ELSEVIER

Carbohydrate Research 265 (1994) 31–39

CARBOHYDRATE RESEARCH

Di-D-fructose dianhydrides from the pyrolysis of inulin ☆

Alan E. Blize, Merilyn Manley-Harris, Geoffrey N. Richards *

Shafizadeh Center for Wood and Carbohydrate Chemistry, University of Montana, Missoula, MT 59812, USA

Received 15 March 1994; accepted in revised form 5 June 1994

Abstract

Inulin was pyrolyzed in air to produce di-D-fructose dianhydrides (DFDAs) in ~26% yield, three of which were identified by MS, NMR, and comparison with literature data. The mass spectra of the per-*O*-trimethylsilyl derivatives of the DFDAs are discussed. A mechanism is proposed for the formation of DFDAs from inulin during pyrolysis.

Keywords: Di-D-fructose dianhydrides; Inulin, pyrolysis of; Fructose dianhydrides, di-D-; Pyrolysis

1. Introduction

A previous study [1] has shown that pyrolysis of inulin gave rise *inter alia* to rather high yields of a mixture of di-D-fructose dianhydrides, one of which was tentatively identified; a mechanism was suggested for the formation of two of the possible isomeric dianhydrides. Di-D-fructose dianhydrides are also significant constituents of a sucrose caramel which has been shown to have a beneficial effect upon intestinal microflora [2]. In order to further our ongoing studies of the metabolism of di-D-fructose dianhydrides by intestinal bacteria, we are attempting to characterize the major components of the dianhydride fraction from pyrolysis of inulin.

2. Results and discussion

Inulin was pyrolyzed in an open test tube with trituration at 200°C for 7 min in an oil bath. The highly-colored residual product from this pyrolysis was fractionated by GPC, Fig.

☆ Part IX of the series: Mechanisms of the Pyrolysis of Polysaccharides.

* Corresponding author.

SSDI 0008-6215(94)00224-X

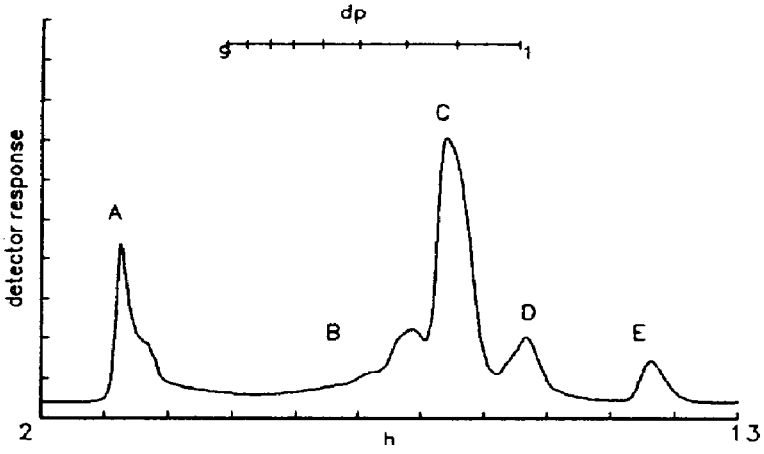


Fig. 1. GPC of inulin thermolysis product on Bio-Gel P-2. Calibration by inulooligosaccharides. (Notations A–E are discussed in the text.)

1. A continuum of polymers and oligomers, A and B, was observed in the GPC trace; these could be fragments of inulin or the products of thermal recombination. The majority of the color was in the region A, which comprises material excluded by the gel. Those portions of the peaks C–E which were amenable to chromatography were identified by LC and by GC–MS of the per-*O*-trimethylsilyl derivatives. Peak C contained di-D-fructose dianhydrides and a trace of 2,6-anhydrofructofuranose, D contained glucose and fructose in similar amounts. Peak E was shown to be 5-hydroxymethyl-2-furaldehyde.

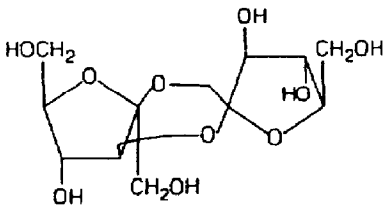
GC–MS of the per-*O*-trimethylsilylated pyrolysis product revealed a cluster of 5 major and several minor peaks whose retention times and mass spectra were consistent with di-D-fructofuranose dianhydrides (~26%, based on inulin). These constituted a major part of the products amenable to GC; 2,6-anhydro-D-fructofuranose (~1%), glucose (~4%), and fructose (~3%) were also present. A cluster of trisaccharides (~15%) was observed.

Table 1
Partial mass spectra of per-*O*-trimethylsilylated **1**, **2**, and **3**

m/z	Abundances (%)		
	1	**2**	**3**
73	100	100	100
103	18	20	14
147	12	13	16
204	0.6	1	1
217	54	23	100
271	2	14	2
361	7	22	5
362	2	8	8
473	0.4	4	0.3
509	6	1	20
563	0.6	9	0.3
653	12	66	0.4
741	0.1		

A fraction containing predominantly trisaccharides with some disaccharides was subsequently isolated by GPC. Mild hydrolysis of this fraction followed by reduction and per-*O*-trimethylsilylation of the products revealed, by GC–FID and GC–MS, the presence of fructose and di-D-fructose dianhydrides in the ratio 1:1.4. The profile of di-D-fructose dianhydrides thus obtained was almost identical with that found in fraction C. This indicates that oligosaccharides are forming either by addition of fructosyl residues to already formed di-D-fructose dianhydrides or by cleavage of a di-D-fructose dianhydride plus a fructosyl residue or residues from a polymer chain as discussed later.

Three components, **1**, **2**, and **3**, made up ~75% of the dianhydrides and these were isolated by preparative LC using successive applications of two different column-solvent systems. Component **1** was identified as α-D-fructofuranose-1,2′:2,3′-β-D-fructofuranose (di-D-fructose anhydride III) by comparison of its ^{13}C NMR spectrum with ref. [3] and by methylation analysis.



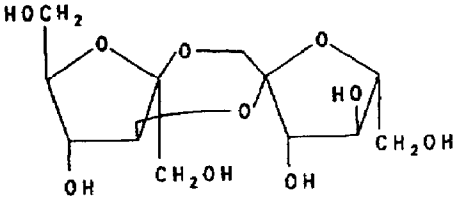
1

Examination of the NMR spectra of **1** indicates that the original tentative assignments of the ^{13}C peaks made in Ref. [3] require considerable amendment. A crystal structure [4] of **1** showed that the central ring was in a skew-boat conformation, with the O-5 and O-5′ substituents essentially axial and with the β-fructofuranose moiety in the unusual $^4T_5$ conformation and the α-fructofuranose in the $^4T_3$ conformation [1]. Examination of a model reveals that the proton H-4′ is brought into fairly close proximity to O-5 of the α-fructofuranose moiety. At the same time, the dihedral angles between the protons on the secondary carbons of both rings approach 140°. It is therefore expected that the ^1H NMR spectrum would show a signal for H-4′ shifted downfield and that all coupling constants between protons on secondary carbons would be ~6–7 Hz. On the basis of this argument a doublet of doublets (1 H) at 4.53 ppm was assigned as H-4′ and a doublet (1 H) coupled to it (*J* 7.1 Hz) at 4.20 ppm was assigned to H-3′. A doublet (1 H) at 3.92 ppm coupled (*J* 13.5 Hz) to another doublet at 3.63 Hz was assigned to the axial (and more compressed) H-1 whilst the upfield signal was that for the equatorial H-1. DQCOSY, one-bond (HMQC) and three-bond (HMBC) ^1H–^{13}C chemical shift correlation experiments were used to assign the remaining ^1H and all ^{13}C resonances.

Methylation analysis of **2** gave a similar result to **1** and the mass spectrum of the per-*O*-trimethylsilyl ether derivative was consistent with a 1,2′:2,3′-linked difructofuranose dianhydride. The optical rotation of **2** indicated that it was probably β-D-fructofuranose-1,2′:2,3′-β-D-fructofuranose dianhydride (difructose anhydride II) the NMR spectrum of which is not in the literature. A crystal structure for this latter compound [5]

[1] For the purposes of this discussion, the atoms of the 2,3-linked fructofuranose moieties in **1** and **2** are distinguished by a prime notation.

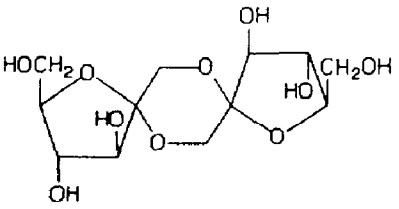
showed that the central 1,4-dioxane ring was in a chair conformation with both furanose ring oxygens axial. The conformation of the 2,3-linked moiety is such that the dihedral angles between H-3′ and H-4′ approach 90° which should give rise to a very small coupling. The dihedral angle between H-4′ and H-5′ also suggest that a weak coupling will exist for this pair of hydrogens. The ^{1}H NMR spectrum of **2** showed a doublet at 3.88 ppm coupled (J 8.3 Hz) to a doublet of doublets at 4.14 ppm. These two signals were assigned to H-3 and H-4, respectively. The absence of a similar pair of signals of the same or larger coupling constant was consistent with the identification of **2** as β-D-fructofuranose-1,2′:2,3′-β-D-fructofuranose dianhydride.



2

A partially resolved doublet at 4.20 ppm and a partially resolved doublet of doublets at 4.10 ppm were assigned to H-3′ and H-4′ respectively. A doublet at 4.04 ppm coupled (J 12 Hz) to a doublet at 3.67 Hz was assigned to the axial H-1 while the upfield signal results from the equatorial H-1. On the basis of a weak coupling between H-4′ and H-5′ a multiplet at 3.98 ppm was assigned to H-5′ and one at 3.91 to H-5. DQCOSY, HETCOR, and COLOC experiments were used to assign the remaining ^{1}H and ^{13}C resonances.

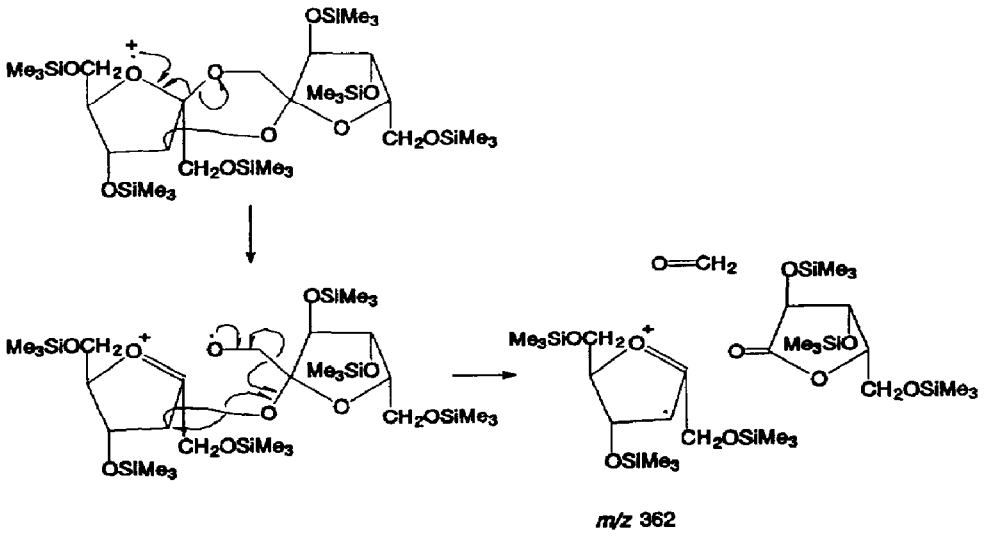
Comparison of **3** by ^{13}C NMR, LC, and GC–MS with an authentic sample [2b] revealed that it was α-D-fructofuranose-1,2′:2,1′-β-D-fructofuranose dianhydride (di-D-fructose anhydride I).



3

The mass spectra of the per-*O*-trimethylsilyl derivatives of these di-D-fructofuranose dianhydrides, which have not been discussed previously, are outlined in Table 1. The spectra of the three methylated derivatives all contained similar relative abundances of analogous ions.

The relative intensities of the ions m/z 204 and 217 indicate that only furanose rings are present [6] and, as would be expected for this type of ring, ions arising from loss of substituents form an important part of the spectra [7]. The decreased abundance of m/z 217 in the spectra of **1** and **2** arises because of the involvement in linkage of C-3 of one of the fructose units and because this ion may arise from carbons 1, 2, and 3 or may involve C-3 and C-4 [8]. The molecular ion is 756 and the ion at 653 is $[M - CH_2OSiMe_3]^+$. The prominence of this particular ion in the spectra of **1** and **2** is probably attributable to the fact that three rather than two primary positions are trimethylsilylated. Subsequent eliminations



Scheme 1.

of Me_3SiOH (90) yield ions 563 and 473. We have previously accounted for the ion m/z 362 in the spectra of related compounds [9]. This ion can form by a fragmentation of the central 1,4-dioxane ring and elimination of formaldehyde. In the case of **1** and **2**, this fragmentation can only occur in one direction as only one primary position is involved in the central ring (Scheme 1).

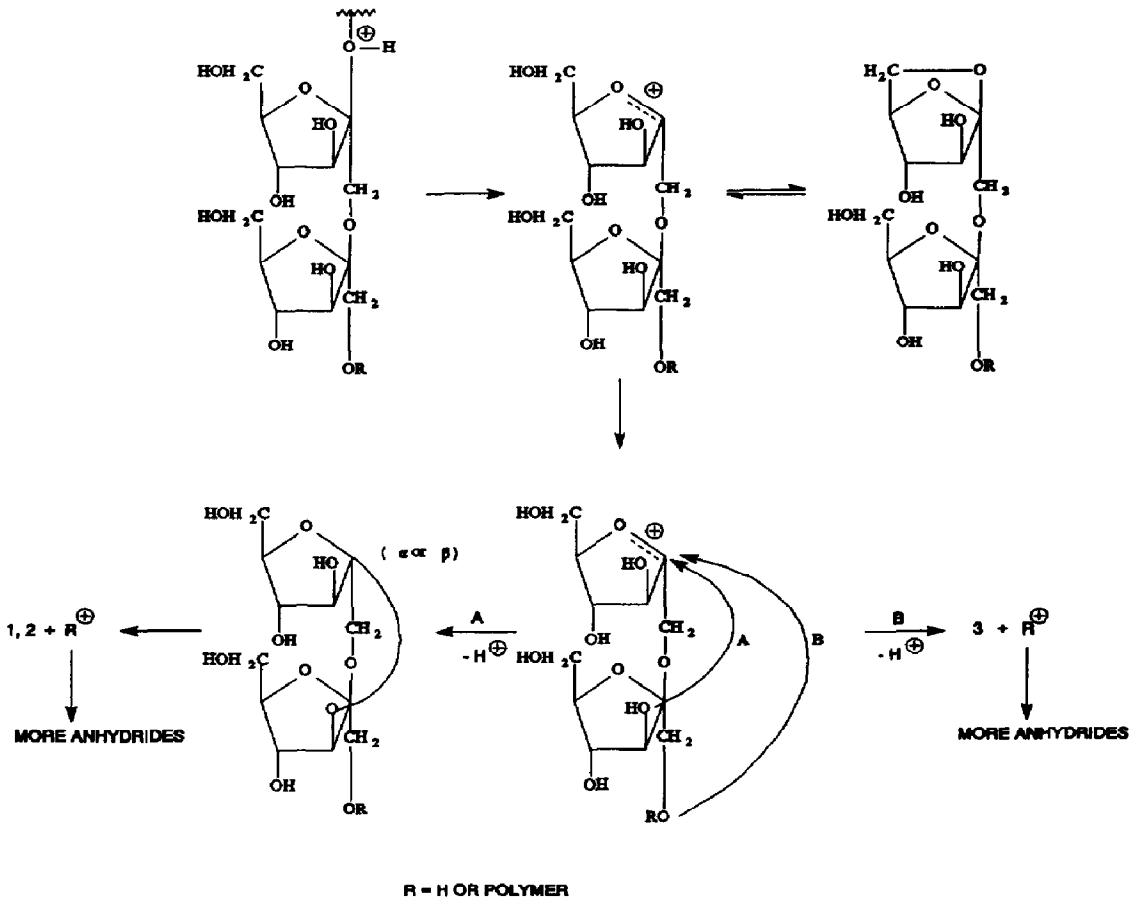
We have previously attributed the presence of the ion m/z 509 to a special case of the formation of a J_1-type ion [9,10]. This type of ion can also form in the case of furanose rings [8] but since it involves a major fragmentation of the ring, it will be less favored than in the dianhydrides containing pyranose moieties. A residue in which C-3 is involved in linkage cannot undergo fragmentation resulting in a J_1-type ion.

The relative abundances of di-D-fructose dianhydride products have been explained by other authors [11–14] in terms of differing reaction conditions permitting more or less equilibration to occur. A survey was made of the ratio of di-D-fructose dianhydrides obtained by the pyrolysis of inulin. The products were assayed as their per-*O*-trimethylsilyl derivatives by GLC–FID, Table 2; the five major peaks were denoted A–E from fastest to slowest

Table 2
Relative abundances of di-D-fructose dianhydride products from inulin pyrolysis

	Peak				
	A (**1**)	B(**2**)	C	D(**3**)	E
t_R [a] (min)	15.7	16.7	16.9	17.3	17.5
Response factor [b]	0.53	0.52	[c]	0.50	[c]
Relative abundance [d]	1.0	0.58	0.27	1.0	0.25

[a] GLC–FID conditions are outlined in the Experimental section.
[b] Measured relative to glucitol as the per-*O*-Me_3Si derivative.
[c] Estimated at 0.52.
[d] Quantified as per-*O*-Me_3Si derivative.



Scheme 2.

running. A, B, and D correspond respectively to **1**, **2**, and **3**; C and E, although they were not isolated, have mass spectra consistent with di-D-fructofuranose dianhydrides. Two small peaks (t_R 17.1 and 17.9 min, relative abundances 0.08 and 0.04) were the only ones to show mass spectral evidence of the presence of furanose-pyranose dianhydrides.

The results in Table 2 are quite different from the mixture of products obtained by the treatment of inulin with anhydrous HF [12], in which the di-D-fructose dianhydride products contained either two or one fructopyranose rings and no difuranose products were observed. Additionally the major products include **1** and **3**, which are not considered to be the most thermodynamically stable of the difuranose dianhydrides [11–14]. This seems to indicate that an equilibrium position has not been attained. It is not possible to investigate this situation further using the current pyrolysis methodology since the high melting point of inulin and the considerable time required for formation of a reactive melt mean that little reliance can be placed upon reproducibility of the thermal history. We are currently investigating the development of more controlled pyrolysis procedures.

Scheme 2 shows an extension of the mechanism that was tentatively postulated in Ref. [1]. Attack by O-1′ or O-3′ upon the C-2 cation occurs within a fructooligosaccharide

Table 3
Results of moderate hydrolysis of a number of per-*O*-methylated di-D-fructose dianhydrides

Per-*O*-methylated substrate	Unhydrolyzed material (%) [a,b]
1	47
2	40
3	5
α-D-Fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride	4
α-D-Fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride	50

[a] Determined by GLC–FID.
[b] Hydrolysis conditions: 1.5% CF_3CO_2H, 60 min, 120°C.

intermediate, which at some unspecified point has cleaved from the polymer chain. Attack by either hydroxyl results in closure of a six-membered ring to form either **1**, **2**, or **3**. Examination of models reveals that attack by the C-3′ hydroxyl resulting in an α-configuration at C-2, as in **1**, will be marginally less hindered than attack resulting in a β-configuration, as in **2**. The formation of polymer or oligomer chains terminating in anhydrofructose is suggested by analogy with the presence of anhydroglucose end-groups found in the pyrolysis of glucose polymers [15]. The presence of such species could possibly be demonstrated by pyrolysis–mass spectrometry; indeed, during the direct chemical ionization mass spectrometry of inulin [16], a series of ions was observed which was interpreted as a series of ammonia adducts of anhydrooligosaccharides. Whether these are in fact due to oligomers terminating in anhydrofructose or to oligomers terminating in di-D-fructose dianhydride units is unknown since the masses would be the same. Examination of the fragmentation of these ions may resolve this.

There are some difficulties attendant upon methylation analysis of DFDAs. These relate to the disparate rates of hydrolysis of the hexa-*O*-methyl DFDAs which may reflect their differing conformational stabilities. Because of the relative lability of fructose derivatives towards acid degradation it is necessary to use the mildest possible conditions for hydrolysis (commensurate with an adequate extent of hydrolysis) in the methylation analysis. Table 3 compares the extents of hydrolysis of a number of per-*O*-methylated DFDAs under conditions of moderate hydrolysis [17]. The results for **1**, **2**, and **3** concur with those obtained by other authors [18]. Another problem related specifically to the hydrolysis of per-*O*-methylated **1** and **2**. For reasons that are still unclear the expected tri-*O*-methylfructoses are not found in a 1:1 ratio; there is significantly less of the 1,4,6-tri-*O*-methylfructose than of the 3,4,6-tri-*O*-methylfructose.

3. Experimental

NMR spectroscopy.—Routine ^1H and ^{13}C NMR spectra were recorded in D_2O at 300 or 75 MHz, respectively, by courtesy of Ribi Immunochem Research, Hamilton, MT. ^1H,^{13}C one dimensional spectra and DQCOSY, HMQC, and HMBC experiments on **1** were carried out by Dr. S. Cheatham of Varian Associates at 400 or 100 MHz. ^1H NMR and DQCOSY of **2** were measured by Dr. A. Sopchik of the University of Utah at 500 MHz. HETCOR and COLOC experiments upon **2** were carried out by Dr. P. Stone of Bruker Instruments

Inc. at 400 or 100 MHz. All chemical shifts are expressed in ppm downfield from external Me_4Si.

Liquid chromatography.—Preparative LC was carried out using three Waters 25 mm × 10 cm Delta-Pak radial compression cartridges in series with H_2O as eluant (10 mL/min). This permitted the separation of two fractions the first containing **1** and **2** and the second containing **3**. Separation of **1** and **2** was achieved by use of a Waters 8 mm × 10 cm μ-Bondapak NH_2 radial compression cartridge eluted with 4:1 MeCN–H_2O at 4 mL/min.

Gas chromatography and mass spectrometry.—GC–FID of trimethylsilyl ether derivatives was carried out using a Hewlett–Packard Ultra 2 (25 × 0.33 mm) cross-linked phenyl methyl silicone fused silica capillary column. Conditions used were 55°C for 1 min, 30°C/min to 220°C, and 10°C/min to 320°C. Glucitol was used as an internal standard. GC–MS was carried out using a Hewlett–Packard Ultra 1 (25 × 0.2 mm) cross-linked methyl silicone fused silica capillary column with direct interface to a Hewlett–Packard 5970 mass spectrometer (70 eV). Conditions used were as above.

Inulin pyrolyses.—Inulin (Sigma, from dahlia tubers) was triturated with a glass rod in an open test-tube while heating in an oil bath at 200°C ± 1°C. After 1 min, the sample formed a white paste or dough which darkened and softened until at 6 min it became a pale-brown fluid. At 7 min considerable foaming and darkening occurred, at which point the sample was removed and cooled. The contents of the tube were almost completely soluble in MeOH. The MeOH solution was taken to dryness (< 40°C).

Mild hydrolysis of the trisaccharide fraction.—A GPC fraction containing trisaccharides and traces of disaccharides was hydrolyzed with HOAc (1 M) at 90°C for 90 min. The hydrolysate was blown dry, reduced ($NaBD_4$), and per-*O*-trimethylsilylated for GC–FID and GC–MS.

Characterization of **1**, **2**, *and* **3**.—These were isolated by LC as described above. Methylation analyses with borodeuteride reduction yielded only 1,2,5 tri-*O*-acetyl-3,4,6-tri-*O*-methyl-2(^{2}H)alditol from **3** and the same residue plus 2,3,5-tri-*O*-acetyl-1,4,6-tri-*O*-methyl-2(^{2}H)alditol from **1** and **2**.

By comparison of the ^{13}C NMR spectrum with Ref. [3], **1** was assigned the identity α-D-fructofuranose-1,2′:2,3′-β-D-fructofuranose dianhydride (di-D-fructose anhydride III); $[\alpha]_D^{20}$ +135.2° (*c* 4.5, D_2O); lit. [19] $[\alpha]_D^{20}$ +138° (*c* 1.12, H_2O). ^{13}C NMR (D_2O): δ 104.6 (C-2), 102.3 (C-2′), 82.2 (C-3), 80.0 (C-3′), 76.4 (C-4), 73.3 (C-4′), 82.9 (C-5), 81.2 (C-5′), 60.0 (C-1), 64.5 (C-1′), 62.1 (C-6), 61.6 (C-6′). ^{1}H NMR (D_2O): δ 3.92 (d, 1 H, $J_{1ax,1eq}$ 13.5 Hz, H-1*ax*), 3.63 (d, 1 H, $J_{1ax,1eq}$ 13.5 Hz, H-1*eq*), 3.57 (d, 1 H, $J_{1',1}$ 12.2 Hz, H-1′), 3.51 (d, 1-H, $J_{1',1}$ 12.2 Hz, H-1′), 4.02 (d, 1 H, $J_{3,4}$ 4.7 Hz, H-3), 4.20 (d, 1 H, $J_{3',4'}$ 7.1 Hz, H-3′), 3.76 (m, 2 H, H-4, H-6′), 4.53 (d of d, H-4′), 3.97 (m, 1 H, H-5), 3.68 (d of d, 1 H, H-6), ~3.57 (H-5′), ~3.53 (H-6), ~3.62 (H-6′).

Compound **2** was similarly assigned the identity β-D-fructofuranose-1,2′:2,3′-β-D-fructofuranose dianhydride (di-D-fructose anhydride II); $[\alpha]_D^{20}$ +13.8° (*c* 1.4, D_2O); lit. [20] $[\alpha]_D^{20}$ +13.85° (*c* 8.993, H_2O). ^{13}C NMR: δ 98.3 (C-2), 104.0 (C-2′), 77.4 (C-3), 73.6 (C-3′), 74.5 (C-4), 76.3 (C-4′), 81.5 (C-5), 85.0 (C-5′), 62.6 (C-1), 63.7 (C-1′), 62.9 (C-6), 62.5 (C-6′). ^{1}H NMR: δ 4.04 (d, 1 H, $J_{1ax,1eq}$ 12 Hz, H-1*ax*), 3.67 (d, 1 H, $J_{1ax,1eq}$ 12 Hz, H-1*eq*), ~3.72 (H-1′), ~3.75 (H-1′), 3.88 (d, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 4.20 (1 H, H-3′), 4.14 (d of d, 1 H, H-4), 4.10 (1 H, H-4′), 3.91 (m, 1 H, H-5), 3.98 (m, 1 H, H-5′), 3.80 (m, 1 H, H-6), ~3.68 (H-6), 3.77 (m, 2 H, H-6′, H-6′).

The ^{13}C NMR spectrum of **3** and the LC and GC retention times were identical with an authentic sample of α-D-fructofuranose-1,2′:2,1′-β-D-fructofuranose (di-D-fructose anhydride I); $[\alpha]_D^{20}$ +27.3° (*c* 2.2, D_2O) ; lit. [21] $[\alpha]_D$ +27.6° (*c* 7.5, H_2O).

Acknowledgements

The authors thank Dr Herman van Halbeek of the Complex Carbohydrate Research Center at The University of Georgia for helpful advice. AEB was supported by a Watkins Scholarship from the University of Montana, which also provided financial support for the project.

References

[1] G.R. Ponder and G.N. Richards, *Carbohydr. Res.*, 244 (1993) 341–359.
[2] (a) M. Manley-Harris and G.N. Richards, *Zuckerindustrie*, in press; (b) M. Manley-Harris and G.N. Richards, *Carbohydr. Res.* 254 (1994) 195–202.
[3] T. Uchiyama, *Carbohydr. Res.*, 101 (1982) 138–140.
[4] T. Taniguchi and T. Uchiyama, *Carbohydr. Res.*, 107 (1982) 255–262.
[5] T. Taniguchi, M. Sawada, T. Tanaka, and T. Uchiyama, *Carbohydr. Res.*, 177 (1988) 13–20.
[6] S. Karady and S.H. Pines, *Tetrahedron*, 26 (1970) 4527–4536.
[7] C.-K. Lee, *Org. Mass Spectrom.*, 22 (1987) 553–556.
[8] N.K. Kochetkov and O.S. Chizhov, *Adv. Mass Spectrom.*, 7 (1978) 1371–1375.
[9] M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 226 (1992) 327–330.
[10] N.K. Kochetkov and O.S. Chizhov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
[11] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 174 (1988) 323–329.
[12] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 136 (1985) 53–65.
[13] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 152 (1986) 89–98.
[14] J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 237 (1992) 223–247.
[15] G.R. Ponder and G.N. Richards *Carbohydr. Res.*, 208 (1990) 93–104.
[16] J.O. Metzger and E. Bruns-Weller *Rapid Commun. Mass Spectrom.*, 6 (1992) 143–146.
[17] M. Manley-Harris and G.N. Richards *Carbohydr. Res.*, 240 (1993) 183–196.
[18] E.J. McDonald and R.F. Jackson *J. Res. Natl. Bur. Stand.*, 24 (1940) 182–204.
[19] T. Uchiyama *Agric. Biol. Chem.*, 47(2) (1983) 437–439.
[20] R.F. Jackson and E. McDonald *J. Res. Natl. Bur. Stand.*, 6 (1931) 709–715.
[21] R.U. Lemieux and R. Nagarajan *Can. J. Chem.*, 42 (1964) 1270–1278.