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Note

Preparation, conformation, and mild hydrolysis of 1-glycosyl-2-acetylhydrazines of the hexoses, pentoses, 2-acetamido-2-deoxyhexoses, and fucose [1]

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Abstract

The title compounds were prepared and their conformations studied by ^{1}H-NMR. Their acid hydrolysis under mild conditions was monitored by ^{1}H-NMR. © 1997 Published by Elsevier Science Ltd.

1. Discussion

Acylhydrazine derivatives of sugars have been prepared [1–5], and some of the acetohydrazide derivatives of monosaccharides (1-glycosyl-2-acetylhydrazines) have been reported [3–5]. The acetohydrazide derivatives of sugars have been of recent interest in this laboratory because of their ease of formation from sugar hydrazones, and their facile hydrolysis. As part of a reaction series enabling monosaccharides to be sequentially removed from the reducing end of oligosaccharides, the highly selective *N*-acetylation of oligosaccharide hydrazones was pursued as a means to quantitatively deprotect them to regenerate a free reducing end [6]. To generalize this deprotection step, the acetohydrazide derivatives of all D-aldohexoses, D-aldopentoses, two 2-acetamido-2-deoxy-D-hexoses, and L-fucose were prepared, and the facility of their hydrolyses studied.

The 1-glycosyl-2-acetylhydrazines were readily prepared in 38–81% isolated yields by treatment of monosaccharides with acetohydrazide in 90% ethanol, followed by LC. Reaction yields were between about 75–90%, depending on the sugar, as analyzed by ^{1}H-NMR of reaction mixtures prior to LC. The derivatives did not give rise to single LC peaks, as monitored by UV at 200 nm. Although one major peak was usually observed, there were 1–3 additional peaks for each compound. The molecules interconverted and each peak gave rise to the others upon rechromatography. All peaks gave the same ^{1}H-NMR spectrum. The molecules were found to exist primarily as cyclic tautomers of the hydrazone products. Previous observations indicated that acylhydrazines do not form osazone (1,2-bishydrazone) derivatives with aldoses under normal reaction conditions [7], and these were not observed.

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[1] Sequential removal of monosaccharides from the reducing end of oligosaccharides, Part III.

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^{1}H-NMR data are presented for the most abundant conformation, or, in the case of lyxose, for those of the two cyclic forms which were approximately equal in quantity (Tables 1 and 2). Conformations were assigned based on J couplings of ring protons and the relatively upfield chemical shift of H-1 for the cyclic compounds. In the case of the idose derivative, two tautomers were nearly equally abundant at equilibrium (Table 1). The open-chain tautomer was assigned by the downfield position of the H-1 signal (δ 7.561 ppm), similar to that previously observed for the open-chain form of D-galactose acetylhydrazone (δ 7.60 ppm) [3] prior to its equilibration to the major β-pyranosyl form. For cyclic forms, it was observed that the acetohydrazide function favors an equatorial orientation, and that pyranose ring forms were dominant. With 2-acetamido-2-deoxy-D-glucose or D-glucose, for example, virtually no observable α-pyranosyl form was observed, which was present in about 45% and 35%, respectively, for the free sugars. This same tendency was observed for the entire hexose series and 2-acetamido-2-deoxyhexoses, the β-pyranosyl ($^4C_1$ conformation) being the major form, usually above 90%, but generally above 75%, with the exception of idose. The acetohydrazide group was also equatorially disposed in the L-fucosyl product which, by nomenclature convention [8] was the $^1C_4$ conformer. In the pentose series, products from xylose and ribose were predominantly (> 85%) β-pyranosyl forms ($^4C_1$ conformation). The product from arabinose was predominantly (> 90%) the α-pyranosyl form ($^1C_4$ conformation), which results in the equatorial orientation of the acetohydrazide derivative, OH-2 and OH-3. The lyxose derivative was unusual in having two conformers of approximately equal proportion. The first of these was the β-pyranosyl form having the $^4C_1$ conformation, the second was the α-pyranosyl form having the $^1C_4$ conformation. The data for the second conformer suggested that the C-3–C-4 bond might be somewhat twisted toward the half-chair conformation, as evidenced by the slightly high $J_{3,4}$ value. Possibly some puckering of the pyranosyl ring occurs to avoid 3,4-diaxial hydroxyl groups. It was surprising that the second conformer was equally favorable to the first, which contains only 1 axial hydroxyl group.

A second feature of these molecules is that the hydrazido nitrogen bonded to the acetyl group (N-1′, Fig. 1) is evidently sp^2 hybridized, and, like amide nitrogens, has a significant double-bond character due to overlap of p orbitals with the carbonyl carbon [9]. Hence, *cis–trans* isomers were found (Fig. 1) which, certainly on the NMR time scale (Fig. 2) and possibly on the chromatographic time scale (35–65 min), interconvert slowly. For example, for every major β-pyranosyl conformer, a minor β-pyranosyl conformer was also present, which, at equilibrium, integrated to about 10–15% of the major form in abundance (Fig. 2).

A third feature of these molecules concerns the nature of the nitrogen attached to the anomeric carbon (N-1). This nitrogen was not protonated upon addition of H_2SO_4 to 0.05M as examined in the NMR spectra (Fig. 2). This was surprising, because acetohydrazide has a pK_a for protonation of 3.24 ($[CH_3CONHNH_3]^+ \rightleftarrows CH_3CONHNH_2 + H^+$) [10], which presumably occurs on this nitrogen. The lack of any significant shifting of ^{1}H-NMR signals upon making the sample 0.05M in H_2SO_4 indicates that the pK_a for protonation of N-1 ($[RNH_2NHAc]^+ \rightleftarrows RNHNHAc + H^+$) is well below 1, probably a negative value. This indicates that the charge distribution of the lone pair of electrons on N-1 is markedly modified from that of a standard sp^3 nitrogen, and that a significant distribution of these electrons occurs in bonds between N-1 and N-1′, and between N-1 and C-1.

Hydrolysis of the acetohydrazide derivatives was quantitative at 35° in 0.05M H_2SO_4, which was performed with all the derivatives in NMR tubes

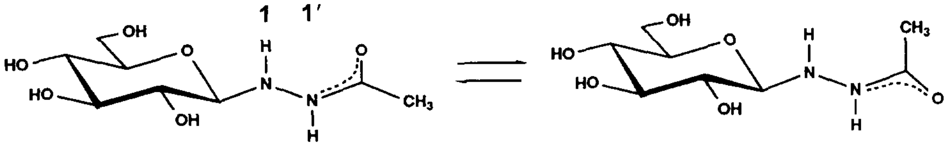

Fig. 1. Structure of 1-(β-D-glucopyranosyl)-2-acetylhydrazine, with nitrogens numbered as described herein. Equilibrium of the *cis/trans* forms of the hydrazide is shown. Other 1-glycosyl-2-acetylhydrazines vary in the configuration of the monosaccharide, but the major conformers for all compounds were pyranosyl forms, except idose, which showed pyranosyl and acyclic tautomers in approximately equal quantities.

Table 1
^{1}H-NMR data of 1-glycosyl-2-acetylhydrazines of hexoses, 2-acetamido-2-deoxyhexoses and fucose [a,b]

Sugar	Chemical shift (δ, ppm)										Coupling constant (Hz)							Conformation [c]
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	N-1'-Ac	N-2-Ac	Methyl	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$	
D-All	4.332	3.483	4.188	3.578	3.714	3.884	3.690	1.977	–	–	9.2	3.0	2.9	9.6	1.3	5.9	−10.1	β-pyr, ${}^4C_1$
D-Alt	4.442	3.926	4.024	3.759	3.715	3.913	3.693	1.963	–	–	≤ 1	3.5	3.3	9.9	2.2	6.6	−10.2	β-pyr, ${}^4C_1$
D-Gal	4.034	3.528	3.675	3.922	3.669	3.777	3.736	1.983	–	–	9.0	9.7	3.2	≤ 1	8.0	4.2	−11.7	β-pyr, ${}^4C_1$
D-Glc	4.093	3.291	3.517	3.363	3.420	3.901	3.712	1.975	–	–	9.0	9.3	8.9	9.7	2.2	5.8	−12.4	β-pyr, ${}^4C_1$
D-Gul	4.333	3.691	4.078	3.805	3.938	3.754	3.723	1.985	–	–	9.5	3.4	3.7	1.2	7.4	4.1	−11.8	β-pyr, ${}^4C_1$
D-Ido (A) [d]	7.561	4.483	3.894	3.744	3.858	3.720	3.648	2.062	–	–	4.8	4.8	4.8	4.2	4.2	6.6	−11.7	acyclic
D-Ido (B)	4.418	3.793	4.041	3.661	3.914	3.848	3.782	1.973	–	–	≤ 1	3.4	3.2	1.2	8.4	3.6	−12.0	β-pyr, ${}^4C_1$
D-Man	4.245	4.046	3.628	3.542	3.342	3.941	3.715	1.960	–	–	≤ 1	3.4	9.6	9.8	2.2	6.8	−12.1	β-pyr, ${}^4C_1$
D-Tal	4.183	3.993	3.744	3.867	3.585	3.861	3.788	1.968	–	–	≤ 1	3.2	3.4	≤ 1	7.8	3.9	−12.0	β-pyr, ${}^4C_1$
L-Fuc	4.017	3.480	3.667	3.751	3.748	–	–	1.982	–	1.235	9.1	9.4	3.3	≤ 1	6.6 [e]	–	–	β-pyr, ${}^1C_4$
D-GalNAc	4.166	4.003	3.734	3.943	3.657	3.794	3.746	1.940	2.030	–	9.5	10.4	3.0	≤ 1	7.9	4.2	−11.6	β-pyr, ${}^4C_1$
D-GlcNAc	4.226	3.800	3.545	3.429	3.423	3.899	3.748	1.924	2.022	–	9.6	10.0	9.1	9.9	1.4	4.8	−12.2	β-pyr, ${}^4C_1$

[a] For a solution in D_2O at 36°, relative to internal acetone at 2.225 ppm.

[b] Chemical shifts and coupling constants of tightly coupled protons were assigned by spectral simulation. These included All and Alt H-5, H-6b; Gul H-6a, H-6b; and Fuc and GlcNAc H-4, H-5 proton pairs.

[c] Deduced conformation of the major conformer, pyranosyl denoted pyr.

[d] The product from idose showed two tautomers (A and B) of approximately equal quantity.

[e] This value refers to the H-5 coupling to the equivalent H-6 methyl protons.

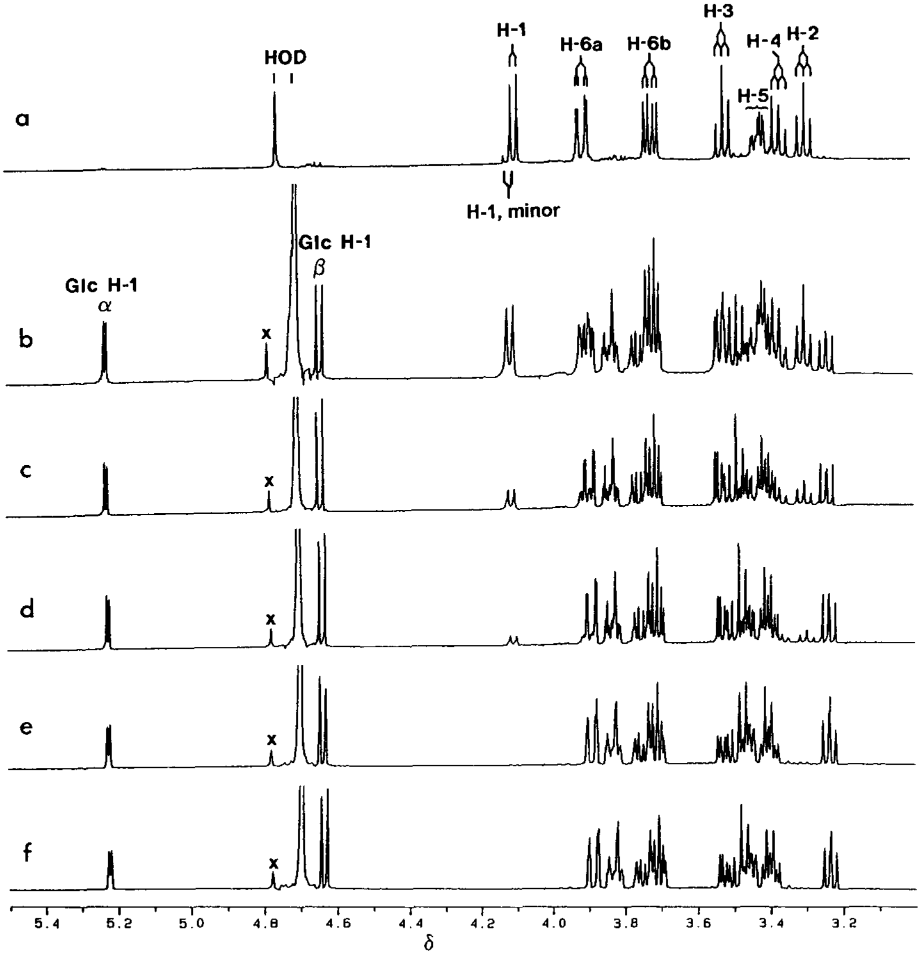

Fig. 2. Hydrolysis of 1-(β-D-glucopyranosyl)-2-acetylhydrazine, as demonstrated by ^{1}H-NMR, showing the downfield regions of spectra. Hydrolysis was performed in D_2O containing 0.05M H_2SO_4 at 35° in an NMR tube. (a) The starting compound, showing assignments of ring protons for the major conformer and the H-1 of the minor conformer resulting from *cis/trans* isomerization of the hydrazide. Both were β anomers. (b–f) Spectra at various times after acidification, showing the appearance of signals corresponding to glucose: (b) 6 min, (c) 13 min, (d) 21 min, (e) 45 min, and (f) 60 min. The peak marked with an X is a spinning sideband.

Table 2
^{1}H-NMR data of 1-glycosyl-2-acetylhydrazines of pentoses [a]

Sugar	Chemical shift (δ, ppm)							Coupling constant (Hz)						Conformation [b]
	H-1	H-2	H-3	H-4	H-5a	H-5b	N-1′-Ac	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5b}$	$J_{5a,5b}$	
D-Ara	3.985	3.555	3.689	3.945	3.900	3.636	1.982	8.7	9.5	3.4	2.2	1.1	−13.1	α-pyr, $^1C_4$
D-Lyx (A) [c]	4.348	3.776	3.994	3.825	3.859	3.749	1.981 [d]	7.8	3.6	4.8	2.2	3.6	−13.0	α-pyr, $^1C_4$ [e]
D-Lyx (B)	4.207	4.038	3.579	3.812	3.962	3.177	1.957 [d]	≤ 1	3.3	9.3	5.4	10.2	−11.4	β-pyr, $^4C_1$
D-Rib	4.291	3.512	4.161	3.816	3.760	3.592	1.972	8.7	3.0	2.6	5.2	10.2	−11.0	β-pyr, $^4C_1$
D-Xyl	4.037	3.276	3.455	3.587	3.932	3.266	1.977	8.8	8.6	9.5	5.4	10.7	−11.4	β-pyr, $^4C_1$

[a] For a solution in D_2O at 36°, relative to internal acetone at 2.225 ppm.

[b] Deduced conformation of the major conformer, pyranosyl denoted pyr.

[c] The product from lyxose showed two conformers (A and B) of approximately equal quantity.

[d] Assignments may have to be interchanged.

[e] $J_{3,4}$ is slightly high for this conformer. Data are compatible with a slight twist about the C-3–C-4 bond toward the half-chair form.

(Fig. 2). Aldopentoses were completely hydrolyzed within 5–6 min, which was the fastest time required to shim NMR samples after addition and mixing of 5 μL of 5.0M H_2SO_4 (in D_2O), to 500-μL samples. Aldohexoses, 2-acetamido-2-deoxyhexoses, and fucose derivatives were quantitatively hydrolyzed within 15 min, with the exception of the D-glucose and 2-acetamido-2-deoxy-D-glucose derivatives, which showed similar rates of hydrolysis, reaching completion after 1 h (Fig. 2). Therefore, these derivatives are exceptionally easily and quantitatively removed to generate the reducing sugars, without any observed epimerization at C-2.

The derivatives are useful for facile deprotection of sugar hydrazones by *N*-acetylation, which occurs highly selectively on the terminal nitrogen. Knowledge of conditions for their cleavage is directly relevant to work in this laboratory involving an overall reaction series for sequential removal of monosaccharides from the reducing end of oligosaccharides, as one step of a repeated series requiring high yields [6]. As described herein, the deprotection of sugar acetohydrazide derivatives is general and quantitative under mild conditions, and avoids the possibility of azine formation, which can accompany carbonyl-deprotecting reagents.

2. Experimental

General methods.—^{1}H-NMR spectra were recorded with either a Bruker AM-500 (University of Washington, Department of Chemistry), or Varian Inova 500 or 600 MHz instruments (University of Colorado). Spectra were recorded in D_2O at 36°, except when hydrolysis studies were performed, which were recorded at 35°. Chemical shifts were assigned relative to internal acetone (δ 2.225 ppm), with an accuracy of ± 0.002 ppm, and coupling constants to an accuracy of ± 0.3 Hz. LC was performed using a Waters HPLC system having a Waters 616 pump and 486 tunable absorbance detector operated at 200 nm. Chromatography was carried out on a column (7.8 × 300 mm) of Waters Glycopak N, eluted isocratically with 17:3 acetonitrile–water, at 1.0 mL/min. Products were concentrated by rotary evaporation at 35°, and exchanged in D_2O by lyophilization before NMR spectroscopy.

Materials.—Monosaccharides were obtained from Sigma Chemical, and all were pure as indicated by NMR, except *N*-acetylgalactosamine, which contained some *N*-acetylglucosamine. It was obtained in pure form from Fluka. Acetohydrazide (acetic hydrazide) was purchased from Aldrich. Other reagents and solvents were obtained in as high a purity as commercially available.

Preparation of 1-glycosyl-2-acetylhydrazines.—To a monosaccharide (0.1 mmol) and acetohydrazide (0.3 mmol) were added EtOH (0.5 mL) and water (50 μL). Samples were heated under N_2 in a 1.5-mL vial at 70° for 16 h. They were concentrated to a stiff syrup, taken up in 5.0 mL of 4:1 MeCN–H_2O, and chromatographed in 0.5-mL batches. UV absorbance detected acetohydrazide at 19–21 min, and peak(s) from 35–65 min, depending on the monosaccharide, which were 1-glycosyl-2-acetylhydrazine derivatives. Products were concentrated and examined by ^{1}H-NMR. Assignments were made through decoupling experiments. In cases where tight or partial tight coupling was observed, chemical-shift values and coupling constants for the tightly coupled proton pair were assigned through spectral simulation of the 7- or 8-proton spin system using the program LAME, a version of the program LAOCOON enabling simulation of magnetically equivalent nuclei [11].

Hydrolysis of 1-glycosyl-2-acetylhydrazines.—Derivatives in 0.5 mL D_2O in an NMR tube were taken to 35° and a t = 0 spectrum recorded. Sulfuric acid (5.0M in D_2O, 5 μL) was added, and the sample was rapidly mixed and immediately reinserted into the magnet. After about 4–5 min, equilibration and resetting of axial shims was complete and spectra were recorded in 8 transients. Additional time-points were taken in about 3-min intervals as 8 transient acquisitions and precise times were recorded midway through acquisitions.

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