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Note

Studies of the stability of reducing sugars towards trifluoroacetolysis: a method for specific elimination of 2-acetamido-2-deoxyhexose residues at reducing ends of oligosaccharides

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In studies of the scope of trifluoroacetolysis in structural carbohydrate chemistry, we have developed a new method for *N*-deacetylation of 2-acetamido-2-deoxy sugar derivatives[1], and also a new technique for the isolation of *N*- and *O*-glycosidically linked carbohydrate chains in glycoproteins[2], based upon the fact that most glycosidic bonds are stable[2] during trifluoroacetolysis and that proteins are degraded by transamidation. We now report on the stability of reducing sugars towards trifluoroacetolysis.

Treatment of pentoses, hexoses, or 6-deoxyhexoses with trifluoroacetic anhydride (TFAA)/trifluoroacetic acid (TFA) in the proportions 1:1 and 50:1, at 100° for 48 h, gave the corresponding pertrifluoroacetates in quantitative yields (Table I). When the proportions were 1:2, degradation occurred; this was more pronounced for the pentoses and the 6-deoxyhexoses, and was probably induced by acid-catalysed elimination of trifluoroacetic acid from the initially formed pertrifluoroacetates. 2-Deoxy-D-*erythro*-pentose and 2-deoxy-D-*lyxo*-hexose were rapidly destroyed under all of the above conditions. 2-Amino-2-deoxy-D-galactose was presumably first converted into its per-*O*- and -*N*-trifluoroacetylated derivative, which was largely stable in 50:1 TFAA/TFA but was degraded when the proportions were 1:1 and 1:2. 2-Acetamido-2-deoxy sugars were converted into their pertrifluoroacetates and then gradually transamidated to give the *N*-trifluoroacetates, which were stable in 50:1 TFAA/TFA but severely degraded by the 1:1 and 1:2 reagents. Thus, the presence of a 2-*O*-trifluoroacetyl group in the pertrifluoroacetates of reducing sugars is essential for stabilisation towards acid-catalysed degradation.

The finding that 2-acetamido-2-deoxy sugars were degraded by TFAA/TFA, under conditions where hexoses, pentoses, and most glycosides were stable, prompted an investigation of the behaviour of oligosaccharides having a 2-acetamido-2-deoxy sugar at the reducing end. Thus, α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-GlcNAc[3] gave pertrifluoroacetylated α-D-Man*p*-(1→3)-D-Man in nearly quantitative yields

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TABLE I

RECOVERY OF SUGARS AFTER TRIFLUOROACETOLYSIS UNDER DIFFERENT CONDITIONS AT 100° FOR 48 h

Sugar	*Recovery (%)*[a]		
	1:2[b]	*1:1*[b]	*50:1*[b]
D-Glucose	85	100	100
D-Mannose	96	100	100
D-Galactose	82	100	99
D-Xylose	82	99	100
D-Ribose	64	100	99
D-Arabinose	65	100	100
L-Fucose	45	100	100
L-Rhamnose	40	100	100
2-Deoxy-D-*lyxo*-hexose	0	0	0
2-Deoxy-D-*erythro*-pentose	0	0	0
2-Acetamido-2-deoxy-D-glucose[d]	8	17	85
2-Acetamido-2-deoxy-D-mannose[d]	9	n.d.[c]	90
2-Amino-2-deoxy-D-galactose[d]	12	n.d.	80

[a]Determined by g.l.c.–m.s. after *O*- and *N*-detrifluoroacetylation by reduction with sodium borodeuteride and reacetylation. [b]Proportions of trifluoroacetic anhydride and trifluoroacetic acid (v/v). [c]Not determined. [d]When analyzed after *O*-detrifluoroacetylation with 50% aqueous acetic acid at room temperature for 1 h, followed by acetylation, only peracetylated 2-deoxy-2-trifluoroacetamido-hexoses were found in g.l.c.–m.s.

after trifluoroacetolysis with the 1:1 and 1:2 reagents, but in only 27% yield with the 50:1 reagent, which gave mainly the per-*O*- and -*N*-trifluoroacetylated trisaccharide derivative. Thus, the 4-*O*-substituted 2-acetamido-2-deoxy-D-glucose residue in the triasaccharide is more labile towards trifluoroacetolysis than is 2-acetamido-2-deoxy-D-glucose. This finding is expected, because the stabilizing effect of the 4-*O*-trifluoroacetyl group is lost. The specific elimination of reducing, 4-linked 2-acetamido-2-deoxy-D-glucose residues from oligosaccharides should be of value in structural carbohydrate chemistry. Preliminary results also suggest that reducing, 3-linked 2-acetamido-2-deoxy sugars are similarly eliminated, whereas the behaviour of 6-linked, reducing 2-acetamido-2-deoxy sugars is more complex.

EXPERIMENTAL

Concentrations were performed under reduced pressure at $\leqslant 40°$ (bath). G.l.c. was performed on a Perkin–Elmer 3920 instrument equipped with a flame-ionization detector and (*a*) glass columns (2 m × 0.25 cm) packed with 3% of ECNSS-M on Chromosorb Q (for alditol acetates at 200°) and (*b*) SE-30 W.C.O.T. glass-capillary columns (25 m × 0.25 mm) (for 2-acetamido-2-deoxy- and 2-deoxy-2-trifluoroacetamido-hexitol derivatives at 220°, and for permethylated oligosaccharide alditols at 250°). For g.l.c.–m.s., a Varian MAT 311A instrument was used fitted with the appropriate column. Mass spectra were recorded at 70 eV with an ionization current of 3 mA, and an ion-source temperature of 150°. The mass spectra were processed on an on-line computer system (Spectrosystem 100, Varian MAT).

Trifluoroacetolysis of sugars. — The sugar (4 mg, accurately weighed) and *myo*-inositol (4 mg, as inert internal standard) were treated with TFAA/TFA (5 ml; 1:2, 1:1, or 50:1) at 100° for 48 h in a sealed glass tube. Each cooled mixture was concentrated to dryness, and a solution of the residue in methanol (5 ml) was concentrated to dryness. A solution of the product in 50% aqueous acetic acid was concentrated to dryness. The residue was then dissolved in water (5 ml) and treated with sodium borohydride (25 mg) to effect simultaneous *N*-detrifluoroacetylation and reduction. The resulting alditols were treated with acetic anhydride and pyridine, to give alditol acetates that were analysed by g.l.c.–m.s. (Table I).

Degradation of α-D-*Man*p-(*1*→*3*)-β-D-*Man*p-(*1*→*4*)-D-*GlcNAc* (**1**). — Mixtures of **1** (2.5 mg) and panose (2.5 mg, as stable internal standard) were treated with TFAA/TFA (5 ml; 1:2, 1:1, 7:1, 20:1, 40:1, and 50:1) at 100° for 48 h in sealed glass tubes. Each reaction mixture was cooled and concentrated. The resulting mixture of trifluoroacetates was treated with methanol and 50% aqueous acetic acid and concentrated to dryness as described above. After reduction with sodium borodeuteride and acetylation, the product was permethylated and analysed by g.l.c.–m.s. (Table II). The identity of permethylated α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-GlcNAc-itol-*1-d* was demonstrated by comparison of retention time and mass spectrum with those of an authentic sample[4]. The identity of permethylated α-D-Man*p*-(1→3)-D-Man-itol-*1-d* was indicated by its mass spectrum, which was typical[5] for a hex*p*-(1→3)-hex-itol-*1-d*.

TABLE II

TRIFLUOROACETOLYSIS[a] OF α-D-MAN*p*-(1→3)-β-D-MAN*p*-(1→4)-GLCNAC (**1**)

TFAA/TFA[b]	**1**[c]	α-D-*Man*p-(*1*→*3*)-D-*Man*[c]
1:2	0	92
1:1	0	96
7:1	17	77
20:1	23	67
40:1	59	37
50:1	67	27

[a]At 100° for 48 h. [b]Relative proportions. [c]Mol % obtained after trifluoroacetolysis, *O*- and *N*-detrifluoroacetylation, and re-*N*-acetylation; analysed as their permethylated alditol derivatives.

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REFERENCES

1 B. NILSSON AND S. SVENSSON, *Carbohydr. Res.*, 62 (1978) 377–380.
2 B. NILSSON AND S. SVENSSON, unpublished data.
3 N. E. NORDÉN, A. LUNDBLAD, S. SVENSSON, P. A. ÖCKERMAN, AND S. AUTIO, *J. Biol. Chem.*, 248 (1973) 6210–6215.
4 A. LUNDBLAD, P. K. MASSON, N. E. NORDÉN, S. SVENSSON, AND P. A. ÖCKERMAN, *Biomed. Mass Spectrom.*, 2 (1975) 285–287.
5 J. LÖNNGREN AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.