Carbohydrate Research, 48 (1976) 1–11

ISOSUCROSE. DEFINITIVE STRUCTURAL ASSIGNMENT BY SPECTRAL CORRELATION TO α,β- AND α,α-SUCROSE OCTAACETATES

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(Received January 19th, 1976; accepted for publication, February 17th, 1976)

ABSTRACT

Three nonreducing disaccharides containing D-glucosyl and D-fructosyl groups, namely, "isosucrose" octaacetate (**3**), sucrose octaacetate (**4**), and "α,α-sucrose" octaacetate (**5**), give qualitatively identical mass spectra and are, therefore, related as diastereoisomers. Spin-coupling values in the 300-MHz n.m.r. spectra of **3**, **4**, and **5** establish that the D-glucopyranosyl group is present in the $^4C_1$(D) conformation, and that the anomeric configuration of this group is α in **4** and **5**, and β in **3**. In the n.m.r. spectra, the H-4 atom of the D-fructofuranosyl group of **3** and **5** resonates 0.5 p.p.m. upfield of the corresponding signal of **4**, from which it was deduced that both **3** and **5** are α-D-fructofuranosides.

INTRODUCTION

Early attempts[1–5] to synthesize sucrose octaacetate by condensation of (*a*) 1,3,4,6-tetra-*O*-acetyl-D-fructofuranose (**1**) with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**2**), or (*b*) **2** with 1,3,4,6-tetra-*O*-acetyl-D-fructofuranosyl halides[2(a),(b)] were unsuccessful. Although sucrose octaacetate was not obtained, an isomeric disaccharide octaacetate, namely ,"isosucrose" octaacetate (**3**), was isolated (<5% yield) together with numerous other compounds‡. "Saccharose D", isolated by Pictet and

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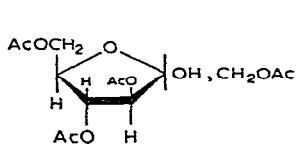
**Based, in part, on the Ph.D. Dissertation of J. D. S., Louisiana State University (Baton Rouge), 1976.
‡Crystalline components isolated[2(b)] were glucose pentaacetate and isotrehalose octaacetate; noncrystalline components included acetylated diglucoses and difructoses, hexose pentaacetates, and at least one additional glucofructose acetate.



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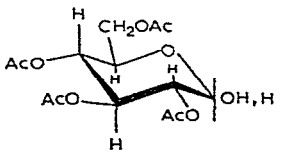

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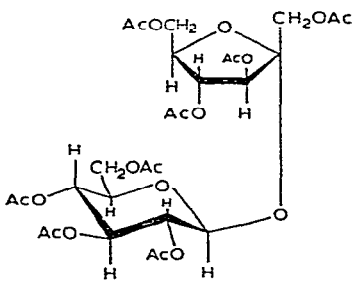
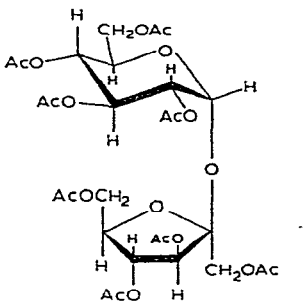
Vogel[6], as well as a compound reported by Schlubach and Middelhoff[7], were probably both impure preparations of 3. Isosucrose has recently been isolated *via* similar condensation procedures[8,9] and characterized as the octaacetate (3).

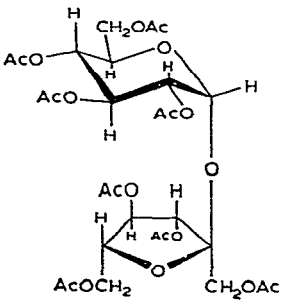
Sucrose has been shown[10] to be α-D-glucopyranosyl β-D-fructofuranoside; the conditions of the condensation reactions were such that isosucrose was presumed to be one of the other three diastereoisomeric disaccharides differing only in anomeric configurations, namely, α,α, β,α, or β,β. The structure accepted for isosucrose[11], namely β-D-glucopyranosyl α-D-fructofuranoside (3), was inferred from the following information: (*a*) the products from the hydrolysis of permethylated isosucrose† are



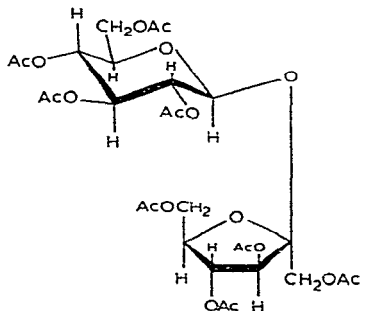
3(β,α)



4(α,β)



5(α,α)



6(β,β)

†Isosucrose was methylated with extreme difficulty; traditional methods afforded mainly hepta-*O*-methylisosucrose, which was further methylated by treatment of the potassium salt with methyl iodide in liquid ammonia[2(e)].

identical to those similarly obtained from octa-*O*-methylsucrose[2(e)]; therefore, they must be isomeric disaccharides differing solely in the configurations of the interglycosidic bond. (*b*) Georg[4] speculated that the anomeric attachment to the D-glucopyranosyl group is β, as higher isolated yields of **3** were obtained when β-**2** (rather than α-**2**, or an equilibrium mixture) was used in the condensation; from this observation, the possibility that the anomeric configuration of the D-fructofuranosyl group is α was rationalized[4] by analogy with the reaction of tetra-*O*-acetyl-D-fructopyranosyl chloride with methanol, which affords methyl tetra-*O*-acetyl-α-D-fructopyranoside, not the usual β-D anomer. (*c*) Isosucrose is unaffected by maltase, invertase, and all of the enzymes in fermenting yeast[4,12]; this observation is inconsistent with an α-D-fructofuranoside linkage. (*d*) The optical rotary values predicted from Hudson's rules of isorotation agree[13] with the experimental values measured for isosucrose, for a second isomer called α,α-sucrose, and for their corresponding octaacetates.

We herein offer direct evidence that isosucrose is, indeed, the β,α isomer[†]. Correlation of n.m.r.-spectral data for **3**, **4**, and **5** also confirms the structural assignment of the α,α isomer.

EXPERIMENTAL

General methods. — Melting points were determined in closed capillary tubes in a Thomas–Hoover Unimelt apparatus, and are uncorrected. Optical rotations were measured with a Schmidt–Haensch polarimeter. Optical rotatory dispersion (o.r.d.) spectra were recorded with a Durrum–Jasco spectropolarimeter, Model J-20. Thin-layer chromatography was performed on Brinkman Silica Gel-HF plates (250 μm, activated for 6 h at 115°); the solvent system used was 9:6:3:1 1-butanol–acetic acid–diethyl ether–water. Nuclear magnetic resonance (n.m.r.) spectra were recorded with a Varian Associates Model HR-300 spectrometer; chemical shifts are given in p.p.m. downfield from tetramethylsilane as the internal standard. Mass spectra were recorded with a Finnigan Model 1015-D electron-impact mass spectrometer at an accelerating potential of 70 eV and a source temperature of 110°, and with a Finnigan Model 3200 chemical-ionization mass spectrometer with methane as the reagent gas at a source pressure of 1 torr. Microanalyses were performed by Mr. R. Seab in these laboratories.

Starting materials. — Inulin acetate, $[\alpha]_D^{20}$ −34° (*c* 1.6, chloroform); lit.[14] $[\alpha]_D^{20}$ −34° (*c* 1.5, chloroform), was prepared from inulin (Calbiochem, Los Angeles, California). 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide [m.p. 88–89° (2-propanol), lit.[15] m.p. 88–89°] was prepared by standard procedures[15].

Sucrose octaacetate (**4**). — Compound **4** was prepared from sucrose by standard procedures[16]: m.p. 87–88°, $[\alpha]_D^{20}$ +60° (*c* 1.5, chloroform) {lit.[16] m.p. 89°, $[\alpha]_D^{20}$ +59.6° (chloroform)}; for the o.r.d. spectrum, see Fig. 1; for the n.m.r. spectrum at 300 MHz, see Table II; for selected m.s. data, see Table I.

[†]The β,β configuration (**6**) has also been proposed for isosucrose [2(b),(d),6].

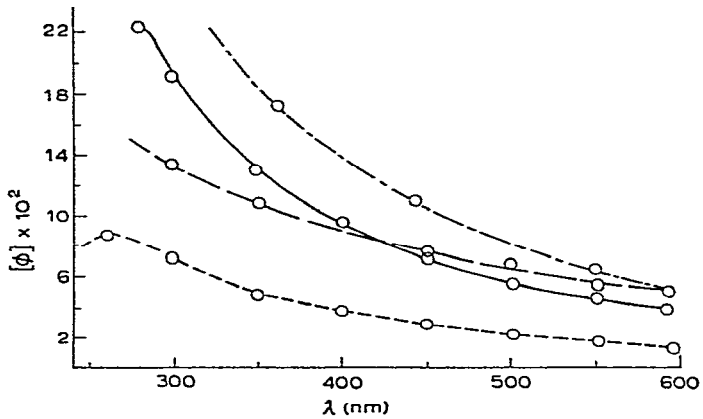


Fig. 1. Optical rotatory dispersion curves of **3** (– – –), **4** (———), **5** (—— ——), and **5** (–––––) [the last set of values obtained from ref. 9] in chloroform.

α,α-Sucrose octaacetate[8] (**5**). — A sample of **5**, generously supplied by Dr. Robert K. Ness of the National Institutes of Health, had m.p. 110–112°, $[\alpha]_D$ +83.5° (*c* 1, chloroform); for the o.r.d. spectrum, see Fig. 1; for the n.m.r. spectrum at 300 MHz, see Table II; for selected m.s. data, see Table I.

2,3,4,6-Tetra-O-*acetyl*-D-*glucopyranose* (**2**). — Water was added dropwise to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (10 g, 24 mmoles) in acetone (50 ml) until the solution became opalescent. Redissolution was accomplished by addition of several drops of acetone, and freshly prepared silver oxide (7 g) was added. The resultant slurry was stirred for 1 h at 0°, filtered with the aid of a Celite pad, and washed with acetone. The filtrate and washings were combined and evaporated, the residual colorless syrup was extracted with benzene (2 × 100 ml), and the extract was washed with water. The aqueous washings were combined, extracted with chloroform (10 × 50 ml), and the extracts were combined, dried (anhydrous magnesium sulfate), and evaporated to afford 8.4 g (99%) of the viscous tetraacetate **2**, $[\alpha]_D^{20}$ +134° (*c* 1.2, chloroform); lit.[17] $[\alpha]_D^{20}$ +135.6 ±0.4° (chloroform).

1,3,4,6-Tetra-O-*acetyl*-D-*fructofuranose* (**1**). — To a stirred solution of inulin acetate (10 g) in glacial acetic acid (100 ml) and acetyl bromide (20 ml) was added a 32% (w/w) solution of hydrogen bromide in acetic acid (10 ml). The solution was stirred for 3 h at room temperature, poured onto ice (100 g), and diluted with a solution of sodium acetate (10 g) in water (1.5 liters). Solid sodium hydrogen carbonate (~170 g) was added to ensure a pH of 5.5. The aqueous solution was extracted with chloroform (10 × 100 ml), and the extracts were combined, dried (anhydrous magnesium sulfate), and evaporated *in vacuo*, affording an orange-red syrup that was dissolved in benzene (100 ml) and washed with water (20 × 100 ml); the washings were combined, and extracted with chloroform (10 × 100 ml), and the extracts were combined, dried, and evaporated *in vacuo* to yield 7.2 g of the pale-yellow tetraacetate **1**: b.p. 115° (1 mtorr), $[\alpha]_D^{20}$ +31.3° (*c* 1.5, chloroform).

Both **1** and **2** were used directly in the preparation of **3**, without additional purification.

β,α-(Iso)Sucrose octaacetate (**3**). — *Method A.* A suspension of **1** (2.5 g, 7 mmoles), **2** (2.5 g, 7 mmoles), and phosphorus pentaoxide (1 g) in anhydrous benzene (150 ml) was mechanically agitated. Additional phosphorus pentaoxide (0.5 g) was added, and vigorous agitation was continued for 24 h. The solution was carefully decanted from the black residue, washed consecutively with water (20 × 100 ml), aqueous sodium hydroxide (1%, 2 × 50 ml), and water (2 × 50 ml), dried (anhydrous sodium sulfate), and evaporated *in vacuo* to afford a light-yellow syrup (900 mg), which was dissolved in ether and kept at −10°. The solid that gradually separated was recrystallized from diethyl ether, to afford 300 mg (5.7%) of isosucrose octaacetate, m.p. 129–130.5° (diethyl ether); $[\alpha]_D^{20}$ +20.0° (*c* 1, chloroform), R_F 0.85; lit.[5] m.p. 131–132°, $[\alpha]_D^{27}$ +20.4° (*c* 4.9, chloroform); for n.m.r. data at 300 MHz, see Table II; for the o.r.d. spectrum, see Fig. 1; for selected m.s. data, see Table I.

Method B[18]. A mixture of **1** (3.2 g, 8.3 mmoles), Linde 4A molecular sieve (10 g), silver(II) oxide[19] (3.05 g), and dichloromethane (15 ml) was placed in a black-coated flask. A single crystal of iodine was added, and the mixture was stirred under nitrogen for 1 h. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.1 g, 10 mmoles) in dichloromethane (15 ml) was then added during 15 min, and the mixture was stirred for 12 h at 25°; filtration through a Celite pad, and evaporation of the filtrate afforded a complex mixture (6.0 g) containing mainly **1** and **2**. Traces (<1%) of **3** were isolated; m.p. 129–130° (diethyl ether), $[\alpha]_D^{27}$ +19.1° (*c* 1.3, chloroform); R_F 0.85.

β,α-(Iso)Sucrose. — A solution of **3** (204 mg, 299 μmoles) in 0.02M methanolic sodium methoxide (20 ml, 0.40 meq) was stirred[20] for 24 h at 25°. The solution was made neutral with an excess of Dry Ice (~2 g) and evaporated *in vacuo*. A solution of the residue in distilled water (3 ml) was added to a mixed-bed column[20] of ion-exchangers (1 g each of Amberlite IR-120-H and Amberlite IR-4B), which was washed with distilled water (100 ml) to elute the disaccharide. The eluate was evaporated *in vacuo*, affording 96 mg (94%) of pure β,α-(iso)sucrose: $[\alpha]_D^{27}$ +52.3° (*c* 0.9, methanol); lit.[2(b)] $[\alpha]_D^{27}$ +50.0° (*c* 2.13, methanol).

DISCUSSION

Synthesis of β,α-sucrose ("isosucrose"). — The general synthesis of the octaacetate **3** followed a modified procedure described by Binkley and Wolfrom[5], in which **1** and **2** were condensed in the presence of phosphoric anhydride under an inert atmosphere (see Experimental section). The yields of **3** varied from 1 to 6%, even under seemingly identical reaction-conditions. Alternative procedures, however, afforded **3** in <1% yield and only after tedious separation from the myriad reaction-products. Saponification of **3** under standard conditions afforded almost quantitative yields of isosucrose, which was identical to an original sample[5].

Acetylation of isosucrose with acetic anhydride in anhydrous pyridine regenerated **3**, which was identical in every respect to the initial sample.

Anomeric configurations of the isomeric sucrose octaacetates. — 1. Mass spectrometric studies. Electron-impact mass spectrometry (e.i.m.s.) generally affords structural insight mainly by virtue of extensive fragmentation-processes, whereas chemical-ionization mass spectrometry (c.i.m.s.) is a complementary tool in which more selective fragmentation generally occurs, and prominent, unfragmented ions are often observed. Selected ions of relative intensity >1% in the e.i. and c.i. mass spectra of **3**, **4**, and **5** are presented in Table I. Relative intensities of ions are expressed as a percentage of that of the most abundant ion (base peak, 100%) within that spectrum. Quantitative discrepancies with relative-intensity data reported[21] for **4** increase with increasing mass number of the ions, in accord with the tendency of quadrupole

TABLE I

ELECTRON-IMPACT AND CHEMICAL IONIZATION (METHANE) MASS-SPECTRAL DATA[a] FOR THE D-GLUCOPYRANOSYL D-FRUCTOFURANOSIDE OCTAACETATES **3–5**

m/e	*Relative intensities (% of base peak)*						*Assignment*[b]
	3		**4**		**5**		
	EI[c]	*CI*[d]	*EI*[c]	*CI*[d]	*EI*[c]	*CI*[d]	
679		0.1		0.1		0.1	MH^+
619		0.9		1.2		1.1	$MH^+ - AcOH$
331	8.4	100	4.5	100	7.0	100	Gl^+
289		0.6		0.4		0.7	
275	0.1		0.1		0.1		
271	0.9	3.5	0.5	10.0	0.8	3.1	$Gl^+ - AcOH$
229	0.4	0.7	0.1	0.7	0.3	0.1	$Gl^+ - AcOH - CH_2CO$
228	0.1		0.1		0.1		$C_4H_3(OAc)_2^+$
211	14.3	34.8	6.4	52.6	9.5	57.0	$Gl^+ - 2AcOH$
187	0.4	1.3	0.2	0.5	0.2	0.1	$Gl^+ - Ac_2O - CH_2CO$
186	0.2		0.1		0.1		*m/e* 228 $- CH_2CO$
169	24.5	31.7	12.8	72.0	18.2	62.3	$Gl^+ - Ac_2O - AcOH$
153		1.3		1.3		4.8	
145	1.4	8.2	0.9	2.3	1.1	1.8	Ac_3O^+
139	1.4	1.9	0.6	2.8	0.9	3.0	acetoxypyrilium
127	5.3	13.3	2.4	4.4	3.5	6.1	$Gl^+ - 2Ac_2O$
126	2.0		0.7		1.0		
115	4.5	4.4	1.8	3.5	3.2	4.8	
109	27.6	20.8	12.9	30.9	19.6	37.2	$Gl^+ - Ac_2O - 2AcOH$
103		5.1		1.2		1.8	Ac_2OH^+
101	3.3	2.1	2.1	2.4	2.6	3.0	$OHC\overset{+}{C}HOAc$
97	5.6	[e]	2.6	[e]	4.0	[e]	hydroxypyrilium
85	2.6		0.9		1.8		
81	2.4		0.9		1.6		pyrilium
73	4.2		3.4		3.4		$AcOCH_2^+$
43	100		100		100		Ac^+

[a]Partial data, including all ions above *m/e* ~100 having >0.2% relative intensity. [b]These designations represent probable identities of the corresponding fragments, in accord with accepted principles, as presented in ref. 21; Gl^+ represents a tetra-*O*-acetylhexosyl (Glycosyl) cation. [c]Ionization by electron impact at a nominal energy of 70 eV. [d]Chemical ionization (methane); source pressure = 1 torr. [e]C.i. mass spectra were measured to *m/e* 100.

mass filters to pass lower-mass ions with relatively greater efficiency than magnetic instruments.

As expected, only slight, quantitative differences are found in the mass spectra of the isomeric sucrose octaacetates; this substantiates the thesis that these three compounds are diastereoisomers. Fig. 2 summarizes decompositions that probably account for the major fragment-ions observed. The c.i. (methane) mass spectra of **3**, **4**, and **5** exhibit a weak (<0.1%) proton-capture [MH^+] ion at *m/e* 679 and a slightly more abundant ion (*m/e* 619, ~0.1%) corresponding to the loss of a molecule of acetic acid from MH^+. Cleavage of the C-2–O-2 bond of D-fructofuranosides to form[21] the resonance-stabilized glycosyl cation (Gl^+) appears to be greatly favored, and presumably accounts for the base peak (*m/e* 331), which further decomposes by sequential loss of molecules of acetic acid (2), ketene (1), and acetic acid (1), to generate less-prominent ions at *m/e* 271, 211, 169, and 109, respectively. Such a mode of decomposition by monosaccharide acetates is well documented[21–24].

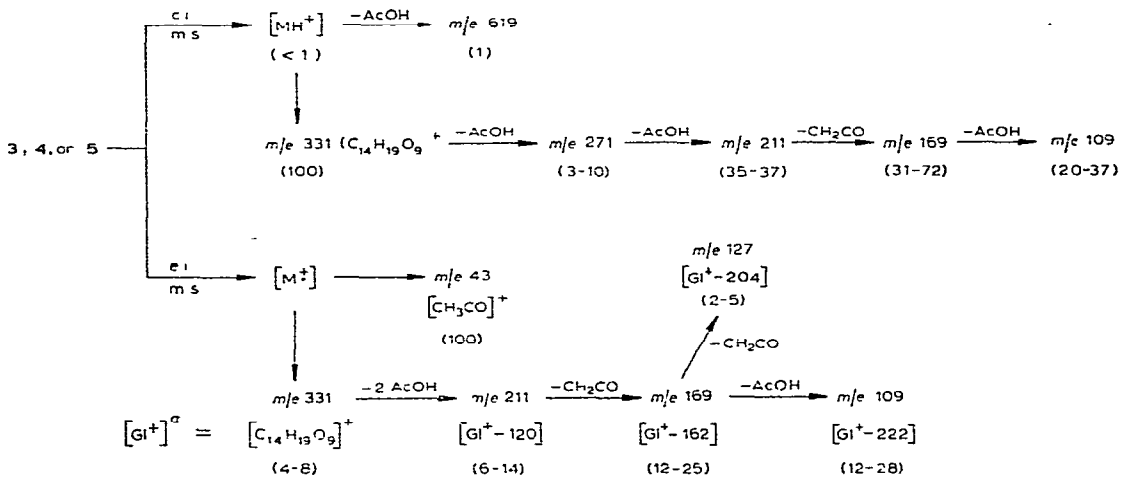


Fig. 2. Generalized scheme of principal mass-spectral fragmentation steps for **3**–**5**. [a]The tetra-*O*-acetylglycosyl radical[21] is represented as Gl.

Similar mass spectra result from e.i. ionization, the principal differences being the relative paucity of higher-mass ions; however, the base peak under these conditions is the acylium ion (*m/e* 43).

2. N.m.r. studies. The n.m.r. spectra of the known, isomeric octaacetates of sucrose have been measured at 300 MHz (see Table II) and, in general, can be analyzed on a first-order basis. N.m.r. spectra reported[25] at 100 and 220 MHz for α,β-sucrose octaacetate (**4**) correspond well with the values presented herein.

A. β,α-Sucrose octaacetate. In the n.m.r. spectrum of **3** in chloroform-*d*, H-2 resonates at δ 5.02 as an apparent triplet in which two vicinal couplings have the same magnitude ($J_{1,2} = J_{2,3} = 10$ Hz). The pattern of two vicinal, diaxial

TABLE II

COMPARATIVE N.M.R.-SPECTRAL DATA FOR METHYLENE AND METHINE PROTON RESONANCES OF SUCROSE OCTAACETATE (4), "ISOSUCROSE" OCTAACETATE (3), AND "α,α-SUCROSE" OCTAACETATE (5), MEASURED AT 300 MHz

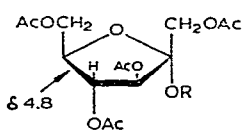
Compound	*Solvent*	*Chemical shifts (δ)*											
		D-*Glucopyranosyl*					D-*Fructofuranosyl*						
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-1*	*H-1'*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*
4	$CDCl_3$	5.70	4.83	5.46	5.06	[a]	[a]	[a]	5.48	5.38	[a]	[a]	[a]
	C_6D_6	5.82	4.99	5.73	5.29	4.47	[a]	[a]	5.68	5.50	[a]	[a]	[a]
	$(CD_3)_2CO$	5.71	4.85	5.44	5.06	[a]	[a]	[a]	5.60	5.41	[a]	[a]	[a]
3	$CDCl_3$	4.96	5.02	5.18	4.98	3.73	3.94	4.35	5.41	4.84	4.57	4.42	4.18
	$(CD_3)_2SO$	4.89	4.81	5.31	5.25	[a]	4.02	4.23	5.21	4.88	4.5[b]	4.38	4.13
5	$CDCl_3$	5.51	5.03	5.53	5.13	[a]	4.05	4.57	5.50	4.82	4.4[b]	4.38[b]	3.90
	C_6D_6	5.47	5.13	5.81	5.38	[a]	3.98	4.79	5.69	4.84	4.45[b]	4.39	3.96
	$(CD_3)_2CO$	5.53	4.97	5.57	5.10	4.4[b]	4.12	4.49	5.46	4.93	4.38	4.43	3.96
		Coupling constants (Hz)											
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$		$J_{1,1'}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	
4	$CDCl_3$	4.0	10.0	10.0	10.0		[a]	5.0	[a]	[a]	[a]	[a]	
	C_6D_6	4.0	10.0	10.0	10.0		[a]	5.0	[a]	[a]	[a]	[a]	
	$(CD_3)_2CO$	4.0	10.0	10.0	10.0		[a]	5.0	[a]	[a]	[a]	[a]	
3	$CDCl_3$	10.0	10.0	9.0	10.0		12.0	2.0	5.0	3.0	5.0	12.0	
	$(CD_3)_2SO$	10.0	10.0	10.0	10.0		12.0	2.0	[a]	3.0	5.0	12.0	
5	$CDCl_3$	3.6	10.0	10.0	10.0		12.0	1.0	3.0	3.0	3.0	10.0	
	C_6D_6	3.7	10.0	10.0	10.0		12.0	1.0	3.0	3.0	2.0	12.0	
	$(CD_3)_2CO$	3.0	10.0	10.0	10.0		12.0	1.0	4.0	4.0	2.0	12.0	

[a]These values could not be extracted from the spectra. [b]These values are approximate.

couplings requires that H-1, H-2, and H-3 be axially disposed on a six-membered ring. This assignment of anomeric configuration (β) and of conformation [$^4C_1$(D)] is verified by the presence of a doublet at δ 4.96 ($J_{1,2}$ 10 Hz). The doublet at lowest field (δ 5.40, J 2 Hz) is assigned to H-3 of the D-fructofuranosyl ring on the basis of its multiplicity. The remaining, low-field signals are observed as doublets of doublets at δ 5.18, 4.98, and 4.84, assigned to H-3 and H-4 of the D-glucosyl group and H-4 of the D-fructosyl group, respectively. The diastereotopic protons on C-1 of the ketose are readily discerned as doublets having a large, geminal coupling ($J_{1,1'}$ 12 Hz).

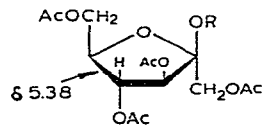
B. α,α-Sucrose octaacetate. The low-field region of the spectrum of **5** in chloroform-*d* can be analyzed on a first-order basis. Doublets at δ 5.51 ($J_{1,2}$ 3.6 Hz) and δ 5.50 ($J_{3,4}$ 1 Hz) are assigned to H-1 of the aldosyl and H-3 of the ketosyl group, respectively. A doublet of doublets at δ 5.03 ($J_{1,2}$ 3.6, $J_{2,3}$ 10 Hz) is assigned to H-2; the coupling values are characteristic of an axially disposed proton vicinally coupled to both an axially and an equatorially attached hydrogen atom[25,26]. Assignments of the remaining chemical shifts and coupling constants are given in Table II.

The data in Table II show that the *anomeric configuration of the* D-*gluco-pyranosyl portion of* **3** *is* β because: (*1*) the large coupling constant observed ($J_{1,2}$ 10 Hz) requires that H-1 and H-2 be antiperiplanar on the D-glucopyranose-$^4C_1$ ring, and (*2*) the strong, upfield shifts ($\Delta\delta$ 0.5–0.7 and 0.3–0.4 p.p.m.) of H-1 and H-3, respectively, for the D-glucopyranosyl portion of **3** relative to the resonance positions of the corresponding protons of **4** and **5** (both of which are α-D-glucopyranosides) confirm that **3** is a β-D-glucopyranoside. Corroboration of the previous[8,9] configurational identification of the D-glucosyl portion of **5** is found in the values of $J_{1,2}$ (3.6 Hz) and $J_{2,3}$ (10 Hz) for this moiety, as the latter indicates the $^4C_1$(D) conformation of the D-glucopyranosyl group, whereas the former shows that H-1 is equatorially attached; because **4** is known[10,20] to be a β-D-fructofuranoside, **5** is necessarily the α-D anomer.



3 R = tetra-*O*-acetyl-β-D-glucopyranosyl

5 R = tetra-*O*-acetyl-α-D-glucopyranosyl



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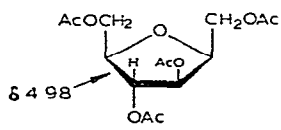

7

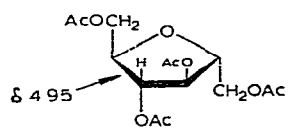

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Fig. 3. Pertinent n.m.r. data for the D-fructofuranosyl groups of compounds **3–5**, **7**, and **8** in chloroform-*d* at 300 MHz.

The anomeric attachment of the D-*fructofuranosyl group in* **3** *may be assigned the* α *configuration* on the basis of the following n.m.r. data. The H-4 resonance of the ketosyl portion of **4** appears at unusually low field (δ 5.38) as a consequence of deshielding resulting from the proximity of the ring-oxygen atom of the D-glucopyranosyl group to this proton; this interaction is possible only when the D-fructofuranosyl group in these sucrose isomers possesses the β-D configuration. Conversely, the corresponding proton of **3** resonates at higher field (δ 4.84), closely approximating the chemical shift (δ 4.82) of H-4 on the α-D-fructofuranoside ring of **5**. Thus, isosucrose is also an α-D-fructofuranoside (see Fig. 3). In order to verify that the D-glucopyranosyl group is a necessary condition for the characteristic deshielding of H-4 in the ketosyl portion of **4**, the n.m.r. spectra[27] of the simplest possible analogues of tetra-*O*-acetyl-α- and -β-D-fructofuranosides, namely, 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-glucitol (**7**) and 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannitol (**8**), were examined. The chemical shifts (δ 4.8–5.0) of H-4 in **7** and **8** are similar to those of **3** and **5**, verifying the singularity of the steric condition in **4**, and thereby confirming the conclusion that the configuration about C-2 of the D-fructofuranosyl group in **3** is the same as that in **5**, and the opposite of that in **4**.

Other solvents (benzene-d_6, Me_2SO-d_6, and acetone-d_6) were used in a quest for better dispersion in the spectrum of the D-fructosyl protons of **3**, **4**, and **5**; little improvement was obtained, and no evidence is present to suggest solvent-dependence of the conformations of **3**, **4**, and **5**.

3. *Enzymic hydrolysis studies.* Isosucrose was incubated with β-D-fructofuranoside fructohydrolase (EC 3.2.1.26) from *Neurospora crassa* under the assay conditions used for sucrose[28]. The enzyme did not release glucose from the disaccharide. Isosucrose was also incubated with β-D-glucosidase (EC 3.2.1.21) from almonds, and α-D-glucosidase (EC 3.2.1.20) from yeast, at the pH optimum of each; neither released glucose from the disaccharide. The inability of the α-D-glucosidase to catalyze hydrolysis of the α-D bond was expected, and the inability of the β-D-glucosidase to hydrolyze the bond is consistent with the marked preference of this enzyme for aryl β-D-glucosides[29]. Isosucrose was hydrolyzed by a crude extract of *Neurospora crassa*. This organism is known to contain both an aryl β-D-glucosidase and a cellobiase[30]. The activities belong to two distinct proteins; hence, the release of glucose from isosucrose is apparently caused by the β-D-glucosidase that has cellobiase activity. These enzymic studies support the conclusions drawn from the spectral studies.

ACKNOWLEDGMENTS

The authors thank Dr. James N. Shoolery (Varian Associates) and Mr. Everett R. Santee (The University of Akron, Institute of Polymer Science) for the 300-MHz n.m.r. spectra, Mr. C. Richard Weisenberger (The Ohio State University) for preliminary mass spectra, Dr. Robert K. Ness (National Institutes of Health) for an authentic sample of "α,α-sucrose" octaacetate, Professor Louis M. Trefonas (Univer-

sity of New Orleans) for attempting a single-crystal, X-ray analysis of 3, Mr. Norman W. Flynn for recording the mass spectra of 3–5, and the Dr. Charles E. Coates Memorial Fund (donated by George H. Coates) of the L. S. U. Foundation for financial aid in the preparation of the dissertation of J. D. S.

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