Carbohydrate Research, 251 (1994) 17–31
Elsevier Science B.V., Amsterdam

Protonic reactivity of sucrose in anhydrous hydrogen fluoride †,‡

Jacques Defaye * and José Manuel García Fernández *,[1]

CNRS and CEA, Département de Recherche Fondamentale sur la Matière Condensée / SESAM, Centre d'Etudes de Grenoble, BP 85 X, F-38041 Grenoble (France)

(Received March 9th, 1993; accepted May 1st, 1993)

ABSTRACT

Sucrose reacts quantitatively, when dissolved at high concentration in anhydrous hydrogen fluoride, to afford a complex mixture of difructose dianhydrides and their glucosylated derivatives. Oligo- and small poly-saccharides up to dp 14 were detected by FABMS. Oligosaccharides up to dp 4, representing ~ 50% of the total mixture, have been isolated and characterized by mass spectrometry, ^{13}C NMR spectroscopy, and comparison with reference oligosaccharides previously obtained by unambiguous synthesis. α-D-Fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride is the main spirodioxanyl pseudodisaccharide entity found in the mixture, either free or glucosylated at C-6 and to a lesser extent at C-3, C-4, C-4′, C-4′ C-6, and C-5′ C-6. Minor spirodioxanyl pseudodisaccharide components are di-β-D-fructopyranose 1,2′:2,1′-dianhydride, which has also been found glucosylated at C-5, α-D-fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride, β-D-fructofuranose β-D-fructopyranose 1,2′:2,3′-dianhydride, and the 6,6′-diglucosylated α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride. A ^{13}C NMR examination of the higher mass oligomeric fraction suggests that it may involve 6-*O*-isomaltooligoglycosyl α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydrides as the main structural components. The reaction of sucrose in anhydrous HF is believed to proceed through initial selective protonic activation of the tertiary anomeric carbon atom of the fructose moiety, resulting in the quantitative formation of difructose dianhydrides, which subsequently suffer electrophilic substitution by glucopyranosyl oxocarbenium ions generated in a second step by action of the HF.

INTRODUCTION

Anhydrous hydrogen fluoride (HF) is an excellent solvent and a strongly protonating and ionizing reagent which has found a number of applications for the activation of carbohydrates at anomeric positions, resulting in either cleavage or

† Dedicated to Professor C.E. Ballou.

‡ Presented in part at the IInd International Workshop on Carbohydrates as Organic Raw Materials, Lyon (France), July 2–3, 1992; abstract in ref 2. Part 16 in the series: Carbohydrate reactivity in anhydrous hydrogen fluoride. For part 15, see ref 1.

* Authors for correspondence.

[1] Present adress: Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41071 Sevilla, Spain.

SSDI 0008-6215(93)E0256-Z

substitution reactions by oxygen as well as sulfur nucleophiles. A quite promising selectivity has been observed in both analytical[3] and synthetic fields[4], which appears to be related to the exceptional flexibility of this reagent in the expression of its protonic availability. Stereoelectronic factors, related to its high dielectric constant, appear furthermore of importance in the outcome of reactions resulting in the formation of heterocyclic systems such as the spirodioxanyl oligosaccharides derived from hexuloses[5], and it was shown that their control could be satisfactorily achieved by modulating temperature and substrate concentration, or by the use of co-solvents[6–8]. Interesting examples of such selectivity were recently found with a series of isomeric glycosylfructoses which resulted, by reaction in either HF (ref 1) or pyridinium poly(hydrogen fluoride) (ref 8), in obtaining a number of selected glycosyl difructose dianhydrides with neither cleavage nor anomerisation of the existing glycosidic linkages, indicating that the protonic activation was restricted to the anomeric tertiary carbon atom of the fructose moiety. It was however to be expected that the reactivity of α-D-glucopyranosyl β-D-fructofuranoside, i.e., sucrose, in anhydrous HF would not be so easy to control, although initial protonic activation would be thought to occur mainly at the fructofuranose anomeric site of the molecule. Such a process has already been described in the patent literature[9], with the aim of obtaining new food products. Formation of glycosyl difructose dianhydrides was noticed, but the characterization of the products was not achieved, owing mainly to the lack of suitable model compounds. The recent synthetic availability[1,8] of a number of glycosyl difructose dianhydrides has now made such an objective practicable, and the lower molecular weight constituents of the mixture have been fully characterized, while a tentative overall structure is given for the higher molecular weight components.

RESULTS AND DISCUSSION

Storage of solutions of sucrose in anhydrous HF for 1 h, using a 2:1 (w/v) sucrose–HF ratio, and subsequent in situ acetylation of the resulting product by addition of 1:1 pyridine–acetic anhydride, gave a crude product mixture which showed, in its FAB-mass spectrum (Fig. 1), peaks at intervals of 288 mass units from m/z 599 to m/z 4056, corresponding to $[M + Na]^+$ pseudomolecular ions for acylated difructose dianhydrides and their derivatives having from 1 to 12 glucosyl residues. The ^{13}C NMR spectrum of this mixture (Fig. 2) displayed prominent signals at 101.5 and 94.9 ppm in the region of anomeric carbon resonances, indicative[6,8] of the presence of a major proportion of α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**1**) compounds, and this was corroborated by signals at 81.2 (C-5), 79.7 (C-3), and 77.9 (C-4) in the nonanomeric carbon range[6,8]. Previously reported results on the relative rates of formation of difructose dianhydrides and their glycosylated derivatives in HF and HF–amine reagents have shown[1,6–8] that this dispirodioxane structure is among the thermodynamically favored.

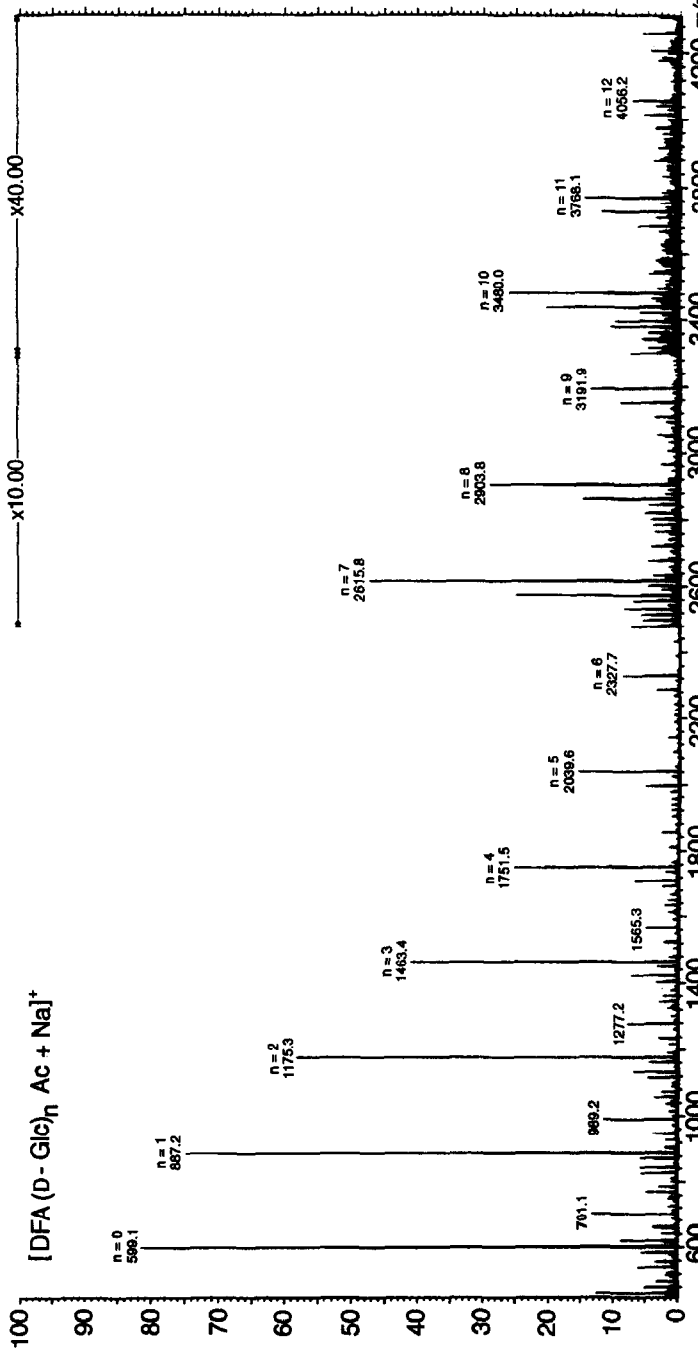


Fig. 1. FAB^+ mass spectrum of the acetylated crude product mixture arising from the action of anhydrous HF on sucrose (glycerol matrix + NaI).

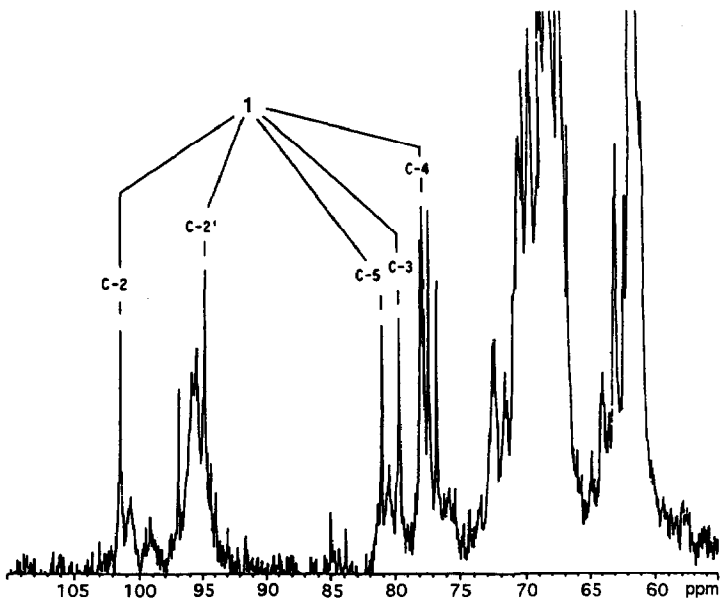


Fig. 2. Partial ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) of the acetylated crude product mixture arising from the action of anhydrous HF on sucrose.

Column chromatography on silical gel of the crude per-*O*-acetylated product, using a hexane–EtOAc gradient, resulted in five fractions amounting to ~ 50% of the total oligosaccharide mixture and comprising, as seen from the FABMS analysis, the di-, tri-, and tetra-saccharidic material. Further elution with more polar solvents gave the higher oligosaccharidic material (Fraction 6). The composition of Fractions 1–6 is shown in Table I and in the formula Scheme.

Fraction 1 (2.3%) and 2 (18.7%) both displayed pseudomolecular ions $[M + Na]^+$ in FABMS at m/z 599, in agreement with difructose dianhydride hexaacetate structures. The ^{13}C NMR spectrum of Fraction 1 showed only 6 signals, indicating a symmetrical arrangement, and their chemical shifts were found identical with those of the known[6] di-β-D-fructopyranose 1,2′:2,1′-dianhydride peracetate **3**. This structural assignment was further confirmed by comparison of the physical data, which agreed with the literature[6].

The ^{13}C NMR spectrum of Fraction 2 was more complex (Fig. 3) since it displayed, in addition to prominent signals at δ 101.5 and 95.0 corresponding to C-2,2′ of acylated α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**1**), minor signals at 97.1, 94.7, and 92.8, and at 103.1 and 94.5, which could be assigned[6] to C-2,2′ of residual **3** and also to C-2,2′ of **5** and **7**, respectively. Further use of an elution gradient for the chromatographic purification of this fraction afforded sequentially residual **3** and the hexaacetates of β-D-fructofuranose β-D-fructopyranose 2,1′:3,2′-dianhydride (**7**), α-D-fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**5**), and α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**1**), with physical data in full agreement with the literature[6].

TABLE I

Products formed by the action of anhydrous hydrogen fluoride on sucrose [a]

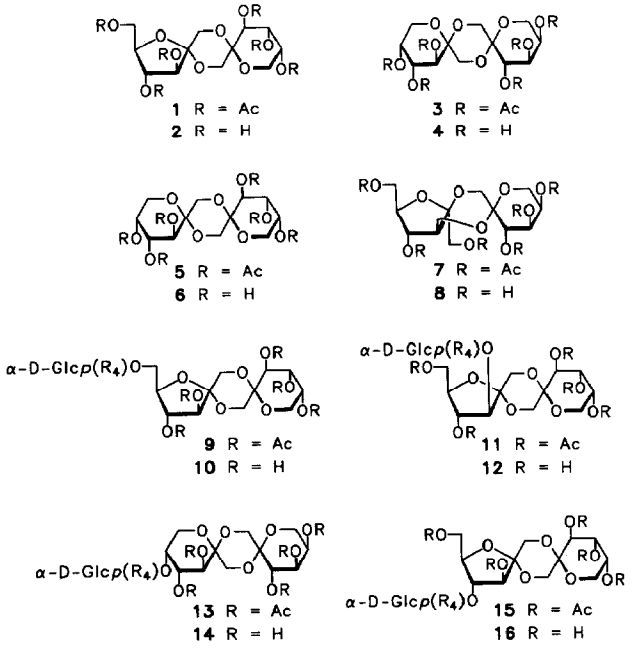
Fraction No. (%) [b]	Products (%) [c]												
	2 [d]	**4** [d]	**6** [d]	**8** [d]	**10** [d]	**12** [d]	**14** [d]	**16** [e]	**18** [d]	**20** [e]	**22** [d]	**24** [d]	**26** [f]
1 (2.3)		100											
2 (18.7)	73	15	9	9									
3 (8.7)					45	37	9						
4 (8.0)					38			32	10	19			
5 (11.3)											16	27	49
6 (45.0) [g]													
Total pseudodisaccharide components (21)	65	13	8	8									
Total pseudotrisaccharide components (16)					43	20	5	16		10			
Total pseudotetrasaccharide components (13)									6		15	25	46

[a] Estimated as their acetates after in situ acetylation. [b] Percentages relative to the total oligosaccharide mixture. [c] Percentages relative to the total corresponding fraction. [d] Assessed by ^{13}C NMR spectroscopy of the corresponding peracetates. [e] Assessed by integration of LC chromatograms. [f] Estimated from the column chromatography of the peracetates. [g] Material having a degree of oligomerization > 4.

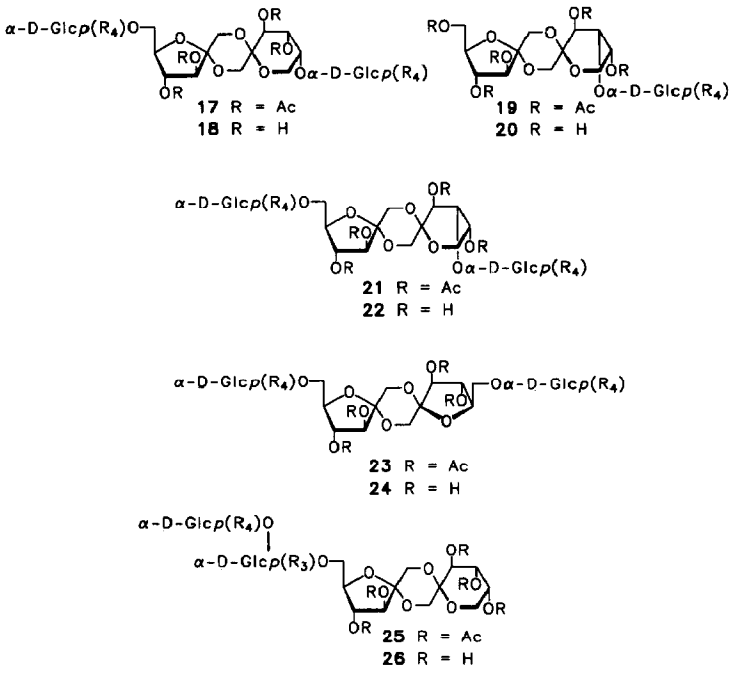
Fraction 3 (8.7%) showed a clean FAB-mass spectrum with a pseudomolecular ion $[M + Na]^+$ at *m/z* 887 indicative of an acylated hexosyl difructose dianhydride structure. The low-field region of its ^{13}C NMR spectrum (Fig. 4) displayed however, in addition to the readily recognizable C-1 signal at δ 96.0 for glycosidically linked, per-*O*-acetylated α-D-glucopyranose residues[10], five signals pointing to some inhomogeneity. This was further confirmed by successive TLC elution, which finally allowed the detection of three constituents. Their separation was scaled up by transposition to a silica gel column using the same solvents in a gradient system. From the signals at δ 102.1–101.5 and 95.0–94.9, a common α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride substructure was to be expected for both major components, while the low-field displacements for the C-6 and C-3 signals as compared to data for **1** suggested that these positions could be glycosylated as in structures **9** and **11**.

This assumption was readily confirmed by comparison of the spectral and physical data with those for authentic peracetates of 6-*O*-α-D-glucopyranosyl-α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**9**) and 3-*O*-α-D-glucopyranosyl-α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**11**) obtained from palatinose and turanose, respectively[8].

The minor component of Fraction 3 showed, in addition to the signal at δ 97.0, five pairs of closely related or superimposed signals together with two distinct



Scheme 1.



Scheme 2.

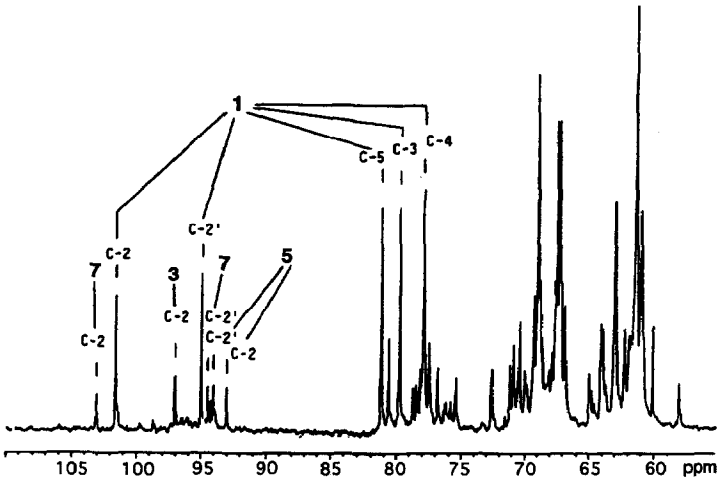


Fig. 3. Partial ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) of Fraction 2.

signals, differing by 6.4 ppm, in the region for fructopyranose ring carbon atom resonances. From the δ value for the anomeric carbon atoms (96.9 and 97.0 ppm) such a finger print was reminiscent of the symetrical di-β-D-fructopyranose dianhydride substructure **3**, which would be asymetrically substituted at C-5. This compound had already been prepared by protonic activation of leucrose[8], and correla-

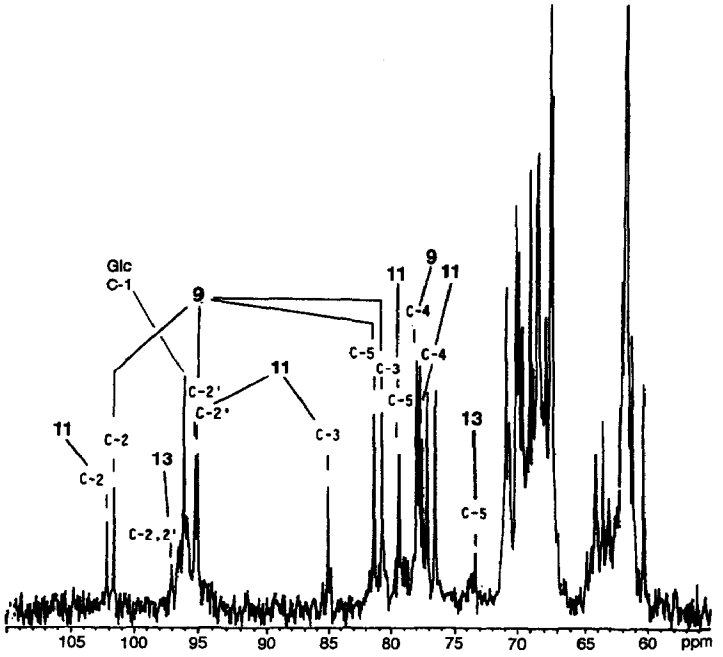


Fig. 4. Partial ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) of Fraction 3.

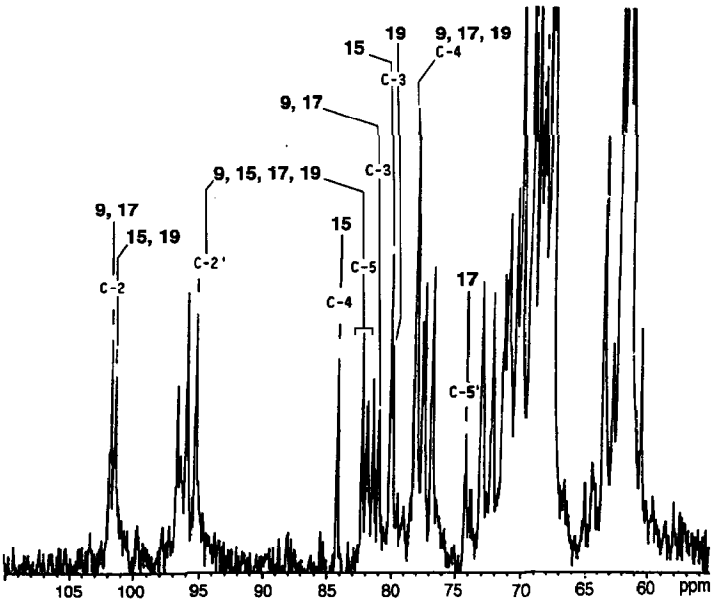


Fig. 5. Partial ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) of Fraction 4.

tion with an authentic sample confirmed that this pseudotrisaccharide was effectively 3,4,3′,4′,5′-penta-*O*-acetyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-di-β-D-fructopyranose 1,2′:2,1′-dianhydride (**13**).

Fraction 4 (8%) displayed two $[M + Na]^+$ pseudomolecular ions in its FAB-mass spectrum at m/z 887 and 1175, indicating the presence of hexosyl- and dihexosyl-dihexulose dianhydride peracetate constituents. The major substructure α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride was inferred from the two sets of signals at ~ 101.5 and 95 ppm in the anomeric region of the ^{13}C NMR spectrum of the crude product (Fig. 5), and the signal multiplicity confirmed the inhomogeneity of this fraction, suggesting glucosylation at several different sites. A more thorough silica gel column chromatography of Fraction 4, using a CCl_4–acetone gradient, proved successful in allowing the separation of the mixture with sequential recovery of residual **9**, a minor proportion of **17**, and then of **15** and **19** still in an admixture. The latter two pseudotrisaccharides could only be separated by LC of their deacetylated derivatives **16** and **20**.

Arguments in favor of structures **15**, **17**, and **19** came, in a first approach, from a comparison of their ^{13}C NMR spectra with that of **1** and its partially glucosylated derivatives. Thus, on the one hand, the resonances for the fructofuranose carbon atoms of **15** are in good agreement with known data for 4-*O*-α-D-glucopyranosyl-α-D-fructofuranose 4-*O*-α-D-glucopyranosyl-β-D-fructopyranose dodecaacetate[8], while values at higher field for the fructopyranose part of the molecule are similar to the corresponding data for **1**, suggesting a glucosylation at C-4 of the fructofura-

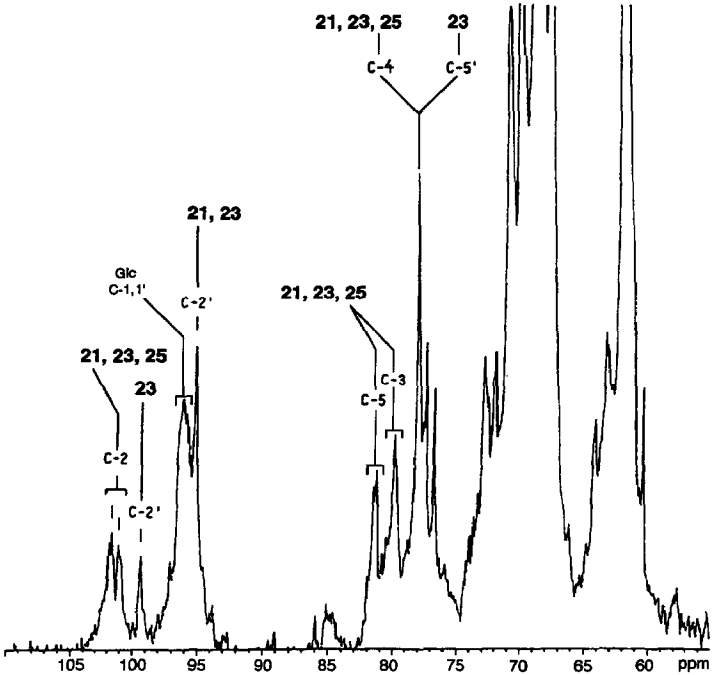


Fig. 6. Partial ^{13}C NMR spectrum (50.3 MHz, $CDCl_3$) of Fraction 5.

nose moiety. The opposite situation was found for **19**, indicating a substitution at C-4′ of the fructopyranose site. On the other hand, the diglucosylated pseudotetrasaccharide **17** showed ^{13}C NMR chemical shifts which could be considered as a superposition of signals for the fructofuranose part of **9** and the β-fructopyranose moiety of 5-*O*-α-D-glucopyranosyl-α-D-fructopyranose 5-*O*-α-D-glucopyranosyl-β-D-fructopyranose 1,2′:2,1′-dianhydride dodecaacetate[8], in agreement with glucosylation sites at C-6 of Fru*f* and at C-5 of Fru*p* in **1**. The postulated structures **15**, **17**, and **19** have now been precisely confirmed by unequivocal synthesis[1] starting from maltulose and D-fructose (for **15** and **19**) and from palatinose and leucrose (for **17**).

Fraction 5 (11.3%) contained only pseudotetrasaccharidic material, as seen from its FAB-mass spectrum. The major substructure α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride was furthermore suggested by the two sets of signals in the anomeric region at ~101.6–101.3 and ~95.0 ppm in its ^{13}C NMR spectrum (Fig. 6). A distinct signal at ~99.3 ppm, relevant to C-2′ of an α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride substructure[6] was also noticed. Further purification of this material resulted in pure samples of the 6,4′-diglucosyl fructofuranose fructopyranose dianhydride **21**, the 6,6′-diglucosyl difuranose isomer **23** and a still impure material which could have an overall 6-diglucosyl structure such as **25**.

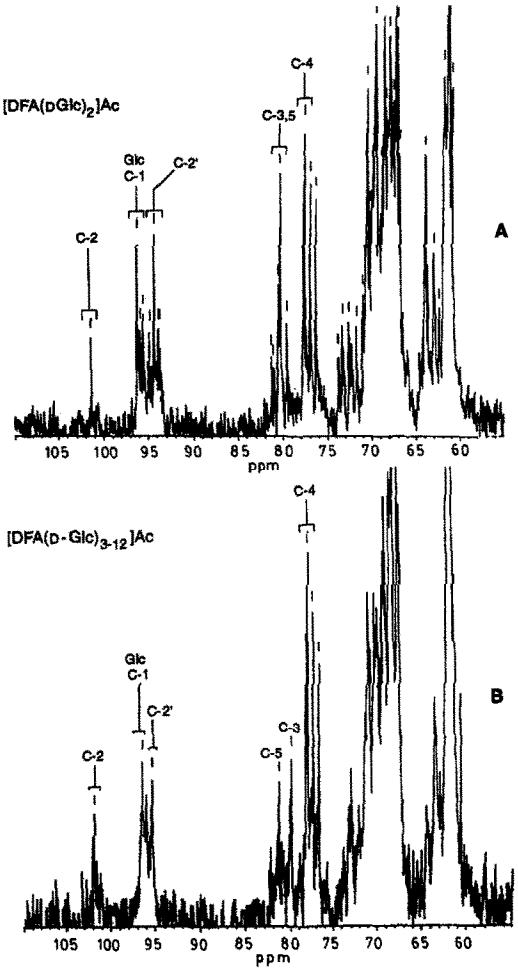


Fig. 7. Partial ^{13}C NMR spectra (50.3 MHz, $CDCl_3$) of: (A) The major constituent of Fraction 5 to which structure **25** is tentatively assigned; (B) Fraction 6.

The structure of **23** was easily confirmed by correlation of its physical and spectroscopic properties with data for the peracetate of 6-*O*-α-D-glucopyranosyl-α-D-fructofuranose 6-*O*-α-D-glucopyranosyl-β-D-fructofuranose 1,2′ : 2,1′-dianhydride, the main product of protonic activation of palatinose[1,8].

The structure assigned to **21** was based on a comparison of ^{13}C NMR data for the pseudotrisaccharides **9** and **19**, and this hypothesis was further confirmed by comparison with an authentic sample of 6-*O*-α-D-glucopyranosyl-α-D-fructofuranose 4-*O*-α-D-glucopyranosyl-β-D-fructopyranose 1,2′ : 2,1′-dianhydride peracetate obtained[1] from the HF catalysed reaction of maltulose and palatinose.

From the signal multiplicity of its ^{13}C NMR spectrum (Fig. 7A) the residual material from Fraction 5, to which the structure **25** is tentatively assigned, was

obviously still a mixture, and attempts to resolve it did not succeed. In the resonance range for anomeric carbon atoms, two groups of signals at ~ 101.5 and 95.0 ppm indicative of an almost exclusive α-D-fructofuranose β-D-fructopyranose 1,2′ : 2,1′-dianhydride substructure were clearly seen, as well as a set of closely related signals at ~ 96.0 ppm which could be assigned to C-1 of α-D-linked glucopyranosidic residues. In the higher field region for furanose carbon atom resonances, the absence of signal in the range 85.0–83.5 ppm precluded any substitution at C-3 or C-4 of the furanose moiety. Since structures such as pseudotetrasaccharides **17** and **21** have already been found, the remaining possible structural assignments for the main component of this pseudotetrasaccharide mixture could either be **25** or a 3′,6-diglucosylated positional isomer. It should be stressed however that the latter structure looks rather improbable in view of the lower stability reported for 3-*O*-α-D-glucopyranosyl D-fructopyranose derivatives in these reaction conditions[1,8].

Fraction 6 (45% of the total material resulting from the action of HF on sucrose) contained the higher molecular weight oligosaccharidic components, with sequential cationized pseudomolecular ions in its FAB-mass spectrum ranging from *m/z* 1463 to *m/z* 4056 at intervals of 288 mass units. Its ^{13}C NMR spectrum (Fig. 7B) showed much similarity with that of **25**, showing on the one hand two sets of signals at 101.5 and 95.0 ppm confirming a basic α-D-fructofuranose β-D-fructopyranose 1,2′ : 2,1′-dianhydride substructure and, on the other hand, a rather tight multiplet at ~ 96.0 ppm pertaining to α-D-linked glucopyranosyl residues, present in numbers from 3 to 12 as seen from the FABMS. Signals for C-3–5 were also clearly seen, and this rather clean and sharp spectrum for such an oligosaccharide mixture further supports the idea that the components of this fraction could have a common structure comprising an α-D-fructofuranose β-D-fructopyranose 1,2′ : 2,1′-dianhydride framework substituted almost exclusively at C-6 with isomaltooligosaccharide segments. Preponderant formation of α-(1 → 6)-linked glucooligosaccharides was also previously noticed[11] from the treatment, at high concentration, of D-glucose or D-glucose-containing polysaccharides such as cellulose or starch with anhydrous HF.

CONCLUSION

Formation of difructose dianhydrides and glucosylated difructose dianhydrides appears to be a main reaction pathway resulting from the protonic activation of sucrose with anhydrous HF. As could be expected from thermodynamic considerations[5,6–8], α-D-fructofuranose β-D-fructopyranose 1,2′ : 2,1′-dianhydride (**2**) is the main dispirodioxanyl structure or substructure present in the mixture, and glucosidation occurs predominantly at C-6 of the fructofuranose moiety as in **10**, **18**, **22** and presumably in the higher molecular-weight oligosaccharide components (**26** and Fraction 6), but also to a lesser extent at C-3 and C-4. Minor spirodioxanyl species are the thermodynamic 2,1′ : 3,2′-dianhydride **8** and the β,β-dipyranose

species **4** and **14**, as well as the presumably kinetic α,β-dipyranose isomer **6**. Intriguing at first sight is the rather high proportion of 6,6′-diglucosylated α-D-fructofuranose-β-D-fructofuranose 1,2′:2,1′-dianhydride **24**, but this is in keeping with the fact that the difructofuranose dianhydride substructure has always been found as a kinetic product in the protonic activation of hexuloses[6,7]. A tentative explanation would be that glucosidation at a C-6 hydroxymethyl group is a fast and irreversible process, at least in the present reaction conditions where HF is diluted by the carbohydrate substrate. This hypothesis is supported by the reaction of palatinose under similar conditions[1], which results almost exclusively in the pseudotetrasaccharide **24**.

The reaction of sucrose in anhydrous HF is then believed to proceed through initial selective protonic activation of the tertiary anomeric carbon atom of the fructose moiety, resulting in the quantitative formation of difructose dianhydrides, which subsequently suffer electrophilic substitution by glucopyranosyl oxocarbenium ions generated in a second step by action of the HF.

Difructose dianhydrides have already been found as byproducts in the industrial processing of fructose syrups from corn wet milling, and they have been proposed as a cause of decreases in crystallisation yields[12]. Difructose dianhydrides are also present in inverted syrups resulting from the action of either HCl or invertase on sucrose[13]. It is likely that glucosylated difructose dianhydrides might be produced, in addition, in these technical processes, and more generally in processes which involve the heat treatment of acidified sucrose-containing materials, at least when the sucrose component is present at high concentration.

EXPERIMENTAL

Materials and methods. — Anhydrous HF was a commercial product obtained in steel cylinders. Prior to use, it was distilled and kept in polyethylene bottles at −25°C. Acetylation was conventionally effected with 1:1 pyridine–Ac_2O as the reagent. For the reaction product resulting from HF treatment, advantage was taken of the catalytic effect of HF in the generation of reactive acylium ions[14], and the acetylating mixture was simply added directly to the product solution in HF.

TLC of peracetates was performed on Silica Gel 60 F_{254} plates (E. Merck), followed by UV light detection and (or) charring with H_2SO_4. Separation experiments were carried out by flash or open column chromatography with the eluent selected by TLC. In several instances, CCl_4–acetone or hexane–EtOAc gradients were used for the separation of positional isomers. LC of unacylated products was performed with a Perkin–Elmer 250 pump fitted to a LiChrosorb RP-18 5-μm (250 × 7.5 mm) column, and an LC-30 refractive index detector. Water was the eluent at 20°C at a flow rate of 1.5 mL/min, after sample injections of 50 μL of 5% (w/v) solutions.

Melting points were determined with a Büchi 535 capillary equipment and are corrected. Optical rotations were measured with a Jobin Yvon Digital Micropo-

larimeter. Microanalyses were obtained from the Service Central de Microanalyse du CNRS in Solaize.

^{13}C NMR spectra were recorded with a Bruker AC-200 instrument, for solutions in $CDCl_3$, using the central peak of the triplet (76.9 ppm) as internal reference. FAB-mass spectra (Cs gun, acceleration potential, 8 kV) were measured in the positive mode with a VG ZAB-SEQ instrument, using *m*-nitrobenzyl alcohol as a liquid matrix. NaI was usually added as a cationizing agent.

Reaction of sucrose in anhydrous HF. — To sucrose (4 g) in a capped polyethylene bottle cooled in a dry ice–acetone bath in order to avoid initial heating, HF (2 mL) was added and the pasty mixture was vigorously stirred with a steel spatula till a homogeneous stiff solution was obtained (~ 5–10 min). The polyethylene bottle was then closed and the mixture was stored at room temperature for 1 h. It was then cooled (dry ice–acetone bath) and 1:1 pyridine–Ac_2O (40 mL) was added. The resulting solution was kept at room temperature overnight and then poured into ice–water (1.5 L) yielding a white precipitate which was filtered, washed abundantly with cold water, and then dried over P_4O_{10} to give a white powder (6 g).

Processing and characterization of the sucrose–HF product. — The crude acetylated mixture arising from the treatment of sucrose in HF followed by acetylation (6 g) was subjected to column chromatography, with elution successively by 2:1, 1:1, 1:1.5, and 1:2 hexane–EtOAc. The separation was monitored by TLC using successive elutions with 1:1 and 1:1.5 hexane–EtOAc. Five fractions (Fractions 1–5) were collected. The column was then eluted with EtOAc and finally with acetone until recovery of the whole starting material was accomplished (Fraction 6). The composition of Fractions 1–6 was assessed by comparison of the intensity of the C-2, C-2′ resonances of the fructosyl residues in ^{13}C NMR spectroscopy. When signal overlapping made this approach not possible, signal intensities from the fructofuranose carbon atoms were considered. In several instances, integration of LC chromatograms was used.

Fraction 1 (0.14 g, 2.3%) crystallized from EtOH yielding 3,4,5,3′,4′,5′-hexa-*O*-acetyl-di-β-D-fructopyranose 1,2′:2,1′-dianhydride (**3**, 0.11 g, 78%); mp 269–270°C; $[\alpha]_D^{20}$ – 194° (*c* 1, $CHCl_3$); lit.[6] mp 269–270°C, $[\alpha]_D$ – 195° (*c* 1.1, $CHCl_3$); lit.[15] mp 267–269°C, $[\alpha]_D$ – 199° ($CHCl_3$).

Fraction 2 (1.12 g, 18.7%) showed a main spot and some minor components on TLC (2:1 then 1:1 hexane–EtOAc in successive elutions). Column chromatography with successive elutions by 2:1, 1.5:1, and 1:1 hexane–EtOAc led successively to residual **3** (22 mg, 2%) and then to:

1,4,6-Tri-O-acetyl-β-D-fructofuranose 3,4,5-tri-O-acetyl-β-D-fructopyranose 2,1′:3,2′-dianhydride (**7**, 45 mg, 4%); mp 121–123°C (EtOH); $[\alpha]_D$ – 85° (*c* 1, $CHCl_3$); lit.[6] mp 122–123°C, $[\alpha]_D$ – 86° (*c* 1.4, $CHCl_3$);

3,4,5-Tri-O-acetyl-α-D-fructopyranose 3,4,5-tri-O-acetyl-β-D-fructopyranose 1,2′:2,1′-dianhydride (**5**, 45 mg, 4%); mp 171–173°C (EtOH); $[\alpha]_D^{20}$ – 55° (*c* 1,

$CHCl_3$); lit.[6] mp 171–173°C, $[\alpha]_D^{20}$ – 57° (*c* 3.5, $CHCl_3$); lit.[16] mp 171–173°C, $[\alpha]_D$ – 59.1° (*c* 1.02, $CHCl_3$), and:

*3,4,6-Tri-*O*-acetyl-α-*D*-fructofuranose 3,4,5-tri-*O*-acetyl-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**1**, 0.56 g, 50%); mp 124–125°C, $[\alpha]_D^{20}$ – 38° (*c* 1.4, $CHCl_3$); lit.[6] mp 124–125°C, $[\alpha]_D$ – 38° (*c* 1.4, $CHCl_3$); lit.[17] mp 123–124°C, $[\alpha]_D$ – 41.5 ($CHCl_3$).

Fraction 3 (0.52 g, 8.7%) showed two major spots and a minor one in TLC (4:1 CCl_4–acetone, several successive elutions). Column chromatography with elution first by 4:1 then 3:1 CCl_4–acetone yielded successively:

*4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-fructofuranose 3,4,5-tri-*O*-acetyl-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**11**, 100 mg, 20%); syrup; $[\alpha]_D^{20}$ + 36° (*c* 1.0, $CHCl_3$); lit.[8] $[\alpha]_D^{20}$ + 36° (*c* 0.9, $CHCl_3$);

*3,4,3′,4′,5′-Penta-*O*-acetyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-di-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**13**, 21 mg, 4%); syrup; $[\alpha]_D^{20}$ – 20° (*c* 1, $CHCl_3$); lit.[8], $[\alpha]_D^{20}$ – 20° (*c* 1, $CHCl_3$), and:

*3,4-Di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-fructofuranose 3,4,5-tri-*O*-acetyl-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**9**, 78 mg, 15%); syrup; $[\alpha]_D^{20}$ + 60° (*c* 1.2, $CHCl_3$); lit.[8] $[\alpha]_D^{20}$ + 60° (*c* 1.2, $CHCl_3$).

Fraction 4 (0.48 g, 8%) showed three spots in TLC (4:1 CCl_4–acetone, several successive elutions). Column chromatography with elution first by 4:1, then 3:1 CCl_4–acetone yielded, besides residual **9** (48 mg, 10%) emerging first, successively:

*3,4-Di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-fructofuranose 3,4-di-*O*-acetyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**17**, 24 mg, 5%) as a syrup; $[\alpha]_D^{20}$ + 55° (*c* 1.0, $CHCl_3$); lit.[1] $[\alpha]_D^{20}$ + 55° (*c* 1, $CHCl_3$); and a mixture of **15** and **19** (0.1 g) which was deacetylated (Zemplén) to **16** and **20**. Semi-preparative LC of this material and conventional reacetylation yielded both:

*3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-fructofuranose 3,4,5-tri-*O*-acetyl-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**15**, 36 mg, 8% relative to Fraction 4); syrup; $[\alpha]_D^{20}$ + 40° (*c* 1.2, $CHCl_3$); lit.[1] $[\alpha]_D^{20}$ + 40° (*c* 1.2, $CHCl_3$) and:

*3,4,6-Tri-*O*-acetyl-α-*D*-fructofuranose 3,5-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-β-*D*-fructopyranose 1,2′:2,1′-dianhydride* (**19**, 24 mg, 5%); syrup; $[\alpha]_D^{20}$ 0° (*c* 1.0, $CHCl_3$; lit.[1] $[\alpha]_D^{20}$ 0° (*c* 1.0, $CHCl_3$).

Fraction 5 (0.68 g, 11.3%) showed two spots in TLC (4:1, then 3:1 CCl_4–acetone in successive elutions). Column chromatography with elution by 4:1, then 3:1 CCl_4–acetone gave successively a mixture (0.3 g, 44%) of **21** and **23**, and then **25** (0.33 g, 49%), which showed a diffuse spot in TLC (3:1 CCl_4–acetone). Further column chromatography of the fastest running fraction with elution first by 4:1, then 3.5:1 CCl_4–acetone yielded successively:

*3,4-Di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-fructofuranose 3,5-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-β-*D*-fructopyra-*

nose 1,2′:2,1′-dianhydride (**21**, 54 mg, 8%); syrup; $[\alpha]_D^{20}$ + 76° (*c* 1, $CHCl_3$); lit.[1] $[\alpha]_D^{20}$ + 76° (*c* 1, $CHCl_3$) and:

3,4-Di-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-α-D-fructofuranose 3,4-di-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-β-D-fructofuranose 1,2′:2,1′-dianhydride (**23**, 81 mg, 12%); mp 186–188°C (ether); $[\alpha]_D^{20}$ + 96° (*c* 0.5, $CHCl_3$); lit.[8] mp 187–188°C, $[\alpha]_D^{20}$ + 96° (*c* 0.5, $CHCl_3$).

The tetrasaccharide mixture **25**, isolated as a syrup, was characterized only by its ^{13}C NMR spectrum (Fig. 7A).

Fraction 6 (2.7 g, 45%), isolated as a syrup, was characterized by its ^{13}C NMR spectrum (Fig. 7B) and by FABMS: *m/z* 4056 (2%, $[DFA(Glc)_{12}Ac + Na]^+$), 3768 (3, $[DFA(Glc)_{11}Ac + Na]^+$), 3480 (10, $[DFA(Glc)_{10}Ac + Na]^+$), 3192 (9, $[DFA(Glc)_9Ac + Na]^+$), 2904 (40, $[DFA(Glc)_8Ac + Na]^+$), 2616 (45, $[DFA(Glc)_7Ac + Na]^+$), 2328 (60 $[DFA(Glc)_6Ac + Na]^+$), 2039 (83, $[DFA(Glc)_5Ac + Na]^+$), 1752 (83, $[DFA(Glc)_5Ac + Na]^+$), and 1463 (100, $[DFA(Glc)_4Ac + Na]^+$).

ACKNOWLEDGMENTS

The authors thank the Scientific Division of NATO and the Ministerio de Educación y Ciencia of Spain for postdoctoral fellowships to J.M.G.F.. This work was carried out within the framework, and with the support, of the Groupement Scientifique Béghin-Say-CNRS "Sucrochemistry".

REFERENCES

1 J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 251 (1994) 1–15, preceeding paper.
2 J.M. García Fernández and J. Defaye, *Zuckerindustrie (Berlin)*, 117 (1992) 481–482.
3 Yu.A. Knirel, E.V. Vinogradov, and A.J. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.
4 J. Defaye and C. Pedersen, *Zuckerindustrie (Berlin)*, 116 (1991) 271–276; J. Defaye, A. Gadelle, C. Pedersen, *Carbohydr. Res.*, 217 (1991) 51–58.
5 K. Bock, C. Pedersen, J. Defaye, and A. Gadelle, *Carbohydr. Res.*, 216 (1991) 141–148.
6 J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 136 (1985) 53–65.
7 J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 152 (1986) 89–98.
8 J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 273 (1992) 223–247.
9 A. Bouchu, J. Chedin, J. Defaye, D. Lafont, and E. Wong, Fr. demande FR 2,599,040; Wo Pat 87-07, 275 (1987); *Chem. Abstr.*, 109 (1988) 95053a.
10 K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.
11 J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 110 (1982) 217–227.
12 J.D. Chu, L.D. Shian, and K.A. Berglund, *J. Cryst. Growth*, 97 (1989) 689–696.
13 V.S. Velasco and J.F. Dowling, *U.S. Agric. Res. Serv. South., Reg., [Rep.] 1975*, ARS-S-88, pp 138–154; *Chem. Abstr.*, 85 (1976) 34931z.
14 I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 26 (1972) 1938–1946.
15 B. Wickberg, *Acta Chem. Scand.*, 8 (1954) 436–442.
16 H.H. Schlubach and C. Behre, *Justus Liebigs Ann. Chem.*, 508 (1933) 16–24.
17 B. Lindberg and B. Wickberg, *Acta Chem. Scand.*, 7 (1953) 969–973.