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Trifluoromethanesulfonic Acid: a Useful Reagent for the Solvolytic Cleavage of Glycosidic Linkages in Structural Analysis of Bacterial Polysaccharides

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Introduction

A common chemical approach in the structural analysis of polysaccharides is the selective cleavage of glycosidic linkages to obtain oligosaccharide fragments. These usually show better-resolved nuclear magnetic resonance (NMR) spectra than the parent polymer and are readily amenable to mass spectrometry (MS) investigations. They are also useful in immunochemical studies and the synthesis of glycoconjugates for elaboration of diagnostic agents and experimental vaccines. Many bacterial polysaccharides contain complex monosaccharides often featuring amide linkages (e.g. N-acyl derivatives of amino sugars and amides of uronic acids). Determination of the structure of these unusual monosaccharides demands their non-destructive isolation, so that they can be analysed directly by chromatography, NMR spectroscopy, MS, and specific optical rotation.

The most common non-specific cleavage method applicable to the production of both oligosaccharides and monosaccharides is acid hydrolysis.\cite{1} The basis of its application for the preparation of oligosaccharides is a difference in stabilities of glycosidic linkages of various monosaccharides, which enables the complete or predominant cleavage of one or more linkages in a polysaccharide with no cleavage of the others. A disadvantage of partial acid hydrolysis is its insufficient selectivity in many cases and, as a result, low yields of desired oligosaccharides. Application of this method to polysaccharides enriched in amino sugars and uronic acids is often complicated by the hydrolytic removal of amide-linked groups. Solvolysis with anhydrous reagents is more selective and generally does not affect amide linkages. Anhydrous hydrogen fluoride has been used in the structural analysis of carbohydrates since the 1980s and found to be a versatile reagent for the solvolytic cleavage of glycosidic linkages.\cite{2} However, the application of this method is limited by the unfavourable physical and chemical properties and high toxicity of the reagent.

In a search for a new solvolytic agent, we tested trifluoromethanesulfonic (triflic) acid, which is used for the deglycosylation of glycoproteins,\cite{3-5} and found it to be an efficient reagent, devoid of the disadvantages of hydrogen fluoride. This review summarizes the initial data on the application of triflic acid for the isolation of complex monosaccharide derivatives and oligosaccharides in the structural analysis of various bacterial polysaccharides.

Technique for Performing Solvolysis

Solvolysis of polysaccharides (20–30 mg) with triflic acid (Fluka, 98\%, 0.5 mL) was performed at –4 to +25°C in a closed glass container. When necessary, the polysaccharide sample and the triflic acid were cooled to the required temperature before mixing. Reaction times varied over a wide range (from 1 h to several days) depending on the stability of glycosidic linkages (i.e. on the type of the constituent monosaccharides) and the purpose of solvolysis (either for the preparation of monosaccharides or oligosaccharides, see below). After completion of the reaction, cold aqueous 25\% ammonia was added at 4°C to neutralize the triflic acid. Aqueous work up resulted in the hydrolysis of unstable glycosyl triflates, which were the primary solvolysis products, into the corresponding sugars with the free anomeric centre. The reaction products were fractionated by gel-permeation chromatography on TSK HW-40 (S), in water or 1\% aqueous acetic acid. In some cases, the solvolysis products were reduced by sodium borohydride prior to chromatography, to improve separation.

Preparation of Monosaccharides

Among bacterial polysaccharides, the O-specific polysaccharides of \textit{Proteus} lipopolysaccharides are particularly rich in complex monosaccharide derivatives, including those with amide linkages, some of which have never been found in other natural carbohydrates.\cite{6} Several monosaccharides of this sort were isolated from the O-polysaccharides using solvolysis with triflic acid. The
stabilities towards solvolysis of the glycosidic linkages of the major constituent monosaccharides (hexoses, N-acetylhexosamines, their 6-deoxy derivatives, and hexuronic acids) were low. As a result, the O-poly saccharides of the *Proteus* strains tested could be cleaved under mild conditions (−4°C, 1–2 h) and monosaccharides were isolated in satisfactory yields.

For instance, together with D-Gal, D-GlcNAc, and D-glucuronic acid (D-GlcA), the linear O-poly saccharide of *Proteus vulgaris* O4 contains 4-amino-4,6-dideoxy-D-glucose N-acetylated with N-[(R)-3-hydroxybutyryl]-L-alanine (1). Treatment of the poly saccharide with triflic acid at −4°C for 1 h followed by gel-permeation chromatography afforded the monosaccharide (1) (7% of the poly saccharide weight) slightly contaminated with GlcNAc.[7] The structure of (1) (Fig. 1) was established by electrospray ionization MS and 1H and 13C NMR spectroscopy.

A new regioisomer of N-acetylmuramic acid, 2-acetamido-4-O-[(R)-1-carboxyethyl]-2-deoxy-D-glucose (2) (Fig. 1), was isolated in a yield of 10% from the O-deacetylated O-poly saccharide of *Proteus vulgaris* O15, after solvolysis with triflic acid at −4°C for 2 h.[8] Similarly, using solvolysis with triflic acid (4°C, 16 h), N-acetylmuramic acid, a known common component of bacterial peptidoglycans,[9] was isolated from the O-poly saccharide of *Providencia alcalifaciens* O16, in a yield of 18% after purification by anion-exchange chromatography.[10]

Solvolysis with triflic acid (−4°C, 1.5 h) of the branched O-poly saccharide from *Proteus mirabilis* O13 was used for the isolation of an amide of D-galacturonic acid (3) (Fig. 1) with an unusual amino acid, Nβ-[(R)-1-ethoxy carbonyl]-L-lysine.[11] The monosaccharide (3) was obtained as the individual compound in a yield of 18% and used for the structural elucidation, and in immunochemical studies, of *Proteus* O-antigens.[12]

![Fig. 1. Monosaccharide derivatives isolated by solvolysis with triflic acid of the O-poly saccharides of *Proteus vulgaris* O4 (1),[13] *Proteus vulgaris* O15 (2),[13] and *Proteus mirabilis* O13 (3).][13]

Preparation of Oligosaccharides

In studies of the O-poly saccharides from *Pseudoalteromonas* sp. and *Vibrio cholerae* O8 enriched in diamino sugars and diamino uronic acids, we were faced with their high stabilities towards anhydrous HF, which could not cleave the poly saccharides even after prolonged treatment at ambient temperature. However, application of triflic acid enabled us to overcome this difficulty and to obtain oligosaccharide fragments in good yields.

One of the poly saccharides (4), isolated from a marine bacterium *Pseudoalteromonas* sp. KMM 634, contained D-GlcA and derivatives of 2,3-diamino-2,3-dideoxy-D-glucuronic acid (D-GlcN3NA), 2,3-diamino-2,3-dideoxy-D-mannuronic acid (D-ManN3NA), and 2,4-diamino-2,4,6-trideoxy-D-glucose (D-Quin4N, bacillosamine). Solvolysis of (4), with triflic acid at ambient temperature overnight, resulted in a disaccharide (5) and a trisaccharide (6) in close yields (20 and 25%, respectively) (Fig. 2).[13] Therefore, the glycosidic linkages of β-GlcA and β-GlcN3NA were cleaved completely, whereas the linkage of β-ManN3NA was stable, and that of α-Quin4N cleaved partially (by about 50%). Both oligosaccharides were used for NMR spectroscopic identification of the diaminouronic acids, which could not be

![Fig. 2. Selective cleavage of the O-poly saccharide of *Pseudoalteromonas* sp. KMM 634 by solvolysis with triflic acid.][13]
isolated as the free monosaccharides. The QuiN4N derivative could be released by mild acid hydrolysis of the trisaccharide (6), but not of the polysaccharide (4), which enabled determination of the positions of the N-acetyl and N-[(S)-3-hydroxybutyryl] groups by gas–liquid chromatography and MS of the corresponding fully methylated alditol.

The other polysaccharide (7a) isolated from *Vibrio cholerae* O8 was composed of derivatives of D-Quin4N, 2,3-diamino-2,3-dideoxy-D-guluronic acid (L-GulN3NA), D-ManN3NA, and D-GlcN3NA. Its peculiar features are the presence of N-formyl-L-alanine as one of the N-acyl substituents, and the amidation of two of three diamino uronic acid residues. Treatment of the polysaccharide with triflic acid at 10°C for 18 h resulted in a non-degraded but modified polysaccharide (7a,b) as the major product, and tetrasaccharides (8a,b) as minor products due to the cleavage of some QuiN4N linkages (ca. 10%) (Fig. 3).[14] In both products, the N-formyl group was partially (by ca. 50%) split off from the alanyl group, whereas the other N-acyl substituents of amino sugars and amide groups of the uronic acids were not affected. Similar partial cleavage of the N-formyl group was also observed when the polysaccharide was treated with anhydrous hydrogen fluoride.[14]

Then, solvolysis of the partially N-deformylated polysaccharide (7a,b) was performed at 22°C for four days to give trisaccharides (9a,b) in a yield of 25% as the only oligosaccharide product, due to the complete selective cleavage of the glycosidic linkages of β-Quin4N and α-GulN3NA (Fig. 3). Analysis of (9) using electrospray ionization MS and NMR spectroscopy showed that GlcN3NA and ManN3NA are amidated and the N-formyl-L-alanyl group (in 9a, the L-alanyl group in 9b) is located at position 3 of GlcN3NA.

Varying the conditions of solvolysis with triflic acid enabled isolation of two different oligosaccharides from the O-polysaccharide of *Proteus vulgaris* O39 (10). This has a trisaccharide repeating unit containing 2-acetamido-2,6-dideoxy-L-galactose (L-FucNAc), a higher sugar 5,7-diacetamido-3,5,7,9-tetradecoxy-L-glycero-1-manno-non-2-ulosonic acid (di-N-acetylpsuedaminic acid, Pse5Ac7Ac), and D-GlcNAc. As expected for a keto sugar,[2] the glycosidic linkage of Pse5Ac7Ac was completely stable towards solvolysis, whereas the glycosidic linkage of FucNAc was most labile and cleaved selectively under rather mild conditions (–4°C, 2 h) to give a trisaccharide (11) in a yield of about 20% (Fig. 4).[15] Under these conditions, the glycosidic linkage of GlcNAc was largely stable, but could be cleaved under more rigorous conditions (20°C, 16 h) to afford a disaccharide (12) in a similar yield. When methanesulfonic acid was used to cleave the polysaccharide at 20°C for 16 h, a 1:2 mixture of (11) and (12) was obtained[13] indicating that methanesulfonic acid is a milder solvolytic reagent than triflic acid. Comparison of the 13C

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**Fig. 3.** Selective cleavage of the O-polysaccharide of *Vibrio cholerae* O8 by solvolysis with triflic acid.[14] R = HCO (a) or H (b).

**Fig. 4.** Selective cleavage of the O-polysaccharide of *Proteus vulgaris* O39 by solvolysis with triflic acid.[15]
NMR chemical shifts of (11) and (12) enabled determination of the position of substitution and the absolute configuration of Pse5Ac7Ac by glycosylation effects.\(^ {[16]}\)

Two more \textit{Proteus vulgaris} \textit{O}-polysaccharides were tested for selective cleavage with triflic acid at \(-4^\circ\mathrm{C}\) for 40–60 min. Together with the monosaccharide (1) (see the previous section), the polysaccharide of \textit{P. vulgaris} O4 (13) gave a disaccharide (14) in a yield of 20\% as a result of cleavage of all glycosidic linkages but not the linkage of glucuronic acid (GlcA).\(^ {[7]}\)

\begin{align*}
&4)-\beta-d\text{-Glc}pA-(1\rightarrow3)-\beta-d\text{-Glc}p\beta\text{-N}Ac-(1\rightarrow2) \\
&\text{-}\beta\text{-Sugp}-(1\rightarrow3)-\alpha\text{-d-Galp}-(1\rightarrow) \quad (13) \\
&\beta\text{-d-Glc}p\beta\text{-A}-(1\rightarrow3)-d\text{-Glc}N\text{Ac} \quad (14)
\end{align*}

(Sug is \(-N\text{-[\(\text{R}\)-3-hydroxybutyryl]-l-alamyl}\)amino-4,6\text{-dideoxy\text{-d-glucose} (1).})

In contrast, in the polysaccharide of \textit{P. vulgaris} O46 (15), the glycosidic linkage of GlcN\text{Ac} was more stable than the linkage of GlcA, and, as a result, solvolyis afforded a disaccharide (16) with GlcN\text{Ac} at the non-reducing end in a high yield of 38\%\(^ {[17]}\). A different behaviour of the GlcN\text{Ac} linkage in the two \textit{P. vulgaris} polysaccharides may be accounted for by its different configurations and/or different neighbouring sugars.

\begin{align*}
&4)-\alpha\text{-d-Glc}p6\text{Ac}-(1\rightarrow3)-\beta\text{-d-Glc}pA4\text{Ac}-(1\rightarrow3)-\alpha\text{-d-Glc}p\beta\text{-N}Ac-(1\rightarrow3)-\beta\text{-d-Glc}pA4\text{Ac}-(1\rightarrow) \quad (15) \\
&\alpha\text{-d-Glc}p\beta\text{N}Ac-(1\rightarrow3)-d\text{-Glc}A \quad (16)
\end{align*}

Conclusions

Although the application of triflic acid has been limited to a few examples, the data obtained show that solvolysis with this reagent is a useful tool for the degradation of polysaccharides into small fragments. Triflic acid does not cleave amide linkages, and is thus applicable for the isolation and concomitant identification of \(N\)-acylated amino sugars and amides of uronic acids. Solvolysis proceeds highly selectively and without significant destruction of sugars, which enables the isolation of monosaccharides and oligosaccharides in satisfactory yields.

The stabilities of glycosidic linkages of various sugars towards triflic acid and anhydrous hydrogen fluoride, which has previously been used for solvolytic cleavage,\(^ {[2]}\) seem to decrease in the same order (5,7-diamino-3,5,7,9-tetradecynonulosonic acids \(\geq 2,3\)-diamino-2,3-dideoxyuronic acids > uronic acids = 2-amino-2-dideoxyhexoses = 2,4-diamino-2,4,6-trideoxyhexoses > 2-amino-2,6-dideoxyhexoses = hexoses). However, the selectivity of the cleavage may be also attributed to other factors, e.g. the nature of neighbouring sugar(s) in the chain, branching, and anomeric configuration, which in some cases complicates reliable prediction of the direction of solvolysis. Varying the temperature and duration of the reaction enables preparation of oligosaccharides of different sizes. Importantly, triflic acid cleaved polysaccharides that were stable towards hydrogen fluoride, and is thus a more efficient reagent. Another advantage of using triflic acid as compared with hydrogen fluoride is that no special equipment is necessary to handle the reaction. We hope that the merits of this new reagent will promote wider application of the method, and aid in determination of structures of complex polysaccharides in many research groups.

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