α-CELLOBIOSE AND α-ISOMALTOSE FROM CRYSTALLINE COMPLEXES WITH SODIUM IODIDE

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ABSTRACT

The crystalline complexes 2NaI·α-cellobiose, NaI·α-cellobiose·$2H_2O$, NaI·α-isomaltose, and NaBr·α-isomaltose have been isolated. Sodium iodide adducts were used to prepare crystalline α-cellobiose (98% α) and amorphous α-isomaltose (90% α), previously inaccessible anomeric forms. The disaccharides and their complexes were analyzed for anomeric content by g.l.c., and characterized by polarimetry and n.m.r. and i.r. spectroscopy. The strong influence of sodium iodide on the optical rotation of a number of carbohydrates suggests that complexation with sodium iodide can affect molecular conformation.

INTRODUCTION

Carbohydrates interact with alkali-metal salts in solution to form adducts whose stability and ease of isolation vary with the solvent and nature of both carbohydrate and salt[1]. Crystalline complexes of alkali-metal salts with monosaccharides, sucrose, and cyclohexaamylose are known[1]; but alkali-metal salt complexes of reducing oligosaccharides have not been reported. Study of complex formation between alkali-metal halides and reducing oligosaccharides has revealed how these complexes can be used to prepare α-cellobiose (4-*O*-β-D-glucopyranosyl-α-D-glucopyranose) and α-isomaltose (6-*O*-α-D-glucopyranosyl-α-D-glucopyranose).

RESULTS AND DISCUSSION

Formation and decomposition of complexes. — A. Cellobiose. Sodium iodide is highly soluble in ethanol, methanol, acetone, 4-hydroxybutanoic 1,4-lactone ("γ-butyrolactone"), and acetonitrile. In these concentrated solutions, the solubility of β-cellobiose is exalted, probably because of complexing with sodium iodide. In dilute sodium iodide solution, where adduct formation would be slight because of weak mass action, the solubility of β-cellobiose is extremely low (solubility in pure ethanol

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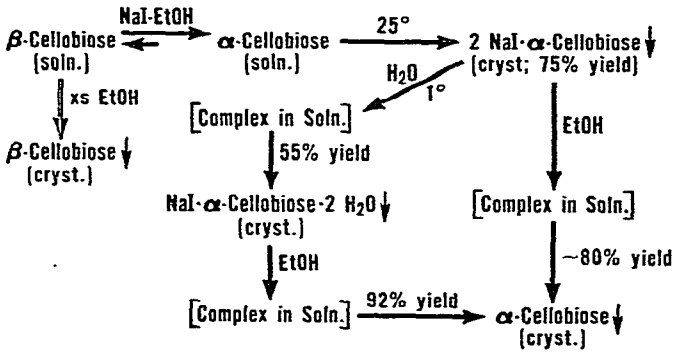
is <0.06% by weight at 20°; <0.3% at 85°). When β-cellobiose is equilibrated in a saturated solution of sodium iodide in ethanol, a high ratio of α to β anomer is produced (Scheme 1). On the one hand, if the saturated sodium iodide solution is



Scheme 1. Solution phenomena in the system cellobiose–NaI.

diluted fivefold with pure ethanol before appreciable anomerization of β-cellobiose has occurred, the solubilizing effect of the salt is lost, and crystalline, uncomplexed β-cellobiose precipitates from solution. On the other hand, if the concentrated solution is not diluted but is kept for several days at 25°, a crystalline 2:1 NaI–α-cellobiose complex is precipitated. This complex is not stable in dilute solution; when it is dissolved in ethanol, slow precipitation of iodide-free, crystalline α-cellobiose soon follows. The free sugar precipitates in 74–81% yields with anomeric purity as high as 96%. The anomeric purity can be increased further (*e.g.*, 96 to 98%) by rapid recrystallization from aqueous ethanol.

α-Cellobiose should be stored in a desiccator because of its tendency to anomerize in a humid atmosphere to the apparently more stable β-form. At 52% relative humidity (r.h.) and 25°, the time for 50% conversion to the β-form is about 70 days. Samples of α(94%)-cellobiose that were stored over calcium chloride for 2 years underwent no conversion.

TABLE I

EFFECT OF REACTION CONDITIONS ON THE GENERATION OF α-CELLOBIOSE FROM 2 NaI·α(94%)-CELLOBIOSE

Solvent	*Wt. of adduct per 100 ml of solvent (g)*	*t* (°C)	*Time* (*h*)	*Iodide-free α-cellobiose*		
				Yield (%)	*M.p.* (°C)	α:β
Methanol	10	1	72	74	216	94:6
Ethanol	0.9	1	20	78	212	96:4
	7.0	25	5	81		95:5
	0.9	40	1.5	33	207	94:6
γ-Butyrolactone	0.8[a]	25	4			92:8

[a]Complete solution of adduct was not obtained.

Table I shows the effect of solvent and temperature on decomposition of the 2:1 adduct to produce free α-cellobiose. Solvent and temperature affect anomeric purity only slightly; however, the lower the temperature, the higher is the purity.

The adduct 2 NaI·α-cellobiose is rapidly converted into crystalline NaI·α-cellobiose·2 H_2O on addition of water. The hydrated 1:1 complex is nonhygroscopic. Like the 2:1 complex, it forms crystalline α-cellobiose in ethanol.

Although acetonitrile and methanol–ether may be used to prepare 2 NaI·α-cellobiose, ethanol is the best solvent and is preferred. Sodium or potassium halides other than sodium iodide do not form isolable complexes with cellobiose, probably because solubilities in nonaqueous solvents are lower (the ease of forming solvates with water and simple alcohols decreases in the order NaI>NaBr>NaCl)[2] and potassium ions do not combine with carbohydrates as easily as sodium ions[1].

B. Isomaltose. Both water and ethanol are excellent solvents for preparing crystalline complexes of isomaltose with sodium iodide and sodium bromide. To provide effective media, nonaqueous systems must contain a high concentration of salt. In aqueous systems, concentration is no problem; a stoichiometric mixture of sugar and salt in water is evaporated to a heavy, viscous syrup, which eventually crystallizes.

Formation of the crystalline sodium bromide complex is slower than that of the sodium iodide complex but stoichiometry is the same; only the 1:1 combining ratio has been observed. Anomeric selectivity occurs in the formation of each complex and leads to a product containing a preponderance of α anomer. Attempts were unsuccessful to prepare crystalline complexes of isomaltose with sodium chloride, sodium acetate, potassium iodide, potassium bromide, potassium chloride, potassium acetate, lithium chloride, lithium iodide, and calcium chloride.

TABLE II

EFFECT OF REACTION CONDITIONS ON THE FORMATION OF NaI·α-ISOMALTOSE

Solvent	*t* (°C)	*Reactant conc. (moles/l)*		*Approx. time for complete crystn.*	*Product*	
		Isomaltose	*NaI*		*% Yield*	*α:β*
Water	25	10	10	1-2 mo	~100	87:13
	45	10	10	⩽2 h	~100	87:13
	55	10	10	⩽2 h	~100	85:15
	70	10	10	<1 h	~100	83:17
EtOH	25	0.3	2.1	6 days	75	93:7
	25	0.07	0.6	6 days	80	86:14
	85	0.1	2.1	<1 day	62	83:17
1-BuOH	85	0.04	1.2	<1 day	78	80:20
	105	0.03	1.1	<1 day	60	74:26
CH_3CN	85	0.05	0.7	<1 day	92	72:28

TABLE III

OPTICAL ROTATION AND ANOMERIC EQUILIBRIA OF CELLOBIOSE AT 20°

Anomeric form used	*Solvent*	*Conc. in moles/l*		$[\alpha]_D^{20}$, *degrees*			*α:β at equil.*[a]	*Approx. time to reach equil.*
		Cellobiose	*NaI*	*α-Cellobiose*	*β-Cellobiose*	*Equil.*		
α	Water	0.12	0	+69.5 (±0.6)[b]	+12.6[b]	+34.7 (±0.3)	39.6:60.4	6 h
β		0.24	0	+68.7[c]	+12.6 (±0.1)[c,d]	+34.7 (±0.1)[d]	[e]	6 h
β		0.09	1.1			+32.5 (±0.1)		6 h
β		0.09	6.6			+23.9 (±0.1)		1 h
α	Me_2SO	0.12	0	+70.5 (±0.3)[b]		+37.3 (±0.3)	46.7:53.3	6 weeks
α		0.12	0.72				48.0:52.0	3 weeks
α		0.12	0.24	+67.5 (±0.6)[b]		+35.8 (±0.3)	46.8:53.2	
β		0.06	0		+11.7[c]	[e]	[e]	
α	Acetone	0.11	1.6	+25 (±0.3)[b]	−26[b,f]	+13 (±0.3)	75:25	3 days
α		0.040	0.53			+17 (±2)	69:31	
α		0.026	0.37	+37 (±1)[b]	−22[b,f]	+18 (±1)	68:32	3 days
α	EtOH	0.015	2.1	[g]	[g]	+20 (±4)		
β		0.015	2.1	[g]	[g]	+20 (±2)	76:24	2 days
β		0.005	0.7	[g]	[g]		62:38	
β		0.0025	0.35	[g]	[g]		62:38	
α	MeOH	0.032	2.8	+46 (±2)[h]		+26 (±1)	67:33	1–2 days
β		0.033	2.8		−15 (±1)[h]	+24 (±1)	[e]	

[a]Samples of methyl sulfoxide and acetone solutions were treated directly with silylating reagents before g.l.c. analysis; aqueous and alcoholic solutions were evaporated to dryness within 5 min at 0.1 mtorr before silylation. The determination of anomeric ratios was not discernibly affected by the presence of NaI. [b]Corrected for the small percentage of β anomer present in the high-α sample; calculation by simultaneous equations from data on α(94.1%)-cellobiose at zero time and at equilibrium. [c]Corrected, by simultaneous equations, for the small amount of α anomer present in the high-β sample. [d]Literature values[3] for β-cellobiose, initial and at equilibrium, are $[\alpha]_D^{20}$ +16.2° and +34.9° (*c* 1), respectively. [e]Assumed to be the same value as that determined with α-cellobiose as starting material. [f]Value could be in error as much as 25%. A more accurate value for β-cellobiose could not be obtained because of its low solubility in NaI–acetone. [g]Extreme slowness in dissolving at 20° prevented a value for either the pure α-form or the pure β-form from being obtained. [h]By approximation, on the basis of data for both anomers.

Data are given in Table II showing the effect of reaction conditions on the formation of NaI·α-isomaltose. Water and ethanol are better solvents for isomaltose and sodium iodide than 1-butanol and acetonitrile. Increased reaction temperature causes a pronounced increase in the rate of complex formation and a small decrease in anomeric purity of the sugar moiety.

Treatment of NaI·α-isomaltose with methanol or ethanol does not decompose it to produce the free solid sugar probably because the solubility of the complex is less than that of the pure sugar. The salt moiety can be removed in a nonaqueous medium, such as dimethylformamide, by means of a cation-exchange resin (Ag^+ form). Noncrystalline high-α isomaltose is isolated from the effluent solution by precipitation with ether. Loss of anomeric purity is no more than a few per cent by this process and 96% recoveries of α-isomaltose are attainable. Undesirable impurities affecting flavor and color can be removed by treating a solution of the high-α isomaltose in 2-methoxyethanol with activated charcoal. The purified sugar is sweet and colorless.

Polarimetric and anomeric equilibration studies. — The specific rotation and position of anomeric equilibrium are greatly affected by the nature of the solvent (Table III). Furthermore, a large concentration of sodium iodide in these systems can alter significantly the rotation and position of equilibrium. In *dilute aqueous* sodium iodide solution, where complex formation between salt and sugar is relatively small, the salt effect is negligible; possibly for a similar reason, the salt effect in methyl sulfoxide is likewise only slight. Data in Tables III and IV show that in acetone–sodium iodide or alcohol–sodium iodide mixtures, the positions of equilibrium for cellobiose, maltose, and isomaltose all lie in the direction of the α anomer. In pure water the β-form preponderates. The kinetics of mutarotation for cellobiose in water are first-order. Mutarotation coefficients k_1+k_2, for the α and β anomer at 20° are 0.0050 (±0.0000) min^{-1} and 0.0048 (±0.0001) min^{-1}, respectively. Equilibrium and rotational data in pure acetone and pure alcohol were not always obtainable because of low solubility of the sugar. However, in methanol, solubility was sufficiently high to permit rotational measurements on α-cellobiose (See Table V).

The difference (45°) between the specific rotation of α-cellobiose in water and in acetone–sodium iodide (1.6M) is remarkable. Even in a relatively dilute sodium iodide solution (0.37M) the difference (33°) is large. A similar, but less pronounced, change in rotation occurs in methanolic sodium iodide solution.

Complexing of either an alkali-metal salt or a solvent molecule with a non-anomeric hydroxyl group attached to an asymmetric carbon atom probably has only a small effect on the rotatory contribution of that particular asymmetric center. The n.m.r. and optical rotation studies of Lemieux, Pavia, and coworkers[6] indicate that large effects of solvent on the optical rotation of certain methyl pyranosides are mainly the result of alterations in chair–chair conformational equilibria and changes in the relative populations of conformers involving rotation of the methoxyl group about the CH_3O–C axis. Their data suggest that water helps to stabilize an orientation of the methoxyl group that is different from the orientation in acetone and certain

TABLE IV

POLARIMETRIC AND EQUILIBRIUM STUDY OF ISOMALTOSE AND MALTOSE AT 20°

Compound	*Solvent*	*Conc. (moles/l)*	$[\alpha]_D^{20}$ *of sugar, degrees*			*α:β at equil.*[a]	*Approx. time to reach equil.*
			α Anomer	*β Anomer*	*Equil.*		
NaI·α(89%)-isomaltose	Water	0.014	+165 (±2)[b]	+98[b]	+122 (±3)[c]	36:64	2 h
	$HCONMe_2$	0.062	+175 (±0.5)[b]	+113[b]	+142 (±0.5)	47:53	3 weeks
Isomaltose (47% α)	$HCONMe_2$	0.062			+142 (±0.5)[d]	47:53	
	EtOH	0.01				47:53	
	2.1M NaI in EtOH	0.011			+128 (±5)	66:34	2 days
Isomaltose (66% α)	1.6M NaI in acetone	0.010			+115 (±2)	64:36	1 day
Maltose (94% α)	Water	0.13	+169[b,e]	+118[b,e]	+137[e]	37:63	
Maltose (>95% β)	Water			+118[f]	+136[f]	37:63	
Maltose (94% α)	1.6M NaI in acetone	0.11	+150 (±0.6)[b]	+106[b]	+137 (±0.3)	68:32	

[a]Samples of alcoholic and aqueous solutions were evaporated rapidly to dryness at 25° and 0.1 mtorr before being silylated for g.l.c. analysis. [b]Corrected, by simultaneous equations, for the small percentage of opposite anomer present in the high-α or high-β sample (see footnote[b], Table III). [c]Lit.[4], $[\alpha]_D^{25}$ +122.0° (*c* 2). [d]The specific rotation is negligibly affected by adding an equimolar amount of NaI. [e]From unpublished data of M. Bardolph developed at this Laboratory. [f]From the data of Hudson and Yanovsky[5].

TABLE V

MOLECULAR ROTATIONS[a] IN VARIOUS SOLVENT MEDIA

Carbohydrate	[M] × 10^{-2}					
	A	*B*	*C*	*D*	*E*	
	Water	*MeOH*	*Acetone*	*2.8M NaI in MeOH*	*1.6M NaI in Acetone*	*E–A*
α-D-Glucose	202^{20}_{4}	$194^{27}_{0.3}$	[b]	$174^{25}_{0.3}$	$197^{25}_{0.3}$	−5
β-D-Glucose	34^{20}_{4}		[b]		$0\ (\pm 2)^{27}_{1}$	−34
Methyl α-D-glucopyranoside	299^{28}_{1}	$321^{25}_{0.6}$	$330^{29}_{0.037}$ (±2%)	$256^{25}_{0.6}$	$219^{25}_{0.6}$	−80
Methyl β-D-glucopyranoside	-64^{27}_{1}	~~-67^{20}_{4}~~	$-67^{27}_{0.16}$	-87^{27}_{1}	-114^{27}_{1}	−50
1,6-Anhydro-β-D-glucopyranose	$-109^{20}_{0.8}$		$-115^{20}_{0.8}$		$-115^{20}_{0.8}$	+6
α-Cellobiose[c]	238^{20}_{4}	$231^{25}_{0.03}$ (±2%)	[b]	148^{20}_{1} (±4%)	81^{20}_{3}	−157
β-Cellobiose[c]	43^{20}_{8}	[b]	[b]	-48^{20}_{1} (±7%)	-84^{20d}_{3}	−127
α-Maltose[c]	578^{20}_{3}		[b]	$546^{28}_{0.7}$	483^{20}_{3}	−95
β-Maltose[c,e]	404^{20}_{3}		[b]	$357^{28}_{0.24}$	342^{20}_{3}	−62
Sucrose	229^{20}_{24}	$237^{25}_{0.3}$	[b]	$235^{25}_{0.3}$	$286^{25}_{0.3}$	+57
α,α-Trehalose[c]	$674^{25}_{0.6}$	$708^{25}_{0.6}$	[b]	$614^{25}_{0.6}$	$557^{25}_{0.6}$	−117
Melezitose[e]	$479^{27}_{0.3}$	$516^{27}_{0.3}$	[b]	$531^{27}_{0.3}$	$555^{25}_{0.3}$	+76
Raffinose[e]	625^{20}_{4}	$638^{27}_{0.36}$	[b]	$564^{27}_{0.36}$	$579^{25}_{0.36}$	−46

[a]Values in nonaqueous media are corrected for refractive index. Superscript and subscript after each rotational value represent, respectively, temperature and concentration of carbohydrate (g/100 ml of solution). Except where otherwise indicated, analytical precision was ±1% or better. [b]Carbohydrate was too insoluble for analysis. [c]Rotational values were corrected for the small amount of opposite anomer present in sugar sample. The value for α-cellobiose in methanol was calculated with the assumption that the molecular rotation of β-cellobiose in methanol is identical with that in water. [d]See Table III, footnote[f]. [e]Polarimetric analyses were made with the crystalline hydrate. Rotational values shown were calculated for the anhydrous sugar.

other nonaqueous solvents. In this laboratory, measurements have been made on solutions of 1,6-anhydro-β-D-glucopyranose in acetone to determine what effect complexing with sodium iodide has on the optical rotation of a nonreducing, pyranoid derivative of fixed ring-conformation. Ebulliometric measurements in acetone showed that at a concentration of 1.6M NaI and 0.05M 1,6-anhydro-β-D-glucopyranose the carbohydrate is entirely in complex form. However, the specific rotation, $[\alpha]_D^{20}$, in the presence of salt (−75.6°) differs only very slightly from that in the absence of salt (−72.1°); and the specific rotation in water (−67.0°) differs little from that in pure acetone. These differences are even smaller when the rotations are corrected for refractive index (see Table V).

Table V presents the results of a study of the effect of methanol, acetone, methanol–sodium iodide, and acetone–sodium iodide media on the molecular rotation of various carbohydrates. For comparative purposes, rotations in media other than water were corrected for refractive index[7,8] by the equation $\alpha^t_{D,H_2O} = \alpha^t_{D,S}(\bar{n}^2_{D,H_2O}+2/\bar{n}^2_{D,S}+2)$, where α^t_{D,H_2O} is the optical rotation referred to the refractive index of water at a fixed temperature t; $\alpha^t_{D,S}$ is the rotation in a different solvent medium of refractive index $\bar{n}_{D,S}$; and $\bar{n}_{D,H_2O}$ is the refractive index of water at the sodium D line. In many instances, measurements were made at temperatures slightly higher than the usual 20°. Because small changes in temperature have an essentially negligible effect on refractive index and on optical rotation of sugars, temperature differences may be ignored in making comparisons between the various rotational values listed in Table V.

Differences between rotations in water, methanol, and acetone are generally small; however at high concentrations of sodium iodide, rotational changes in the nonaqueous media are often large. In some instances (such as with methyl α-D-glucopyranoside and α,α-trehalose), the direction of rotational change where methanol or acetone is substituted for water is opposite from that where methanol–sodium iodide or acetone–sodium iodide is substituted. Of the carbohydrates studied in salt-containing media, α- and β-cellobiose and α,α-trehalose (a nonreducing disaccharide) are the most strongly influenced. 1,6-Anhydro-β-D-glucopyranose and α-D-glucose are only weakly affected.

The large influence of sodium iodide on the optical rotation of α-cellobiose cannot be satisfactorily explained on the basis of mere interaction between salt and anomeric hydroxyl group. Were such an interaction responsible for the effect, α-D-glucose would be expected to behave similarly; but it does not. I suggest, therefore, that the large changes in the optical rotation of cellobiose and certain other oligosaccharides are caused primarily by changes in ring conformation or by alteration of the torsional angles about the C′–O and O–C bonds at the glycosidic linkage. Of the two possibilities, the latter is perhaps the more probable, in view of the results of recent conformational studies by Rees[9], who has obtained considerable evidence that optical rotation in oligosaccharides is very sensitive to conformation of the linkage. Conceivably, an alteration in linkage conformation could result from chelation of sodium iodide with the proper donor-groups in the carbohydrate molecule.

N.m.r. and i.r. spectra. — Proton resonance spectra of α-cellobiose and α-isomaltose show that, in Me_2SO, the pyranoid rings of both sugars are predominantly in the *C1* (D) conformation. Chemical shifts and coupling constants are given in Table VI for the anomeric hydroxyl protons and the H-1 protons. In the spectrum of

TABLE VI

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF α-CELLOBIOSE AND α-ISOMALTOSE IN METHYL SULFOXIDE-d_6[a]

Disaccharide	*Axial 1-OH Doublet*[b]		*H-1 Doublet*[b]			
			Equatorial		*Axial*	
	τ (*p.p.m.*)	J (*Hz*)	τ (*p.p.m.*)	J (*Hz*)	τ (*p.p.m.*)	J (*Hz*)
α-Cellobiose						
(*a*) Without NaI	3.76	4.5	5.13[c]	3.0[c]	5.79	7.0
(*b*) As 1:1 NaI adduct[d]	3.75	4.5			5.80	7.0
(*c*) As 2:1 NaI adduct[d]	3.72	4.5	5.12[c]	3.0[c]	5.79	7.0
α-Isomaltose						
(*a*) As 1:1 NaI adduct	3.77	4.5	5.11[c]	3.0[c]	5.36[c]	3.0[c]

[a]Solutions contained approximately 34 mg of sugar/ml of solvent. [b]*J* and τ were estimated to the nearest 0.5 unit and 0.01 unit, respectively. [c]Value was obtained with D_2O in the solution. [d]An identical spectrum is obtained when sugar and salt are dissolved individually, rather than as a complex.

α-cellobiose, unassigned hydroxyl-proton signals appear as doublets at τ 4.84 (J = 4.0 Hz) and τ 5.44 (J = 7.0 Hz). High concentrations of sodium iodide cause a significant displacement of certain hydroxyl signals toward lower field, whereas the H-1 signals are unaffected. For example, when a 0.1M solution of α-cellobiose is made 0.2M with respect to sodium iodide, the hydroxyl doublets at τ 3.76 and τ 5.44 shift to τ 3.72 and τ 5.28, respectively; however, neither the hydroxyl doublet at τ 4.84 nor the H-1 signals are noticeably affected. The effect of salt on hydroxyl resonance-frequencies indicates that salt–hydroxyl group interactions (ion–dipole) do occur, even in Me_2SO solution wherein the hydroxyl protons are bound to the solvent.

The i.r. spectrum of crystalline α-cellobiose in mineral oil shows the following bands that indicate the α-D-glucopyranose constituent: 910 (strong), 843 (moderate, with shoulders), and 760 cm^{-1} (strong)[10]. Similarly, crystalline NaI·α-isomaltose shows bands at 910 (strong), 855 (moderate), and 760 cm^{-1} (strong). Neither α-cellobiose, NaI·α-isomaltose, NaI·2 α-D-glucose, nor NaI·α-D-mannose absorbs significantly in the region of 1600–1650 cm^{-1}. On the other hand, the sodium iodide adducts of α-cellobiose absorb moderately in this region, even after being subjected to rigorous dehydrating conditions (130° at 0.1 mtorr for 18 h) to remove any water of hydration. Perhaps mere traces of moisture (≤0.5% by wt), held tightly and irremovably by the adducts, are responsible for the unusual absorption.

X-Ray diffraction. — α-Cellobiose, β-cellobiose, 2 NaI·α-cellobiose, and

NaI·α-cellobiose·2 H_2O each show clear, distinctive X-ray powder patterns, providing confirmatory evidence that the adducts are real and not simply intimate mixtures of sugar and salt.

EXPERIMENTAL

Isomaltose, in highly purified form[4], was obtained from Allene R. Jeanes. Anhydrous β-maltose was prepared by dehydrating β-maltose·H_2O at 56° and 0.1 mtorr. High-α maltose was prepared by the method of Hodge and Rendleman[11]. Details concerning this preparation will be discussed in a manuscript now in preparation. Other carbohydrates, inorganic salts, and solvents were commercial and were used without further purification.

Analyses. — Water of hydration, ethanol of solvation, and halide ion were determined by Karl Fischer titration, Zeisel alkoxyl analysis, and silver nitrate titration, respectively. Anomeric ratios (or anomeric purities) were determined by g.l.c. of the trimethylsilyl ethers performed isothermally at 215° on an F & M Series 700* instrument equipped with a flame-ionization detector. The 6 ft × 1/8 in stainless-steel column was packed with Gas Chrom Q support (80–100 mesh) with 3% liquid phase. The precision in determining the percentage of an anomeric form in a mixture of two anomers was about ±0.5%. For each sugar studied, the retention time of the β anomer was greater than that of the α anomer. I.r. spectra were of mineral oil mulls on silver chloride plates. Optical rotations were made with either a manual Bellingham and Stanley instrument or an automatic Bendix polarimeter, with a relative precision of ±1% or better in most cases. High concentrations of sodium iodide often decreased the light transmission and lowered the precision of measurements made manually. N.m.r. spectra were recorded with a Varian Associates HA-100 spectrometer, with tetramethylsilane ($\tau = 10.00$) as the internal standard; coupling constants were approximated to the nearest 0.5 Hz.

The melting points of α- and β-cellobiose were taken, in a thin-wall capillary tube, as the lowest temperature at which the sugars would melt completely within 2–3 sec after placement in a bath held at constant temperature.

Ebulliometric measurements were performed as described in an earlier paper[12].

Cellobiose. — A. Preparation of 2 NaI·α-cellobiose. β-Cellobiose (9.9 g, 94% β, m.p. 153°) was dissolved in a hot, nearly boiling, solution of NaI (34 g) in ethanol (100 ml). The cooled solution was kept at 25° until precipitation of adduct was complete (1 week). The product was washed with ethanol–ether, ether, and then dried under vacuum at 25°. The nearly colorless, crystalline, hygroscopic compound retained traces of water and ethanol. Yield, 13.9 g.

Anal. Calc. for $C_{12}H_{22}O_{11}$·2 NaI: I^-, 39.6. Found: I^-, 38.2; H_2O, 0.70; EtOH, 1.05; α:β, 93.5:6.5.

The content of α anomer of the cellobiose moiety in the complex was decreased

*Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

by raising the reaction temperature. For example, at 85°, α(83%)-cellobiose was combined.

B. Preparation of NaI·α-cellobiose·2H₂O. A sample of 2NaI·α-cellobiose (1.00 g; α:β, 94:6) was wet with 0.3 ml of ice–water at 1°. As soon as solution was complete, the entire mixture suddenly crystallized. After 2 h at 1°, the crystalline solid was blotted on filter paper, washed thoroughly with 1:1 ethanol–ether, and finally dried for 0.5 h under 1-torr vacuum; yield, 0.45 g.

Anal. Calc. for $C_{12}H_{22}O_{11}$·NaI·2 H_2O: I, 24.1; H_2O 6.82. Found: I, 24.7; H_2O, 6.78; EtOH, <0.5; α:β, 95.2:4.8.

When heated for 1 h at 95° and 0.1 mtorr, the adduct lost 2 H_2O (determined by measuring the weight loss) to become an anhydrous, crystalline adduct of high anomeric purity (94.5% α).

C. Generation of α-cellobiose. (i) From 2:1 adduct. Table I summarizes the reaction conditions and results. A sample of 2 NaI·α(94%)-cellobiose was dissolved with rapid stirring in methanol or ethanol and the resulting solution was kept until precipitation of α-cellobiose was complete. The precipitate (small, colorless spherocrystals) was washed with ethanol, ether, and then dried under vacuum. In γ-butyrolactone, the adduct needed to be finely pulverized to ensure transformation.

Prolonged treatment of high-α cellobiose with ethanol for 20 h at 0–25° caused no measurable change in α-anomeric content. Higher temperatures, however, may lower the α-anomeric content. For example, when α(94%)-cellobiose (5 mg) was stirred in 0.2 ml of ethanol at 70° for 20 h, α(86%)-cellobiose (4 mg) was isolated by centrifugation.

The anomeric purity of high-α cellobiose was increased by recrystallization: α(94%)-cellobiose (20 mg) was quickly dissolved in a minimum volume of ice–water (~0.3 ml). Addition of ethanol (3 ml at 25°) gave a homogeneous solution that was placed in an ice bath, seeded with α(94%)-cellobiose, and kept for 0.5 h to ensure complete precipitation; yield, 6 mg; m.p., 214°; α, 98.2.

(ii) From 1:1 adduct. NaI·α(95%)-cellobiose·2 H_2O (15 mg) was dissolved in 1.8 ml of ethanol at 1°. Precipitation of crystalline α-cellobiose began within 5 min and was complete in about 30 min. The crystals were washed with 1:1 ethanol–ether, ether, and dried under vacuum; yield, 9.7 mg (92%); m.p. 210°, α, 96; EtOH, <1.4.

Isomaltose. — (A). Preparation of NaI·α-isomaltose. (i) In aqueous media. Equimolar amounts of amorphous isomaltose (α:β, 47:53) and NaI were dissolved together in a minimum volume of water. In the experiments at 25°, the solutions were evaporated in a desiccator to a thick, viscous syrup, which was then exposed to air (r.h. <30%) until crystallization was complete (~3 weeks). At higher temperatures, the sugar–salt solutions were placed in open-mouth test tubes and then seeded with a trace of crystalline adduct; evaporation of solvent and formation of colorless, nonhygroscopic, microcrystalline aggregates occurred simultaneously (Table II).

(ii) In ethanol. When isomaltose (47% α) was dissolved in a saturated solution of NaI in ethanol at 25°, NaI·α-isomaltose crystallized from solution.

Anal. Calc. for $C_{12}H_{22}O_{11}$·NaI: I, 25.8. Found: I, 26.6. Dilution of the original

solution with ethanol before crystallization began, or the use of a much higher reaction temperature, caused a small decrease in anomeric purity (Table II).

(*iii*) *In 1-butanol or acetonitrile.* Preparations in these media were similar to those in ethanol (Table II).

(*B*). *Preparation of NaBr·α-isomaltose.* Although the 1:1 NaBr adduct can be prepared in an aqueous medium at 25° by the method used for the NaI adduct, crystallization in this medium was incomplete. Totally crystalline NaBr adduct was prepared by keeping an ethanolic solution of isomaltose (47% α) and NaBr, saturated with respect to each component, for several weeks at 25°.

Anal. Calc. for $C_{12}H_{22}O_{11}$·NaBr: Br, 18.0. Found: Br, 16.6; α, 82.

The crystals are nonhygroscopic and similar in appearance to those of the NaI adduct prepared in ethanol.

C. Generation of α-isomaltose from 1:1 NaI adduct. A solution of NaI·α(93%)-isomaltose (69 mg) in 1 ml of *N,N*-dimethylformamide was shaken with 0.8 g of cation-exchange resin (Ag^+ form of Dowex-50W X8) for 15 min at 25°. The AgI and resin were then removed by centrifugation. To the centrifugate was added 5 ml of ethanol, followed by 80 ml of ether to precipitate α-isomaltose. The ether-washed product was colorless, amorphous, and hygroscopic above 30% r.h.; yield, 46 mg (96%); α, 90.

To remove impurities that affected organoleptic evaluation, activated carbon (2 g) and ethanol (15 ml) were added to crude α-isomaltose (2 g) dissolved in 2-methoxyethanol (25 ml). The mixture was stirred for 5 min at 25° and then centrifuged. Ether (1–2 l, or enough to cause maximum precipitation of sugar) was added to the centrifugate, which was stirred rapidly to induce coagulation of the colloidal particles (~5 min). The sugar was then washed with ether and dried under vacuum. Yields were ~85%; and loss of anomeric purity was no greater than 3%.

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