Carbohydrate Research, 132 (1984) 45–50
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE REACTIVITY OF URONIC AND ALDONIC ACID DERIVATIVES TOWARDS TRIFLUOROACETOLYSIS. A NEW METHOD FOR SPECIFIC ELIMINATION OF REDUCING 4-*O*-SUBSTITUTED HEXOSE RESIDUES

ALF GUNNARSSON AND SIGFRID SVENSSON*
Department of Carbohydrate Chemistry, Chemical Center, University of Lund, Lund (Sweden)
(Received January 10th, 1984; accepted for publication, March 7th, 1984)

ABSTRACT

Trifluoroacetolysis of D-glucuronic acid and methyl α-D-glucopyranosiduronic acid resulted in an initial phase of degradation followed by stabilisation of the compounds as their 6,3-lactones. The methyl ester of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid was largely stable towards trifluoroacetolysis. Aldonic acids substituted at O-3 or O-6 were stable towards trifluoroacetolysis because of the formation of γ-lactones. Aldonic acids substituted at O-4, and incapable of forming γ-lactones, were converted into the trifluoroacetylated enol of 3-deoxy-2-hexulosonic acid. Treatment of the 3-deoxy-2-hexulosonic acid with mild base eliminated the substituent at O-4.

INTRODUCTION

Trifluoroacetolysis is a versatile reaction in studies of glycoconjugates and has been used for the *N*-deacetylation of 2-acetamido-2-deoxy sugars[1], for the isolation of *N*- or *O*-glycosylically linked carbohydrates from glycoproteins[2–4], and to release carbohydrates from glycosphingolipids[5,6]. The usefulness of the trifluoroacetolysis reaction depends on the electron-attracting character of the *O*-trifluoroacetyl groups introduced into the sugar moieties, which dramatically retards the rates of solvolysis of glycosidic bonds[7–11]. We now report on the reactivity of uronic and aldonic acid derivatives under trifluoroacetolysis conditions.

MATERIALS AND METHODS

Methyl α-D-glucopyranosiduronic acid was prepared by catalytic oxidation (Pt/O_2) of methyl α-D-glucopyranoside[12]. Methyl 3-*O*-methyl-α-D-glucopyranoside was prepared by Purdie methylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[13] and debenzylidenation with trifluoroacetic acid–trifluoroacetic anhydride (1:50, 50°, 1 h). Catalytic oxidation of the primary hydroxyl group with freshly

*To whom correspondence should be addressed.

0008-6215/84/$ 03.00

reduced Adams' catalyst[14] and oxygen then gave methyl 3-*O*-methyl-α-D-glucopyranosiduronic acid[15]. Methyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate and maltotriose were commercial products, isomaltotriose was obtained by partial acid hydrolysis of dextran[16], and laminaritriose by partial acid hydrolysis of laminarin[17]. The aldonic acids of the trisaccharides were obtained by oxidation with hypoiodite[18,19].

Analytical methods. — G.l.c. was performed with a Perkin–Elmer 3920 gas chromatograph equipped with a flame-ionisation detector and an SE-30 W.C.O.T. glass-capillary column (25 m × 0.25 mm) at 140–180° (for perethylated uronic acid derivatives) and at 180–330° (for permethylated aldonic acid derivatives). G.l.c.–m.s. was performed on a Varian MAT 311A instrument fitted with the appropriate column. The spectra were recorded at 70 eV, with an ionisation current of 3 mA and an ion-source temperature of 120°. The spectra were processed by an on-line computer system (Spectrosystem 100, Varian MAT).

Trifluoroacetolysis. — Compounds were treated with mixtures of trifluoroacetic acid and trifluoroacetic anhydride in proportions varying from 1:1 to 1:50 (2 mL/mg of oligosaccharide) at 100° for 48 h (*caution*, corrosive mixture under pressure). Each cooled mixture was concentrated to dryness and a solution of the residue in methanol (5 mL) was again concentrated to dryness. The residue was dissolved in glacial acetic acid (5 mL), water (5 mL) was added, and, after storage for 30 min at 100°, the solution was concentrated to dryness.

Treatment with base. — The products of trifluoroacetolysis were stirred with 0.05M NaOH (5 mL) overnight at room temperature. Each mixture was neutralised with Dowex 50 (H^+) resin and concentrated to dryness, and the residue was reduced with $NaBD_4$. The reduction was stopped with Dowex 50 (H^+) resin, and the mixture was concentrated to dryness after adding methanol. The product was permethylated[20], and analysed by g.l.c.[21] and m.s.[22].

RESULTS AND DISCUSSION

Treatment of D-glucuronic acid (D-Glc*p*A) and methyl α-D-glucopyranosiduronic acid (Me α-D-Glc*p*A) with a mixture of trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA) in various proportions at 100° gave, after perethylation of the product and analysis by g.l.c., the time curves shown in Fig. 1. Initial, fast reaction was followed by slower degradation of D-Glc*p*A and Me α-D-Glc*p*A. The first phase reflects the degradation of the trifluoroacetylated derivatives *via* eliminations from an acylium ion, and the second phase reflects the formation of 6,3-lactones which are degraded more slowly (Scheme 1). The 6,3-lactones are in equilibrium with an acylium ion from which elimination can take place, but the formation of lactones is favoured and they are stable towards trifluoroacetolysis.

In order to verify this interpretation, methyl 3-*O*-methyl-α-D-glucopyranosiduronic acid (Me 3-α-D-Glc*p*A) and the methyl ester of methyl 4-*O*-

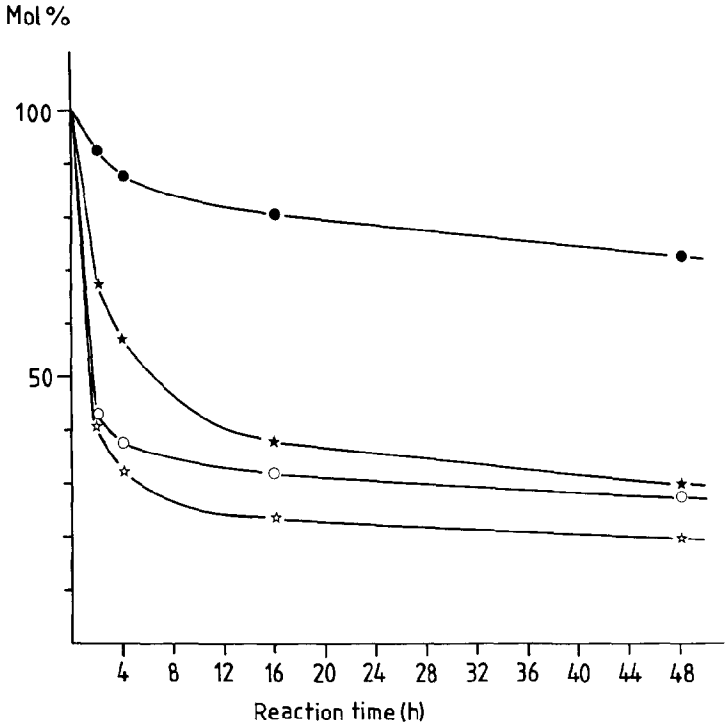
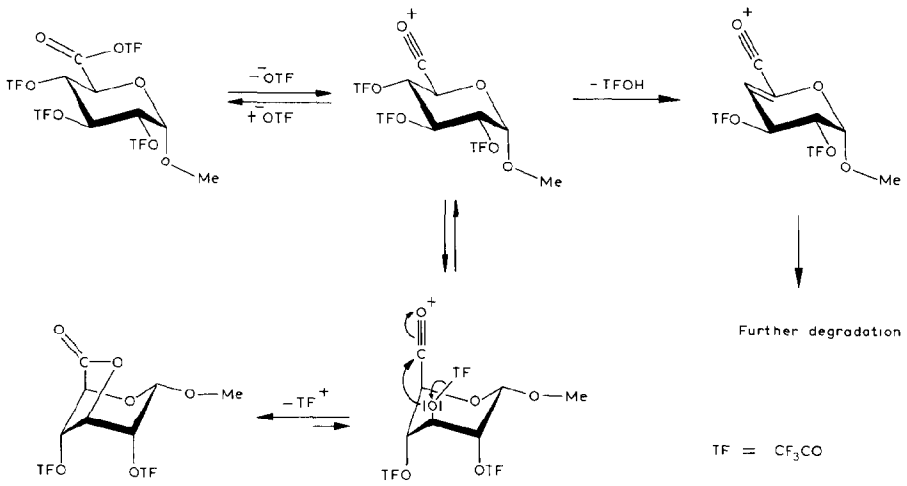


Fig. 1. Time curve of the degradation of D-glucuronic acid with 1:50 TFA/TFAA (—○—) and 1:1 TFA/TFAA (—☆—), and methyl α-D-glucopyranosiduronic acid with 1:50 TFA/TFAA (—●—) and 1:1 TFA/TFAA (—★—).



Scheme 1

TABLE I

TREATMENT OF URONIC ACIDS WITH TFA/TFAA AT 100° FOR 48 h

Compound	*Recovery (%)*[a]			
	TFA/TFAA			
	1:1	*1:4*	*1:15*	*1:50*
Me α-D-Glc*p*A	35	58	65	77
Me 3-α-D-Glc*p*A	1	7	25	56
Me (Me 4-α-D-Glc*p*)uronate	85	85	90	93

[a]Determined by g.l.c –m.s. after *O*-detrifluoroacetylation and perethylation.

methyl-α-D-glucopyranosiduronic acid [Me (Me 4-α-D-Glc*p*)uronate] were subjected to trifluoroacetolysis. In 1:1 TFA/TFAA, 35% of Me α-D-Glc*p*A was recovered, whereas Me 3-α-D-Glc*p*A was completely degraded because it cannot form a 6,3-lactone (Table I).

When the carboxylic acid group is esterified, no acylium ion can be formed and no elimination can take place. The glycosidic bond is stabilised by the inductive effect of the *O*-trifluoroacetyl groups. The recovery of Me (Me 4-α-D-Glc*p*)uronate after treatment with 1:1 TFA/TFAA is slightly lower than that[8] of Me 4-α-D-Glc*p*, because of the absence of a 6-*O*-trifluoroacetyl group in the former compound, but is approximately the same as that[8] of Me 4,6-α-D-Glc*p*. When the carboxylic acid is not esterified, an acylium ion can be formed and elimination can occur. However, elimination will be prevented if a 6,3-lactone can be formed.

The role of a free carboxylic acid and the inability to form a 6,3-lactone is reflected by the recoveries of Me 3-α-D-Glc*p*A (1%, Table I) and Me 3-α-D-Glc*p* (97%)[8] after treatment with 1:1 TFA/TFAA. A decrease in the proportion of TFA in the trifluoroacetolysis mixture resulted in higher recoveries of the D-Glc*p*A derivatives (Table I) and was most marked for those compounds (Me α-D-Glc*p*A and Me 3-α-D-Glc*p*A) which contained a free carboxylic acid group, possibly because the formation of acylium ion was progressively disfavoured.

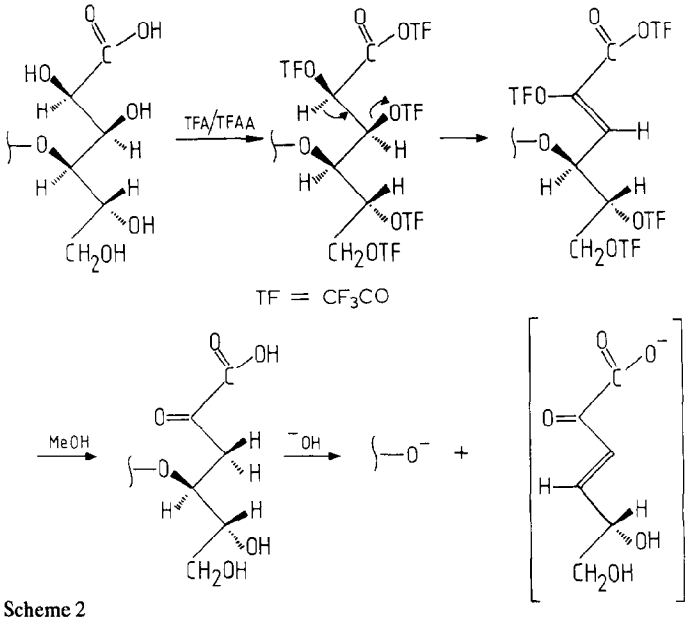
The above results prompted an investigation of the possibility of specific elimination of a 4-*O*-substituted hexose residue at the reducing end of appropriate oligosaccharides. The aldonic acids (**1–3**) of isomaltotriose, laminaritriose, and maltotriose were treated severally with 1:50 TFA/TFAA for 48 h at 100°, together with gentiobiose as an inert internal standard. The reaction products were *O*-detrifluoroacetylated, reduced with sodium borodeuteride, permethylated, and subjected to g.l.c.–m.s. As can be seen from the data in Table II, **1** and **2** were recovered almost completely (as mixtures of free aldonic acid or lactone), whereas **3** was converted into the trifluoroacetylated enol of 3-deoxy-2-hexulosonic acid (Scheme 2). Treatment of the 3-deoxy-2-hexulosonic acid, after *O*-detrifluoroacetylation, with mild base eliminated the substituent at O-4 (Scheme 2)

TABLE II

TREATMENT OF ALDONIC ACIDS **1–3**[a] WITH 1:50 TFA/TFAA, AND WITH 1:50 TFA/TFAA AND THEN BASE (0.05M NaOH)

Compound	*Recovery (%)*[b]					
	TFA/TFAA (1:50)			*TFA/TFAA (1:50) and then base*		
	A[c]	*B*	*C*	*A*	*B*	*C*
1	90	8	—	78	6	—
2	87	7	trace	57	10	7
3	—	70	28	—	—	101

[a]Derived from isomaltotriose, laminaritriose, and maltotriose, respectively. [b]Determined by g.l.c.–m.s., as permethylated oligosaccharide-alditols after *O*-detrifluoroacetylation. [c]Key: A, aldonic acid and corresponding lactone; B, hexulosonic acid and corresponding lactone; C, disaccharide eliminated from the reducing end.



Scheme 2

and gave a quantitative yield of the resulting disaccharide (Table II). Treatment of the aldonic acids **1** and **2** with mild base did not release the corresponding disaccharide, because of the formation of γ-lactones (Table II) that were stable towards trifluoroacetolysis. The lower recovery of **2** after treatment with base is due to a peeling reaction from the reducing end.

Thus, after oxidation of the reducing terminus of an oligosaccharide to an aldonic acid, 4-*O*-substituents can be eliminated specifically therefrom by trifluoroacetolysis followed by treatment with base. Oligosaccharides with base-labile

linkages released in this process can be protected from a peeling reaction by including sodium borohydride in the base[23].

ACKNOWLEDGMENTS

The authors thank Mr. S. Strömberg for skilful technical assistance, and the Swedish Medical Research Council (3X-4956) and the Medical Faculty, University of Lund, for financial support.

REFERENCES

1 B. NILSSON AND S. SVENSSON, *Carbohydr. Res.*, 62 (1978) 377–380.
2 B. NILSSON AND S. SVENSSON, *Carbohydr. Res.*, 72 (1979) 183–190.
3 B. LINDBERG, B. NILSSON, T. NORBERG, AND S. SVENSSON, *Acta Chem. Scand., Ser. B*, 33 (1979) 230–231.
4 L.-E. FRANZÉN, S SVENSSON, AND O. LARM, *J. Biol. Chem.*, 255 (1980) 5090–5093.
5 B. NILSSON AND D ZOPF, *Arch. Biochem. Biophys.*, 222 (1983) 628–648.
6 A. GUNNARSSON, J. LUNDSTEN, AND S. SVENSSON, *Acta. Chem. Scand., Ser. B*, in press.
7 B. NILSSON AND S. SVENSSON, *Carbohydr. Res.*, 69 (1979) 292–296.
8 L.-E. FRANZÉN AND S. SVENSSON, *Carbohydr. Res.*, 73 (1979) 309–312.
9 L.-E. FRANZÉN AND S. SVENSSON, *Carbohydr. Res.*, 79 (1980) 147–150.
10 L.-E. FRANZÉN AND S. SVENSSON, *Acta Chem. Scand., Ser. B*, 34 (1980) 133–135.
11 L.-E. FRANZÉN AND S. SVENSSON, *Acta Chem. Scand., Ser. B*, 34 (1980) 171–175.
12 S. A. BARKER, E. J. BOURNE, AND M. STACEY, *Chem. Ind. (London)*, (1951) 970.
13 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021–1037.
14 R. ADAMS AND R. L. SHRINER, *J. Am. Chem. Soc.*, 46 (1924) 1683–1693.
15 C. A. MARSH AND G. A. LEVVY, *Biochem. J.*, 68 (1958) 617–621.
16 L. W. GEORGES, I L. MILLER, AND M. L. WOLFROM, *J. Am. Chem. Soc.*, 69 (1947) 473.
17 W. A. P. BLACK, E. T. DEWAR, AND F. N. WOODWARD, *J. Sci. Food Agric.*, 12 (1955) 754–763.
18 P. A. LEVENE AND R. S. TIPSON, *J. Biol. Chem.*, 115 (1936) 731–747.
19 R. SCHAFFER, *J. Res. Natl. Bur. Stand., Sect. A*, 65 (1961) 507–512.
20 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
21 J. S. SAWARDEKER, J. H. SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
22 L. S. GOLOVKINA, O. S. CHIZHOV, AND N. S. WULFSON, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1966) 1915–1926.
23 A. GUNNARSSON, B. NILSSON, AND S. SVENSSON, unpublished data.