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Carbohydrate Research 280 (1996) 345–350

CARBOHYDRATE RESEARCH

Note

A one-pot procedure for the quantitative conversion of glycosides into acetylated glycosyl fluorides

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Received 19 August 1994; accepted 1 September 1995

Keywords: Glycosyl fluoride per-*O*-acetates; Glycosides; HF solvolysis

Glycosyl fluorides remain popular for a variety of synthetic and experimental applications largely because they are much more stable than other glycosyl halides. This stability results from the high energy of the glycosidic C–F bond relative to its brominated or chlorinated counterparts [1], and permits a wide variety of uses. For example, glycosyl fluorides have been shown to be useful intermediates in the synthesis of oligosaccharides and glycoconjugates [2], and have been used as probes in studies of enzyme specificity and mechanism [3]. We have recently become intrigued with the possibility of using acetylated glycosyl fluorides as the final analytes in investigations of difficult-to-analyze carbohydrate polymers in environmental samples [4], and so wished to study the conversion of methyl glycosides into acetylated glycosyl fluorides as an analog to reaction termination in solvolysis experiments [5].

We report here an efficient micro-scale procedure for converting methyl glycosides into glycosyl fluoride per-*O*-acetates. The conversion is effected by treatment of the methyl glycosides with liquid anhyd HF followed by direct reaction with acetic anhydride. The procedure is a variation on the classical method of synthesis published by Brauns over seventy years ago [6]. In that study glucose and xylose peracetates were treated with anhyd liquid HF in sealed platinum vessels to form the 1-fluorinated counterparts. The reaction scheme is also similar to that presented by Wiesner [7] wherein per-*O*-acylated sugars were treated with a mixture of acetic anhydride and HF.

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SSDI 0008-6215(95)00328-2

For the method reported here, the glycosyl fluoride is first formed from an *O*-unprotected starting material by reaction of HF directly with a monosaccharide methyl glycoside. Cold acetic anhydride is then added to the liquid anhyd HF solution, resulting in the immediate formation of acetyl fluoride and acetic acid, and the complete elimination of HF from solution. The acetyl fluoride in turn reacts with the hydroxyl moieties on the sugar to form the glycosyl fluoride peracetate [5].

The advantage of conducting the preparation in this way is that the conversion efficiencies are higher than the yields previously reported in comparable synthetic schemes [1]. In particular, the conversion of pentoses is about as good as that of hexoses. For most comparable synthetic methods, pentose yields are lower, usually less than 65% of those obtained from hexoses [1]. For the experiment reported here, the conversion of the methyl glycosides of both glucose and mannose into acetylated glycosyl fluorides was greater than 99%. Conversion of the methyl glycosides of xylose and arabinose was slightly lower at 98 and 95% respectively. The minor products were the corresponding α- and β-glycosyl acetates. The degree of conversion into per-*O*-acetylated glycosyl fluorides was found to be dependent on both the amount of acetic anhydride added and the temperature of both the HF reaction mixture and the added acetic anhydride, with colder temperatures and higher relative amounts of the anhydride favoring the formation of the desired product. The efficiency of conversion reported here for methyl glucoside is higher than reported previously for similar reactions using cellulose as a starting material [5]. A greater amount of acetic anhydride as well as its more rapid addition at reaction termination (owing to greater facility in use of the apparatus) are probable reasons for the observed increase.

Identification of the products and measurement of the degree of conversion were routinely accomplished by gas-chromatographic separation of the glycosyl fluoride tetraacetate (or triacetate) from the minor products, with EI mass spectrometric detection and integration of the total ion chromatogram. No residual methyl glycosides were detected even though they are resolvable under these conditions. The completeness of the reaction was explored in two ways in order to ensure that substantial amounts of dimers or oligomers were not formed during the HF treatment [8] or acetic anhydride addition, and then not chromatographically resolved. First, a ^{1}H-NMR study of pooled samples from five experiments (prior to pyridine–acetic anhydride treatment) using methyl glucoside as a starting material provided a spectrum consistent with relatively pure glucopyranosyl fluoride tetraacetate, with the exception of a peak corresponding to residual acetic anhydride [9]. The spectrum was free of peaks corresponding to *O*-glycosides, which would have indicated the presence of starting material or condensation products. Second, a similar high degree of conversion in single runs with each glycoside was established by comparison to the response of coinjected acetylated methyl glycoside. However, since no pure standard material was available for verification of the ^{1}H-NMR spectra and determination of relative chromatographic response under analytical conditions, absolute degrees of conversion were not established. Determination of true synthetic yield was beyond the scope of the experiment described here.

In general, the EI fragmentation patterns of both hexopyranosyl fluoride tetraacetates and pentopyranosyl triacetates are typical of carbohydrates: the parent ion is not observed and the product ions indicate characteristic fragmentation by losses of acetic

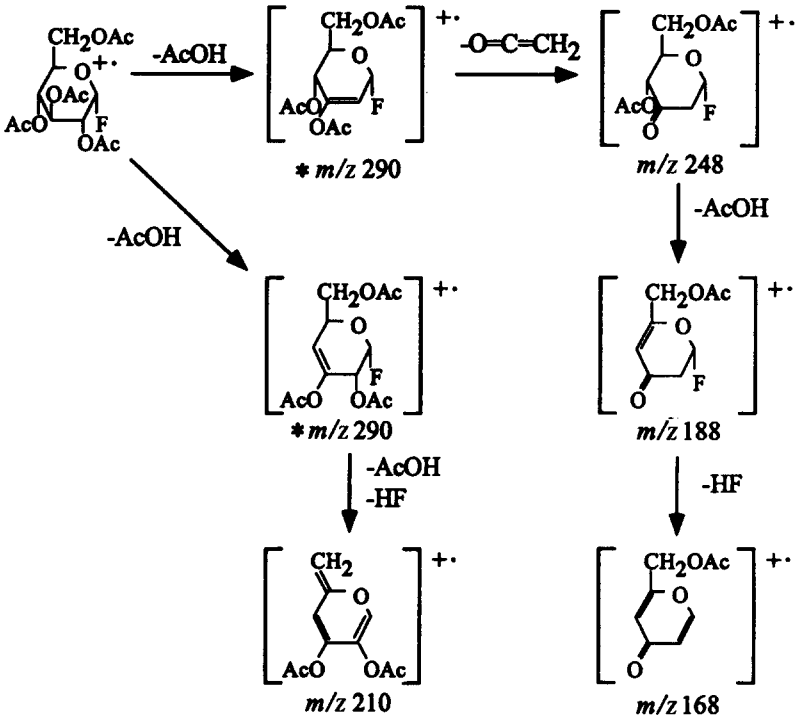
Table 1
The ten most abundant and selected additional high mass fragments resulting from EI-MS analysis of conversion products

		1	2	3	4	5	6	7	8
Hexopyranosyl fluoride	*m/z*	168	188	115	103	126	116	145	97
tetraacetate	%	100.0	95.0	75.2	61.5	61.4	57.6	56.4	34.6
Pentopyranosyl fluoride	*m/z*	115	156	103	114	116	86	128	85
triacetate	%	100.0	72.9	37.3	33.2	32.9	25.5	23.4	20.6
		9	10						
Hexopyranosyl fluoride	*m/z*	117	189	205	169	210	248	187	
tetraacetate	%	30.9	27.5	21.4	18.0	16.7	11.1	9.7	
Pentopyranosyl fluoride	*m/z*	102	71	145	158	216	198	170	
triacetate	%	18.1	17.5	15.2	13.3	11.6	10.8	9.5	

acid (60 mass units), ketene (42 mass units), and hydrogen fluoride (20 mass units) from the parent structure. The mass spectra of hexopyranosyl fluoride tetraacetates have been recorded for chemical ionization using a variety of ionization gases [10]. The results obtained here for EI-MS (Table 1) resemble those previously described. The most obvious and unusual characteristic of the EI mass spectra of pentosyl and hexosyl fluoride peracetates, as compared to those of per-*O*-acetylated sugars and to previous results, is the preponderance of even-mass fragments. For hexopyranosyl fluoride tetraacetates, fragmentation apparently followed one of two prominent paths (Scheme 1). The major fragmentation probably proceeds by the elimination of acetic acid and ketene, to result in the peak at mass 248, then further loss of an additional acetic acid to form the mass 188 ion. Finally, loss of HF may result in the formation of the fully conjugated major ion at 168 mass units. The mass 210 fragment may result from a secondary fragmentation pathway via elimination of two acetic acid units and HF from the parent ion.

In contrast to the situation in the hexose series, mass spectra for acetylated pentopyranosyl fluorides (Table 1) have not been previously reported. These spectra were consistent with eliminations similar to those seen in the hexose series (Scheme 2), except that no single pathway seemed to predominate. The observed higher mass fragments probably result from the elimination of acetic acid and ketene to form the mass 176 fragment, loss of hydrogen fluoride and ketene to form the mass 216 fragment, or loss of acetic acid and hydrogen fluoride to form the mass 198 fragment. Loss of ketene from the mass 198 fragment, or HF from the mass 176 fragment, would result in a mass 156 ion. The mass 176 fragment may also eliminate acetic acid to give the mass 116 fragment.

Our results indicate that formation of the glycosyl fluoride prior to acetylation increases conversion into glycosyl fluoride per-*O*-acetate, particularly for pentose sugars. In principle the experiment may be scaled up to produce larger quantities of acylated glycosyl fluorides by using a larger apparatus [7]. Another finding is that pentopyranosyl fluoride triacetates are stable and well behaved during EI mass spectrometry, giving



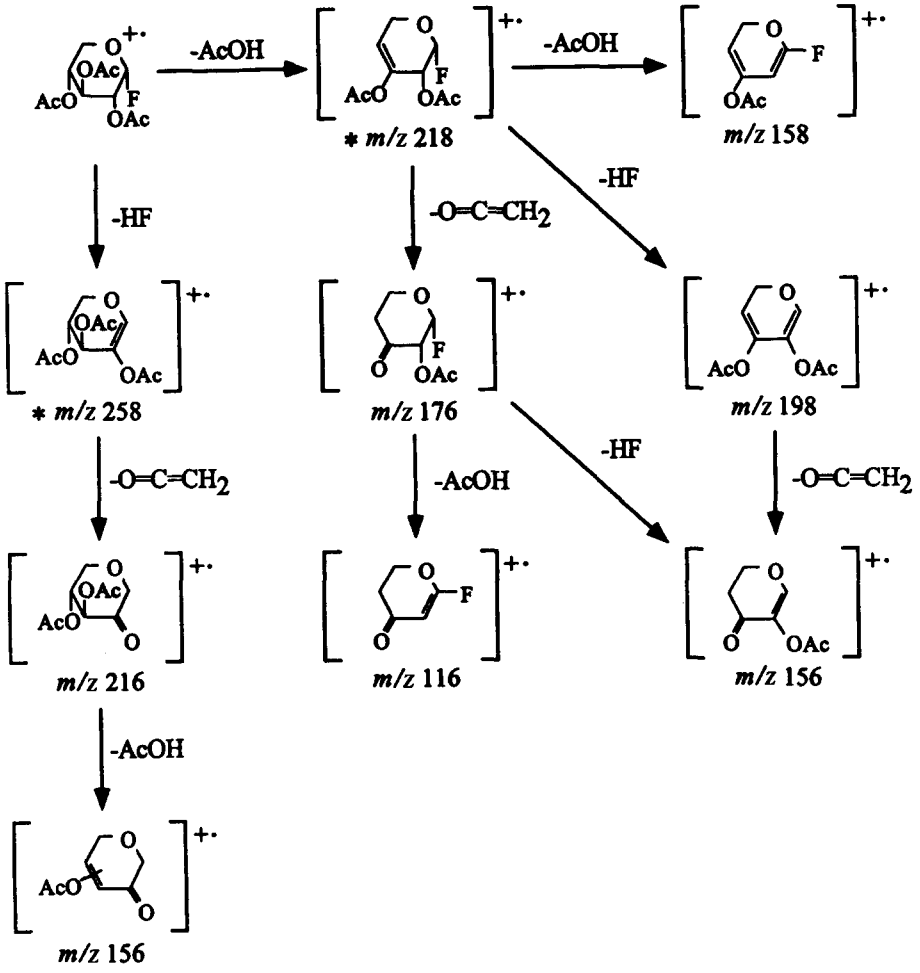
Scheme 1. Proposed fragmentation scheme for hexopyranosyl fluoride tetraacetate. An asterisk (*) indicates a hypothetical intermediate not observed in the mass spectrum.

spectra consistent with fragmentations via elimination of acetic acid and HF, similar to those of their hexose counterparts.

1. Experimental

Materials and apparatus.—The experiments reported here were conducted with an apparatus specially constructed for HF solvolysis experiments [5]. Briefly, it comprises six independent, fully closed and pressurized Teflon reaction vessels, along with a network of valves which enable the precise measurement of liquid HF into each reaction vessel and permit the addition of measured volumes of reagents directly to the closed vessels without depressurizing them. The arrangement of the apparatus also permits rapid and precise temperature control by immersion of the reaction vessels in a thermostatted bath.

Hydrogen fluoride was obtained from Air Products (Allentown, PA) in a 2.3-kg cylinder (size D). Methyl β-D-arabinopyranoside, methyl β-D-xylopyranoside, methyl α-D-glucopyranoside, methyl α-D-galactopyranoside, and methyl α-D-mannopyranoside, along with pyridine and acetic anhydride, were purchased from Aldrich Chemical (Milwaukee, WI).



Scheme 2. Proposed fragmentation scheme for pentopyranosyl fluoride triacetate. An asterisk (*) indicates a hypothetical intermediate not observed in the mass spectrum.

Experimental procedure.—In a typical experiment, an accurately weighed sample of 10–20 mg of methyl glycoside was placed directly into a reaction vessel, a Teflon micro stir bar was added, and the reaction vessel was capped and installed in the solvolysis apparatus. The cluster of six reaction vessels (on a single plate) was placed directly into a −45 °C temperature-controlled bath. The reaction vessels were allowed to equilibrate for approximately 15 min, the integrity of each vessel was tested, and 0.5 mL of liquid HF added to each in turn. The reaction vessels were then transferred to a 0 °C bath and the samples were solvolyzed for 2 h, followed by return to the −45 °C bath for 15 min. While they were still in the bath, 12 mL of cold (−70 °C) acetic anhydride was added to each vessel.

Once the acetic anhydride additions were complete, the sample vials were removed from the solvolysis apparatus and the contents were concentrated under vacuum. In runs quantified by coinjection, an underivatized standard in pyridine was added directly to the vial using a lambda pipette. To ensure complete conversion into per-*O*-acetate derivative and also to derivatize the standard, the mixture was dissolved in 0.5 mL pyridine and 0.5 mL acetic anhydride, and the vial was securely capped and placed in a heating/stirring module at 80 °C for 1 h. GC and NMR analysis of selected samples prior to this step indicated an already substantially complete conversion into peracetylated derivatives. However, a smoother chromatographic baseline was attained by the addition of this step. The treated samples were dried a second time under vacuum to remove the acetic anhydride, and taken up in 0.5 mL pyridine for analysis by GC–MS as previously described [5]. Peaks were identified by comparison of retention times and mass spectral characteristics to those of authentic standards, or by analysis of the mass spectral fragmentation patterns as discussed above.

Acknowledgements

The authors wish to thank Dr Ken Mopper and the UW MOG group for their assistance, and Dr Tom Pedersen for performing the NMR analyses. The research was supported by National Science Foundation Grants OCE9401903 and OCE9101908, and the Royalty Research Fund of the University of Washington. This is contribution number 2144 from the School of Oceanography, University of Washington.

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