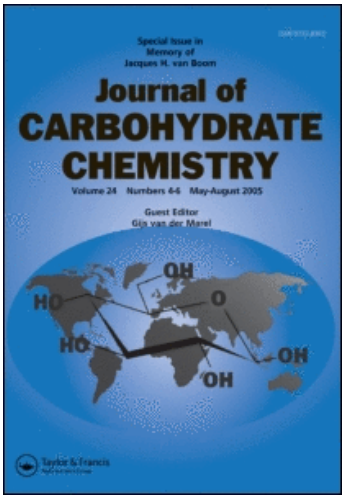

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PLATINUM CATALYSED OXIDATION OF SUCROSE

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ABSTRACT

The platinum catalyzed oxidation of sucrose by oxygen has been studied. With platinum on carbon at 100°C and at constant neutral pH, the oxidation is highly specific for the primary hydroxyls at the 6- and 6′- positions of sucrose. There was no evidence of oxidation of the 1′ hydroxyl. The 6,6′-dicarboxysucrose was resistant to attack by invertase. The 6- and 6′-carboxysucroses could not be resolved from each other, but the former component was hydrolysed rapidly by invertase and subsequently the 6′-carboxysucrose was isolated. The ^{13}C spectrum of 6-carboxysucrose was then assigned by difference.

INTRODUCTION

Heyns and Paulsen have comprehensively reviewed the literature on catalytic oxidation of carbohydrates,[2,3] but the catalytic oxidation of sucrose is briefly mentioned in only one[3a] of the two reviews and has not been reported since. The references are to Reiners' patent,[4] wherein sucrose is used as a chemical feedstock

to produce glucuronic acid, and to the authors' own unpublished results. The main body of Reiners' patent claims the oxidation of glucosides to glucuronosides and suggests that sucrose is a suitably protected glucoside. The possibility of oxidation of the fructose moiety was not mentioned. Heyns and Paulsen[3a] stated that, with platinum as a catalyst, the primary hydroxyl groups of sucrose (at C6, C1′, and C6′) are all oxidised at similar rates and that the resulting product is a complex mixture of all the expected mono- and dicarboxylic acids but the reaction conditions were not reported. Liston's Master of Sciences Thesis[5] describes the platinum catalysed oxidation of sucrose with the aim of producing "sucronic acids" (the products of sucrose oxidation at only one of the three primary hydroxyl groups, with the glycosidic bond remaining intact). No purified products were isolated. There has been a recent claim[6] to have isolated a 40% yield of a sucrose tricarboxylic acid from this type of oxidation, but no details were provided and at the time of writing (Sept., 1990) no detailed publication has appeared.

RESULTS AND DISCUSSION

Catalytic Oxidation of Sucrose in Alkaline Solution; Choice of Base. All previously reported Pt-catalytic oxidations of non-reducing sugars were carried out in solutions containing one or more equivalents of a base per mole of carbohydrate present at the beginning of the reaction. Thus pH decreases during oxidation as carboxylic acid products form. In our initial experiments at 100°C we used this approach. $NaHCO_3$ gave the fastest oxidation rate as indicated by disappearance of sucrose. After a further batch addition of $NaHCO_3$ at 120 min, sucrose oxidation recommenced (presumably due to restoration of the alkaline pH) and continued to 95% completion. A similar oxidation of sucrose in Na_2CO_3 proceeded at a comparable rate to that in $NaHCO_3$ but only after a lag phase where the pH of the solution decreased, without measurable sucrose oxidation. Presumably the lag phase is due to the loss of carbon dioxide from the reaction solution and the conversion of carbonate to bicarbonate. Sucrose oxidations using $Ca(OH)_2$ and $CaCO_3$ as bases were very sluggish and catalyst poisoning by calcium ions has been reported previously.[7] In both cases where there was extensive oxidation, i.e., with $NaHCO_3$

and with Na_2CO_3 as base, glucuronic acid and another product, apparently a disaccharide acid, were tentatively identified by TLC. Glucuronic acid could result from alkaline scission of the glycosidic linkage following oxidation both of C6 to carboxyl and of the C4 hydroxyl group in the fructose moiety to a ketone which would then undergo β-alkoxycarbonyl elimination at the fructoside linkage. Alkaline scission of the glycosidic linkages in unoxidised sucrose under these relatively mild alkaline conditions is considered unlikely (cf.[8]). Evidently these oxidations in alkaline solution are much less specific than those at constant pH 7 described below.

Sucrose Oxidation at pH 7.0. De Wilt *et al.*[9,10] have applied a pH-stat technique to the platinum catalysed oxidation of glucose to gluconic acid, and claimed that increase in pH effected faster oxidation rates but reduced selectivity. The previous application of this technique to the catalytic oxidation of carbohydrates is limited to the oxidation of glucose (aldehyde group to carboxylic acid). De Wilt *et al.* utilised relatively low temperatures (25 to 65 °C). At the higher temperatures employed in this investigation however, pH measurements are drastically affected not only by the change in water activity (p_w = 12.29 at 100 °C), but also by a change in the characteristics of the electrode. In our investigation the pH electrode was calibrated at 25 °C and pH measurements were made at 100 °C. Thus the differences between calibration and operation temperatures certainly resulted in some error in the pH value at 100 °C. In fact the Radiometer Gk2402B combined pH electrode gave stable pH readings in boiling alkaline solutions, but the relationship between these observed and reported pH values and the actual hydrogen ion activities at 100 °C was not determined. Therefore the pH values measured at 100 °C are considered to be nominal or approximate values only. However, the manufacturers claim that at temperatures from 10 to 100 °C, this electrode has a stable isopotential point, and that pH measurement is "almost hysteresis-free". Therefore in this investigation the error associated with electrode performance is considered small, and differences in nominal pH values are considered to represent corresponding differences in actual pH.

Sucrose was oxidised under reaction conditions which preliminary experiments had indicated would optimise the formation of disaccharide acids (*viz.* pH 7.0, Pt on carbon, 100 °C). The reaction progress was followed by HPLC analysis and NaOH

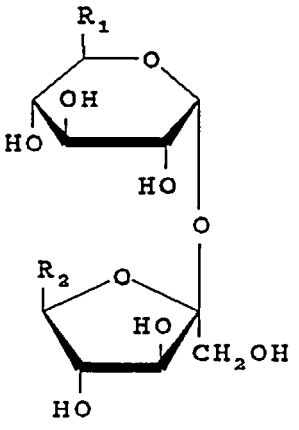
TABLE 1. ^{13}C NMR Shifts and Assignments of the Disaccharide Carboxylic Acid Products of Sucrose Oxidation.

δppm	Sucrose[a]	6,6′-Dicarboxysucrose	6′-Carboxysucrose	6-Carboxysucrose[b]
C1	92.94	92.93	92.96	92.95
C2	72.03	72.74	71.80	72.03
C3	73.58	73.44	73.68	73.65
C4	70.19	71.60	70.24	70.41
C5	73.27	73.14	73.24	72.77
C6	61.12	177.25[c]	61.46	177.03
C1′	63.34	62.54	62.09	62.40
C2′	104.41	105.23	104.99	104.59
C3′	77.39	77.45	77.45	76.63
C4′	74.96	76.79	75.12	73.65
C5′	82.21	80.42	80.46	82.29
C6′	63.35	178.01[c]	177.59	63.20

a. Bax *et al.*[12]

b. The shifts of 6-carboxysucrose were determined by the difference of the monocarboxylic acid mixture and the pure 6′-carboxysucrose ^{13}C NMR spectra.

c. Based on the shifts of 6′-carboxysucrose and 6-carboxysucrose carbonyl groups.



	R_1	R_2
1	COOH	CH_2OH
2	CH_2OH	COOH
3	COOH	COOH

SCHEME 1

titre; the oxidation of sucrose at constant pH values will be described in greater detail in a subsequent publication. After *ca.* 80% of the sucrose was consumed the reaction was stopped and the acid products were isolated as ammonium salts (yield: 68%) by ion exchange. These were separated into three components by preparative paper chromatography (PC) (*viz.* slow band [A], intermediate band [B], and fast band [C]). Band C contained a complex mixture of acids, including glucuronic acid and was not further investigated. The ^{13}C NMR spectrum of band A showed only twelve peaks. This product was 6,6′-dicarboxysucrose (3) (Scheme 1) and peak assignments are shown in Table 1. The acid hydrolysate of 3 did not contain glucose or fructose (HPLC), but contained both D-glucuronic acid and another unidentified acid. The retention time of the latter acid (ion exchange HPLC) was identical with the acid product obtained by hydrolysis of inulin after platinum catalysed oxidation and tentatively assumed to be 5-ketomannonic acid.

The ^{13}C NMR spectrum of band B from preparative PC resolved 21 resonances; the relative peak heights suggested that resonances at δ 73.65 ppm and 92.95 ppm might represent combinations of three and two carbon nuclei respectively. This product was tentatively identified as a mixture of two monocarboxylic acid derivatives of sucrose based on four resonances (δ 61.42, 62.09, 62.40, and 63.20 ppm) in the

CH_2OH region of the spectrum, two resonances (δ 177.03, and 177.59 ppm) in the carbonyl region of the spectrum, and a total of 24 resonances (assuming the above coincident resonances at δ 73.65 and 92.95 ppm). The mixture yielded glucose and fructose in approximately equal proportions on hydrolysis. The hydrolysis conditions caused greater loss of fructose than glucose by acid degradation and the ratio of fructose to glucose was corrected by extrapolation to zero time.[11] Both D-glucuronic acid and the supposed 5-ketomannonic acid were detected by HPLC, but there was no evidence of 2-ketogluconic acid in the hydrolysate. Based on these analyses B was identified as a mixture of 6-carboxysucrose (1) and 6′-carboxysucrose (2) in approximately equal proportions.

Invertase Hydrolysis of the Disaccharide Acid Products of Sucrose Oxidation. The rates of the invertase catalysed hydrolysis of sucrose and of the sucrose oxidation products were compared. Sucrose hydrolysis was complete in *ca.* 20 min. In the case of the invertase catalysed hydrolysis of the 6-carboxysucrose/6′-carboxysucrose mixture under the same conditions, fructose was the only neutral sugar product. With two further additions of invertase the fructose concentration reached a maximum after 9 h, with an HPLC fructose yield of 22% by weight of the original mixed product. Predictably, after 9 h (with two further additions of invertase at 90 min, and at 7 h) neutral sugars were not detected in the invertase treated 6;6′-dicarboxysucrose solution. TLC analysis confirmed that 6,6′-dicarboxysucrose was not hydrolysed by invertase, and that the monocarboxylic acid mixture was only partly hydrolysed. The surviving disaccharide acid component of the invertase treated 6-carboxysucrose/6′-carboxysucrose mixture was purified by preparative PC. The ^{13}C NMR (75MHz) spectrum of this disaccharide showed twelve peaks whose assignments are shown in Table 1, implying the identification of this invertase-resistant monocarboxylic acid as 6′-carboxysucrose. The recent unambiguous assignment of the ^{13}C NMR spectrum of sucrose by Bax *et al.*[12], determined by double-quantum coherence techniques is also shown in Table 1 for comparison. The chemical shifts of 6-carboxysucrose are based on the differences between the spectra of the monocarboxylic acid mixture (band B above) and the 6′-carboxysucrose isolated after invertase treatment. The assignments of the two carboxyl resonances of 6,6′-dicarboxysucrose are derived from assignments of the carboxyl resonances of

the two monocarboxylic acid derivatives. It should be noted that comparison of the ^{13}C NMR spectra of sucrose and of its carboxylic acid derivatives affords unambiguous assignment of the CH_2OH resonances in sucrose, and is in agreement with the assignments made by Bax *et al.*[12]

Thus, only two of the three possible monocarboxylic acid derivatives (*viz.* 6-carboxysucrose, and 6′-carboxysucrose), and one of the three possible dicarboxylic acid derivatives of sucrose (*viz.* 6,6′-dicarboxysucrose) were identified in the products of platinum catalysed sucrose oxidation. The reluctance of the C-1′ hydroxyl group to undergo oxidation is consistent with the earlier conclusions of Heyns[3b] *et al.* on the order of reactivity to catalytic oxidations of hydroxyl groups in partially protected ketoses (*viz.* "Prim. 6-OH (furanoses) > 5-OH_{ax} = 4-OH_{ax} ≳ 3-OH_{ax} ≳ prim. 1-OH > sek. OH (furanoses)"). We intend to describe in a subsequent publication the catalytic oxidation of sucrose at higher pH which induces in addition to all of the above products, oxidation of the C-1′ hydroxyl group. Since invertase treatment of 6,6′-dicarboxysucrose did not result in hydrolysis, it is not surprising that this sucrose-like disaccharide is an inhibitor of invertase, and the detailed investigation of this effect will be described in a subsequent publication. Contrary to a previous report,[5] 6′-carboxysucrose was not significantly hydrolysed by invertase, and the possibility that this disaccharide is an inhibitor of invertase is also being investigated.

The assignments of the ^1H NMR resonances of sucrose,[13] 6′-carboxysucrose, and 6,6′-dicarboxysucrose are shown in Table 2. For 6′-carboxysucrose, and 6,6′-dicarboxysucrose, assignment of resonances of the C5-H and C5′-H nuclei (α to the carboxyl groups) are made by comparison of the spectra of the two acids. All other chemical shifts are assigned by analogy with sucrose.

EXPERIMENTAL

General Methods. β-D-Fructofuranosidase (EC 3.2.1.26) was purchased from Sigma Chemical Co. Reverse phase (8C1810 and Dextropak) and amine (8MBNH10) HPLC columns were Millipore Waters radial compression cartridges used with the Z-module radial compression system. The ion exchange column was

TABLE 2. Assignments of ^1H NMR Shifts of Sucrose, 6′-Carboxysucrose, and 6,6′-Dicarboxysucrose.

	Chemical Shift, ppm (multiplicity); *J* Values, Hz		
	Sucrose[a]	6,6′-Dicarboxysucrose	6′-Carboxysucrose
C1 -H	5.18 (d)	5.35 (d)	5.16 (d)
C2 -H	3.31 (dd)	3.39 (dd)	3.31 (dd)
C3 -H	3.52 (t)	3.50 (t)	3.35 (t)
C4 -H	3.26 (t)	3.25 (t)	3.26 (t)
C5 -H	3.63 (m)	3.85[b] (d)	3.63 (m)
C6 -H_2	3.58 (d)	------	3.57 (d)
C1′-H_2	3.43 (s)	3.48 (s)	3.48 (s)
C3′-H	3.98 (d)	4.13 (d)	4.13 (d)
C4′-H	3.81 (t)	3.97 (t)	3.95 (t)
C5′-H	3.65 (m)	4.15[b] (d)	4.15[b] (d)
C6′-H_2	3.58 (d)	------	------
$J_{1,2}$	3.7	3.7	4.2
$J_{2,3}$	9.7	9.9	9.6
$J_{3,4}$	9.6	9.9	9.5
$J_{4,5}$	9.6	9.8	9.5
$J_{5,6}$	N.D.	------	7.2
$J_{3',4'}$	8.4	8.4	8.4
$J_{4',5'}$	8.3	8.5	8.5
$J_{5',6'}$	N.D.	------	------

a. Shifts and *J* values from this investigation, assignments after Hull.[13]

b. Assignments by comparison of the spectra of the two acid derivatives.

N.D., not determined.

a stainless steel column (300 mm x 4 mm i.d.), slurry-packed with Aminex A-28 resin (Bio-Rad Labs) in the formate form, as described by Blake *et al.*[14] For the sucrose oxidation products the eluent was 0.6 M ammonium formate at pH 5.0, 60 °C, 0.5 mL min^{-1}. Quantitative results were determined by comparing peak areas with those of standards in separate consecutive injections. Solvent systems for TLC (cellulose) and PC (Whatman No.1) were: solvent 1, ethyl acetate:pyridine:water:butan-1-ol:acetic acid (5:4:4:10:2); solvent 2, pyridine:ethyl acetate:acetic acid:water (12:5:1:6). Development sprays: spray 1, *p*-aminohippuric acid, thiobarbituric acid[15]; spray 2, silver nitrate, sodium hydroxide[16].

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Bruker AM-300 Spectrometer on samples pre-exchanged with D_2O. The spectra were recorded in D_2O, using 1,4-dioxane-d_8 as an internal standard (for ^{13}C NMR 67.4 ppm, and for ^{1}H NMR 3.55 ppm). All ^{13}C NMR were completely decoupled and run with a relaxation delay of 3.0 sec for the carboxylic acids.

Apparatus for Catalytic Oxidations. In the preliminary investigation oxidation reactions were performed in a 250 mL side arm Quickfit conical flask with a condenser fitted to the neck. Oxygen flowed into the reaction solution through a glass tube with a No 1 sinter turned upward at the outlet, fitted to the side arm of the conical flask. The reactions were stirred and heated to 100 °C with a magnetic stirrer/hot plate. The gas bubbler was removed, temporarily halting oxygen flow while reaction samples were removed by pipette through the side arm.

The oxidation reaction at pH 7.0 was performed in a 700 mL cylindrical (75 mm i.d.) wide neck flat flanged reaction vessel with a clamped five neck Quickfit lid. A condenser, gas bubbler, thermometer, burette tip and a Radiometer combined pH electrode (GK2402B) were fitted to the five necks. The pH electrode and the burette tip were attached to a Radiometer Automatic titration System (PHM82 standard pH Meter, TTT80 Titrator, ABU80 Autoburette). The reaction was stirred and heated with a magnetic stirrer/hot plate. The pH electrode was standardised at 25 °C and corrections for higher operating temperatures were made at the pH meter (*viz.* correction for the change in water activity). The pH values measured at 100 °C do not represent accurate hydrogen ion activities, but are nominal values only, as discussed above. Constant pH was maintained in the reaction solution by continuous

addition of sodium hydroxide solution (1.0 M) through the autoburette, while titration volume was recorded continuously. The reaction solution was sampled at intervals through the thermometer port. In all cases oxygen flow of 600 mL min^{-1} was maintained.

Catalytic Oxidation of Sucrose in Alkaline Solution. Sucrose (1.0 g) and a base ($NaHCO_3$, Na_2CO_3, $CaCO_3$ or $Ca(OH)_2$; expressed as molar equivalents with respect to sucrose in Table 1) were dissolved in water (100 mL) at 100°C. The reactions were started by bubbling oxygen and adding platinum on activated carbon (0.1 g, 10% Pt). Samples (1 mL) were removed from the reactions at intervals and sucrose concentrations determined by reverse phase HPLC. The 120 min reaction samples were treated with Amberlite IR120(H) ion exchange resin. TLC (solvent 1, spray 1) of the $NaHCO_3$ and Na_2CO_3 reactions showed three bands: $R_{SUCROSE}$ 0.3, 0.5 and 1.0 with the middle band corresponding to glucuronic acid. The samples from oxidation in $CaCO_3$ and $Ca(OH)_2$ showed only unchanged sucrose.

Sucrose Oxidation at pH 7.0 and Recovery of Acid Products. Sucrose (5 g, in 500 mL water, at 100°C) was oxidised with platinum (10% Pt on carbon, 0.5 g) as catalyst at pH 7.0. Reaction progress was followed by HPLC and NaOH titre and stopped after *ca.* 80% of the sucrose was consumed (240 min, 1.24 moles NaOH/mole sucrose oxidised). The yields of the products described below, determined by ion exchange HPLC and assuming the same response factor as sucrose, and based on sucrose consumed, were 14% for 6,6′-dicarboxysucrose **3** (21.0 min) and 49% for a mixture of 6-carboxysucrose **1** and 6′-carboxysucrose **2** (7.35 min). Acid hydrolysis of the latter mixture and HPLC subsequently indicated that **1** and **2** were present in approximately equal amount. The solution from the oxidation was filtered, cooled to room temperature, then eluted through an Amberlite IRA400(OH) ion exchange column (50 mL). The neutral sugars were washed from the resin column with water (*ca.* 150 mL). Ammonium carbonate solution (1.0 M, 200 mL) was then passed through the column and the fractions containing carbohydrate (2-20) were combined, filtered (Millipore, 0.45 μm) and concentrated to dryness to yield the ammonium salts of the oxidation products (yield: 3.4 g). PC with solvent 2 and spray 2 revealed products at $R_{SUCROSE}$ 0.36 (band A), 0.79 (band B) and 1.16 (band C), which were isolated by preparative PC. TLC

(solvent 2, spray 2) confirmed that band A (R_F 0.19) and band B (R_F 0.41) were single spots, but that band C (R_F 0.61) was contaminated with band B (R_F 0.41). The ^{13}C NMR spectrum of band A is summarised in Table 1 assigned as 6,6′-dicarboxysucrose (3). The spectrum of band B resolved 21 resonances and apparently represented a mixture of 6-carboxysucrose (1) and 6′carboxysucrose (2). The spectrum of band C was very complex and was not further investigated.

Acid Hydrolysis of the Disaccharide Acid Products of Sucrose Oxidation. In separate experiments samples of band A and band B (0.15 g) were hydrolysed in water (1.5 mL) containing Amberlite IR120(H) ion exchange resin (0.5 mL) at 100°C. HPLC analysis (amine column) of the band A hydrolysates failed to detect glucose or fructose. The glucose to fructose weight ratios from band B hydrolysis were determined by HPLC and showed an increase in the ratio of glucose to fructose with time. Extrapolation of this ratio to zero time (to allow for acid decomposition of fructose) gave a glucose/fructose yield ratio of *ca.* 1.05 wt/wt. Analysis of the final hydrolysate solutions from band A by ion exchange HPLC (conditions as reference[13]) revealed a major product peak at 28.1 min (D-glucuronic acid) and a smaller peak at 25.6 min (identical with the supposed 5-ketomannonic acid from inulin oxidation, see below).

Platinum Catalysed Oxidation of Inulin. Inulin (β-D-fructofuranosyl-2-[→1-β-D-fructofuranosyl-2-]$_n$→1-α-D-glucopyranoside, 5.0 g in 500 mL water) was oxidised using 0.5 g 10% Pt on carbon (600 mL min^{-1} O_2) at constant pH 7.0 and 100°C. After 8.5 h the NaOH titration indicated *ca.* 7% oxidation of primary hydroxyl groups of the polysaccharide, and the reaction was stopped. The reaction solution was filtered and concentrated to *ca.* 100 mL. An aliquot portion of the concentrate (20 mL) was hydrolysed with Amberlite IR120(H) (5 mL) at 100°C for 1 h. The acidic products were absorbed on Amberlite IRA400 (acetate), then recovered by elution with 10% acetic acid (25 mL) (yield: 17.8 mg). Analysis of this product by ion exchange HPLC[13] revealed a major product peak at 25.7 min (presumed to be 5-ketomannonic acid, but not further confirmed at this stage), and a minor peak at 28.1 min (corresponding to authentic D-glucuronic acid).

Invertase Hydrolysis of the Disaccharide Acid Products of Sucrose Oxidation. In separate experiments sucrose (0.3 g), 6,6′-dicarboxysucrose (0.3 g), and the

6-carboxysucrose/6′-carboxysucrose mixture (0.3 g) were each dissolved in ammonium formate buffer (3.9 mL, 0.6 M, pH 5.0). The sugar solutions were equilibrated (15 min) in a water bath (55°C) before the addition of invertase solution (0.1 mL, 2.5 mg/mL). A further 0.2 mL of invertase solution was added to the sucronic acid solutions after 90 min, and after 7 h. The hydrolysis was followed by HPLC. The fructose concentration in the 6-carboxysucrose/6′-carboxysucrose solution reached a maximum after 9 h reaction (with a peak area of 0.22 relative to initial sucrose in the pure sucrose hydrolysis experiment). After 9 h incubation the solutions were filtered through an Amicon Centricon 10 microconcentrator to remove the enzyme, concentrated to dryness, then redissolved in water (1.5 mL). TLC (solvent 2, spray 2) of the 6,6′-dicarboxysucrose showed a single spot of R_F 0.20, while the 6-carboxysucrose/6′-carboxysucrose product showed three spots at R_F 0.41, R_F 0.59, and R_F 0.72. Preparative PC of the product from 6-carboxysucrose/6′-carboxysucrose yielded 2 major bands and the ^{13}C NMR spectrum of the slower band is shown in Table 1, designated as 6′-carboxysucrose.

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