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Heterocyclic derivatives of sugars: An NMR study of the formation of 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles from hydrazones

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Abstract

Hydrazones were prepared by treatment of monosaccharides and disaccharides with hydrazine hydrate and converted in high yield to mixtures of 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles by reaction with pentan-2,4-dione (acetylacetone). The isomeric products were separated by HPLC and characterized by NMR spectroscopy. This represents a new approach to the introduction of a heteroaromatic label into sugars under nonacidic and nonreducing conditions, and it is a process likely to be especially useful for glycan hydrazones obtained from glycoproteins by hydrazinolysis or beta elimination in the presence of hydrazine. © 1997 Elsevier Science Ltd.

Keywords: Sugar hydrazone; Equilibria; Pyrazole; Sugar labelling; NMR spectroscopy

1. Introduction

The preparation of aldose hydrazones by treatment of the sugars with anhydrous hydrazine or hydrazine hydrate is well documented [1–3]. As with a reducing sugar, an aldose or ketose hydrazone can exist either in an acyclic (true) hydrazone **7**, or in isomeric cyclic forms such as **1**, **4**, **9**, **12**, which can be considered to be glycosylhydrazines. The distribution between the isomeric forms is expected to be a subtle function of the stereochemistry of the parent sugar and rate of establishment of the equilibrium. It has been demonstrated [3] that aldose hydrazones are formed initially as acyclic hydrazones that undergo slow cyclisation in unbuffered aqueous solution at pH > 8. At pH < 7, however, the mixture equilibrated more rapidly, forming predominantly β-D-glucopyranosylhydrazine (**4**) [4].

We report herein studies of the formation and properties of monosaccharide hydrazones and their conversion to heterocyclic derivatives, the 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles. The simple two-step method for the introduction of an ultraviolet chromophore is effected in almost quantitative yield from the parent sugars without the use of heat or acidic conditions. It offers an alternative to the standard procedure of reductive amination at the reducing

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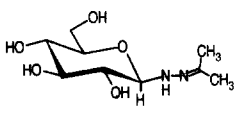
terminus [5,6] for use in the analysis of glycans, and it is likely to be particularly suitable when glycan hydrazones are already available as products of hydrazinolysis of a glycoprotein or beta elimination in the presence of hydrazine [7–9].

2. Results

Monosaccharides were converted to their hydrazones by treatment with aqueous hydrazine at room temperature. Analysis by ^{1}H NMR spectroscopy of glucose hydrazone in D_2O (Fig. 1a) showed that it consists predominantly of acyclic isomers **7**. Fresh glucose hydrazone was dissolved in deuterium oxide at an apparent pD of 6.0 and examined at intervals by ^{1}H NMR spectroscopy. The resonances of H-1 for the acyclic *E*- and *Z*-glucose hydrazones **7** were 7.24 (d, 6.5 Hz) and 6.68 ppm (d, 6.5 Hz), respectively, and 4.03 ppm (d, 9.0 Hz) for the β-D-glucopyranosylhydrazine **4**. At pD 6.0, a preponderance of **4** was rapidly established (Fig. 2), followed by slow hydrolysis to glucose until, in the limit, only 7% of **4** remained. Adding acetone to an unbuffered solution of the fresh hydrazone, however, produced cyclisation within 6 min (Fig. 1b). The main species present was probably **15**, which has a very similar NMR spectrum to **4**, except for a downfield shift of H-1. The small doublet at 7.22 ppm, corresponding to 2–4% of total glucose, is assigned to H-1 of the acyclic form of the azine **16** [1].



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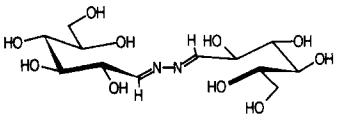

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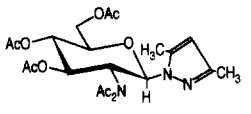

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When the reaction of glucose hydrazone with pentan-2,4-dione (acetylacetone) was studied by ^{1}H NMR spectroscopy, it was found that there was rapid formation of 1-D-glucosyl-3,5-dimethyl-1*H*-pyrazoles (Figs. 1c and 3). The only significant intermediate was **5**, with similar ^{1}H NMR properties to **15**. Pure 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles were obtained by treatment of aqueous solutions of the fresh hydrazones with excess acetylacetone, followed by fractionation by reversed-phase HPLC. To improve their chromatographic properties, the 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles from 2-amino-2-deoxyaldoses were peracetylated before separation. Unexpectedly, significant amounts of *N*,*N*-bisacetyl derivatives, such as **17**, were obtained. These showed an increase of 42 in mass, as indicated by ESIMS, doubled acetyl resonances in the NMR spectra and an absence of H-2–NH coupling. For 1-(2-deoxy-2-*N*,*N*-bisacetylamino-β-D-glucopyranosyl)-3,5-dimethylpyrazole, further confirmation was provided by the $^3J_{CH}$ interactions between H-2 and two of the ^{13}C nuclei (at 175.3 and 175.0 ppm), which were demonstrated by selective proton decoupling. Such an *N*,*N*-bisacetyl derivative has been obtained by acetylation of a derivative of 2-amino-2-deoxy-D-glucitol [10]. On treatment of the peracetates with dimethylamine in methanol [11] to effect *O*-deacetylation, one of the *N*-acetyl groups was also lost.

The 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles and their peracetates were characterised by NMR spectroscopy (Tables 1–5); pyranosyl and furanosyl isomers were distinguished by the effects of peracetylation upon their ^{1}H NMR spectra (Tables 3 and 4). When resonances were obscured by the HDO signal, or were strongly overlapping, a trace of acid was added to induce small changes in the chemical shifts of H-1, H-2, and H-3. The chemical shifts reported (Tables 1, 2 and 4) were measured at neutral pH. Resonances were assigned using proton connectivities obtained with the aid of homonuclear decoupling and spectral simulation where required. All ^{13}C NMR assignments were confirmed by heteronuclear chemical shift correlation spectroscopy (HETCOR).

The anomeric configurations of the 1-glycopyranosyl-3,5-dimethyl-1*H*-pyrazoles, except for the derivatives of mannose, rhamnose, and fructose, were apparent from their $J_{1,2}$ values. The 3,5-dimethyl-1-L-rhamnopyranosyl-1*H*-pyrazole isomers were assigned by (*i*) measurement of the anomeric $^1J_{CH}$ couplings (161 Hz for α and 153 Hz for β) [12], and (*ii*) the ^{13}C chemical shift for C-5, which occurs at 3.1 ppm lower field for the α anomer, similar to the difference of 3.7 ppm observed for the parent sugars [13]. The ^{1}H and ^{13}C spectra of the 3,5-dimethyl-1-L-rhamnopyranosyl-1*H*-pyrazoles are very similar to those of the corresponding D-mannopyranosyl isomers. The assignments for the fructopyranosyl derivatives are more tentative, as the literature ^{13}C chemical shift values [13] for the parent sugars and their methyl glycosides are incomplete.

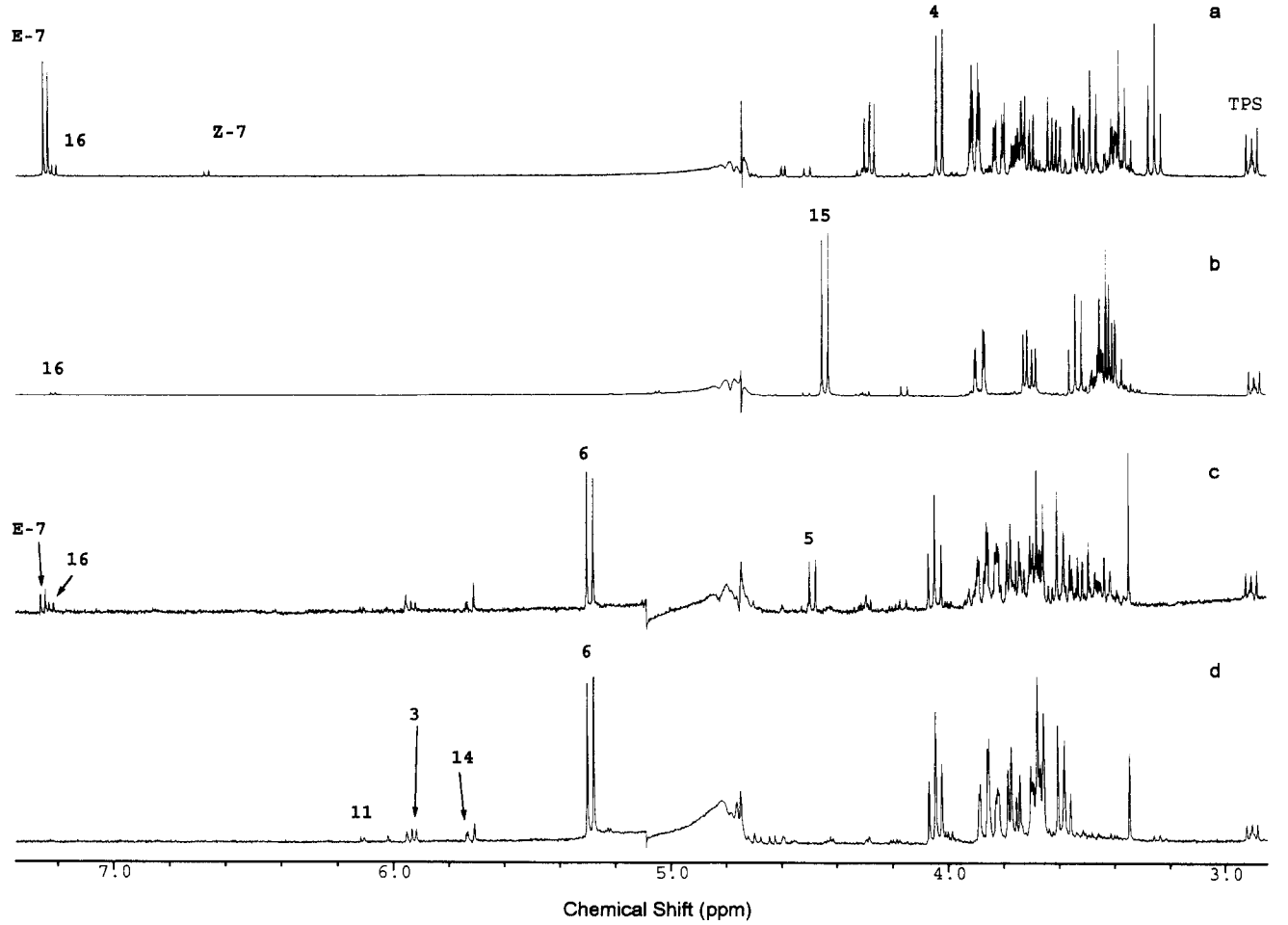


Fig. 1. ^{1}H NMR spectrum of the glucose hydrazone at 27 °C (a) immediately after dissolution in D_2O; (b) 6 min after addition of acetone; (c) 8 min after addition of acetylacetone; (d) 16 h after addition of acetylacetone. The labeled peaks correspond to H-1 of the corresponding structures.

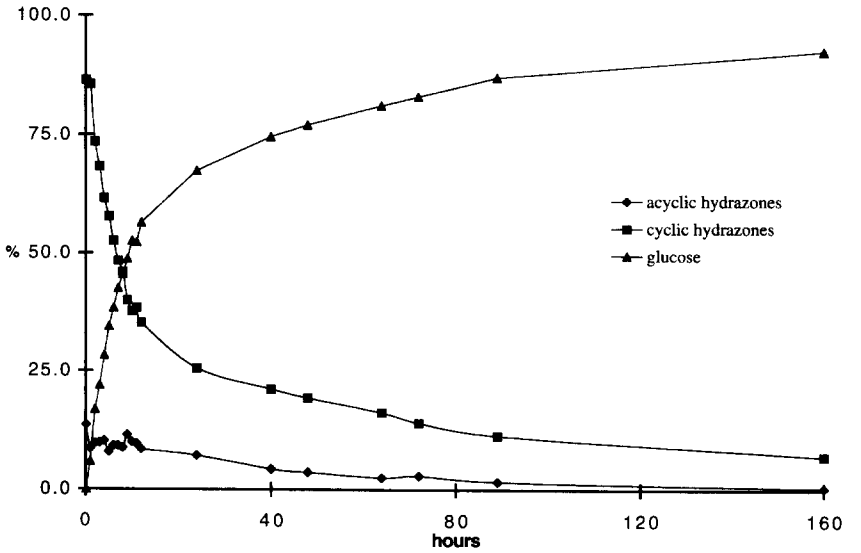


Fig. 2. Time course of equilibration of glucose hydrazone at pD 6.0 and 27 °C.

Table 1
^{1}H NMR data for 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles [a,b]

	α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl
Glucosyl					*Galactosyl*					*Mannosyl*				
H-1	5.94	5.29	6.12	5.74	H-1	5.96	5.22	5.99	5.68	H-1	5.75	5.54	5.79	
H-2	4.01	4.04	4.57	4.60	H-2	4.24	4.31	4.51	4.75	H-2	4.56	4.31	4.86	
H-3	4.70	3.67	4.43	4.30	H-3	4.76	3.82	4.62	4.33	H-3	4.44	3.83	4.46	
H-4	3.52	3.58	4.44	4.27	H-4	4.06	4.04	4.04	4.12	H-4	3.79	3.80	4.33	
H-5	3.7	3.67	3.96	3.99	H-5	3.82	3.92	3.94	3.84	H-5	3.44	3.61	3.96	
H-6	3.68	3.76	3.71	3.62	H-6	3.65	3.75	3.66	3.60	H-6	3.76	3.77	3.60	
H-6′	3.72	3.86	3.83	3.76	H-6′	3.65	3.75	3.66	3.64	H-6′	3.83	3.91	3.67	
$J_{1,2}$	6.3	9.3	4.5	2.0	$J_{1,2}$	6.4	9.0	6.5	6.9	$J_{1,2}$	4.1	1.0	7.7	
$J_{2,3}$	9.7	9.3	3.1	1.6	$J_{2,3}$	10.4	9.8	8.5	8.0	$J_{2,3}$	3.7	2.8	4.2	
$J_{3,4}$	9.3	9.5	4.5	4.0	$J_{3,4}$	3.8	3.4	8.3	8.1	$J_{3,4}$	7.4	9.0	2.5	
$J_{4,5}$	9.6	9.3	8.3	8.8	$J_{4,5}$	1.2	1.0	2.5	2.7	$J_{4,5}$	7.5	9.5	9.0	
$J_{5,6}$	5.0	5.0	2.9	5.8	$J_{5,6}$	6.2	5.6	6.4	7.4	$J_{5,6}$	2.9	5.8	5.9	
$J_{5,6'}$	2.0	2.2	5.8	2.8	$J_{5,6'}$	6.2	5.6	6.4	5.1	$J_{5,6'}$	6.9	2.3	3.0	
$J_{6,6'}$	12.8	12.4	12.2	12.1	$J_{6,6'}$	[c]	[c]	[c]	11.4	$J_{6,6'}$	12.5	12.4	12.5	
Xylosyl					*Arabinosyl*					*Ribosyl*				
H-1	5.83	5.21	6.07	5.74	H-1	5.16	5.77	5.72	6.01	H-1	5.59	5.40	6.09	5.74
H-2	4.03	4.06	4.50	4.56	H-2	4.34	4.23	4.78	4.52	H-2	4.36	4.26	4.65	4.66
H-3	4.53	3.62	4.69	4.33	H-3	3.84	4.46	4.23	4.55	H-3	4.07	4.33	4.18	4.35
H-4	3.71	3.77	4.55	4.36	H-4	4.06	4.17	4.19	4.03	H-4	3.93	4.00	4.35	4.11
H-5	3.66	3.53	3.78	3.78	H-5	3.90	3.79	3.73	3.84	H-5	3.78	3.82	3.68	3.72
H-5′	3.81	4.02	3.83	3.89	H-5′	4.00	3.93	3.82	3.90	H-5′	3.91	3.82	3.72	3.81
$J_{1,2}$	5.0	9.0	5.8	3.4	$J_{1,2}$	9.1	3.5	6.6	6.1	$J_{1,2}$	3.2	9.2	6.4	5.9
$J_{2,3}$	7.6	9.2	6.0	3.4	$J_{2,3}$	9.8	6.8	6.8	7.3	$J_{2,3}$	3.2	2.9	6.4	5.7
$J_{3,4}$	7.6	9.2	6.0	5.2	$J_{3,4}$	3.5	7.0	6.8	7.9	$J_{3,4}$	3.2	2.8	2.4	4.0
$J_{4,5}$	8.4	11.0	5.8	6.7	$J_{4,5}$	0	7.1	4.3	4.3	$J_{4,5}$	3.2	8.7	4.5	5.1
$J_{4,5'}$	3.8	5.4	3.8	4.1	$J_{4,5'}$	1.8	3.7	2.7	2.6	$J_{4,5'}$	6.0	8.7	3.7	3.6
$J_{5,5'}$	10.7	10.9	12.3	12.1	$J_{5,5'}$	12.9	14.3	11.8	10.0	$J_{5,5'}$	14.0	[c]	12.4	12.5
Fucosyl					*Rhamnosyl*					*Fructosyl*				
H-1	5.91	5.20	6.00	5.69	H-1	5.71	5.49	5.76		H-1	3.93	3.89	3.90	4.02
H-2	4.21	4.28	4.50	4.66	H-2	4.56	4.41	4.92		H-1′	3.95	3.94	3.90	4.14
H-3	4.76	3.82	4.55	4.23	H-3	4.42	3.79	4.49		H-3	4.32	4.18	4.90	4.30
H-4	3.91	3.85	3.81	3.95	H-4	3.82	3.59	4.14		H-4	3.96	4.12	4.11	4.19
H-5	4.19	4.02	4.07	3.96	H-5	3.73	3.66	4.06		H-5	4.11	3.98	3.97	4.02
H-6	1.14	1.25	1.27	1.24	H-6	1.29	1.34	1.24		H-6	4.14	3.52	3.78	3.69
$J_{1,2}$	6.3	8.8	4.0	6.8	$J_{1,2}$	4.0	0.6	7.6		H-6′	4.57	3.88	3.91	3.88
$J_{2,3}$	10.3	9.6	7.5	7.4	$J_{2,3}$	3.8	3.2	4.2		$J_{1,1'}$	13.4	12.3	[c]	12.6
$J_{3,4}$	3.7	3.3	7.6	7.4	$J_{3,4}$	7.5	9.3	3.1		$J_{3,4}$	9.4	10.5	5.1	7.2
$J_{4,5}$	0.9	0	4.0	3.8	$J_{4,5}$	7.4	9.3	8.4		$J_{4,5}$	3.4	3.3	7.0	7.4
$J_{5,6}$	6.5	6.4	6.8	6.2	$J_{5,6}$	6.2	6.1	6.3		$J_{5,6}$	0	1.3	5.7	5.2
										$J_{5,6'}$	0	2.1	2.9	3.0
										$J_{6,6'}$	13.8	12.1	12.5	12.5

[a] The resonance of pyrazole H-4′ was at 6.0 ± 10.2 ppm 3′- and 5′-methyl groups at 2.2 ± 0.02 and 2.3 ± 0.02 ppm.
[b] Solvent D_2O.
[c] Geminal protons coincident.

Table 2
^{13}C NMR data for 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles [a]

	α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl
Glucosyl					*Galactosyl*					*Mannosyl*				
C-1			87.1	90.7	C-1			84.3	87.9	C-1	83.0	82.7	88.1	
C-2			76.0	80.1	C-2			76.8	78.3	C-2	68.5	71.3	75.2	
C-3			77.2	75.7	C-3			73.4	74.5	C-3	71.6	73.6	71.5	
C-4			79.9	81.9	C-4			81.4	81.8	C-4	68.4	66.7	80.4	
C-5			69.9	69.5	C-5			71.2	70.7	C-5	76.5	79.0	69.4	
C-6			63.6	63.6	C-6			63.4	63.0	C-6	60.7	61.3	63.3	
Xylosyl					*Arabinosyl*					*Ribosyl*				
C-1			85.3	89.4	C-1			87.8	84.3	C-1	81.8	81.4	85.6	88.4
C-2			77.0	79.6	C-2			78.5	77.0	C-2	69.7	68.8	71.0	73.4
C-3			75.2	75.6	C-3			74.2	73.2	C-3	68.4	71.2	70.9	70.6
C-4			80.2	82.5	C-4			82.8	82.3	C-4	67.8	66.6	87.9	84.9
C-5			61.0	60.9	C-5			61.1	61.7	C-5	65.9	64.8	62.2	62.2
Fucosyl					*Rhamnosyl*					*Fructosyl*				
C-1			84.1	87.7	C-1	82.9	82.4	88.0		C-1	65.1	63.8	64.4	63.1
C-2			77.1	78.6	C-2	68.7	71.4	75.2		C-2	94.8	93.7	99.7	96.7
C-3			74.2	75.1	C-3	71.6	73.4	71.4		C-3	71.6	70.3	82.7	79.2
C-4			85.4	85.7	C-4	73.0	72.1	84.8		C-4	70.4	70.1	77.0	74.4
C-5			67.6	67.2	C-5	72.2	75.1	65.4		C-5	68.0	69.4	83.0	82.4
C-6			19.4	18.6	C-6	16.9	17.2	19.5		C-6	57.2	65.7	61.5	61.6

[a] Solvent D_2O.

Table 3
^{1}H NMR data of peracetylated 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles [a]

	α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl
Glucosyl					*Galactosyl*					*Mannosyl*				
H-1	6.08	5.40	6.12	5.66	H-1	6.13	5.38	6.06	5.78	H-1	5.68	5.55	5.80	
H-2	5.18	5.71	5.20	5.94	H-2	5.38	5.82	5.28	6.09	H-2	5.81	5.77	6.17	
H-3	6.47	5.38	6.05	5.36	H-3	6.46	5.18	6.23	5.27	H-3	6.41	5.21	5.83	
H-4	5.21	5.24	5.02	4.38	H-4	5.64	5.50	4.15	4.55	H-4	5.38	5.37	4.65	
H-5	4.51	3.89	5.27	5.42	H-5	4.73	4.12	5.46	5.33	H-5	3.84	3.88	5.31	
H-6	3.94	4.15	4.12	4.08	H-6	3.98	4.14	4.22	4.16	H-6	3.95	4.22	4.05	
H-6′	4.29	4.26	4.58	4.52	H-6′	4.06	4.19	4.46	4.30	H-6′	4.31	4.30	4.51	
$J_{1,2}$	6.2	9.3	5.9	3.4	$J_{1,2}$	6.2	9.3	6.5	3.7	$J_{1,2}$	1.6	1.0	5.6	
$J_{2,3}$	9.9	9.4	5.8	1.4	$J_{2,3}$	10.7	10.3	7.5	4.0	$J_{2,3}$	4.0	3.2	4.7	
$J_{3,4}$	9.6	9.4	4.2	4.3	$J_{3,4}$	3.6	3.4	7.5	6.8	$J_{3,4}$	9.8	10.0	3.7	
$J_{4,5}$	10.3	9.4	8.0	9.1	$J_{4,5}$	1.0	1.2	5.6	4.3	$J_{4,5}$	10.0	9.8	8.9	
$J_{5,6}$	1.8	2.3	6.4	4.7	$J_{5,6}$	6.2	6.2	5.8	7.2	$J_{5,6}$	2.3	6.0	6.1	
$J_{5,6'}$	3.5	5.0	2.6	4.2	$J_{5,6'}$	7.5	6.9	4.0	4.4	$J_{5,6'}$	4.8	2.5	2.3	
$J_{6,6'}$	12.5	12.4	12.3	12.4	$J_{6,6'}$	11.2	11.0	12.2	11.7	$J_{6,6'}$	13.4	12.2	12.3	
Xylosyl					*Arabinosyl*					*Ribosyl*				
H-1	5.99	5.31	6.12	5.64	H-1	5.28	6.08	5.76	6.08	H-1	5.73	5.65	6.26	5.88
H-2	5.10	5.67	5.26	5.37	H-2	5.88	5.47	6.10	5.30	H-2	5.58	5.68	5.57	5.84
H-3	6.51	5.38	6.19	6.02	H-3	5.19	6.41	5.31	6.08	H-3	5.36	5.89	5.23	5.74
H-4	5.07	5.17	5.03	4.48	H-4	5.37	5.52	4.61	4.16	H-4	5.22	5.02	4.83	4.31
H-5	3.74	3.48	4.10	4.30	H-5	3.83	3.91	4.26	4.48	H-5	4.14	3.9	4.22	4.02
H-5′	4.03	4.23	4.26	4.38	H-5′	4.16	4.40	4.32	4.64	H-5′	4.19	3.97	4.38	4.35
$J_{1,2}$	6.0	9.1	6.2	4.0	$J_{1,2}$	9.2	6.0	3.3	6.4	$J_{1,2}$	1.4	8.1	5.2	2.4
$J_{2,3}$	9.6	9.5	6.4	2.6	$J_{2,3}$	10.3	10.3	4.3	7.6	$J_{2,3}$	3.7	2.8	6.7	5.1
$J_{3,4}$	9.6	9.3	7.1	5.0	$J_{3,4}$	3.4	3.8	7.2	6.7	$J_{3,4}$	3.7	3.0	7.7	5.4
$J_{4,5}$	5.7	9.3	4.0	6.8	$J_{4,5}$	1.0	2.7	5.1	8.2	$J_{4,5}$	1.6	5.0	5.1	5.2
$J_{4,5'}$	10.6	5.7	5.2	5.9	$J_{4,5'}$	2.1	1.4	3.2	3.3	$J_{4,5'}$	1.8	9.4	3.1	3.8
$J_{5,5'}$	10.9	9.4	12.2	11.8	$J_{5,5'}$	13.2	12.9	12.4	11.8	$J_{5,5'}$	13.3	11.3	12.1	11.1
Fucosyl					*Rhamnosyl*					*Fructosyl*				
H-1	6.09	5.37	6.06	5.79	H-1	5.8	5.82	5.94		H-1	4.68	4.41	4.36	4.70
H-2	5.37	5.18	5.28	6.08	H-2	5.77	5.72	6.16		H-1′	4.72	4.54	4.46	5.10
H-3	6.45	5.80	6.13	5.26	H-3	6.26	5.24	5.78		H-3	6.16	5.94	6.68	5.53
H-4	5.47	5.34	3.96	3.94	H-4	5.06	5.09	4.38		H-4	5.31	6.00	5.30	5.32
H-5	4.68	4.00	5.29	5.13	H-5	3.63	3.95	5.06		H-5	5.34	5.34	4.23	4.46
H-6	1.07	1.25	1.29	1.29	H-6	1.10	1.25	1.22		H-6	3.96	3.32	4.27	4.36
$J_{1,2}$	6.3	10.1	6.4	3.9	$J_{1,2}$	1.4	1.1	5.1		H-6′	4.15	3.85	4.42	4.39
$J_{2,3}$	10.7	10.1	7.3	3.9	$J_{2,3}$	4.1	3.3	5		$J_{1,1'}$	12.0	12.1	11.6	11.8
$J_{3,4}$	3.6	3.3	7.3	6.4	$J_{3,4}$	10.0	10.1	3.9		$J_{3,4}$	7.1	10.5	2.3	3.5
$J_{4,5}$	1.2	0	6.1	4.5	$J_{4,5}$	9.9	10.0	8.6		$J_{4,5}$	0.8	3.5	3.3	3.2
$J_{5,6}$	6.5	6.4	6.5	6.5	$J_{5,6}$	6.2	6.1	6.4		$J_{5,6}$	3.3	1.6	5.0	6.4
										$J_{5,6'}$	6.5	2.4	6	5.8
										$J_{6,6'}$	12.3	13.1	11.7	12.3

[a] Solvent: $CDCl_3$.

Table 4
^{1}H NMR data for 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles from 2-amino-2-deoxyaldoses

	α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		α-pyranosyl	β-pyranosyl	*N*,*N*-bisacetyl β-pyranosyl	α-furanosyl	β-furanosyl	*N*,*N*-bisacetyl β-furanosyl
Glucosaminyl [a]					*Glucosaminyl peracetate* [b]						
H-1	5.96	5.41	6.18	5.81	H-1	5.94	6.10	6.42	6.13		6.04
H-2	4.33	4.22	4.58	4.53	H-2	4.66	4.37	4.8	4.82		5.48
H-3	HDO	3.78	4.86	4.39	H-3	6.24	5.69	5.91	5.97		5.59
H-4	3.60	3.66	4.47	4.13	H-4	5.09	5.05	5.17	4.68		4.78
H-5	3.75	3.68	4.02	4.04	H-5	3.78	4.04	4.15	5.22		5.34
H-6	3.71	3.82	3.75	3.63	H-6	3.92	4.08	4.1	4.04		4.04
H-6′	3.74	3.9	3.84	3.77	H-6′	4.16	4.22	4.24	4.53		4.52
					N–H	6.7	7.3		7.7		
$J_{1,2}$	6.2	9.5	6.5	3.0	$J_{1,2}$	6.0	9.6	8.7	6.7		6.9
$J_{2,3}$	10.4	10.0	6.5	1.9	$J_{2,3}$	10.8	9.5	10.6	6.8		3.1
$J_{3,4}$	9.0	9.1	6.1	3.9	$J_{3,4}$	9.2	10.2	9.0	6.8		5.2
$J_{4,5}$	9.0	9.8	6.1	8.8	$J_{4,5}$	10.3	9.9	10.1	6.5		8.8
$J_{5,6}$	4.6	4.4	6.0	5.6	$J_{5,6}$	2.4	2.4	2.3	6.6		5.8
$J_{5,6'}$	2.2	1.9	3.2	2.8	$J_{5,6'}$	5.0	5.2	5.1	2.7		2.5
$J_{6,6'}$	13.5	12.6	12.2	12.1	$J_{6,6'}$	12.3	12.5	12.1	12.1		12.2
					$J_{2,N-H}$	9.0	7.8		7.8		
Galactosaminyl [a]					*Galactosaminyl peracetate* [b]						
H-1	5.99	5.36	6.1	5.73	H-1	6.06	5.98	6.36	6.00	6.05	6.33
H-2	4.53	4.46	H-DO	4.9	H-2	4.79	4.57	5.02	4.92	5.13	5.68
H-3	4.85	3.96	4.61	4.42	H-3	6.18	5.6	5.86	6.05	5.54	5.82
H-4	4.09	4.07	4.12	4.24	H-4	5.49	5.47	5.54	4.2	4.4	4.36
H-5	4.07	3.92	3.99	3.87	H-5	4.23	4.3	4.44	5.2	5.38	5.55
H-6	3.68	3.80	3.70	3.63	H-6	3.95	4.04	4.08	4.1	4.15	4.22
H-6′	3.69	3.80	3.70	3.67	H-6′	4.04	4.13	4.16	4.29	4.24	4.28
N-Ac		1.83	1.81	1.97	N–H	6.9	7.11	–	6.8	7.6	–
$J_{1,2}$	6.3	9.5	6.9	7.3	$J_{1,2}$	5.9	9.6	8.7	7.1	6.6	7.3
$J_{2,3}$	11.1	10.9	6.9	8.6	$J_{2,3}$	11.6	11.1	11.2	9.4	6.7	7.2
$J_{3,4}$	3.6	3.2	9.4	8.1	$J_{3,4}$	3.6	3.5	3.6	7.8	6.6	5.5
$J_{4,5}$	0.5	0	2.5	2.5	$J_{4,5}$	0.9	1.1	0.9	5.4	4.5	5.3
$J_{5,6}$	3.6	6.0	6.0	7.6	$J_{5,6}$	6.8	6.8	6.9	6.4	7.2	6.8
$J_{5,6'}$	8.8	6.0	6.0	5.1	$J_{5,6'}$	6.5	6.0	6.0	3.9	4.4	4.3
$J_{6,6'}$	12.5	[c]	[c]	11.7	$J_{6,6'}$	11.2	11.3	11.3	12.1	11.8	11.9
					$J_{2,N-H}$	7.4	7.7	–	7.8	7	–

[a] Solvent D_2O.
[b] Solvent acetone-d_6.
[c] Geminal protons coincident.

Table 5
^{13}C NMR data for 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles from 2-amino-2-deoxyaldoses

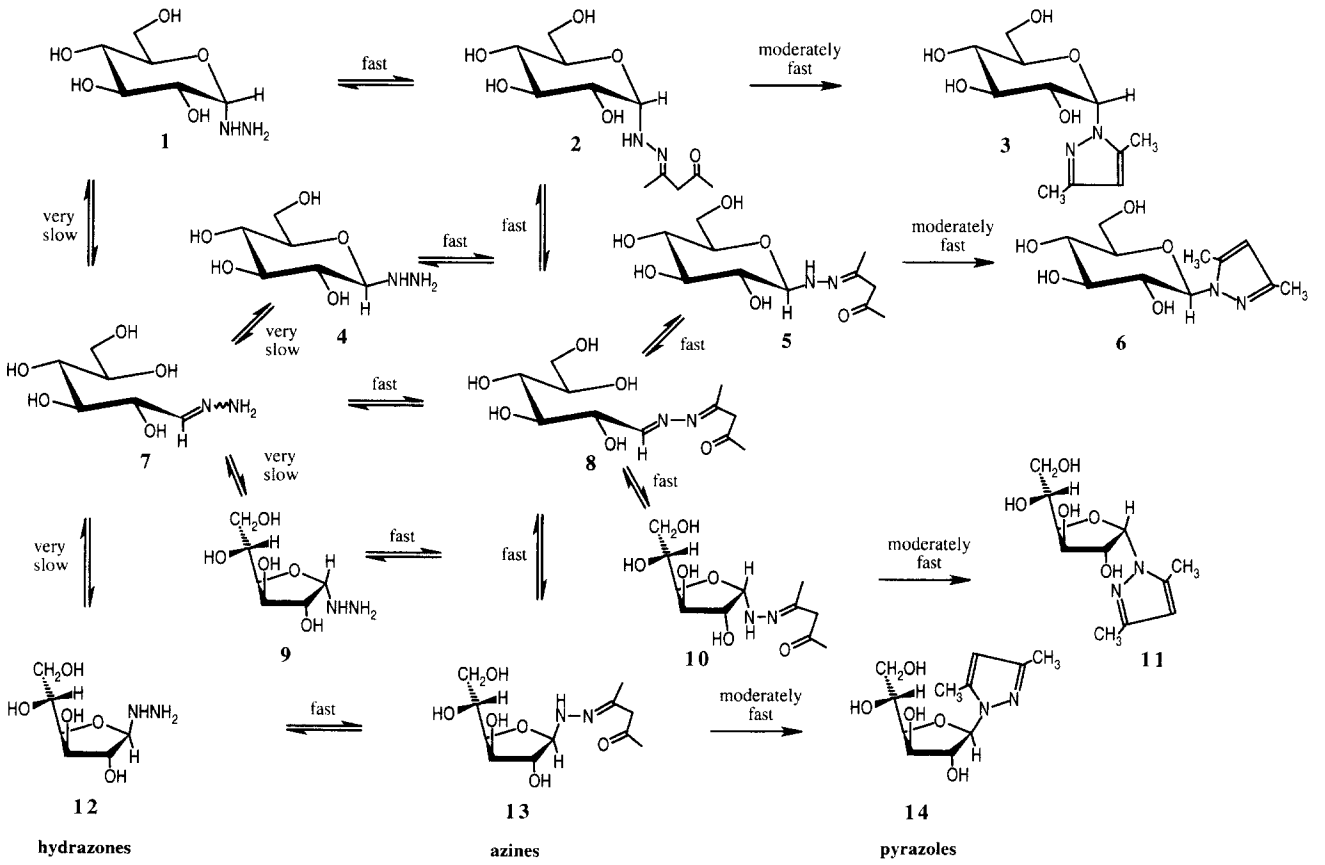
	α-pyranosyl	β-pyranosyl	α-furanosyl	β-furanosyl		β-pyranosyl	bisacetyl β-pyranosyl	α-furanosyl	β-furanosyl	bisacetyl β-furanosyl
Glucosaminyl [a]					*Glucosaminyl peracetate* [b]					
C-1	78.9	82.7		88.2	C-1	83.5	82.3	84.0		85.9
C-2	52.6	54.6		61.4	C-2	54.8	60.5	58.4		67.4
C-3	70.9	73.3		73.2	C-3	73.2	71.5	76.6		76.4
C-4	69.8	68.8		80.7	C-4	69.8	70.3	76.8		77.3
C-5	73.2	77.6		68.5	C-5	74.7	74.9	69.7		69.1
C-6	59.9	57.0		62.6	C-6	63.0	63.0	63.2		63.4
N-Ac	21.1	21.2		21.1						
Galactosaminyl [a]					*Galactosaminyl peracetate* [b]					
C-1	79.7	84.0	83.4	86.7	C-1	85.0	82.4		87.0	84.5
C-2	49.5	52.0	58.9	59.8	C-2	62.5	62.3		60.2	65.5
C-3	68.3	71.2	71.1	72.7	C-3	70.9	69.2		76.0	73.8
C-4	67.8	68.2	81.8	82.1	C-4	68.2	68.6		79.9	80.3
C-5	73.4	77.6	70.8	70.1	C-5	73.7	73.9		70.6	70.7
C-6	61.4	61.0	63.2	62.8	C-6	50.8	57.5		63.1	63.0
N-Ac	21.7	21.9	21.2	22.0						

[a] Solvent D_2O.
[b] Solvent acetone-d_6.

Table 6
Distribution of cyclic isomers of 1-glycosyl-3,5-dimethyl-1*H* pyrazoles (%)

	α-furanosyl-	β-furanosyl-	α-pyranosyl-	β-pyranosyl-
Arabinose	29	7	58	6
Ribose	12	40	11	37
Xylose	13	26	13	48
Galactose	9	39	5	47
Glucose	3	5	6	86
Mannose	31	< 1	13	56
Fucose	3	12	3	80
Rhamnose	39	< 1	18	41
Galactosamine	5	45	2	50
Glucosamine	2	2	4	92
Fructose	55	28	10	7
Maltose	0	0	12	88
Cellobiose	0	0	12	88
Isomaltose	6	6	7	81

In general, the coupling constants are similar to those of the parent sugars [14–17], except that the $J_{1,2}$ values for the 1-(α-D-glycopyranosyl)-3,5-dimethyl-1*H*-pyrazoles from glucose, galactose, fucose, rhamnose, mannose, and xylose are somewhat larger than expected. This suggests some distortion of the $^4C_1$ conformation. The coupling constants for 3,5-dimethyl-1-(β-D-ribopyranosyl)-1*H*-pyrazole are consistent with the $^4C_1$ conformation, rather than the mixture of conformations found for the parent sugar [15]; no conclusions about the conformation of 3,5-dimethyl-1-(α-D-ribopyranosyl)-1*H*-pyrazole could be drawn from the coupling constants. The coupling constants of 1-(α-D-arabinopyranosyl)-3,5-dimethyl-



Scheme 1. Steps in the conversion of glucose hydrazones to 1-(D-glucosyl)-3,5-dimethyl-1*H*-pyrazoles.

1 *H*-pyrazole are consistent with the same $^1C_4$ conformation as the parent sugar [17], while the β anomer appears to be conformationally unstable. The observation that 1-(β-glycopyranosyl)-3,5-dimethyl-1 *H*-pyrazoles in the $^4C_1$ conformation have H-3 resonances significantly upfield from those of the corresponding α anomers (Tables 1 and 4), possibly as a result of deshielding by the heterocyclic ring, may be diagnostically useful.

The anomeric configurations of the 1-furanosyl-3,5-dimethyl-1 *H*-pyrazoles were assigned from the relative chemical shifts of the anomeric carbons (Table 2), which occur at lower field for the β anomers of the parent sugars [13]. There were similar relationships between other carbons. The proportions of isomeric glycosylpyrazoles (Table 6) are broadly as expected from their relative stabilities. A 1,2-*trans* configuration in furanosyl and pyranosyl rings and a 3,4-*trans* configuration in furanosyl rings were preferred. The exceptions to this rule were the mannopyranosyl and rhamnopyranosyl derivatives.

3. Discussion

Protected glycosylhydrazines have been converted to glycosylpyrazoles [19,20], by condensation with a 1,3-diketone such as acetylacetone but, when the hydrazones of unprotected ribose or glucose were treated in this way [19,20], 3,5-dimethyl-1 *H*-pyrazole was formed, with regeneration of the starting monosaccharides. Subsequently, unprotected hydrazones were converted to simple mixtures of 1-glycosyl-3,5-dimethyl-1 *H*-pyrazoles by treatment with acetylacetone in boiling methanol [18]. At variance with these reports, we have found that monosaccharide and oligosaccharide hydrazones are converted efficiently to glycosylpyrazoles under mild conditions, and that the products are typically complex mixtures of isomers (Table 6).

It is possible that the mixtures of isomeric products were simplified by controlling the distribution of isomers in the precursor hydrazones. As formation of the pyrazole would require prior closure of the sugar ring, we pre-equilibrated the precursor glycosylhydrazines in aqueous buffers, but obtained only minor changes in the isomer distributions of the glycosylpyrazoles, and a reduced yield because of regeneration of reducing sugar (Fig. 2).

The addition of a small amount of acetone to an aqueous solution of glucose hydrazone, however, has a striking effect on the rate of cyclisation (Fig. 1b), and the half-life for the formation of 1-glucosyl-3,5-dimethyl-1 *H*-pyrazole (Fig. 1c) is < 15 min, suggesting that similar catalysis of cyclisation occurs in the presence of acetylacetone (Scheme 1). The intermediate **8**, equilibrates rapidly with **2**, **5**, **10**, and **13**, and cyclizes irreversibly to **3**, **6**, **11**, and **14** (Scheme 1). It is therefore likely that the observed proportions of products are the result of a complex equilibrium before the formation of the glycosylpyrazole products, and do not correspond in any simple way to the initial proportions of the glycosylhydrazine isomers **1**, **4**, **9**, and **12**. The detailed mechanism for the formation of 1-substituted 1 *H*-pyrazoles from methylhydrazine has been studied [21,22]. By analogy it might be expected that acetylacetone would couple first to the unsubstituted nitrogen, and then cyclize on to the substituted nitrogen, before loss of water. The slightly different mechanism (Scheme 1), in which water is lost immediately, appears more likely in view of the observed chemical shift of the anomeric proton of the main intermediate **5**, which corresponds to that of the acetone adduct **15**.

This report demonstrates that 1-glycosyl-3,5-dimethyl-1 *H*-pyrazoles are formed efficiently from aldoses (such as those obtained by beta-elimination of some O-linked glycoproteins), 6-deoxyhexoses, 2-amino-2-deoxyaldoses (which are the reducing terminal residues of N-linked glycans of glycoproteins), three disaccharides, and a ketose. The chemistry provides a convenient strategy for the introduction of a heteroaromatic label under very mild conditions, but, compared with existing methods [5,6], has the disadvantage of leading to isomeric products. The proportions of the products (Table 6) depend on the precursor monosaccharide, and may be simpler for higher oligosaccharides, particularly when there is linkage to the 4-position of the reducing terminal residue, such as in maltose (Table 6), when the formation of a furanosyl ring is not possible. Further studies are in progress, particularly in relation to the analysis of glycans released from glycoproteins in the presence of hydrazine [7–9].

4. Experimental

General.—All materials were obtained from commercial sources and used without further purification. Preparative reversed-phase high-performance liquid chromatographic (RP-HPLC) purification of monosaccharide 1-glycosyl-3,5-dimethyl-1 *H*-pyrazoles was carried out using a Jordi divinylbenzene

column (100 × 10 mm, Alltech, Deerfield, USA) with detection at 220 and 240 nm using a Shimadzu series 10 HPLC system (Kyoto, Japan). The elution conditions were adjusted to suit each monosaccharide. For example, the 3,5-dimethyl-1-D-ribosyl-1*H*-pyrazoles were separated by elution at 1.5 mL/min with 13:87 acetonitrile–water for 8 min, a gradient to 25:75 over 12 min, then to 60:40 over 6 min. Peracetylated pyrazoles were eluted with 45:55 acetonitrile–water for 5 min, a gradient to 70:30 over 15 min, then to 80:20 over 2 min. Disaccharide pyrazoles were fractionated by normal-phase HPLC on a 5 μm aminopropylsilica column (220 × 4.6 mm, Brownlee, Foster City, USA), eluted isocratically at 1 mL/min with 84:16 acetonitrile–water.

NMR spectra were acquired at 27 °C on a Varian XL-400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C observation. D_2O was used as solvent for sugar hydrazones and glycosylpyrazoles, with referencing to HDO at 4.75 ppm (^1H) or MeOH at 49.3 ppm (^{13}C). Spectra of acetylated 1-glycosyl-3,5-dimethyl-1*H*-pyrazoles were acquired in $CDCl_3$ (and referenced to MeOH) or acetone-d_6 (and referenced to acetone-d_6 at 29.8 ppm). Buffers for NMR studies were prepared by titrating 0.1 M D_3PO_4 with satd NaOD to the appropriate apparent pH. NMR kinetic experiments were referenced to sodium 3-(trimethylsilyl)propionic acid, and peak intensities were normalized to this internal standard. The data from kinetic studies were processed with a Lorentzian line broadening of 1.0 Hz. Electrospray ionisation mass spectrometry (ESIMS) was performed with a Fisons VG Quattro spectrometer coupled to a Hewlett–Packard 1090 liquid chromatograph (Palo Alto, CA, USA).

Preparation of glucose hydrazone [3].—Hydrazine hydrate (400 μL) was added to 100 mg of glucose and allowed to stand overnight. The soln was diluted to 2 mL, then dispensed into vials and evaporated in a centrifugal evaporator. The dry samples were stored at room temperature.

NMR study of the equilibration of glucose hydrazone in buffered deuterium oxide.—Two dried samples of glucose hydrazone, prepared from 10 μmol of glucose, were prepared. One sample was dissolved in deuterium oxide (0.60 mL), a deuterium oxide soln of sodium 3-(trimethylsilyl)propionic acid (0.04 M, 50 μL) added as an internal standard, and the ^1H NMR spectrum was measured to establish the initial proportions of components [3]. The second sample was dissolved in 0.1 M deuterated phosphate buffer (pD 6, 0.60 mL), the internal standard added, and the soln was maintained at 27 °C and analyzed at intervals by ^1H NMR spectroscopy. Peak intensities were normalized relative to the resonances of the internal standard at 2.9 ppm. *E*-D-Glucose hydrazone (**1**) ^1H NMR: 3.54 (dd, 1 H, $J_{4,5}$ 8.5 Hz, H-4), 3.62 (dd, 1 H, $J_{6,6'}$ 11.6 Hz, H-6), 3.75 (ddd, 1 H, $J_{5,6}$ 6.2 Hz, $J_{5,6'}$ 2.9 Hz, H-5), 3.82 (dd, 1 H, H-6'), 3.91 (dd, 1 H, $J_{3,4}$ 1.7 Hz, H-3), 4.28 (ddd, 1 H, $J_{2,3}$ 7.4 Hz, H-2), 7.24 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1). β-D-Glucopyranosylhydrazine (**3**) ^1H NMR: 3.26 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.36 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.42 (ddd, 1 H, $J_{5,6}$ 5.6 Hz, $J_{5,6'}$ 2.2 Hz, H-5), 3.49 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 3.72 (dd, 1 H, $J_{6,6'}$ 12.3 Hz, H-6), 3.91 (dd, 1 H, H-6'), 4.03 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1).

NMR study of the kinetics of reaction of glucose hydrazone with acetone.—A sample of fresh glucose hydrazone (ca. 20 μmol) was dissolved in D_2O (0.6 mL) containing internal standard and analyzed by ^1H NMR spectroscopy. The sample was withdrawn from the spectrometer, an aliquot of acetone-d_6 (20 μL, ca. 10-fold excess) in D_2O added, mixed, and reanalyzed. *N*-β-D-glucopyranosyl-*N'*-isopropylidenehydrazine (**15**) ^1H NMR: 3.40 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.43 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.46 (ddd, 1 H, $J_{5,6}$ 5.6 Hz, H-5), 3.54 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 3.71 (dd, 1 H, $J_{6,6'}$ 12.2 Hz, H-6), 3.90 (dd, 1 H, $J_{5,6'}$ 2.2 Hz, H-6'), 4.44 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1).

NMR studies of the formation of isomeric 1-glycosyl-3,5-dimethyl-1H-pyrazoles [18].—(a) Kinetics of formation of 1-D-glucosyl-3,5-dimethyl-1H-pyrazoles.—A soln of fresh glucose hydrazone (20 μmol) was dissolved in deuterium oxide (0.60 mL) containing sodium 3-(trimethylsilyl)propionic acid (4 mmol) and analyzed by ^1H NMR spectroscopy to establish the initial proportions of components. Acetylacetone in D_2O (10% v/v, 80 μL, ca. 80 μmol) was added, and the soln was maintained at 27 °C and analyzed at intervals by ^1H NMR spectroscopy (Fig. 3). The chemical shift of H-1 of the main intermediate species **5** was 4.48 ppm ($J_{1,2}$ 8.8 Hz), corresponding to **15**.

(b) 1-Aldosyl-3,5-dimethyl-1H-pyrazoles.—To a dispersion of aldose (4.5 g, 25 mmol) in water (10 mL) was added hydrazine monohydrate (10 mL), and the soln was allowed to stand overnight at room temperature. The soln was rotary evaporated to give a gum, which was redispersed in water (50 mL) and re-evaporated. The gum was dissolved in water (100 mL) and acetylacetone (15 mL) was added with swirling and allowed to stand for 2 h before extracting with $CHCl_3$ (3 × 25 mL) to remove excess acety-

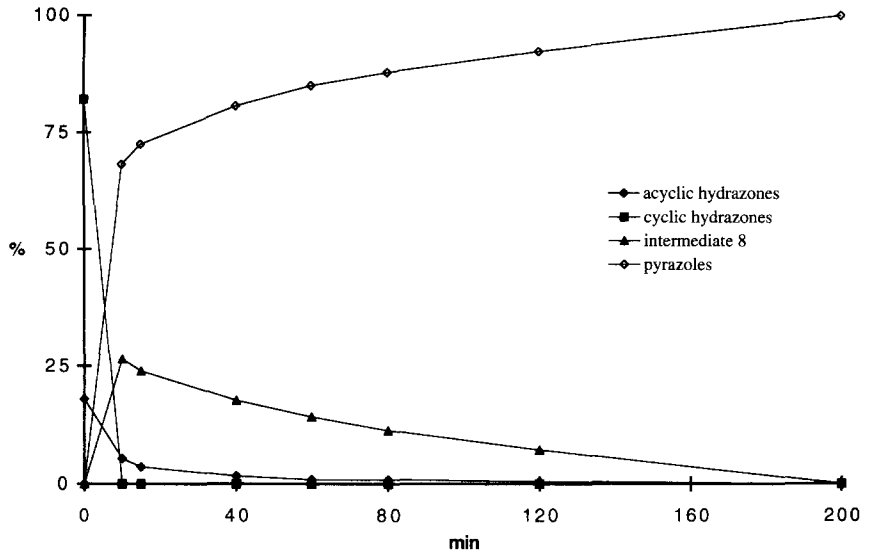


Fig. 3. Time course of formation of 1-(D-glucosyl)-3,5-dimethyl-1*H*-pyrazoles from glucose hydrazone and acetylacetone in unbuffered D_2O at 27 °C.

lacetone and contaminating 3,5-dimethyl-1*H*-pyrazole (formed from hydrazine present in the crude hydrazone). The aq layer was evaporated to yield a hygroscopic solid. Aliquots of this were submitted to preparative RP-HPLC, and the isomeric products were characterized by NMR spectroscopy (Tables 1 and 2).

(c) 1-(2-Deoxy-2-acetamidoaldosyl)-3,5-dimethyl-1H-pyrazoles.—Glucosamine or galactosamine hydrochloride (1 g, 4.6 mmol) was dispersed in water (2 mL) and hydrazine hydrate (2 mL), allowed to stand overnight, rotary evaporated, and treated with acetylacetone (4 mL) for 2 h. After extraction with $CHCl_3$ (3 × 1 mL) and evaporation of the aq layer to dryness, the crude product was acetylated in Ac_2O (5 mL) and pyridine (15 mL) at reflux for 2 h. The mixture was poured into 100 mL of water, extracted into $CHCl_3$ (2 × 10 mL), the extracts were evaporated to dryness. Aliquots of this were submitted to preparative RP-HPLC and analysis by NMR spectroscopy (Table 4).

Peracetylation of 1-glycosyl-3,5-dimethyl-1H-pyrazoles.—An aq soln of a 1-glycosyl-3,5-dimethyl-1*H*-pyrazole fraction was lyophilized and acetylated with pyridine (0.2 mL) and Ac_2O (0.2 mL) at 100 °C for 2 h. After addition of water (5 mL) and extraction into $CHCl_3$ (1 mL) the product was evaporated to dryness in a stream of nitrogen and analyzed by NMR spectroscopy (Table 3).

O-*Deacetylation of isomeric 3,5-dimethyl-1-glycosyl-1H-pyrazole peracetates [11].*—The sample was dissolved in MeOH (0.5 mL), treated with 40% aq dimethylamine (50 μL) and allowed to stand overnight. It was then evaporated in a stream of nitrogen, redissolved in water (0.4 mL) and lyophilized to remove dimethylacetamide.

Preparation of isomeric 3,5-dimethyl-1-glycosylpyrazoles from disaccharides.—The same general method was used as for the monosaccharide derivatives.

(a) Maltose.—^{1}H NMR, 3,5-dimethyl-1-(α-maltopyranosyl)-1*H*-pyrazole: 3.58 (dd, 1 H, H-2′), 4.05 (dd, 1 H, $J_{1,2}$ 6.3 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.51 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.94 (d, 1 H, H-1), 6.05 (s, 1 H, pyrazole H-4). 3,5-dimethyl-1-(β-maltopyranosyl)-1*H*-pyrazole: 3.61 (dd, 1 H, $J_{1',2'}$ 3.8 Hz, H-2′), 4.08 (dd, 1 H, $J_{1,2}$ 9.1 Hz, $J_{2,3}$ 9.3 Hz, H-2), 5.32 (d, 1 H, H-1), 5.47 (d, 1 H, H-1′), 6.04 (s, 1 H, pyrazole H-4).

(b) Cellobiose.—^{1}H NMR, 1-(α-cellobiopyranosyl)-3,5-dimethyl-1*H*-pyrazole: 3.34 (dd, 1 H, $J_{1',2'}$ 8.1 Hz, H-2′), 4.06 (dd, 1 H, $J_{1,2}$ 6.1 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.56 (d, 1 H, H-1′), 5.94 (d, 1 H, H-1), 6.04 (s, 1 H, pyrazole H-4). 1-(β-cellobiopyranosyl)-3,5-dimethyl-1*H*-pyrazole: 3.29 (dd, 1 H, $J_{1',2'}$ 7.9 Hz, H-2′), 4.04 (dd, 1 H, $J_{1,2}$ 9.1 Hz, $J_{2,3}$ 9.1 Hz, H-2), 4.52 (d, 1 H, H-1′), 5.28 (d, 1 H, H-1), 5.98 (s, 1 H, pyrazole H-4).

(c) Isomaltose.—The mixed 3,5-dimethyl-1-isomaltosyl-1*H*-pyrazoles were not fractionated, and the proportions of products were estimated by ^{1}H NMR spectroscopy, by comparison of the signals for the anomeric protons with those of the corresponding glucose derivatives: 1-(α-isomaltofuranosyl)-3,5-dimethyl-1*H*-pyrazole 6.08 ppm ($J_{1,2}$ 4.4 Hz), 1-(β-

isomaltopyranosyl)-3,5-dimethyl-1*H*-pyrazole 5.3 ppm ($J_{1,2}$ 9.0 Hz), 1-(β-isomaltofuranosyl)-3,5-dimethyl-1*H*-pyrazole 5.73 ppm ($J_{1,2}$ 2.0 Hz).

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