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Note

Highly selective cleavage of furanosyl linkages in liquid hydrogen fluoride

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Earlier experiments indicated that anhydrous liquid HF at Dry Ice–acetone temperatures was nearly specific for cleavage of arabinofuranosyl linkages in plant cell-walls[1]. Proof of this indication became necessary because of our need for a method to remove arabinofuranosyl residues from the plant cell-wall protein "Extensin[2]", in order to render the protein susceptible to proteolysis by trypsin, without cleavage of any other sugar linkages associated with the protein. Early experiments showed incomplete specificity for furanose linkages. However, recently we have found that our normal procedures for deactivating HF, forming a relatively unreactive complex with ether, is highly exothermic[3]. We now report that, if precautions are taken to ensure that during the reaction the HF is maintained at temperatures below −70°C and that during quenching of the reaction equally low temperatures are maintained, only furanosyl linkages are cleaved. We have tested the lability of three different furanosides in several different compounds by comparing the ^{1}H and/or ^{13}C NMR spectra of the compounds before and after such an HF treatment.

Fructofuranosides.—Sucrose and related oligomers are readily available examples containing fructofuranosyl linkages. These oligomers contain the α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside linkage, which we presumed would be labile at the very low temperature conditions being tested. The higher oligomers raffinose, stachyose, and melezitose contain additional less-labile linkages which could illustrate the specificity of HF at such a low temperature. Table I summarizes our results with four oligomers of this type. Fig. 1 shows the ^{1}H NMR spectrum of one of them, raffinose, before and after the HF treatment. In all cases

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the signal at 5.42 ppm arising from H-1 of the α-glucosyl residue completely disappeared after 1 h in HF at −73°C, indicating that the linkage between the glucose and fructose residues was completely cleaved. New signals appeared at 5.23 ppm ($^3J_{H1,H2}$ 3.4 Hz) from reducing α-glucose and 4.66 ppm ($^3J_{H1,H2}$ 8 Hz) from reducing β-glucose. The resonance from H-3 (4.22 ppm, $^3J_{H3,H4}$ 8 Hz) of the fructofuranose residue in sucrose is well resolved in the ^{1}H spectrum and is completely absent in the spectrum of the treated sucrose. The signals expected[4] for H-3 and H-4 of free β-fructofuranose at 4.08 ppm may be seen in the product. However, many minor signals that are present are not from any of the tautomers of free fructose[4]. The ^{13}C spectrum of the treated sucrose shows that the fructose residue has been converted into several different forms. Free fructose appears to be a major portion of the product from the similarity of relative intensities of the signals for C-2 of fructose at 97.8, 101.4, and 104.3 ppm representing the β-pyranosyl, β-furanosyl, and α-furanosyl forms[5]. The remainder of the fructose is most probably in the form of fructose dianhydrides, which have been produced previously and characterized by Defaye et al.[6].

There was no trace of α-glucosyl fluoride among the products: this would show a characteristic doublet of doublets at 5.72 ppm ($^2J_{H,F}$ 53 Hz, $^3J_{H1,H2}$ 3.4 Hz) in the ^{1}H spectrum and a doublet at 107.9 ppm of $^1J_{C,F}$ 226 Hz in the ^{13}C spectrum[7]. As α-glucosyl fluoride is very stable, this result indicates that the cleavage of sucrose (and similar oligomers) was via formation of the fructosyl fluoride, which is presumably too unstable to be isolated during an aqueous workup. In an attempt to isolate fructosyl fluoride, the reaction product was dissolved in dry dimethyl sulfoxide-d_6 rather than in water and the ^{13}C spectrum was immediately recorded. Surprisingly, we found a large proportion of α-glucosyl fluoride (C-1 107.9 ppm, $^1J_{C,F}$ 226 Hz) and an even more complex set of signals between 90 and 105 ppm that we presume to be mostly from the fructose dianhydrides[6]. Reaction of glucose in HF at −73°C for 1 h followed by quenching with ether and dissolution of the products in D_2O showed that glucose was totally inert under these conditions. No trace of glucosyl fluoride was seen in the ^{1}H NMR spectrum. The only explanation of the formation of the glucosyl fluoride is that, in Me_2SO, the fructosyl fluorides are so reactive that they are all converted into nonfluorinated products, releasing HF into the Me_2SO which then reacts with the glucose to produce the glucosyl fluoride. We propose that this exchange of fluoride does not take place in the HF–ether complex formed during quenching of the HF, since the addition of water rather than Me_2SO leads to no glucosyl fluoride.

In all of the oligosaccharides tested we obtained no evidence to indicate that linkages other than that of the fructofuranoside were cleaved.

Arabinofuranosides.—Our main interest is in arabinofuranoses linked to the cell-wall protein Extensin. This protein is not available in large enough quantities for rapid NMR analysis. However, larch arabinogalactan and beet arabinan are readily available and both contain arabinofuranoses. Beet arabinan is thought to be composed entirely of arabinofuranose residues[8]. The ^{13}C NMR spectrum of our

TABLE I

^{1}H NMR chemical shifts and relative intensities of resolved protons in untreated and HF-treated oligosaccharides containing fructofuranosides

Compound [a]	Chemical shift (ppm)	Coupling constant (Hz)	Intensity	Chemical shift (ppm)	Coupling constant (Hz)	Intensity	Chemical shift (ppm)	Coupling constant (Hz)	Intensity	Chemical shift (ppm)	Coupling constant (Hz)	Intensity
Sucrose							α-glucose H-1			β-fructose H-3		
Structure 1							5.42	3.4	1	4.22	8	1
HF-treated							α-glucose H-1			fructose		
Sucrose							5.23	3.4	0.35	Multiple signals		
								β-glucose H-1				
							4.63	8	0.64			
Raffinose				α-galactose H-1			α-glucose H-1			β-fructose H-3		
Structure 2				4.99	3.4	1	5.42	3.4	1	4.22	8	1
							α-glucose H-1			fructose		
				α-galactose H-1			5.23	3.4	0.38	Multiple signals		
				4.98	Complex	1	β-glucose H-1					
							4.66	8	0.61			
Stachyose	α-galactose H-1			α-galactose H-1			α-glucose H-1			β-fructose H-3		
Structure 3	5.00	3.4	1	5.00	3.4	1	5.42	3.4	1	4.2	8	1
HF-treated	α-galactose H-1			α-galactose H-1			α-glucose H-1			fructose		
Stachyose	5.98	Unresolved	1	5.98	Unresolved	1	5.23	3.4	0.32	Multiple signals		
							β-glucose H-1					
							4.66	~8	0.68			
Melizitose				α-glucosyl (1-3) fructose H-1			α-glucosyl (1-2) fructose H-1			β-fructose H-3 and H-4		
Structure 4				5.19	3.4	1	5.46	3.4	1	4.30	Unresolved	2
HF-treated				α-glucosyl (1-3) fructose H-1			α-glucose H-1			fructose		
Melizitose				5.32 and	3.4		5.23	Unresolved		Multiple signals		
				~5.2		1	β-glucose H-1					
					Unresolved		4.66	8				

[a] 1, α-D-Glc*p*-(1 → 2)-β-D-Fru*f*; 2, α-D-Gal*p*-(1 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*; 3, α-D-Gal*p*-(1 → 6)-α-D-Gal*p*-(1 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*; 4, α-D-Glc*p*-(1 → 3)-β-D-Fru*f*-(2 → 1)-α-D-Glc*p*.

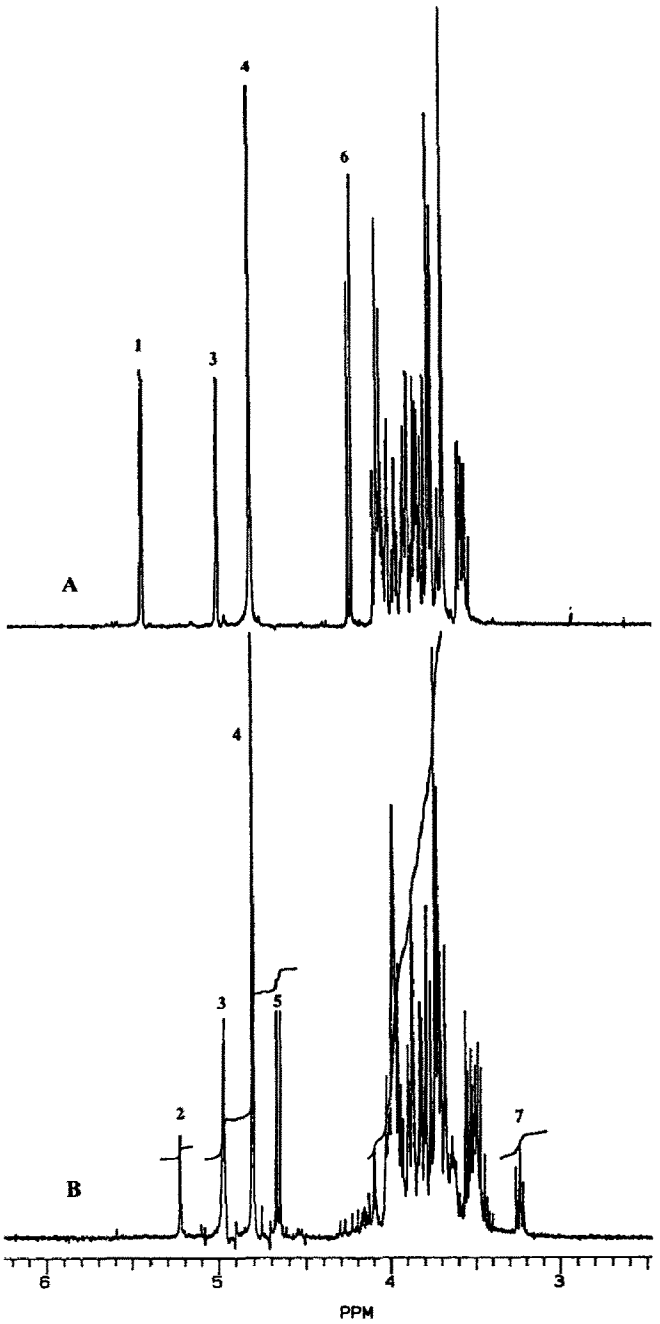

Fig. 1. ^{1}H NMR spectrum of raffinose before (A) and after (B) HF treatment at −73°C for 1 h. The numbered resonances are identified as: in (A) 1, H-1 of α-Glc*p*; 3, H-1 of α-Gal*p*; 4, HOD; 6, H-3 of β-Fru*f*; in (B) 2, H-1 of reducing α-Glc*p*; 3, H-1 of α-Gal*p* linked to either reducing α- or β-Glc*p*; 4, HOD; 5, H-1 of reducing β-Glc*p*; 7, H-2 of β-Glc*p*.

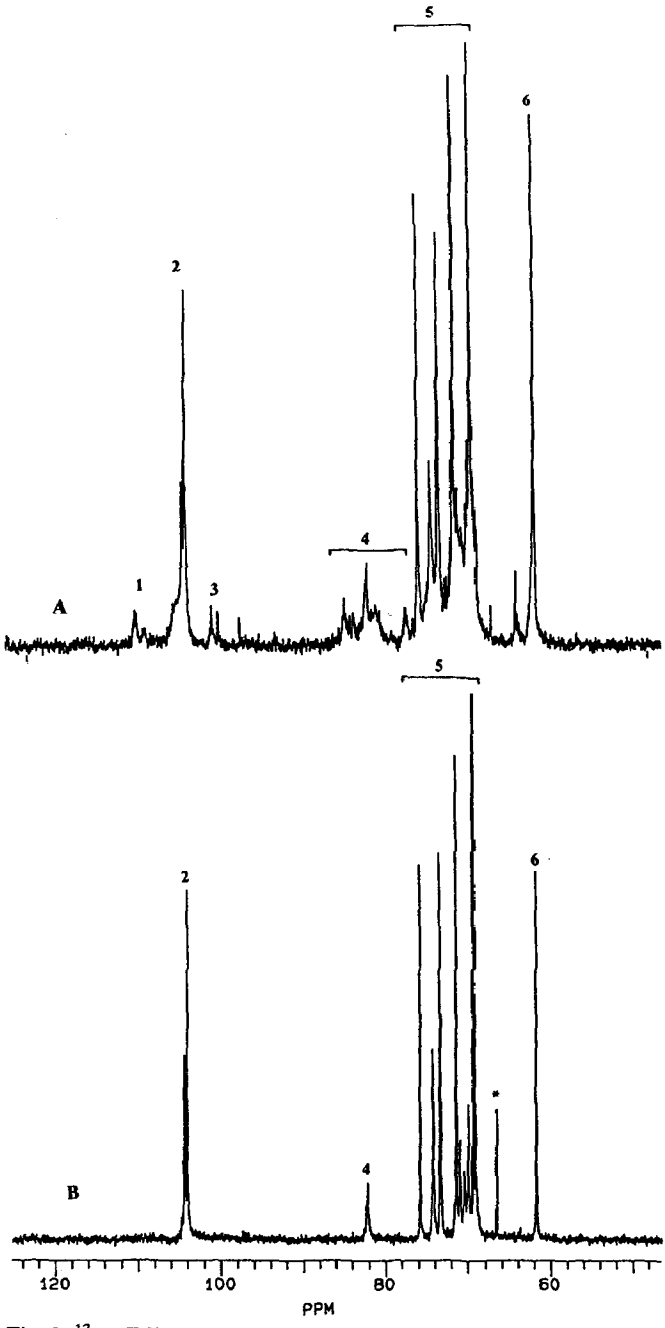

Fig. 2. ^{13}C NMR spectrum of larch arabinogalactan before (A) and after (B) HF treatment at −73°C for 1 h. The numbered resonances are identified as: in (A) 1, C-1 of Ara*f*; 2, C-1 of Gal*p*; 3, C-1 Ara*p*; 4, ring carbons of Ara*f* plus C-3 of 3-linked Gal*p*; 5, ring carbons of Gal*p*; 6, C-6 of Gal*p* and C-5 of nonreducing terminal Ara*f*; in (B) 2, C-1 Gal*p*; 4, C-3 of 3-linked Gal*p*; 5, ring carbons of Gal*p*; 6, C-6 of Gal*p*; * residual ether.

sample only showed signals at 107.9 and 108.3 ppm in the 90–120 ppm region, confirming that it too contained only L-arabinofuranoses.

After treating a sample of the polymer at −73°C in HF and quenching the reaction with cooled ether, all of the product remained soluble in the HF–ether mixture. We find that only monomers and, to a small extent, dimers, are soluble in this solvent-system. The ^{13}C NMR spectrum of the product showed no signals for furanoses and a predominance of free arabinopyranoses. The ^{1}H NMR spectrum also indicated a predominance of those for free arabinopyranoses (major signals at 5.22 ppm $^{3}J_{H1,H2}$ 3.4 Hz for β-L-arabinopyranose and at 4.48 ppm $^{3}J_{H1,H2}$ 8 Hz for α-L-arabinopyranose, but also a complex group of smaller signals between 4.9 to 5.3 ppm). The lower-intensity signals are probably from reversion products formed from condensation of arabinosyl fluorides, which were present at ~ 10 mg/mL in the liquid HF, to form randomly connected oligosaccharides. In the case of glucose this concentration of glucosyl fluorides would not lead to reversion[9], but arabinosyl fluorides must be more reactive, as they were not observed in the final products. Evidence for the concentration of the arabinose affecting the formation of the reversion products was obtained during experiments with larch arabinogalactan in which no reversion was observed (see later). This polymer is ~ 20% arabinose and 80% galactose. The arabinose is thought to exist in the polymer at the termini of branches on a 6- and 3-, 6-linked branched galactan[10]. Around 85% of the arabinose is in furanosyl linkages, with the remainder as single pyranosyl linkages to arabinofuranoses. Thus, selective cleavage of all of the furanosyl linkages should lead to the production of a pure polymeric galactan and mono- and di-saccharides of arabinose. When this polymer was treated with HF at −73°C, ~ 20% of its weight became soluble in the HF–ether complex. The only sugar detected by GLC in the soluble fraction was arabinose. The soluble component could be separated into a major and minor fraction on an HW 40S gel-filtration column. The major fraction eluted in the included volume and by ^{1}H NMR appeared to be essentially pure arabinose monosaccharide. The minor fraction eluted a little earlier from the column; its ^{1}H spectrum indicated it to be a disaccharide of arabinose because of the presence of three major signals in the anomeric region, two from a reducing arabinose (5.23 ppm β-L-arabinopyranose and 4.51 ppm $J_{1,2}$ 8 Hz α-L-arabinopyranose) and a complex signal at 5.08 ppm that is probably from β-arabinopyranose residues linked to either the α- or β-reducing arabinose. The ^{13}C NMR spectrum of the disaccharide showed from the signals at 95.5 and 96.0 ppm that there was a pyranosyl linkage between the two sugars. No signals were observed downfield of these, showing the absence of furanosides. Thus, it appears that the arabinopyranosyl linkages in the arabinogalactan are stable in HF at −73°C.

Fig. 2 shows the ^{13}C NMR spectra of the larch arabinogalactan before and after HF treatment. There were no detectable signals from the arabinose residues in either the furanosyl or pyranosyl forms in the treated sample.

Compositional analysis by GLC of the polymeric part of the arabinogalactan

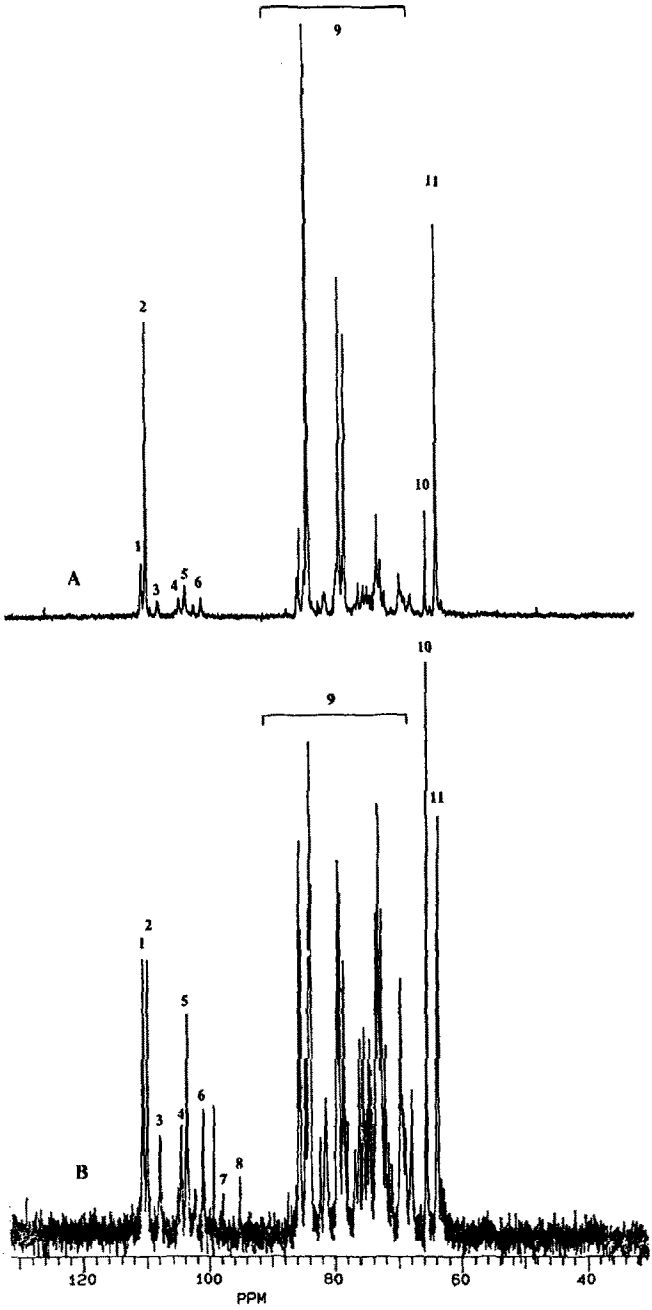

Fig. 3. ^{13}C NMR spectrum of peptidophosphogalactomannan before (A) and after (B) HF treatment at −73°C for 1 h. Signals were identified by comparison to the report of Unkefer and Gander[11]. TSP was used as reference. 1, C-1 of t-β-Gal*f*; 2, C-1 of 5-linked β-Gal*f*; 3, C-1 of other β-Gal*f*; 4, C-1 of 6-linked α-Man*p*; 5, C-1 of 2-linked α-Man*p* linked to a 2-linked α-Man*p*; 6, C-1 of 2-linked α-Man*p* linked to a 6-linked α-Man*p*; 7, C-1 of free β-Gal*p*; 8, C-1 of free α-Gal*p*; 9, C-(2-5) Gal*f* and Man*p*; 10, C-6 of t-β-Gal*f*; 11, C-6 of 5-linked β-Gal*f* and C-6 of 2-linked α-Man*p*.

after treatment showed ~2% of arabinose and, as mentioned, the portion soluble in the ether–HF quenching mixture contained no galactose.

Galactofuranosides.—Galactofuranosyl residues are often found in polysaccharides. Thus, it seemed important to determine whether galactofuranosyl linkages are of similar lability to cold HF as are fructofuranoses and arabinofuranoses. A well-studied example of a galactofuranoside-containing polymer is the peptidophosphogalactomannan produced by the fungus *Penicillium charlesii*[11]. This polymer is composed of three major polymeric components, a core peptide to which is attached ~10 mannan chains. To each mannan are attached several linear galactofuranosyl polysaccharide segments. Fig. 3 shows the ^{13}C NMR spectrum of the polymeric material before and after HF treatment at −73°C. About 35% of the sample was soluble in the quenching mixture and, thus, is not seen in the second spectrum. Only the galactose component was soluble in the HF–ether quenching mixture, although some of the galactose remained insoluble. This observation corresponds well with the ^{13}C NMR spectra, which show a dramatic decrease in the signal for the 5-linked galactofuranosyl linkages at 109.8 ppm but not complete abolition.

The treated material appeared to contain an almost equal proportion of nonreducing terminal galactofuranosyl residues (C-1, 110.5 ppm; C-6, 65.5 ppm) and 5-linked galactofuranosyl residues (C-1, 109.8 ppm; C-6, 63.8 ppm). A smaller signal at 107.7 ppm, probably from galactofuranose residues in a different linkage, is almost unchanged in intensity in comparison to the original t-galactofuranose signal. All of the signals from the mannan component of the complex appear to be unchanged in their relative intensities.

Derivatized sucrose.—Anhydrous HF has been used on several occasions[9] to cleave derivatized polysaccharides and has been used frequently to prepare derivatized sugar fluorides[12]. The literature indicates that the linkages between peracetylated sugars in small oligosaccharides are considerably more stable than those between nonderivatized sugars[12]. In one case there were indications that even a single acetyl substituent on a sugar could increase the stability of the associated glycosidic linkage significantly[13]. The behavior permethylated of oligo- and polysaccharides is almost unknown, except that HF has been used at room temperature to generate methylated monosaccharides from aqueous acid-resistant methylated polysaccharides[9].

We treated octa-*O*-acetylsucrose with HF at −73°C and found by ^{13}C and ^{1}H NMR that absolutely no reaction occurred. Repeating the experiment at −31°C gave the same result. Inulin acetate was also found to be completely unreactive at −73°C, even after 90 min. After treatment at 0°C, a complex mixture was obtained, as expected from the results of Defaye et al.[6] On the other hand, treatment of octa-*O*-methylsucrose at −30°C did lead to formation of tetra-*O*-methyl-α-D-glucosyl fluoride (C-1 104.81 ppm $^{1}J_{C,F}$ 226 Hz) and a mixture of products from the fructose. Even treatment of octa-*O*-methylsucrose at between −73 and −75°C for 1 h led to the production of free 2,3,4,6-tetra-*O*-methyl-D-glu-

cose, tetra-*O*-methylglucopyranosyl fluoride and various methylated fructose isomers.

A similar effect of acetylation was seen on the stability of the inter-residue linkage in octa-*O*-acetylmaltose. Treatment at ~ −20°C in HF for 30 min led to ~ 85% conversion into hepta-*O*-acetylmaltosyl fluoride and 15% of unreacted starting material. Reaction of nonacetylated amylose under these conditions leads exclusively to glucosyl fluoride[14].

CONCLUSIONS

Anhydrous HF at temperatures below −70°C can be used for extremely specific cleavage of furanosyl linkages in all sugars tested. However, some furanosyl linkages are resistant to cleavage under these conditions. The resistance is probably dependent on the nature of the alcohol to which the furanoside is linked. Peracetylation of the furanoside confers complete resistance to low temperature HF solvolysis. Permethylation does not affect the susceptibility of the linkage.

EXPERIMENTAL

Sugar samples.—Sucrose, raffinose, stachyose, melizitose, and larch arabinogalactan were purchased from Sigma Chemical Co. (St. Louis MO). Beet arabinan was a gift from Dr. Bruno Moerschbacher (Aachen, Germany) and the peptidophosphogalactomannan was a gift from Dr. John Gander (Gainesville, FL). Sucrose and maltose octaacetate were prepared by acetylation of sucrose and maltose, respectively, with Ac_2O in pyridine and were crystallized from EtOH[15]. Octa-*O*-methylsucrose was prepared[16] by reaction of sucrose in *N*,*N*-dimethylformamide with MeI and Ag_2O.

HF solvolysis.—HF solvolysis of the sugars was performed using the apparatus described in Fig. 2 of ref 17. A detailed description of the procedure is given in ref 1. However, it is briefly described here.

The desired volume of liquid HF was distilled into an equilibration vessel and then cooled to −73°C with a Dry Ice–acetone bath. An EtOH bath using an immersion cooler/heater apparatus, or a Dry Ice–acetone bath was used to equilibrate the reaction vessel at −73°C. The reaction was started by forcing the liquid HF from the equilibration vessel by a light N_2 pressure into the reaction vessel through Teflon tubing connecting the vessels. To terminate the reaction, the reaction vessel was immersed in liquid N_2 until the HF solidified. Ether cooled with Dry Ice was then introduced into the reaction vessel and the HF allowed to thaw slowly with frequent swirling to carry away the heat generated by the exothermic formation of the HF–ether complex. After reaching close to room temperature the HF–ether mixture was evaporated under vacuum into a liquid N_2-cooled vessel on the HF apparatus.

NMR spectroscopy.—^1H and ^{13}C NMR spectra were recorded with either a

Varian XL 400 or XL 300 instrument. For unacylated sugars D_2O was the solvent unless otherwise stated, and the ^1H NMR chemical shifts were referenced to internal sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP) at 0 ppm. ^{13}C NMR chemical shifts were referenced to acetone at 31.07 ppm except for the case of the peptidophosphogalactomannan in which TSP was used as reference for ease of comparison to the published spectra. For derivatized sugars, deuteriochloroform was the solvent and Me_4Si the internal reference.

Sugar compositions of the various fractions were determined by formation of the trimethylsilyl methyl glycosides by methanolysis followed by trimethylsilylation and capillary gas–liquid chromatography[18].

Gel filtration chromatography.—Oligo- and mono-saccharides from larch arabinogalactan were separated using Toyo Pearl HW-40S beads packed into a 50 × 1.27 cm (i.d.) stainless-steel column in 50 mM ammonium acetate buffer (pH 5.2) flowing at 1 mL/min and detected by a refractive-index detector (Waters R401).

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