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TAUTOMERISM OF SACCHARIDE HYDRAZONES IN SOLUTION AND THEIR REACTION WITH NITROUS ACID*

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ABSTRACT

N.m.r. spectroscopy (^{13}C and ^{1}H) has been used to show that saccharide hydrazones in solution in hydrazine exist mainly as the *syn* acyclic-tautomer. In deuterium oxide solutions, slow tautomerisation occurs to give glycosylhydrazines; at pH 6 tautomerism is rapid and the latter preponderate; exceptions are the hydrazones of D-ribose and L-arabinose which give almost equal proportions of acyclic and cyclic tautomers at pH 6. Reaction of representative saccharide hydrazones with nitrous acid gives a mixture of the corresponding saccharide and the β-glycosyl azide, the latter being formed from the glycosylhydrazine tautomer.

INTRODUCTION

Hydrazones of saccharides have been less well studied than *N*-substituted hydrazones[1–3]; phenylhydrazones, for example, are intermediates in the formation of the well known phenylosazones. Interest in the unsubstituted hydrazones arose in 1977 in connection with model studies of the hydrazinolysis of glycoproteins and glycopeptides. The reaction is used to cleave the amide linkage between 2-acetamido-2-deoxy-β-D-glucopyranose and asparagine, and also to *N*-deacetylate the *N*-acetylhexosamines and *N*-acetylneuraminic acid residues. Our model studies[4] showed that the product of hydrazinolysis was the hydrazone of 2-amino-2-deoxy-D-glucose and that this could not be easily hydrolysed under weakly acidic conditions to give 2-amino-2-deoxy-D-glucose. N.m.r. studies showed that, in weakly acidic solution, the hydrazone of 2-amino-2-deoxy-D-glucose existed mainly as the β-D-glucopyranosylhydrazine tautomer.

Early studies of aldose hydrazones by Haas and coworkers[5,6], using polarography, led to the conclusion that these compounds existed mainly in a non-reducible cyclic form in aqueous medium at pH 2.3 and that the equilibrium favoured the acyclic form at higher pH. Several crystalline aldose hydrazones are known[7], although the yields reported for some of the preparations were very low[8].

2,3-*O*-Isopropylidene-D-ribose hydrazone was found by p.m.r. measure-

*Saccharide Hydrazones, Part I.

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TABLE I

^{13}C-NMR DATA FOR SACCHARIDE HYDRAZONES[a]

Saccharide hydrazone	*Chemical shifts of C-1 In hydrazine*	*Ratio Acyclic:cyclic*	*Chemical shifts of C-1*	
			In D_2O	*In D_2O, pH 6*
D-Galactose[b]	150.3, (149.6),83 4	5:1	151.3 93.7	precipitation
D-Mannose[b]	(150 8), 150.1,83 6	12:1	150.0,(93.7),91.6	occurred
D-Glucose	148 3, 82.8	10:1	149.1,94 1,(92 1)	(92 2), 90.7
L-Arabinose	149.6, (149.0),83 2	7.5:1	150 6,95.0,89.0	151 2, 148.4, 91.8, 91.6
L-Fucose	149 9, (149.3),83 2	7:1	151 6,94.4	(148 4, 93.1, 91 6), 90.8
D-Ribose	148.6, (148.5),(83 1)	>14 1	149 6, (148.7,92.3, 91 1)	complex[c]
D-Xylose	148.7, (148.3),82 7	9:1	149 9, (148.7),95.0	147 3, 92 0, 91.2
Lactose	148.9, (146.8)	∞	149.8 93.8	90.4
Cellobiose	148.9, (147 4)	∞	149.6 93.8	90.3

[a]Figures in parentheses refer to signals of low, relative intensity. [b]D_2O solutions were not clear. [c]150.4, 147 9, 147 0, 89 4, 88 6, 88.1, and 87.8.

ments to exist mainly (90%) as the hydrazone in dimethyl sulphoxide[9], but no detailed studies of the tautomerism of unsubstituted saccharide hydrazones have been reported. A study of the tautomerism of several saccharide hydrazones in solution is now reported together with a discussion of their reaction with nitrous acid.

RESULTS AND DISCUSSION

^{13}C-N.m.r. spectroscopy is a convenient method for determining the tautomeric equilibrium of saccharide hydrazones, especially for solutions in hydrazine for which p.m.r. measurements are not possible. The signal for C-1 of aldose hydrazones is at a characteristic low-field (~150 p.p.m.) and the anomeric carbons of the cyclic tautomers resonate between 80 and 100 p.p.m. (see Experimental). In solution in hydrazine, all of the monosaccharide and disaccharide hydrazones studied existed mainly as one of the acyclic tautomers; a weak signal for the second acyclic tautomer could also be detected (see Table I) together with weak signals for the cyclic tautomers*, except for the disaccharide hydrazones whose spectra contained unassigned weak signals at 187, 150, and 96 p.p.m. These signals were absent when the spectra were measured without first keeping the solutions overnight.

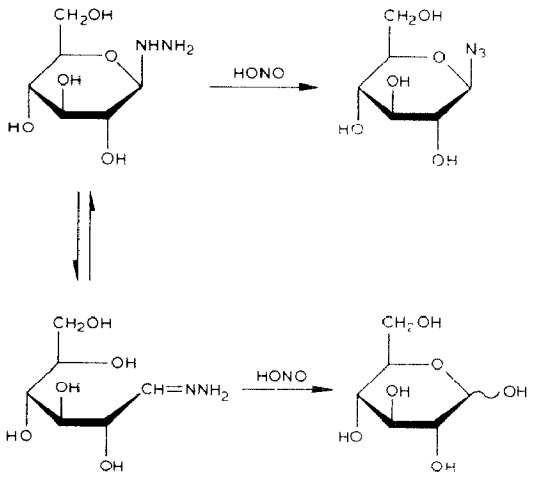
After removal of the hydrazine (at room temperature), the hydrazones were dissolved in deuterium oxide, and ^{1}H- and ^{13}C-n.m.r. spectra were measured immediately and after 24 h. The ^{1}H spectra contained a strong doublet at δ 7.3 and a weak doublet or multiplet at δ 6.8 for H-1 of the aldose hydrazones; the former signal is assigned to the *syn* isomer by analogy with the oximes of D-glucose and D-arabinose[10]. These signals slowly decreased in intensity as tautomerisation occurred to give the glycosylhydrazines. When the pH was adjusted to 6 with CD_3COOD, rapid tautomerisation occurred to give mainly the glycosylhydrazines. The solvent absorption frequently obscured the anomeric region; although solvent suppression and other techniques could reduce this problem, the anomeric carbon-signals were readily detected in the ^{13}C spectra. The anomeric carbons of the glycosylhydrazine were shielded by 3.0–3.8 p.p.m. after the adjustment of pH, as a result of protonation of the NH_2 group.

The tautomerisation in aqueous solution could also be followed by measurements of optical rotation. Thus, crystalline L-fucose hydrazone, which is reported here for the first time, mutarotated to a constant optical rotation in 2 days. The pH of the unbuffered solution was between 8 and 9. When a buffer of pH 7.0 was used, the mutarotation was very rapid.

Schmidt and co-workers[9] have reported that attempts to synthesise nucleoside analogues from D-ribose hydrazone were unsuccessful. However, it is pos-

*The integrated ratios for the hydrazine solutions are subject to error caused by the differential values of T_1 and η. However, these errors are expected to be small, since the T_1 values of C-1 of D-xylose hydrazone and β-D-xylosylhydrazine are both <1 s and the n.O.e. factor (η) is expected to be similar for the two carbon nuclei.

sible to utilise the glycosylhydrazine tautomer in irreversible reactions, as illustrated by the reaction with nitrous acid. This reaction was investigated as a possible route to glycosyl azides from aldoses in two steps. Hydrazones are known to react with nitrous acid to form the corresponding carbonyl compound[11]. Glycosylhydrazines would be expected to form glycosyl azides[12]. The yield of azide would depend on the relative rates of the four reactions shown in Scheme 1. When a solution of D-glucose hydrazone in dilute acetic acid was treated with sodium nitrite, ^{13}C-n.m.r. measurements showed the presence of β-D-glucopyranosyl azide, and n.m.r. analysis of the product, after deionisation, showed that β-D-glucopyranosyl azide and D-glucose were present in approximately equal amounts. The estimated yield (38%) of the azide (from D-glucose) compares favourably with the overall yield (44%) for the four-step synthesis *via* tetra-*O*-acetyl-α-D-glucopyranosyl bromide[13]. However, the method has the disadvantage that the azide has to be separated from the saccharide which is also formed. Glycosyl azides were also detected in the products of nitrosation of L-fucose hydrazone and lactose hydrazone, but the yields have not been optimised (for example, by controlling the pH*) in any of these experiments, because an efficient synthesis of glycosyl azides by the reaction of aldose peracetates with trimethylsilyl azide is now available[15].



Scheme 1

Further reactions of saccharide hydrazones and the significance of their reaction with nitrous acid, in the context of the sequential hydrazinolysis and nitrosation of glycoproteins and glycopeptides, will be discussed elsewhere.

*It has been suggested[14] that nitrosations at lower pH would favour azide formation.

EXPERIMENTAL

Melting points were measured on a Kofler hot-stage and are corrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. The ^{1}H- and ^{13}C-n.m.r. spectra were recorded with Varian HA 100 and XL 100 spectrometers, respectively. ^{13}C Spectra were recorded with proton-noise decoupling and, for those spectra that were integrated, a digital resolution of 3 data points per Hz (in the transformed spectrum) and a (30°) pulse-repetition time of 3 s were used; otherwise, 1 data point per Hz and a pulse-repetition time of 1.6 s were used. ^{13}C Spectra of hydrazine solutions were measured using a co-axial tube containing a solution of sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate (T.S.P.) in D_2O, which provided the deuterium-lock signal. The coupling constants cited were the directly observed line-spacings, except when spin simulation was used. Chemical shifts were measured downfield from T.S.P. Spin simulation was carried out by using a Varian L100 computer and the programme of C. W. F. Kort and M. J. A. de Bie.

Preparation of saccharide hydrazones. — The mono- or di-saccharide (300 mg) was dissolved in anhydrous hydrazine (2 mL), and the solution was kept overnight. The ^{13}C-n.m.r. spectrum was then measured and integrated, and hydrazine was removed *in vacuo* over conc. H_2SO_4. The syrupy residue was dissolved in D_2O, and the ^{1}H- and ^{13}C-n.m.r. spectra were measured immediately. Because of the slow tautomerisation, the relative intensity of the glycosylhydrazine absorptions in the ^{13}C spectrum depends on the duration of the measurement. Most measurements took 1–4 h, but the disaccharide spectra were acquired overnight. The pH of these solutions was 8–9, and measurements were repeated after neutralisation (to pH 6) by the dropwise addition of CD_3COOD. The hydrazones were now either absent or present in relatively small proportions, except for the pentoses for which the hydrazones constituted 25–50% of the mixture.

L-*Fucose hydrazone.* — A solution of L-fucose (513 mg) in hydrazine (3 mL) was kept overnight, and the hydrazine was removed *in vacuo* over conc. H_2SO_4, to give a solid residue (536 mg). Recrystallisation from methanol gave L-fucose *syn*-hydrazone (291 mg); $[\alpha]_D^{21}$ +13° (3 min), +12° (51 min), −2° (20.6 h), −4.5° (45.1 h) (*c* 0.9, water); in 25 mM phosphate buffer (pH 7.0), the rotation attained after 45.1 h by the unbuffered solution was reached after only 3 min. ^{1}H-N.m.r. data (D_2O): δ 7.37 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1), 4.45 (dd, 1 H, $J_{2,3}$ 2.5 Hz, H-2), 4.10 (dq, 1 H, H-5), 3.74 (dd, $J_{3,4}$ 8.9 Hz, H-3), 3.50 (dd, 1 H, $J_{4,5}$ 2 Hz, H-4), and 1.25 (d, 1 H, $J_{5,6}$ 6.6 Hz, H-6). This spectrum was recorded on the XL 100 spectrometer with solvent suppression[16] (using a delay of 2.3 s between the π and $\pi/2$ pulses), and the parameters and assignments reported were checked by spin simulation.

Anal. Calc. for $C_6H_{14}N_2O_4$: C, 40.4; H, 7.9; N, 15.7. Found: C, 39.9; H, 8.7; N, 15.1.

A more convenient preparation involved dissolving L-fucose (0.5 g) in hydrazine hydrate (1 mL) with gentle warming, and keeping the solution overnight.

Addition of ethanol (4–5 mL) initiated crystallisation of the hydrazone, a yield of 322 mg being obtained after the mixture had been kept in a refrigerator for 1 day.

Nitrosation of D-*glucose hydrazone.* — The syrupy hydrazone prepared from D-glucose (310 mg) was dissolved in D_2O (2 mL), and the pH was adjusted to 6 with CD_3COOD. A ^{13}C-n.m.r. spectrum showed that the glucosylhydrazine constituted >90% of the mixture. The liberated T.S.P. acid was removed by ether extraction, after dilution with water. The solution was cooled in ice, and the pH was adjusted to 4.5 with CD_3COOD. Sodium nitrite (0.35 g) was added in three portions during 20 min. After 1 h, the solution was deionised with Amberlite IR-120 (H^+) resin (35 mL), purged with N_2, and evaporated to dryness under reduced pressure. The dried residue weighed 246 mg. Paper-chromatographic analysis (solvent: 1-butanol–pyridine–water, 4:3:4) gave two major components (detected with silver nitrate) which had the same mobilities as D-glucose and β-D-glucopyranosyl azide. ^{13}C-N.m.r. data (D_2O): 98.5 and 94.6 (C-1 of β- and α-D-glucose), 92.6 (C-1 of β-D-glucopyranosyl azide), 80.4, 78.4, 78.3, 76.7, 76.4, 75.4, 74.0, 72.2, 72.0, 71.8, 63.4, and 63.2 p.p.m. Integration of the anomeric carbon region gave a ratio of 52:48 for D-glucose and D-glucosyl azide.

*β-*D-*Glucopyranosyl azide.* — This compound was prepared from β-D-glucose penta-acetate according to the literature procedure[13]. The overall yield from D-glucose was 44%, assuming that the acetylation of D-glucose is quantitative. N.m.r. data (D_2O): ^1H, δ 4.73 (d, J 8 Hz, H-1); ^{13}C, 92.7, 80.6, 78.5, 75.6, 71.9, and 63.3 p.p.m.

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