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Note

The stability of partially methylated methyl α-D-glucopyranosides towards trifluoroacetolysis

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Under trifluoroacetolysis conditions that will effect transamidation[1], most glycosides[2] and reducing sugars[3] are stable, being converted into their pertrifluoroacetylated derivatives. These findings have enabled us to develop procedures for the isolation of *O*- and *N*-glycosylically-linked carbohydrate chains in glycoproteins[4] and glycopeptides[5]. It has also been demonstrated that the glycosidic bond to the sphingosine moiety in glycolipids can be cleaved by trifluoroacetolysis[6]. A prerequisite for the stability of glycosidic bonds in oligo- and poly-saccharides is that enough *O*-trifluoroacetyl groups are introduced close to the glycosidic oxygens, in order to withdraw electrons and thereby prevent solvolysis.

We now report studies of the stability towards trifluoroacetolysis of glucosides having a limited number of free hydroxyl groups.

Partially methylated methyl α-D-glucopyranosides having hydroxyl groups in all possible combinations were treated with trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA) in proportions varying from 1:1 to 1:50 at 100°C for 48 h. The reaction mixtures were analysed after *O*-detrifluoroacetylation, reduction ($NaBD_4$), and acetylation. The results are summarized in Table I.

The reagent TFA/TFAA contains, in addition to TFA and TFAA, the ionic species CF_3CO^+ and CF_3COO^- formed by disproportionation of TFAA. When treated with the reagent, free hydroxyl groups are rapidly trifluoroacetylated. *O*-Trifluoroacetyl groups are strongly electron-attracting and, when positioned close to the acetal oxygen atoms of a glycoside, render the glycosidic bond stable towards solvolysis by TFA/TFAA. However, if the hydroxyl groups close to the glycosidic bond are blocked, the glycoside may be solvolysed by TFA/TFAA, and the pertrifluoroacetylated sugar formed may be further degraded by acid-catalysed elimination reactions. Anomerisation may also occur.

As can be seen from Table I, all of the partially methylated methyl α-D-glucopyranosides having HO-2 unsubstituted are stable in 1:50 TFA/TFAA. When HO-2 is blocked by a methyl group, minor degradation takes place with the 2,3-di-*O*-methylglucoside in 1:50 TFA/TFAA. Under the same conditions, the trimethylated

TABLE I

TRIFLUOROACETOLYSIS OF PARTIALLY METHYLATED METHYL α-D-GLUCOPYRANOSIDES USING TFA/TFAA (1:1, 1:4, 1:15, AND 1:50) AT 100°C FOR 48 h

Location of methyl group(s)	*Recovery (mol%)*[a]															
	Starting material				*Anomerised product*				*Free sugar*				*Total recovery*			
	1:1	*1:4*	*1:15*	*1:50*	*1:1*	*1:4*	*1:15*	*1:50*	*1:1*	*1:4*	*1:15*	*1:50*	*1:1*	*1:4*	*1:15*	*1:50*
2	89	n.d.[b]	n.d.	103	12	n.d.	n.d.	—[c]	2	n.d.	n.d.	—	103	n.d.	n.d.	103
3	97	n.d.	n.d.	99	< 1	n.d.	n.d.	—	< 1	n.d.	n.d.	—	97	n.d.	n.d.	99
4	100	n.d.	n.d.	102	< 1	n.d.	n.d.	—	< 1	n.d.	n.d.	—	100	n.d.	n.d.	102
6	102	n.d.	n.d.	103	< 1	n.d.	n.d.	—	< 1	n.d.	n.d.	—	102	n.d.	n.d.	103
2,3	52	71	88	92	7	9	5	4	31	14	5	3	90	94	98	99
2,4	49	75	94	102	13	12	3	< 1	34	13	5	< 1	96	100	102	102
2,6	61	84	99	97	11	7	2	< 1	28	8	3	< 1	100	99	104	97
3,4	87	99	103	101	5	1	—	—	6	1	—	—	98	101	103	101
3,6	91	97	98	98	3	< 1	—	—	5	< 1	—	—	99	97	98	98
4,6	92	100	103	97	2	< 1	—	—	5	< 1	—	—	99	100	103	97
2,3,4	—	6	51	66	—	< 1	7	9	—	13	28	23	—	19	86	98
2,3,6	1	17	64	79	< 1	2	4	3	21	41	29	16	22	60	97	98
2,4,6	—	1	26	59	—	< 1	3	2	—	15	31	26	—	16	60	87
3,4,6	45	81	94	97	4	4	1	< 1	33	10	3	< 1	82	95	98	97
2,3,4,6	—	n.d.	n.d.	—	—	n.d.	n.d.	—	—	n.d.	n.d.	—	—	n.d.	n.d.	—

[a]Determined after *O*-detrifluoroacetylation, reduction ($NaBD_4$), and acetylation; [b]n.d., not determined; [c]—, not detectable.

glucosides having a hydroxyl group at C-3, C-4, or C-6 could only be recovered in 60–80% yield, the remainder being anomerised or solvolysed. The total recovery of the 2,4,6-tri-*O*-methylglucoside was not quantitative even in 1:50 TFA/TFAA, demonstrating that the solvolysed products were further degraded, probably *via* acid-catalysed eliminations. When methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside was treated with 1:50 TFA/TFAA, no glucoside or free sugar could be recovered. This finding is expected as the fully methylated glucoside should be rapidly solvolysed and the resulting trifluoroacetate further degraded *via* acid-catalysed elimination reactions.

When the partially methylated glucosides were treated with 1:1 TFA/TFAA, all those containing three hydroxyl groups were stable, except for the one having the 2-position blocked, which underwent ~10% anomerisation. For glucosides containing two hydroxyl groups, there was also a marked difference in stability in 1·1 TFA/TFAA between those having the 2-position blocked and those having a free hydroxyl group at C-2. When only one hydroxyl group was unsubstituted, all glucosides were completely solvolysed, except for a trace of that having HO-4 unsubstituted and ~50% of that having HO-2 unsubstituted. The solvolysed products were also degraded further and, after *O*-detrifluoroacetylation, the free sugar could only be obtained from the trimethylated glucosides having HO-2 or HO-4 unsubstituted.

The foregoing results suggest that glycosidic bonds of oligosaccharides and polysaccharides containing hexopyranose residues should be stable in 1:50 TFA/TFAA, provided that no hexose residue is more than disubstituted. 3,4,6-Trisubstituted hexopyranose residues, with HO-2 unsubstituted, should also be stable. Since the glycosidic bonds in oligo- and poly-saccharides may be further stabilised by the hydroxyl groups of the aglycon residue, many of them should be stable even in 1.1 TFA/TFAA.

EXPERIMENTAL

Concentrations were performed at reduced pressure with bath temperatures not exceeding 40°C. G.l.c.–m.s. was performed with a combined gas chromatograph–mass spectrometer (Varian MAT 311A). Separations were performed at 180°C on glass-capillary columns (25 m × 0.25 mm) wall-coated with SE-30 or OV101 (LKB-Products, Sweden). Quantitative analyses with these columns were performed with a Perkin–Elmer 3920 gas chromatograph equipped with a flame-ionisation detector.

Partially methylated methyl α-D-glucopyranosides[7] were obtained by standard synthetic procedures. The identity and purity of the compounds were established by g.l.c.–m.s.[8] of the corresponding acetates.

Trifluoroacetolysis experiments. — The partially methylated methyl α-D-glucopyranoside (15 mg) and 10 mg of methyl α-D-glucopyranoside or methyl α-D-mannopyranoside (as stable internal standard[2]) were dissolved in methanol (5 ml), and 1 ml of the mixture was analysed by g.l.c.–m.s. after acetylation, to obtain response factors. Other portions (1 ml) were dried, and solutions of the residues in TFA/TFAA (1:1, 1:4, 1:15, and 1:50; 4 ml) were then heated in sealed glass tubes at 100°C for 48 h

(*caution:* corrosive mixture under pressure). The mixtures were then cooled to room temperature and evaporated to dryness. The residues were dissolved in methanol (2 ml), and the solutions were evaporated to dryness. The residues were then dissolved in ethanol–water (2:1; 2 ml) and reduced with sodium borodeuteride (10 mg). The reduced products were acetylated, and analysed by g.l.c.–m.s. The identity of each component was established by its mass spectrum[8] and retention time in g.l.c., in comparison with authentic samples.

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