and A. S. Jones, *J. Chem. Soc.* 3837 (1960); W. E. Jensen, A. S. Jones and G. W. Ross *Ibid.* 2463 (1965).

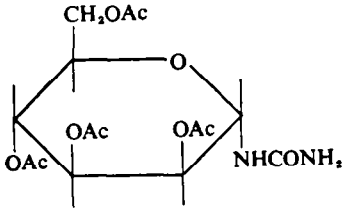
[2] M. N. Schoorl, *Rec. Trav. Chim.* **22**, 31 (1903).

two glycosylureas by the action of acetic anhydride in pyridine, and from the galactosylurea a pentacetate was obtained by the action of acetic anhydride and zinc chloride at 100°.

The two glycosylureas were shown to be pyranosylureas by the fact that they consumed two moles of periodate at 2° in the dark, and negligible amounts of formaldehyde were produced. These results exclude both furanose and open chain structures.

When equimolar solutions of the N-D-mannopyranosylurea, the N-D-galactopyranosylurea and N-β-D-glucopyranosylurea were treated with periodate, the optical rotations of the resulting solutions were identical. As the periodate destroyed the asymmetry at C_2, C_3 and C_4, and as the three compounds were all of the D series, this indicated a β-configuration at C_1 in each of the three cases. This result was not entirely conclusive, however, because chromatography of the products of the reactions with periodate, showed that, although they were identical, several compounds were present in each case.

In order to confirm the assignment of the β-configuration to the D-mannopyranosylurea, tetra-O-acetyl-D-mannopyranosyl chloride was treated with silver cyanate to give the corresponding isocyanate. This, on treatment with methanolic ammonia at low temperature gave a tetra-O-acetyl-D-mannopyranosylurea. This must have been the α anomer (III), because in the replacement of the chlorine of the tetra-O-acetyl-D-mannopyranosyl chloride by isocyanate, the latter takes up an orientation which is *trans* to the acyloxy group at C_2 irrespective of the orientation of the chlorine at C_1.



III

Compound III differed from the tetra-O-acetate obtained from II in m.p. optical rotation and IR spectrum. Hydrolysis of III with aqueous ammonia gave a mannosylurea which was chromatographically different from II. Hence it was concluded that II was N-β-D-mannopyranosylurea.

This assignment of configuration is supported by the optical activity of the compounds. Comparison of the optical rotation of N-β-D-glucopyranosylurea ($[M]_D$ −52·5°) with those of methyl β-D-glucopyranoside ($[M]_D$ −62°) and methyl α-D-glucopyranoside ($[M]_D$ +312°) showed that the ureido group had a very similar effect on optical activity to the methoxyl group in this case. The optical activities of the tetra-O-acetyl-α- and β-D-mannopyranosylureas ($[M]_D$ +178° and −122° respectively) were similar to those of the corresponding methyl tetra-O-acetyl-α-and β-D-mannopyranosides ($[M]_D$ +178° and −170° respectively). Because of this correlation in the glucose and mannose series it seemed justified to use this type of evidence to confirm the structure of the galactopyranosylurea. The optical rotation ($[M]_D$) of this is +22° whereas that of methyl β-D-galactopyranoside is −0·82° and methyl α-D-galactopyranoside is +345°. It may be concluded, therefore, that the D-galactopyranosylurea has a β-configuration.

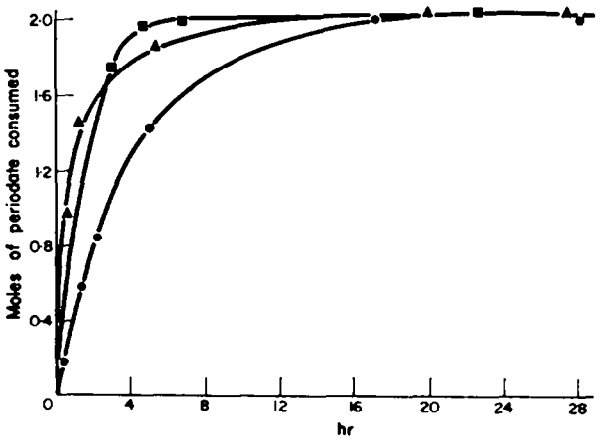


FIG. 1. Reaction of periodate with glycosylureas at 2° in the dark:
●—● N-β-D-glucopyranosylurea; ▲—▲ N-β-D-mannopyranosylurea;
■—■ N-β-D-galactopyranosylurea.

EXPERIMENTAL

Paper chromatography. Chromatograms were run on either Whatman No. 1 or No. 4 paper in butan-1-ol–EtOH–water (4:1:5). The components were detected by the use of the $AgNO_3$[3] or the *p*-dimethylaminobenzaldehyde[4] sprays. Mobilities are quoted with respect to urea (R_U value).

N-β-Galactopyranosylurea. D-Galactose (24 g) and urea (24 g) were allowed to react in 1% (v/v) H_2SO_4aq (120 ml) at 50° for 3 days. Paper chromatography of the reaction mixture on Whatman No. 4 paper for 22 hr showed the presence of 5 components in addition to galactose and urea. These had the following R_U values and gave the following colours with the $AgNO_3$ spray: A, 0·04, brown; B, 0·08, light brown; C, 0·13, brown; D, 0·18, white with brown halo; E, 0·25, brown. The reaction solutions were neutralized with $BaCO_3$, decolourized with charcoal and concentrated to a syrup. This was solidified by trituration with EtOH and then with hot MeOH. The solid so obtained was extracted 4 times for 3–6 hr with boiling EtOH (75 ml) to give a white powder (8 g) which decomposed at 182°; this contained mainly component D. A portion of this solid (1 g) was crystallized from MeOH, but the product was contaminated with a little A. It was purified by chromatography on a column (16·2 × 2 cm diam) of charcoal–celite by the use of a gradient of EtOH in water (0 → 1% in 1 l.) and the fraction which was eluted between 75 ml and 200 ml of eluant, collected and evaporated to dryness to give a deliquescent solid (0·267 g). This was dried by adding dry MeOH and distilling off the solvent, and then crystallized from MeOH to give needles of chromatographically pure *N-β-D-galactopyranosylurea.* (Found: C, 37·57; H, 6·41; N, 12·8. $C_7H_{14}N_2O_6$ requires: C, 37·83; H, 6·34; N, 12·6%). The compound decomposed at 189° and then melted at 194°; $(\alpha)_D^{26}$ +10·0° (*c*, 1·74 in H_2O).

N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)urea. N-β-D-galactopyranosylurea (0·33 g) was treated with Ac_2O (1·3 ml) and dry pyridine (2·3 ml) with shaking at room temp for 40 hr. The reaction mixture was cooled to 4° and the crystalline material (70–80% yield) crystallized from MeOH to give prisms, m.p. 221–223° (d); $(\alpha)_D^{26}$ +9·09° (*c*, 0·66 in C_5H_5N). (Found: C, 46·54; H, 5·66; N, 7·58. $C_{15}H_{22}N_2O_{10}$ requires: C, 46·15; H, 5·68; N, 7·18%.)

N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N-acetylurea. Anhydrous $ZnCl_2$ (0·4 g) was dissolved in Ac_2O (11 ml) and N-β-D-galactopyranosylurea (1 g) added and the mixture heated at 100° for 10 min. Part of the Ac_2O was removed by distillation *in vacuo* and the residual solution poured into water (300 ml). The mixture was neutralized with $NaHCO_3$, extracted with $CHCl_3$ (4 times), the extract dried over Na_2SO_4 and the $CHCl_3$ removed by distillation under red. press. The resulting syrup (which gave one component on paper chromatography which gave a yellow colour only slowly

[3] W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature, Lond.* **166,** 444 (1950).
[4] H. J. Hubener, F. Bode, H. J. Mollatt and M. Wehner, *Z. Physiol. Chem.* **290,** 136 (1952).

with Ehrlich's reagent) was dissolved in AcOEt, decolourized with charcoal and evaporated to dryness to give a colourless powder which sintered at 80° and melted at 110°; $(\alpha)_D^{23}$ +4·0° (*c*, 2·0 in $CHCl_3$). (Found: C, 47·24; H, 5·79; N, 6·12. $C_{17}H_{24}N_2O_{11}$ requires: C, 47·22; H, 5·59; N, 6·48%.)

N-β-D-*Mannopyranosylurea*. To a solution of D-mannose (24 g) and urea (24 g) in water (111 ml) there was added 25% (v/v) H_2SO_4 (5 ml) and the solution kept at 50° for 7 days. It was then brought to pH 6 by the addition of $BaCO_3$ and the resulting suspension filtered. The filtrate was decolourized with charcoal and a sample subjected to paper chromatography on Whatman No. 4 paper. In addition to D-mannose and urea, 4 components were detected, which had the following R_U values and colours with the $AgNO_3$ spray: A, 0·06, brown; B, 0·12, brown; C, 0·27, white with brown halo; D, 0·37, brown. The bulk of the solution was concentrated to dryness *in vacuo*. The semi-solid residue crystallized upon extraction with boiling MeOH to give a product (21 g) which consisted of mainly component C and urea, but which also contained small amounts of A and D. This material was repeatedly extracted with boiling dry EtOH (total volume, 520 ml) and the EtOH extract evaporated to dryness to give a residue (1·7 g) which was crystallized from MeOH to give N-β-D-*mannopyranosylurea–urea adduct* (0·23 g), m.p. 160–160·5° (d); $(\alpha)_D^{23}$ −34·9°. (Found: C, 34·16; H, 6·85; N, 19·3. $C_7H_{14}O_6N_2$. CH_4N_2O requires: C, 34·04; H, 6·43; N, 19·8%.) Attempts to obtain large quantities of this compound gave an impure product, so a portion (5·8 g) of the residue which had not dissolved in EtOH was dissolved in water (20 ml) and applied to a column (46 cm × 3·6 cm diam) of charcoal–celite and eluted with EtOH–water (1:99); 25 ml fractions were collected. Each 4th fraction was examined by paper chromatography on Whatman No. 4 paper. The major component was present in almost pure form in fractions 358 to 626. These were combined, concentrated to dryness and the residue (3·5 g) crystallized from MeOH to give chromatographically pure N-β-D-*mannopyranosylurea*, m.p. 163° (d); $(\alpha)_D^{23}$ −41·3°(*c*, 0·45 in H_2O). (Found: C, 37·54; H, 6·58; N, 12·9. $C_7H_{14}N_2O_6$ requires: C, 37·83; H, 6·34; N, 12·6%.)

N-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-*urea*. A mixture of N-β-D-mannopyranosylurea (1 g), Ac_2O (4 ml) and dry pyridine (7 ml) was shaken at room temp for 23 hr. The reaction mixture was filtered and the filtrate concentrated *in vacuo* to a small bulk. The crystalline material which separated was filtered off and crystallized from MeOH to give N-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-*urea* (61%) m.p. 213–215° (d); $(\alpha)_D^{26}$ −31·4° (*c*, 0·51 in C_5H_5N). (Found: C, 46·26; H, 5·48; N, 7·23. $C_{15}H_{22}N_2O_{10}$ requires: C, 46·15; H, 5·68; N, 7·18%.) The compound had R_F value of 0·68 on paper chromatography.

Periodate oxidations

The two glycosylureas and N-β-D-glucopyranosylurea were treated with 0·02M sodium metaperiodate at 2° in the dark and at intervals the amount of periodate consumed determined by Fleury and Lange's method.[5] The results are shown in Fig. 1. The reaction mixtures obtained from the galactosyl- and the mannosylurea were examined for the presence of formaldehyde by the dimedone method. At the same time a control experiment was carried out on the products of the reaction of periodate with mannitol. In the case of the galactosylurea and mannosylurea a very small precipitate was formed with the dimedone corresponding to 0·11 and 0·04 moles of formaldehyde respectively. In neither case did this precipitate have the m.p. of the dimedone derivative of formaldehyde. From the experiment with mannitol the amount of precipitate corresponded to 2·02 moles of formaldehyde per mole of mannitol and the m.p. of the product was 189–191°.

Equimolar solutions of the 3 glycosylureas were treated with 0·2M sodium metaperiodate at 2° in the dark until 2 moles of periodate had been consumed and then the optical rotations of the solutions were measured. All three solutions had a rotation of +0·04 in a 0·5 dm tube. The periodate and iodate were removed by the addition of $Ba(OH)_2$ (to pH 8·4), the Ba-salts filtered off and the filtrate examined by paper chromatography on Whatman No. 1 paper in phenol–water (4:1) and in the butanol–EtOH–water solvent. The reaction mixtures from the 3 glycosylureas gave an identical array of spots, but the reaction mixtures were complex, there being three components which gave yellow spots and three which gave blue spots with the Ehrlich spray in each case.

2,3,4,6-*Tetra*-O-*acetyl*-α-D-*mannosyl isocyanate*. 2,3,4,6-Tetra-O-acetyl-D-mannosyl chloride (5 g) was dissolved in dry toluene (24 ml), finely divided, dry AgCNO (3·5 g) was added and the mixture

[5] P. Fleury and J. Lange, *J. Pharm. Chim.* **17**, 107 (1933).

heated on a boiling water bath in the dark with stirring for 2·5 hr. The suspension was filtered, the solid residue washed with toluene (2 × 5 ml) and the combined filtrate and washings added to light petroleum (b.p. 40–60°; 35 ml). The viscous gum which separated was allowed to settle and to the clear supernatant liquid there was added a further quantity of light petroleum (16 ml) and the liquid allowed to stand at 0°. After the addition of more light petroleum (20 ml) and standing at 0° for 3 days, a crystalline product was obtained. This was filtered off and dried (11 g). It sintered at 60° and melted at 100°. $(\alpha)_D^{27}$ +36·0 (*c*, 1·0 in C_5H_5N). (Found: C, 48·63; H, 5·42; N, 3·44. $C_{15}H_{19}NO_{10}$ requires: C, 48·26; H, 5·13; N, 3·75% IR carbonyl peak at 1745 cm^{-1}, isocyanate at 2275 cm^{-1}.) A second crop of isocyanate (0·44 g) was obtained giving a total yield of 31%.

The viscous gum which separated upon the first addition of light petroleum was crystallized with difficulty from a mixture of AcOEt and ether. The product (1·96 g) differed from the isocyanate with respect to its IR spectrum and in that it gave a yellow colour on paper chromatograms with the Ehrlich reagent whereas the isocyanate did not. It was possibly an octa-O-acetyl-dimannosylurea. (Found: C, 48·33; H, 5·65; N, 4·01; acetyl, 47·5. $C_{39}H_{40}N_2O_{19}$ requires: C, 48·33; H, 5·60; N, 3·89; acetyl, 47·8%.)

N-(2,3,4,6-*Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-*urea*

Tetra-O-acetyl-D-mannopyranosylisocyanate (0·5 g) was dissolved in dry $CHCl_3$ (5 ml) and methanolic ammonia (0·5 ml containing 0·13 g of NH_3) and the mixture shaken at 2° for 30 min. The solvent was then distilled off *in vacuo* and the residual syrup crystallized from MeOH to give N-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-*urea*, m.p. 209° (d) $(\alpha)_D^{23}$ +45·7 (*c*, 1·4 in C_5H_5N). (Found: C, 46·04; H, 5·78; N, 7·35. $C_{15}H_{22}N_2O_{10}$ requires: C, 46·15; H, 5·68; N, 7·18%.) The material gave one spot with R_f 0·68 on paper chromatography and gave colours typical of a glycosylurea with the Ehrlich and the $AgNO_3$ sprays. When mixed with the corresponding β-anomer the mixture sintered and decomposed at 198°. The IR spectrum of this compound differed markedly from that of the β-compound in the region 800–1000 cm^{-1}.

Deacetylation of N-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-*urea*. The tetra-O-acetate (5 mg) was suspended in aqueous ammonia (s.g., 0·88) at room temp. It dissolved in a few minutes. Paper chromatography of the solution gave a single spot of R_G 0·37 which gave a yellow colour with the Ehrlich spray. When the chromatogram was run on the same paper as the products of the acid-catalysed reaction of mannose with urea, this spot did not correspond with the main component, N-β-D-mannopyranosylurea, but did correspond with component D in the reaction mixture.

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