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Esterification of select polyols with D-glucaric acid as model reactions for esterification of starch

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Abstract—Aqueous solutions of D-glucaric acid and model polyols xylitol, methyl α-D-glucopyranoside or β-cyclodextrin were freeze dried, then heated, and the product mixtures analyzed by instrumental methods that included GC–MS, electrospray ionization-mass spectrometry (ESIMS), and NMR. The thermal process and analyses were carried out with these polyols in order to determine to what extent multiple acylations of the alcohol functions occurred with D-glucaric acid lactones serving as acylating agents, the extent to which acylations occurred at the 1° alcohol sites, and the relative tendency for acylations to occur at the C1 or C6 end of the glucaryl unit. The results of these studies showed an overwhelming preference for 1° alcohol acylation and preferred acylation occurring at the C1 end of the glucaryl unit.

Keywords: D-Glucaric acid lactones; Transesterification; α-Methyl D-glucopyranoside; Cyclomaltoheptaose (β-cyclodextrin)

1. Introduction

The objective of the research described here was to determine to what extent some select polyols, as structural models for starch, could be directly esterified with D-glucaric acid which in aqueous solution is in equilibrium as the acyclic diacid and the 1,4- and 6,3-D-glucaro lactones;[1] under heat/vacuum conditions, D-glucaro-1,4:6,3-dilactone is formed (Fig. 1). Such esterifications of starch, if successful, would give a modified starch with potentially useful properties and a structural architecture with pendant carbohydrate based carboxylic acid and/or lactone functions available for further modification.

Starch has been previously esterified using a variety of esterifying agents, including the lactones, β-propiolactone, β-butyrolactone and γ-valerolactone in aqueous slurry or semi-dry conditions.[2] Interestingly, modification of pH and temperature affected whether esterification or etherification occurred with such lactones. In contrast, α-angelica lactone yielded only starch esters of levulinic acid.[2] Modified starches are used as adhesives, binders, paper sizing materials, some structural products, and cosmetics.[3,4] As far as these investigators are aware, no definitive information has been reported describing acylation of starch with D-glucaric acid.

Given the structural complexity of starch it was decided that, in order to study systematically starch esterifications of this kind, it was first necessary to determine which hydroxyl groups of the α-D-glucosyl units of starch were more susceptible to esterification under the thermal conditions employed, whether there was an observable difference in the reactivity of the two D-glucaric acid carboxylic acid functions, and to what extent the appended glucaryl units had carboxylic acid and/or lactone functionality. Some experimental results that address these points are reported here.

2. Results and discussion

Freeze drying of a concentrated syrup of D-glucaric acid resulted in the formation of a solid foam consisting of a

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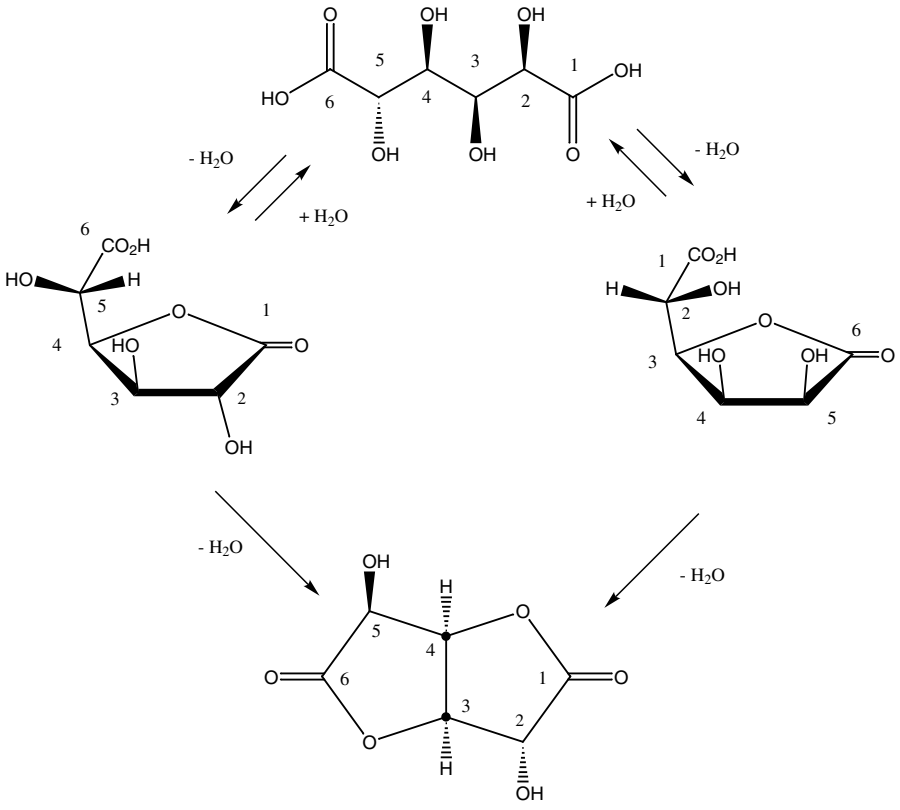

Figure 1. Structural forms of D-glucaric acid in aqueous solution and under drying conditions.

mixture of 1,4- and 6,3-monolactones and 1,4:6,3-dilactone (lactone mix). More protracted freeze drying increased the proportion of the dilactone in this mixture. GC–MS of the lactone mix revealed that only trace amounts of D-glucaric acid remained unlactonized. This mixture melted readily at ~90 °C.

2.1. Thermolysis of xylitol with lactone mix

A solution of xylitol and lactone mix was freeze-dried and the resulting solid foam heated at 90 °C during which it melted. Examination by GC–MS of the melt products as their per-*O*-trimethylsilyl (per-*O*-TMS) ethers revealed dimeric products. ESIMS of the products gave the series of ions, which are listed, together with assigned structures (Scheme 1). The most intense ion in the ESIMS spectrum was that of the lactone–xylitol dimer (*m*/*z* 365). Minor ions (*m*/*z* 1039, 1365, and 1691) were observed for the hexamer, octamer, and decamer with two terminal and one branching D-glucarolactone residue; that is, these ions correspond to the ions 865, 1191, and 1517 to each of which has been appended another, branching lactone giving a change of mass of 174. The absence of the ion *m*/*z* 713 appears to indicate that the branching lactone links through an acid rather than through a xylitol or lactone moiety.

Examination by ESIMS of the freeze-dried material prior to heating revealed the same series of ions up to *m*/*z* 1517 but ESIMS of the solution prior to freeze drying revealed only monomers. It has been reported that polymerization of hydroxycarboxylic acids occurs in situ during field desorption ionization,[5] however the latter experiment served to demonstrate that the observed ions are not an artifact of the ionization procedure. No dimers were observed in the GC–MS of the melt product of lactone mix and *meso*-inositol, which has five equatorial and one axial secondary alcohol functions.

From these results it was concluded that transesterification of D-glucaric acid lactones occurs readily to unhindered primary hydroxyls of polyols, and that the pendant D-glucaric groups are in the appropriate lactone form rather than open-chain acids. These pendant lactones can further transesterify to form linkages between different polyol molecules. Acylation of secondary hydroxyl groups with D-glucaric acid lactones occurs only with difficulty. The ease of the reaction is in accord with the literature reports[2,6] and the apparent selectivity for primary hydroxyls is probably a result of steric effects since the pK_a values of the various OH groups of xylitol are essentially equal.[7]

2.2. Thermolysis of methyl α-D-glucopyranoside with lactone mix

Melt thermolysis of a freeze-dried mixture of lactone mix and methyl α-D-glucopyranoside resulted in the

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[XYL-LACT]K^+=365
[LACT-XYL-LACT]K^+=539
[LACT-XYL-ACID-XYL]K^+=691
[LACT-XYL-ACID-XYL-LACT]K^+=865
[LACT-XYL-ACID-XYL-ACID-XYL]K^+=1017
[LACT-XYL-ACID-XYL-ACID-XYL-LACT]K^+=1191
[LACT-XYL-ACID-XYL-ACID-XYL-ACID-XYL]K^+=1343
[LACT-XYL-ACID-XYL-ACID-XYL-ACID-XYL-LACT]K^+=1517
[LACT-XYL-ACID-XYL-ACID-XYL-ACID-XYL-ACID-XYL]K^+=1669
[LACT-XYL-ACID-XYL-ACID-XYL-ACID-XYL-ACID-XYL-LACT]K^+=1843

MINOR IONS

[LACT-XYL-ACID-XYL-LACT]K^+ =1039 LACT

[LACT-XYL-ACID-XYL-ACID-XYL-LACT]K^+ =1365 LACT

[LACT-XYL-ACID-XYL-ACID-XYL-ACID-XYL-LACT]K^+ =1691 LACT

NOT OBSERVED [LACT-XYL-LACT]K^+ =713 LACT

Scheme 1. Oligomeric ions observed in LC–MS of thermolysis product of lactone mix and xylitol (XYL = xylitol; LACT = D-glucaric acid lactone; ACID = D-glucaric acid).

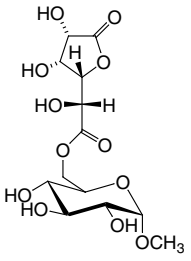
formation of dimeric products which were observed by GC–MS. The two principal dimers were isolated by preparative HPLC on Cl8 reversed phase and characterized by NMR (Table 1) and by high resolution ESIMS. The assigned structures are methyl 6-*O*-[D-glucaro-6,3-lactone-(1′→6)]-α-D-glucopyranoside, **1** and methyl 6-*O*-[D-glucaro-l,4-lactone-(6′→6)]-α-D-glucopyranoside, **2**. Compounds **1** and **2** formed in the ratio 1.46:1.00 ± 0.04 (mean of five separate determinations). These products would be produced by a reaction of the principal

Table 1. NMR assignments of **1**–**4**

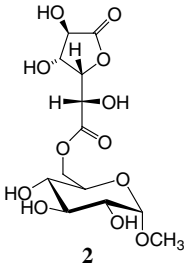
Carbon[a]	**1**		**2**		**3**		**4**	
	C	H	C	H	C	H	C	H
1	99.72	4.52 $J_{1,2}$ = 2.8 Hz	99.59	4.53 $J_{1,2}$ = 3.6 Hz	102.18	4.93	101.96	4.93
2	71.79	3.22	71.95[b]	3.21	72.64	3.43	72.43	3.41
3	73.26	3.36	73.09	3.38	73.26	3.74	73.07	3.73
4	70.38	3.06	70.32	3.08	81.85	3.46	81.61	3.46
5	69.53	3.55	69.36	3.55	72.25	3.67	72.05	3.66
6	64.32	4.35, 4.08 $J_{6a,6b}$ = 11.6 Hz, $J_{6b,5}$ = 6.8 Hz	64.07	4.39, 4.01 $J_{6a,6b}$ = 11.8 Hz, $J_{6b,5}$ = 6.6 Hz	60.16	3.75	59.96	3.74
OMe	54.58	3.25	54.36	3.26				
1′	170.08	—	175.36	—	170.19	—	175.48	—
2′	69.62[b]	4.25	71.70[b]	4.33	69.71	4.42	72.05	4.45
3′	80.12	4.44	73.09	4.27	79.98	4.58	73.38	4.38
4′	69.35[b]	4.25	78.77	4.76	69.50	4.37	78.73	4.92
5′	70.33	4.47	68.56	4.35	70.46	4.59	68.84	4.5
6′	175.99	—	170.62	—	176.05	—	170.63	—
1^I					102.49	5.00	102.41	5.05
4^I					82.08	3.58	82.0	3.55
5^I					68.96	3.98	68.93	3.96
6^I					64.03	4.40, 4.36	63.59	4.58, 4.20

[a] C′ indicates a carbon in the lactone; C^I indicates a carbon in the cyclodextrin residue to which the lactone is attached.
[b] Assignments may be interchanged.

D-glucaric acid source present in the reaction mixture, D-glucaro-1,4:6,3-dilactone, through transesterification involving one or the other lactone function, or via the individual lactone–acids as minor mixture components. Consequently, **1** must be formed by acylation with the glucaro-1,4-lactone unit of the dilactone, and **2** must similarly be formed from the glucaro-6,3-lactone unit of the dilactone. These results indicate that the 1,4-lactone is some 50% more reactive than the 6,3-lactone, in keeping with similar differential activity which occurs in ring-opening during aminolysis.[8]



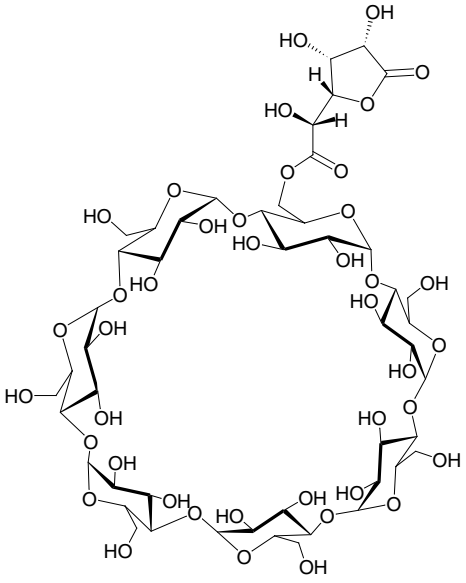
1



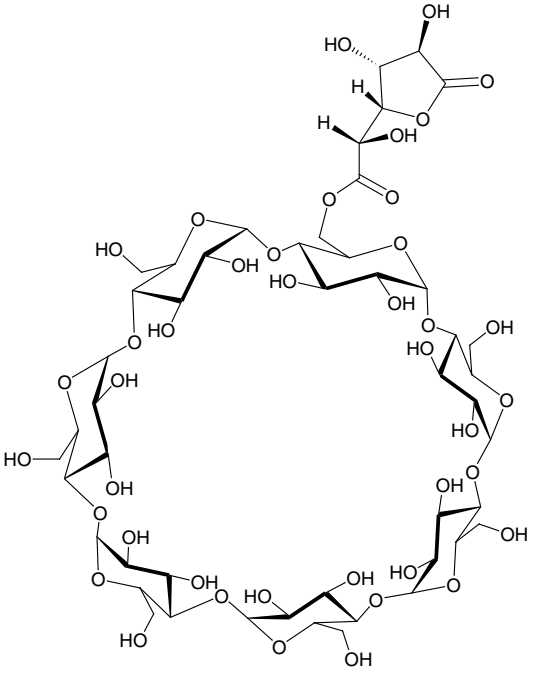
2

2.3. Thermolysis of β-cyclodextrin with lactone mix

The melt product of β-cyclodextrin was analyzed by ESIMS and ions *m*/*z* 1173, 1347, 1521, 1695, 1869 were observed corresponding to $[M+K]^+$ for cyclodextrin and cyclodextrin with the addition of one, two, three and four ester–lactone units, respectively. A physical mixture of β-cyclodextrin and lactone mix showed only the ion 1173 but a freeze-dried mixture showed 1347 and 1521 in addition to 1173. A multiplicity of products are possible for this reaction, which corresponds to the case of a 'free necklace'[9] described as N′(7,3) when the lactone adds only to the primary position of a glucose; in this case the total number of possible products is 198. The assumption that addition occurs principally at C6 is based upon the results for methyl glucopyranoside, in which only two major products were observed by GC–MS; addition at other positions would only increase the number of possible products. Notwithstanding this, it was possible to isolate fractions by preparative HPLC which corresponded to the two possible monosubstituted products, **3** and **4**, when analyzed by ESIMS. These two products, characterized from their NMR (Table 1) and high resolution mass spectra, proved to be 6^{I}-*O*-[D-glucaro-6,3-lactone-(1′→6^{I})]cyclomaltoheptaose (**3**) and 6^{I}-*O*-[D-glucaro-l,4-lactone-(6′→6^{I})]-cyclomaltoheptaose (**4**), respectively. Compounds **3** and **4** were isolated in the approximate ratio 2:1 presumably due again to the greater reactivity of the 1,4-lactone as indicated above.



3



4

In the carbon NMR spectra of cyclodextrin, the effect of a substitution on the glucose residues is to remove the

equivalency of the atoms in these glucose residues so that the carbon spectrum shows clusters of peaks for the carbons in the six unsubstituted glucose residues. It was, however, possible to distinguish C-6^{I}, C-5^{I}, C-4^{I} and C-1^{I} of the substituted glucose residue and an HMBC crosspeak was observed from C-1′ (C-6′) of the lactone to H-6^{I}a and H6^{I}b in both **3** and **4**. Despite the fact that the carbon NMR spectra showed an almost pure compound for **4**, the ESIMS revealed significant quantities of the disubstituted and unsubstituted β-cyclodextrin. This indicates that the ionization conditions of the ESIMS encourage disproportionation by transfer of the lactone. As expected,[10] substitution of the β-cyclodextrin markedly increased its water solubility.

We conclude that D-glucaric acid lactones can readily be grafted onto primary positions of polyols or polysaccharides under mild non-toxic conditions. This can be expected to modify the physical properties of the substrate molecule providing potential for modifications of starch for the food industry as well as for other applications.

3. Experimental

3.1. General methods

Xylitol, α-methyl D-glucopyranoside and cyclomaltoheptaose (β-cyclodextrin) were purchased from Sigma and used as such. Freeze drying was carried out with a Labconco FreeZone 4.5 freeze dry system. Monopotassium glucarate was prepared at the Shafizadeh Center using a standard procedure.[11]

3.1.1. Gas chromatography–mass spectrometry (GC–MS). GC–MS of samples as per-*O*-trimethylsilyl (per-*O*-TMS) derivatives was carried out using an Agilent 6890 N GC fitted with a Zebron ZB5 fused silica column (30 m × 0.25 mm × 0.25 μm) interfaced to an Agilent 5973 mass selective detector. The temperature program was 120 °C + 30 °C/min to 320 °C.

3.1.2. Electrospray-mass spectrometry (ESIMS). ESIMS of methyl α-D-glucopyranoside derivatives was carried out using a Micromass LCT time-of-flight mass spectrometer. ESIMS of β-cyclodextrin derivatives was carried out using a Bruker Daltonics MicrOTOF time-of-flight mass spectrometer.

3.1.3. Nuclear magnetic resonance spectrometry (NMR). NMR of methyl α-D-glucopyranoside derivatives was carried out using a Varian Unity *plus* 400 MHz spectrometer. NMR of β-cyclodextrin derivatives was carried out using a Bruker DRX400 400 MHz spectrometer. Samples were dissolved in DMSO-d_6 and referenced thereto.

3.1.4. Preparative liquid chromatography (LC). LC was carried out using three Waters Delta Pak 25 × 10 radial compression cartridges eluted at 15 mL/min and with refractive index detection. For α-methyl D-glucopyranoside the eluent was water and for β-cyclodextrin the eluent was 8.5% aqueous methanol.

3.2. Preparation of D-glucaric acid lactones (lactone mix)

To a suspension of monopotassium glucarate (20 g, 0.086 mol) in deionized water (250 mL) in a 2.0 L flask was added Dowex 50WX8-100 ion exchange resin (H^+, 70 mL) that had been pre-washed with deionized water until the wash water was colorless. The mixture was stirred on a radial shaker (4 h) during which time the monopotassium glucarate gradually dissolved. The resin was removed by vacuum filtration and the filtrate evaporated to an amber syrup under reduced pressure. The syrup was freeze-dried to yield an amorphous solid determined by GC–MS to be a mixture of acyclic D-glucaric acid, D-glucaro-l,4-lactone, D-glucaro-6,3-lactone and D-glucaro-1,4:6,3-dilactone (acid/lactone mix).

3.3. Preparation and reaction of model compounds

Xylitol and α-methyl D-glucopyranoside were each dissolved in water together with acid/lactone mix in approximately 1:1 mol ratio, β-cyclodextrin was dissolved with acid/lactone mix in the ratio 1:5 by weight. The resulting solutions were freeze-dried. The freeze-dried products were heated in air for 30 min at 90 °C for xylitol and 120 °C for the other model compounds. The melt products were allowed to cool, open to air at room temperature.

3.3.1. Xylitol. A sample of the xylitol melt product was taken directly for GC–MS and the remainder dissolved in water for ESIMS.

3.3.2. α-Methyl D-glucopyranoside. Analysis by GC–MS of the melt product showed two products, which were subsequently shown to be dimers **1** and **2**, with retention times 12.3 and 11.8 min, respectively, and yields by GC-FID of 17.8% and 12.5%, respectively, plus other minor products. The melt product was dissolved in water and fractionated by preparative LC yielding products **1** and **2** with retention times of 29 and 42 min, respectively. Compound **1** was characterized by NMR (Table 1) and by high resolution ESIMS of the ion $[M+Na]^+$ ($C_{13}H_{20}O_{12}Na$; found: 391.0885, calcd: 391.0852). These data were consistent with a structure of compound **1** as methyl 6-*O*-[D-glucaro-6,3-lactone-(1′→6)]-α-D-glucopyranoside. Compound **2** was similarly characterized by NMR (Table 1) and high resolution ESIMS ($C_{13}H_{20}O_{12}Na$; found: 391.0892,

calcd: 391.0852). Compound **2** was designated as methyl 6-*O*-[D-glucaro-l,4-lactone-(6′→6)]-α-D-glucopyranoside.

3.3.3. β-Cyclodextrin. The melt product of β-cyclodextrin (24 g containing 4 g β-cyclodextrin) was extracted with MeOH (5 × 200 mL) and the insoluble material analyzed by ESIMS, which demonstrated the presence of singly and multiply substituted cyclodextrin. Preparative LC using 8.5% MeOH yielded, inter alia, **3** (188 mg) and **4** (87 mg) which eluted at 21 and 31 min, respectively compared to unreacted β-cyclodextrin, which eluted at 51 min. NMR (Table 1) and high resolution ESIMS of the ion $[M+Na]^+$ ($C_{48}H_{76}O_{41}Na$; found: 1331.3770, calcd: 1331.3754) showed **3** to be (β-cyclodextrin monosubstituted with D-glucaric acid 6,3-lactone linked (1′-6^I) to one of the glucose residues (6^I-*O*-[D-glucaro-6,3-lactone-(1′→6^I)]-cyclomaltoheptaose). Similarly NMR, Table 1, and high resolution ESIMS of the ion $[M+Na]^+$ ($C_{48}H_{76}O_{41}Na$; found: 1331.3794, calcd: 1331.3754) showed **4** to be β-cyclodextrin monosubstituted with D-glucaric acid 1,4-lactone linked (6′-6^I) to one of the glucose residues (6^I-*O*-[D-glucaro-l,4-lactone-(6′→6^I)]-cyclomaltoheptaose).

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