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^{1}H-Nuclear magnetic resonance spectroscopy of reducing–residue anomeric protons of pertrifluoroacetylated carbohydrates

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ABSTRACT

Eight monosaccharides (L-arabinose, L-fucose, D-galactose, D-glucose, D-lyxose, D-mannose, L-rhamnose, and D-xylose), eight disaccharides (cellobiose, gentiobiose, isomaltose, lactose, maltose, nigerose, sophorose, and xylobiose), and three trisaccharides (isomaltotriose, maltotriose, and xylotriose) were derivatized with *N*-methylbis-(trifluoroacetamide) in pyridine solution to form trifluoroacetylated derivatives. These were analyzed by ^{1}H-n.m.r. spectroscopy to determine the characteristics of the spectra and distributions of the reaction products. Peaks corresponding to reducing-residue anomeric protons were located significantly downfield of all others, and were in general 0.4 p.p.m. or more downfield of equivalent signals from the same carbohydrates when they were free or derivatized with other groups. Neither the location of anomeric proton peaks relative to each other nor the degree of spin–spin coupling between H-1 and H-2 varied greatly with type of derivatization. Spin–spin coupling, however, decreased for some β-pyranose forms of xylobiose and the three trisaccharides. In all examples except some where H-2 was oriented equatorially to a pyranose ring, the proportion of the α-pyranose was either enhanced or not changed in concentration by trifluoroacetylation.

INTRODUCTION

This article continues investigations into pertrifluoroacetylated (TFA) carbohydrates. An earlier article described the capillary gas chromatography of TFA-trisaccharides[1], noting that these derivatives were of extremely high volatility and that mixtures of them could be almost completely separated by this technique. However, we and others before us[2–4] found that these mixtures had distributions that could be different from the known mutarotational equilibrium ratios of the starting carbohydrates, and that varied with reaction conditions, suggesting that the derivatization reaction was relatively slow. This uncertainty in product distribution renders what would be a highly useful derivatization method for trisaccharides and potentially for longer oligosaccharides less attractive than it otherwise would be. Despite these drawbacks, we were quite successful, after proper standardization, in using the method to

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measure the kinetics and equilibria of reactions catalyzed by glucoamylase to produce trisaccharides from disaccharides and D-glucose[5]. The ^{1}H-n.m.r. work described in this article was carried out to determine the spectra and product distributions resulting from the pertrifluoroacetylation of mono-, di-, and tri-saccharides. Apparently there have been no previous ^{1}H-n.m.r. studies on TFA-carbohydrates.

MATERIALS AND METHODS

Materials. — The monosaccharides L-arabinose, L-fucose, D-galactose, D-glucose, D-lyxose, D-mannose, L-rhamnose, and D-xylose were purchased from various laboratory suppliers and were of reagent grade. The disaccharides cellobiose [*O*-β-D-glucopyranosyl-(1→4)-D-glucose], gentiobiose [*O*-β-D-glucopyranosyl-(1→6)-D-glucose], isomaltose [*O*-α-D-glucopyranosyl-(1→6)-D-glucose], lactose [*O*-β-D-galactopyranosyl-(1→4)-D-glucose], maltose [*O*-α-D-glucopyranosyl-(1→4)-D-glucose], nigerose [*O*-α-D-glucopyranosyl-(1→3)-D-glucose], and xylobiose [*O*-β-D-xylopyranosyl-(1→4)-D-xylose] were obtained from Sigma (St. Louis, MO, U.S.A.), while sophorose [*O*-β-D-glucopyranosyl-(1→2)-D-glucose] came from Adams Chemical (Round Lake, IL, U.S.A.). The trisaccharides isomaltotriose [*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose] and maltotriose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose] came from Sigma, while xylotriose [*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose] was made in our laboratory by acidic hydrolysis of xylan followed by chromatographic separation[6].

N-Methylbis(trifluoroacetamide) (MBTFA) and pyridine (silylation grade) were purchased from Pierce (Rockford, IL, U.S.A.). Dichloromethane-d_2 (CD_2Cl_2) came from Aldrich (Milwaukee, WI, U.S.A.).

Derivatization. — A quantity of 10–20 mg of carbohydrate was added to 0.8 mL of pyridine (1.3 mL if the solid did not fully dissolve) and the mixture was allowed to come to mutarotational equilibrium at 45° overnight. Polarimetric measurements of a number of mono- and di-saccharides at room temperature showed that all had come to equilibrium within this time. Derivatization was with 1 mL of MBTFA for 1 h at 65°, following the procedure of Sullivan and Schewe[4]. Pyridine was largely removed by vacuum aspiration at room temperature.

^{1}H-Nuclear magnetic resonance. — ^{1}H-n.m.r. spectra were obtained at room temperature with a Bruker WM-300 spectrophotometer locked on deuterium of the solvent CD_2Cl_2. The chemical shifts (δ) are relative to tetramethylsilane.

RESULTS AND DISCUSSION

Identification of n.m.r. peaks. — The ^{1}H-n.m.r. spectroscopy of free and derivatized carbohydrates has been studied extensively. It has been found uniformly that peaks representing H-1 atoms of free aldoses and those derivatized with other than acyl groups are located significantly downfield from all other peaks, as these protons are linked to a carbon atom attached to two oxygen atoms[7]. However, the H-1 atoms of

TABLE I

^{1}H-n.m.r. chemical shifts (δ) and coupling constants ($J_{1,2}$) of anomeric hydrogen atoms of monosaccharides

Carbohydrate	*Configuration*[a]	*This work*			*Published data*					
		Pertrifluoroacetylated sugar in CD_2Cl_2			*Free sugar in D_2O*			*Peracetylated sugar in $CDCl_3$*		
		δ (p.p.m.)	δ^{*b} (p.p.m.)	$J_{1,2}$ (Hz)	δ (p.p.m.)	δ^{*b} (p.p.m.)	$J_{1,2}$ (Hz)	δ (p.p.m.)	δ^{*b} (p.p.m.)	$J_{1,2}$ (Hz)
L-Arabinose	α-p	6.17	−0.56	6.6	4.52[c]	−0.72	7.8[c]	5.68[d]	−0.67	6.4[d]
	β-p	6.73		3.4	5.24[c]		3.4[c]	6.35[e]		2.9[d]
	α-f	6.58	−0.15	[f]	5.26[g]	0.02	1.0[g]	6.21[h]	−0.14	0.8[i]
	β-f	6.52	−0.21	3.4	5.27[g]	0.03	4.1[g]	6.35[h]	0.00	4.0[j]
		6.40[k]	−0.33	[f]						
L-Fucose	α-p	6.65	0.67	3.6	5.19[l]	0.66	3.0[l]			
	β-p	5.98		8.0	4.53[l]		7.5[l]			
	α-f	6.45	0.47	3.7	5.20–5.26		[l, m]			
	β-f	6.52	0.54	[f]						
D-Galactose	α-p	6.80	0.49	3.8	5.27[c]	0.69	3.6[n]	6.36[e]	0.62	3.3[n]
	β-p	6.31		7.8	4.58[c]		7.8[n]	5.74[e]		7.5[e]
	α-f	6.76	0.45	4.3	5.19[g]	0.61	3.5[g]			
	β-f	6.64	0.33	[f]	5.26[g]	0.68	2.0[g]			
D-Glucose	α-p	6.72	0.47	3.7	5.22[g]	0.57	3.7[n]	6.34[e]	0.58	3.3[e]
	β-p	6.25		7.9	4.65[g]		7.9[n]	5.76[e]		6.7[e]
D-Lyxose	α-p	6.57	0.14	3.9	5.03[p]	0.14	4.1[p]	6.00[d]		3.1[d]
	β-p	6.43		1.4	4.89[p]		1.4[p]			2.5[d]
	α-f	6.70	0.27	4.6	5.26[g]	0.37	4.0[g]	6.28[h]		2.1[h]
	β-f				5.24[g]	0.35	4.5[g]	6.36[h]		4.6[h]
		6.48[k]	0.05	[f]						
D-Mannose	α-p	6.51	0.14	[f]	5.17[g]	0.29	1.9[n]	6.09[e]	0.16	1.8[q]
	β-p	6.37		1.4	4.88[g]		1.1[n]	5.93[e]		1.4[q]
L-Rhamnose	α-p	6.48[k]		[f]	5.07[l]	0.26	1.7[l]	6.02[r]	0.17	1.4[r]
	β-p	6.45[k]		[f]	4.81[l]		1.1[l]	5.85[s]		1.5[s]
		6.40[k]		[f]						
D-Xylose	α-p	6.65	0.36	3.7	5.19[l]	0.62	3.6[l]	6.27[e]	0.53	3.3[e]
	β-p	6.29		7.4	4.57[l]		7.8[l]	5.74[d]		6.6[d]
	α-f				5.39[g]	0.82	4.0[g]	6.41[h]	0.67	4.6[h]
	β-f				5.19[g]	0.62	[m]	6.15[h]	0.41	0.4[h]

[a] p = pyranose, f = furanose. [b] δ^* = δ(α-p, α-f, or β-f) − δ(β-p). [c] Ref. 11. [d] Ref. 12 (peracetylated α-D-arabinopyranose, α-D-lyxopyranose, and β-D-xylopyranose in $CDCl_3$; peracetylated β-D-arabinopyranose and β-D-lyxopyranose in acetone-d_6). [e] Ref. 13. [f] Single peak. [g] Ref. 14 (using D-arabinose instead of L-arabinose). [h] Ref. 15 (using D-arabinose instead of L-arabinose). [i] Ref. 16 (using D-arabinose instead of L-arabinose). [j] Ref. 17 (using D- and L-arabinose). [k] Unidentified peak. [l] Ref. 18. [m] Not determined. [n] Ref. 19. [o] Ref. 20 (using acetone-d_6 instead of $CDCl_3$). [p] Average of refs. 21 and 22. [q] Ref. 23 (using CCl_4 or C_6D_6 instead of $CDCl_3$). [r] Ref. 24. [s] Ref. 25. [t] Ref. 26.

TABLE II

^{1}H-n.m.r. chemical shifts (δ) and coupling constants ($J_{1,2}$) of reducing-residue anomeric hydrogen atoms of oligosaccharides

Carbohydrate	*Configuration*[a]	*This work*			*Published data*					
		Pertrifluoroacetylated sugar in CD_2Cl_2			*Permethylated sugar in acetonitrile-d_3*[b]			*Pertrimethylsilylated sugar in acetone-d_6*		
		δ (p.p.m.)	δ^*[c] (p.p.m.)	$J_{1,2}$ (Hz)	δ (p.p.m.)	δ^*[c] (p.p.m.)	$J_{1,2}$ (Hz)	δ (p.p.m.)	δ^*[c] (p.p.m.)	$J_{1,2}$ (Hz)
Cellobiose	α-p	6.53	0.47	4.2	4.77	0.63	3.5	5.02	0.47	3.5[d]
	β-p	6.06		7.9	4.14		7.6	4.55		7.0
Gentiobiose	α-p	6.70	0.46	4.4	4.77	0.65	3.4	5.03	~0.48	3.0[d]
	β-p	6.24		8.0	4.12		7.5	~4.55		7.0
Isomaltose	α-p	6.67		3.7	4.76	0.63	3.5	4.97	0.46	3.3[e]
	β-p				4.13		7.5	4.51		7.5
Lactose	α-p	6.54	0.47	3.7	4.75	0.62	3.4	5.00	~0.45	3.2[d]
	β-p	6.07		7.2	4.13		7.6	~4.55		7.3
Maltose	α-p	6.64	0.37	3.8	4.77	0.61	3.4	~5.09	~0.43	3.5[d]
	β-p	6.27		7.7	4.16		7.4	4.66		7.2
Nigerose	α-p	6.62	0.47	3.8						
	β-p	6.15		7.4						
Sophorose	α-p	6.67	0.55	4.1	4.74	0.53	2.8	5.17	0.22	3.3[d]
	β-p	6.12		7.7	4.21		6.8	4.95		7.6
Xylobiose	α-p	6.62	0.44	3.5				4.95	0.49	3.1[e]
	β-p	6.18		5.5				4.46		7.1
Isomaltotriose	α-p	6.66	0.57	4.4						
	α-p	6.55	0.46	3.6						
	β-p	6.09		4.8						
Maltotriose	α-p	6.62	0.50	3.6						
	α-p	6.50	0.38	3.6						
	β-p	6.30	0.18	5.8						
	β-p	6.12		7.6						
Xylotriose	α-p	6.53	0.51	3.7						
	β-p	6.02		5.2						

[a] p = pyranose. [b] Ref. 27. [c] $\delta^* = \delta(\alpha\text{-p}) - \delta(\beta\text{-p})$. [d] Ref. 28. [e] Ref. 29.

nonreducing sugar residues of oligosaccharides derivatized with acyl groups are strongly shielded, leaving the H-1 peaks of the reducing residue isolated[8]. In many cases, anomeric configurations have been assigned to the species giving rise to the absorptions of the various anomeric protons. Protons oriented equatorially to a pyranose ring in the chair form are more deshielded than those oriented axially[7,9]. Furthermore, $J_{1,2}$ couplings are highest when the angle between the two protons approaches 0° or 180° and are lowest[7,9] when the angle is near 90° (the Karplus correlation)[10].

This prior work permits straightforward assignment of configurations to the carbohydrates corresponding to the reducing-residue anomeric-proton peaks listed in Tables I and II. For TFA-D-glucose and the seven TFA-disaccharides having a reducing D-glucose residue, chair-form pyranose rings overwhelmingly dominate and there are only two anomeric-proton peaks. The anomeric-proton peak having $\delta > 6.5$ p.p.m. and $J_{1,2} < 4.5$ Hz may be assigned to the α-pyranose (equatorial H-1 and axial H-2). The other peak, present in all cases except TFA-isomaltose, has δ usually ~ 0.45 p.p.m. less than that of the α-pyranose form and $J_{1,2} > 7$ Hz, and clearly results from the β-pyranose, which has H-1 and H-2 axial. These results vary little from earlier ones shown in Tables I and II, except that peaks of trifluoroacetylated derivatives, because of the strong electron-withdrawing nature of the trifluoroacetyl group, are ~ 1.5, 0.4, 1.9, and 1.6 p.p.m. downfield from those of free, peracetylated, permethylated, and pertrimethylsilylated sugars, respectively.

Similar n.m.r. behavior is exhibited by TFA-D-xylose, which is homomorphic with D-glucose about C-1–C-4, and by the TFA-disaccharide xylobiose (Tables I and II). In addition, the reducing-residue anomeric protons of both TFA-sugars show n.m.r. peaks similar to those generated previously by both free and derivatized forms (Tables I and II), indicating that the peaks are produced by their α- and β-pyranose tautomers. The only variation is that the reducing-residue anomeric proton of the β-pyranose form of TFA-xylobiose yields a doublet having $J_{1,2} = 5.5$ Hz, significantly lower than expected, suggesting that in this derivative the geometric relationship of the C-1–H-1 and C-2–H-2 bonds differs from that of the other β-pyranoses because of subtle conformational differences. Greater variations occur with the three TFA-trisaccharides studied (Table II). With TFA-isomaltotriose, two α-pyranose and one β-pyranose forms appear, while with TFA-maltotriose there are two of each, suggesting that incompletely derivatized forms have survived, and that longer derivatization times, perhaps at higher temperatures, should be used with trisaccharides and compounds of longer chain-lengths. As with TFA-xylobiose, some of the β-pyranose forms yield doublets having $J_{1,2}$ values lower than usual. No sure identification of the incompletely derivatized forms can be made with the data available. However, it may be surmised that that the α-pyranose forms with the lower δ values, which are found in significantly lower concentrations, have an underivatized hydroxyl group, whereas the ones present in higher concentrations are fully derivatized. If forms missing a trifluoroacetyl group were found in highest concentrations, others missing two groups should be present also.

Significantly more difficulty in assigning configuration occurs when more than two tautomeric forms occur, as with L-arabinose, L-fucose, D-galactose, D-lyxose, and

L-rhamnose, and when H-2 of the pyranose form is oriented equatorially, as with L-arabinose, D-lyxose, D-mannose, and L-rhamnose, rather than axially, as it is with L-fucose, D-galactose, D-glucose, and D-xylose. D-Galactose and L-fucose are considered first, by comparing the ^{1}H-n.m.r. spectra gathered from the TFA-derivatives with those of the same materials in free form and when peracetylated (Table I).

N.m.r. spectra of various tautomers of D-galactose have established that the anomeric-proton peak furthest upfield and having the highest $J_{1,2}$ value is that of the β-pyranose, as with D-glucose and D-xylose. With the free sugar, the second-largest $J_{1,2}$ is that of the α-furanose, the third largest the α-pyranose, and the smallest the β-furanose. This leads to assignment of the peaks at 6.80, 6.76, 6.64, and 6.31 p.p.m. to the α-pyranose, α-furanose, β-furanose, and β-pyranose tautomers of TFA-D-galactose, respectively.

The same reasoning applies with L-fucose, which except for its nonhydroxylated C-6 is the mirror image of D-galactose. Although less prior evidence is available, it seems likely that the anomeric-proton peaks at 6.65, 6.52, 6.45, and 5.98 p.p.m. are generated by the α-pyranose, β-furanose, α-furanose, and β-pyranose forms, respectively. These assignments are supported by the fairly close agreement of the four n.m.r. fractional peak-areas for TFA-L-fucose with those of the corresponding peaks of TFA-D-galactose (Table III).

The anomeric-proton n.m.r. peaks of the four monosaccharides that have H-2 oriented equatorially in the pyranose form may be identified as follows. With D-mannose, prior work (Table I) indicates that two peaks, each a doublet having a very small $J_{1,2}$ value, appear, and the one identified with the α-pyranose is located downfield from that of the β-pyranose. Given these findings, we may assign the peak at 6.51 p.p.m. to the α-pyranose and the one at 6.37 p.p.m. to the β-pyranose.

D-Lyxose has the same structure as D-mannose except that it lacks C-6. A large anomeric-proton peak furthest upfield, at 6.43 p.p.m. and having a small $J_{1,2}$ value, corresponds to the β-pyranose, following previous work (Table I). A second large peak at 6.57 p.p.m. with a $J_{1,2}$ value of 3.9 Hz is assigned to the α-pyranose. Peaks corresponding to furanose forms are located downfield from the pyranose peaks, with the peak corresponding to the β-furanose having a larger $J_{1,2}$ value in both the free sugar and the acetylated derivative than that of the α-furanose. While it therefore is likely that the anomeric-proton peak at 6.70 p.p.m. is associated with the former, the absence of a neighboring downfield peak leaves this assignment in doubt, and in fact it is possible that the α- and β-furanose peaks overlap. The very small, unsplit peak at 6.48 p.p.m. remains unidentified.

N.m.r. spectra of TFA-L-rhamnose yielded three closely spaced, unsplit peaks. They differ in shape and location from those obtained previously (Table I), and cannot be identified with the data available.

Finally, for TFA-L-arabinose, we can assign, by analogy with earlier work (Table III), the anomeric-proton peak at 6.17 p.p.m. to the α-pyranose; it is located well upfield of the four other peaks and has the largest $J_{1,2}$. Two peaks, a very small one at 6.52 p.p.m. and a very large one at 6.73 p.p.m., have $J_{1,2}$ values of 3.4 Hz (Table I), suggesting

TABLE III

Comparison of fractional n.m.r. peak areas with tautomeric equilibria of monosaccharides measured by various techniques

Carbohydrate	*Configuration*[a]	*This work*	*Published equilibria*	
		Ave. n.m.r. peak area	*Free sugar in water*	*Free sugar in pyridine*
L-Arabinose	α-p	0.06[b]	0.60[c]	0.33[d]
	β-p	0.64	0.355	0.33
	α-f	0.24	0.025	0.21
	β-f	0.04	0.02	0.13
		0.02[e]		
L-Fucose	α-p	0.86	0.28[f]	
	β-p	0.03	0.67	
	α-f	0.03	0.05	
	β-f	0.08		
D-Galactose	α-p	0.66[g]	0.325[h]	0.33[d]
	β-p	0.07	0.638	0.48
	α-f	0.13	0.012	0.07
	β-f	0.14	0.025	0.12
D-Glucose	α-p	0.90[b]	0.388[i]	0.47[j]
	β-p	0.10	0.609	0.50
	α-f	0.00	0.001	0.03
	β-f	0.00	0.002	
D-Lyxose	α-p	0.40	0.70[c]	0.825[d]
	β-p	0.52	0.28	0.15
	α-f	0.05	0.015	0.02
	β-f		0.005	0.004
		0.03[e]		
D-Mannose	α-p	0.92	0.63[k]	0.78[j]
	β-p	0.08	0.36	0.22
	α-f	0.00	0.006	0.00
	β-f	0.00	0.003	0.00
L-Rhamnose	α-p	0.61[e]	0.60[f]	
	β-p	0.19[e]	0.40	
		0.20[e]		
D-Xylose	α-p	0.76	0.365[c]	0.45[d]
	β-p	0.24	0.64	0.53
	α-f	0.00	<0.01	0.01
	β-f	0.00		0.01

[a] p = pyranose, f = furanose. [b] Average of two determinations. [c] Ref. 14. D-Arabinose, D-lyxose, and D-xylose measured by ^{1}H-n.m.r. in D_2O at 31°. [d] Ref. 30. Measured by ^{1}H-n.m.r. in C_5D_5N at 80° (L-arabinose) and 25° (D-galactose, D-lyxose, and D-xylose). [e] Unidentified peak. [f] Ref. 18. Measured by ^{1}H-n.m.r. in D_2O at 31°. [g] Average of three determinations. [h] Ref. 31. Measured by gas chromatography of trimethylsilyl derivatives of samples equilibrated in aqueous buffer at 25°. [i] Ref. 32. Measured by ^{13}C-n.m.r. in pH 4.8 aqueous buffer at 27°. [j] Ref. 33. Measured by gas chromatography of trimethylsilyl derivatives of samples equilibrated in pyridine at room temperature. [k] Ref. 34. Measured by ^{13}C-n.m.r. in H_2O at 36°.

TABLE IV

Comparison of fractional n.m.r. peak areas with tautomeric equilibria of oligosaccharides measured by various techniques

Carbohydrate	*Configuration*[a]	*This work*	*Published equilibria*	
		Ave. n.m.r. peak area	*Free sugar in water*	*Free sugar in pyridine*[b]
Cellobiose	α-p	0.40[c]	0.41[d]	0.49
	β-p	0.60	0.59	0.51
Gentiobiose	α-p	0.39	0.29[e]	0.32
	β-p	0.61	0.71	0.68
Isomaltose	α-p	1.00	0.23[e]	0.44
	β-p	0.00	0.77	0.56
Lactose	α-p	0.89[c]	0.41[d]	0.48
	β-p	0.11	0.59	0.52
Maltose	α-p	0.92[f]	0.39[d]	0.45
	β-p	0.08	0.61	0.55
Nigerose	α-p	0.61	0.56[g]	0.60
	β-p	0.39	0.44	0.40
Sophorose	α-p	0.73	0.74[e]	0.67
	β-p	0.27	0.26	0.33
Xylobiose	α-p	0.54		
	β-p	0.46		
Isomaltotriose	α-p	0.22		
	α-p	0.13		
	β-p	0.65		
Maltotriose	α-p	0.62		
	α-p	0.32		
	β-p	0.05		
	β-p	0.02		
Xylotriose	α-p	0.92		
	β-p	0.08		

[a] p = pyranose. [b] Ref. 29. Measured by gas chromatography of trimethylsilyl derivatives of samples equilibrated in pyridine at 40°. [c] Average of two determinations. [d] Average of refs. 35 and 36. Measured by gas chromatography of trimethylsilyl derivatives of samples equilibrated in water at room temperature. [e] Ref. 35. [f] Average of three determinations. [g] Ref. 36.

the β configuration. While there is little to differentiate between the β-pyranose and β-furanose forms from previous values of δ or $J_{1,2}$, prior studies of acetylations under various conditions[17] suggest that the β-pyranose should have the higher concentration. Therefore the peak at 6.73 p.p.m. is assigned to the β-pyranose and the other peak to the β-furanose. Of the two unsplit peaks, the large one at 6.58 p.p.m. has a shift appropriate for the α-furanose, leaving the small peak at 6.40 p.p.m. unidentified.

Relative amounts of anomers formed during TFA derivatization. — Inspection of Tables III and IV indicates that in all but two cases where sufficient evidence is available, the α-pyranose form of the carbohydrate is either increased or remains unchanged in concentration during TFA derivatization of carbohydrates initially at mutarotation equilibrium in pyridine (Table IV). The only exceptions appear to be L-arabinose and D-lyxose, whose 2-hydroxyl groups are oriented axially. This phenomenon of increase in concentration of the α-pyranose form when hydroxyl groups are being derivatized with strongly electron-withdrawing substituents may be explained by the anomeric effect[37], where the axial orientation is favored at the anomeric center of an aldopyranose.

These results demonstrate that the pertrifluoroacetylation reaction with MBTFA is relatively slow, especially with carbohydrates of longer chain-length, and that preservation of mutarotation equilibria upon derivatization cannot be expected. Nevertheless, as demonstrated earlier[5], with appropriate precautions the method can be satisfactorily used in the gas-chromatographic analysis of carbohydrates.

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