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THE STRUCTURE OF D-GLUCOSYLUREAS

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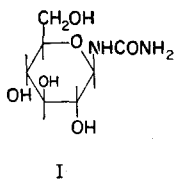
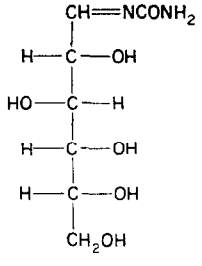
Abstract—Evidence from studies of periodate oxidation and from I.R. and U.V. spectroscopy is presented to support the conclusion that the mono- and di-D-glucosylureas formed by the acid-catalysed reaction of D-glucose with urea are N-β-D-glucopyranosylurea and N,N'-di-β-D-glucopyranosylurea respectively.

THERE is considerable evidence[1] that the mono-D-glucosylurea formed by the reaction of D-glucose with urea in acid solution[2,3] is N-β-D-glucopyranosylurea (I). Thus tetra-*O*-acetyl-α-D-glucopyranosyl bromide on treatment with silver cyanate and then with ammonia gave an identical monoglucosylurea.[4] Complete acetylation gave a penta-acetate while acetylation with pyridine as catalyst gave a tetra-acetate.[5] Treatment of this tetra-acetate with nitrous acid at 0° gave 2,3,4,6-tetra-O-acetyl-D-glucopyranose.[6] Furthermore the monoglucosylurea consumed 2 moles of periodate at 4° in the dark[6] and during periodate oxidation only a trace of formaldehyde was produced.[7] These results indicate that the monoglucosylurea does not exist in the open chain form (II) proposed by Schoorl.[2]



I



II

It has been briefly reported, however, that mono-D-glucosylurea consumed 5 moles of periodate in 24 hours at 25°[8] and from evidence from infra-red spectroscopy it has been suggested[9] that mono-D-glucosylurea does exist in the open chain form. In view of these conflicting conclusions we have re-examined both the periodate oxidation, and infra-red and ultra-violet spectra of mono-D-glucosyl- and di-D-glucosyl-urea.

[1] I. Goodman, *Advanc. Carbohyd. Chem.* **13**, 215 (1958).
[2] M. N. Schoorl, *Rec. Trav. Chim.* **22**, 31, (1903).
[3] A. Hynd, *Biochem. J.* **20**, 195, 205, (1926).
[4] E. Fischer, *Ber. Dtsch. Chem. Ges.* **47**, 1377, (1914).
[5] B. Helferich and W. Kosche, *Ber. Dtsch. Chem. Ges.* **59**, 69, (1926).
[6] M. H. Benn and A. S. Jones, *J. Chem. Soc.* 3837 (1960).
[7] P. R. Steyermark, T. R. Steadman and P. R. Germann, *Industr. Engng Chem.* **53**, 212 (1961).
[8] I. Goodman and F. B. Hayes, *Advanc. Carbohyd. Chem.* **13**, 229 (1958).
[9] L. Segal, R. T. O'Connor and F. V. Eggerton, *J. Amer. Chem. Soc.* **82**, 2807, (1960).

Mono-D-glucosylurea consumed 2·08 moles of periodate at 4° in the dark after 24 hours and only a very slight increase in consumption occurred during the following 7 days. At 25° the reaction was initially somewhat more rapid and there was a slow continued uptake of periodate so that after about 7 days 3·22 moles had been consumed. In view of the slowness of this reaction and the results obtained at 4° this could be ascribed to "over oxidation". If the monoglucosylurea were in the open chain form (II), a rapid uptake of 5 moles of periodate would have been expected. In bright sunlight, however, there was a much increased uptake of periodate (7 moles after 68 hours), but it is known that under these conditions non-specific oxidation occurs.[10] This might explain the results obtained by Goodman and Hayes.[8]

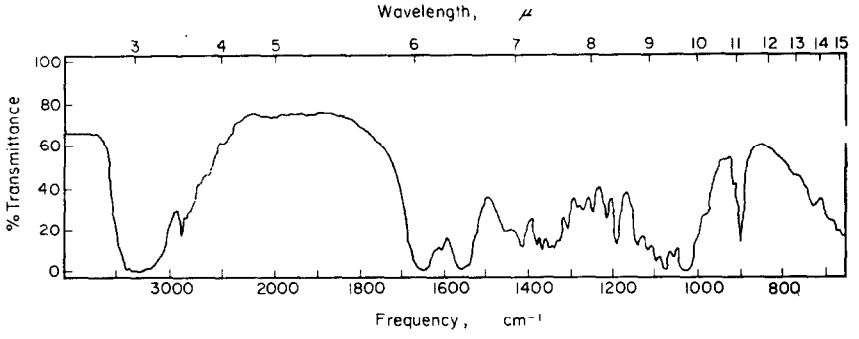
Application of the criteria suggested by Barker *et al.*[11] to the infra-red spectrum of mono-D-glucosylurea (Fig. 1) shows that it is consistent with the β-D-glucopyranosyl structure (I). Thus there is present a strong type 2b absorption at 900 cm^{-1} and a weak type 3 absorption at 780 cm^{-1} Type 2a absorption at 844 cm^{-1} is absent, showing the absence of the α-form. The moderate absorption at 917 cm^{-1} can be ascribed to a ring vibration (ref 11 gives 920 ± 5 cm^{-1}).

The conclusion arrived at by Segal *et al.*[9] that mono-D-glucosylurea has an open chain structure arose from two misinterpretations. In the first place the spectra were compared with that of the α-form of D-glucose so that the differences noted were merely those associated with the α- and β-forms. The second argument was based on the claim that an absorption peak at 1145 cm^{-1} (8·75 μ) indicates a ring structure and that the spectrum of mono-D-glucosylurea has no distinct peak at this frequency. Apart from the difficulty of making any definite correlations in a region of complex absorption, this observation is at variance with the spectrum of chromatographically purified mono-D-glucosylurea used in this work. This shows an absorption peak at 1145 cm^{-1}; only in the spectrum of the unpurified material (Fig. 3), which is identical in this region with that published by Segal *et al.*[9] the peak is not discernible. Di-D-glucosylurea is known to occur as an impurity[6] and has a spectrum (Fig. 2) which has a minimum at 1145 cm^{-1}. Furthermore, the spectrum of β-methyl-D-glucopyranoside (Fig. 4) does not show a peak at 1145 cm^{-1} so that the absence of a peak in this position cannot be taken as an indication of the absence of a β-pyranose ring structure.

Further evidence against the open chain structure II is the absence of any absorption in the ultra-violet region, 190–300 mμ which would be expected if the structure —CH=N—$CONH_2$ is present. The evidence from both infra-red and ultra-violet spectroscopic studies is in accord therefore with the chemical evidence, so that I is the correct structure.

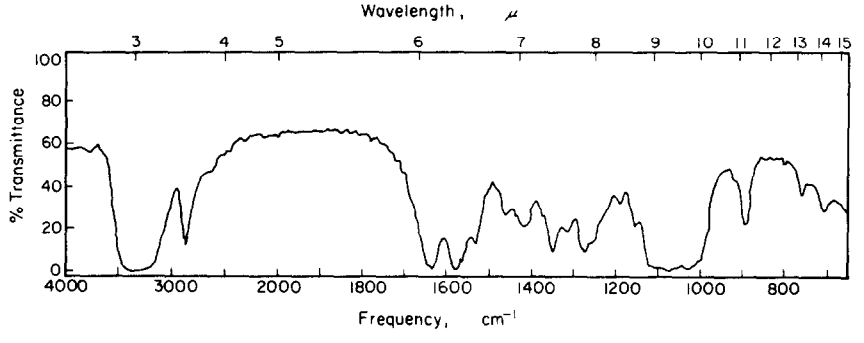
Similarly there is chemical evidence that the structure of the di-D-glucosylurea obtained by the acid-catalysed condensation of 2 moles of D-glucose with one mole of urea has the di-β-D-glucopyranosyl structure (III). Thus a compound identical to III was obtained by the action of aqueous ammonia on an octa-acetyl-di-D-glucosylurea which had been obtained by the action of aqueous acetone on tetra-O-acetyl-β-D-glucopyranosyl isocyanate.[12] It has also been shown[6] that di-D-glucosylurea consumed 4 moles of periodate at 4° in the dark.

[10] See references cited by J. R. Dyer, *Methods of Biochemical Analysis* Vol. III, p. 120. Interscience, New York (1956).
[11] S. A. Barker, E. J. Bourne and D. H. Whiffen, *Methods of Biochemical Analysis* Vol. III, p. 213. Interscience, New York (1956).
[12] T. B. Johnson and W. Bergmann, *J. Amer. Chem. Soc.* **54**, 3360, (1932).



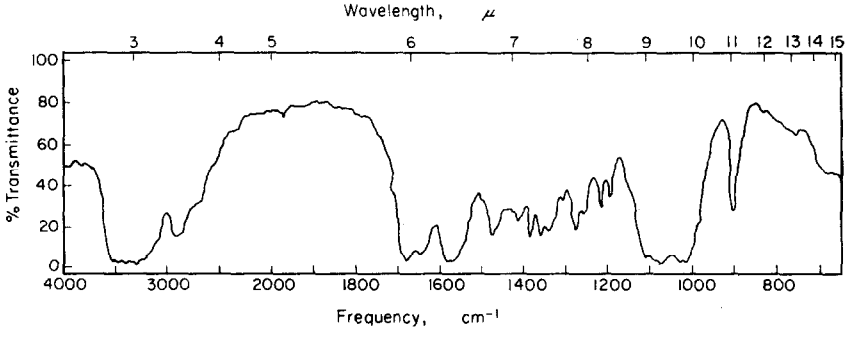
N-β-D-Glucopyranosyl urea

FIG. 1.



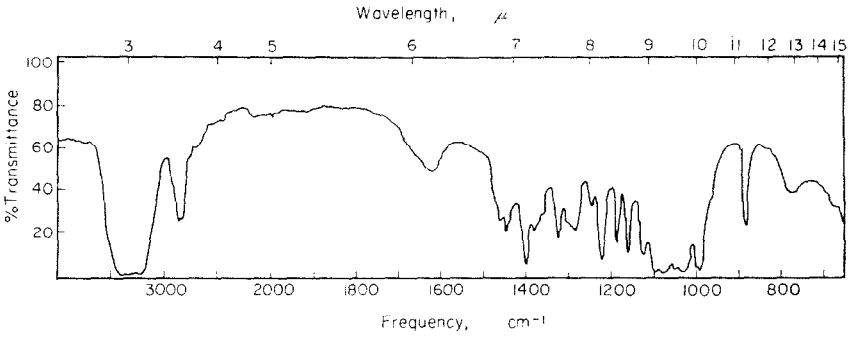
NN'-di-β-D-Glucopyranosyl urea

FIG. 2.



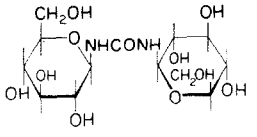
Crude Glucosyl urea

FIG. 3.



β-Methyl - D-Glucopyranoside

FIG. 4.



III

The infra-red spectrum of the diglucosylurea (Fig. 2) is in accord with this evidence. Thus strong type 2b absorption at 890 cm^{-1} is present and type 2a absorption at 844 cm^{-1} is absent. There is also present a weak absorption at 915 cm^{-1} which may be assigned to a ring vibration. The moderate intensity type 3 absorption at 760 cm^{-1} is inconclusive. As in the case of the monoglucosylurea the absence of absorption in the ultra-violet region at 190–300 mμ indicates that the open chain form is not present. It can be concluded, therefore that III represents the structure of the diglucosylurea.

EXPERIMENTAL

Paper chromatography. Descending chromatograms were run on Whatman No. 4 paper for 16–18 hr. with n-butanol–ethanol–water (4:1:5) as the solvent. The components were detected by spraying with an acetone solution of silver nitrate (0·6% w/v) and then with ethanolic sodium hydroxide solution (2% w/v).

N-β-D-Glucopyranosylurea. This was prepared by the acid-catalysed condensation of D-glucose with urea with subsequent purification by column chromatography on charcoal as described by Benn and Jones.[6] The product was twice recrystallized from water and had m.p. 208–209°. (Found: C, 37·6; H, 6·45; N, 12·8. Calc. for $C_7H_{14}N_2O_6$: C, 37·9; H, 6·3; N, 12·6%). Paper chromatography showed the presence of only traces of di-D-glucosylurea.

N,N'-Di-β-D-glucopyranosylurea. This was prepared by the acid catalysed condensation of 2 moles of D-glucose with 1 mole of urea as described by Benn and Jones.[6] The product turned brown at 248–251° but did not melt even at 350°. (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%). Paper chromatograms showed the presence of only traces of mono-D-glucosylurea.

β-Methyl-D-glucopyranoside. This was a sample available in this Department. It was twice recrystallized from ethanol and had m.p. 104·5° $[\alpha]_D^{20}$ −16° in water (*c*, 2·0). (Found: C, 41·3; H, 7·25. Calc. for $C_7H_{14}O_6 \cdot \frac{1}{2}H_2O$: C, 41·4; H, 7·39%). The low rotation was due to the presence of about 15% of the α form. Comparison of the I.R. spectrum of this compound with the published spectrum[13] showed that this impurity had only a slight effect and none at 1145 cm^{-1}.

[13] G. N. Bollenback, *Methyl Glucoside* p. 27. Academic Press, New York (1958).

Infra-red absorption spectra. These were determined by the potassium bromide disc technique with a Perkin-Elmer 21 spectrophotometer.

Ultra-violet absorption spectra. These were carried out in aqueous solution (0·1% w/v) on a Cary 14 recording spectrophotometer. Both N-β-D-glucopyranosylurea and N,N′-di-β-D-glucopyranosylurea gave no detectable absorption in the region, 190–300 mμ.

Periodate oxidations. N-β-D-Glucopyranosylurea (50 mg) was dissolved in 0·02M sodium metaperiodate (100 ml) and allowed to stand at 4° in the absence of light. Similar solutions were allowed to stand at 25° in the dark and at room temp in bright sunlight. At intervals samples were withdrawn and the amount of periodate consumed was determined by Fleury and Lange's method.[14] The results were as follows:—

	Time (hr)											
Mols. of periodate consumed at:	1·75	2·0	5·75	19·5	23·5	43·5	48	67	94	119	170	220
4° in the dark	—	—	1·75	—	2·08	—	2·09	—	1·99	2·25	—	2·10
25° in the dark	1·78	—	—	1·98	—	2·22	—	2·56	—	2·66	3·22	—
Room temp in bright sunlight	—	2·66	—	3·14	—	4·47	—	7·11	—	7·09	7·14	—

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