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PREPARATION OF CARBONATES OF POLYSACCHARIDES AND CYCLOAMYLOSES

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ABSTRACT

The preparation of water-insoluble carbonates of cellulose, diethylaminoethylcellulose, nigeran, and xylan, containing *trans*-2,3-carbonate groups, is described. The occurrence of a carbonyl peak in the i.r. spectrum of inulin carbonate at 1820 cm^{-1}, in addition to one corresponding to acyclic carbonate (*O*-ethoxycarbonyl, 1750 cm^{-1}), was attributable to formation of the strained *trans*-4,6-carbonate group on the fructofuranose residues of the inulin chain, in addition to the formation of the *trans*-2,3-carbonate group on the relatively small number of terminal D-glucopyranose residues. The relative contents of acyclic carbonate of the products appeared to be a function of the reaction conditions rather than the availability of a free hydroxyl group at C-6. The presence of carboxyl groups in carboxymethylcellulose and alginic acid prevented the formation of *trans*- and *cis*-2,3-carbonate groups, respectively, but derivatisation of alginic acid propylene glycol ester was successful. Specialised procedures were required for the isolation of cyclohexa-amylose and cyclohepta-amylose carbonates.

INTRODUCTION

trans-Cyclic carbonates have been prepared by the action of ethyl chloroformate on pyranoid compounds in the presence of triethylamine[1,2]. Thus, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside gives the 2,3-carbonate, methyl 2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside gives the 3,4-carbonate, and methyl 2,3-di-*O*-methyl-α-D-glucopyranoside gives the 4,6-carbonate. Preparation of sugar carbonates has generally been confined to monosaccharides, since it is easier to prepare exclusively *trans*-cyclic carbonates from suitably protected monosaccharides. However, for polysaccharides, similar protection can prove difficult, and heterogeneous reactions usually occur. Thus, in the preparation of polysaccharide carbonates, the products from unprotected starting materials usually contain a mixture of cyclic and acyclic carbonate (*O*-ethoxycarbonyl) groups, *e.g.*, in the case of dextrin[3] and cellulose[4] carbonates. Dextran, where the C-6 positions are blocked by glycosidic linkage, gave dextran *trans*-2,3-carbonate[3], and 6-*O*-tritylamylose gave 6-*O*-tritylamylose *trans*-2,3-carbonate[1], which contained acyclic carbonate groups. The preparation of 6-*O*-

tritylamylose *trans*-2,3-carbonate, containing exclusively cyclic carbonate groups, *via* the thionocarbonate, has also been reported[5].

Polysaccharide carbonates are usually water soluble. However, the optimal conditions for preparing cellulose carbonate[4] had been sought with a view to providing a water-insoluble matrix in which the nucleophilic susceptibility of the *trans*-2,3-carbonate group could be utilised for the formation of active, water-insoluble, covalent derivatives of enzymes, immunoglobulins, etc. This work led to the successful formation of such derivatives of, for example, β-D-glucosidase[6] (E.C. 3.2.1.21). Cellulose *trans*-2,3-carbonate has also been used for the insolubilisation of antibodies to human immunoglobulin IgE, the active product being suitable for the determination of human myeloma forms of immunoglobulin IgE by radioimmunoassay[7]. The industrial interest shown in cellulose carbonate prompted an investigation of the formation of *trans*-carbonate groups in polysaccharides of other types with a view to determining their potential for insolubilisation of biologically active molecules. The results of an investigation of cyclic carbonate formation in certain neutral, acidic, and basic polysaccharides, and in cycloamyloses are now reported.

EXPERIMENTAL

The conditions established for the preparation of cellulose carbonate having the highest degree of substitution of cyclic carbonate[4] were adopted for these preparations. Quantitative infrared spectroscopy was performed in the range 2000–1600 cm^{-1}, using KBr discs and a Perkin–Elmer Model 21 double-beam infrared spectrophotometer as previously described[4].

Preparation of carbonates of polysaccharides insoluble in methyl sulphoxide. — Cellulose (Sigmacell, particle size 38 μm, Sigma Chemical Co., 0.2 g) and filter paper (Whatman No. 1) in sheet and shredded form (50 mg) were separately suspended in dry mixtures of methyl sulphoxide (2.0 ml), 1,4-dioxane (0.3 ml), and triethylamine (1.6 ml), and stirred for 5 min at 0°. Ethyl chloroformate (3.2 ml) was added dropwise during 12 min with stirring and the mixture was left to react with stirring for a further 10 min. The products were immediately washed with dry 1,4-dioxane (10 × 15 ml), dry ethanol (3 × 15 ml), and ethyl ether (3 × 15 ml), dried, and stored *in vacuo* over phosphorus pentaoxide at 20° and assessed by i.r. spectroscopy (Table I).

Carboxymethylcellulose (CM32, microgranular form, Whatman, 0.2 g), diethylaminoethylcellulose (DE32, microgranular form, Whatman, 0.2 g), and xylan (Koch–Light Laboratories Ltd., 0.2 g) were derivatised, purified, and assessed as above (Table I).

Preparation of carbonates of polysaccharides soluble in methyl sulphoxide. — (*a*) *Nigeran and inulin carbonates.* Nigeran (Koch–Light, 0.1 g) and inulin (Koch–Light, 0.1 g) were separately dissolved in dry mixtures of methyl sulphoxide (1.0 ml) and 1,4-dioxane (0.15 ml), dry triethylamine (0.8 ml) was added, and the solutions were stirred at 0° for 5 min. Ethyl chloroformate (1.6 ml) was added dropwise during 12 min with stirring and left to react for a further 10 min. The products were washed on

sintered-glass funnels with dry 1,4-dioxane (6 × 10 ml), dry ethanol (3 × 10 ml), and ethyl ether (3 × 10 ml), and stored as described above (Table I).

TABLE I

QUANTITATIVE INFRARED SPECTROSCOPY OF POLYSACCHARIDE AND OLIGOSACCHARIDE CARBONATES

Carbohydrate	*Ratio of absorbances[a] at 1750 and 1810 cm^{-1}*	*Relative substitution[b]*
Cellulose, microcrystalline	0.36	100[c]
, sheet paper	0.55	4
, shredded paper	0.46	20
Carboxymethylcellulose	—	1
Diethylaminoethylcellulose	0.10	11
Xylan	0.32	65
Nigeran	0.75	39
Inulin, direct filtration	2.71	18
, precipitation before filtration	1.38	79
, ex filtrate	1.02	n.d.[d]
Cyclohexaamylose *via* dialysis	2.0	1
via extraction	1.27	18
via ion-exchange	0.66	30
Cycloheptaamylose *via* dialysis	3.3	1
via extraction	1.17	27
via ion-exchange	0.74	63
Sephadex G-25	—	0
Sephadex G-100	—	0
Alginic acid	—	0
Sodium alginate	—	0
Calcium alginate	—	0
Alginic acid propylene glycol ester	1.20	10

[a]Absorbance at 1750 cm^{-1} (acyclic carbonate or *O*-ethoxycarbonyl) relative to absorbance at ~1810 cm^{-1} (cyclic carbonate). [b]Cyclic carbonate content relative to that of cellulose carbonate (100) prepared under standard conditions[4]. [c]This preparation of cellulose carbonate possessed a degree of substitution of 0.54 in terms of cyclic carbonate groups. [d]Not determined.

From the low yield of inulin carbonate, it appeared that some product had been lost in the reaction solvent mixture. The derivatisation was repeated and the reaction mixture was treated with excess of 1,4-dioxane before filtering and washing (Table I). Concentration of the filtrate by rotary evaporation until only methyl sulphoxide remained, followed by addition of ether, precipitated a small amount of material (Table I).

(*b*) *Cycloamylose carbonates.* Cyclohexa-amylose (Schardinger α-dextrin) (0.3 g) and cyclohepta-amylose (Schardinger β-dextrin) (0.3 g) were derivatised as described above, and the final reaction mixtures, which contained insoluble material, were divided into three equal aliquots. The first aliquots were dialysed against running water for 2 h and then evaporated to dryness, and the residue was dried as usual. The second aliquots were air-dried on a sintered-glass funnel and washed once with dry

1,4-dioxane (10 ml) at 20°, and the filtrate was discarded. The solids were then washed with dry 1,4-dioxane (6 × 10 ml) pre-warmed to 60°, and the filtrates were collected, combined, and evaporated to dryness (yields up to 90%). The third aliquots were air-dried, washed with dry 1,4-dioxane (6 × 10 ml) at 20°, and suspended in dry 1,4-dioxane (15.0 ml). To the suspensions, Dowex-AG50W x8 (H^+) resin (200–400 mesh) was added gradually until the precipitates dissolved. The filtered solutions were then evaporated to dryness (yields up to 40%).

Preparation of carbonates of polysaccharides which swell in methyl sulphoxide. — (*a*) Sephadex G-25 (Pharmacia Ltd., coarse, 0.1 g) and Sephadex G-100 (Pharmacia Ltd., ordinary size, 0.1 g) were separately suspended and derivatised, as described for xylan (Table I).

(*b*) Alginic acid (0.1 g), sodium alginate[8] (0.1 g), calcium alginate (0.1 g, prepared by treatment of the sodium salt with calcium chloride), and Manucol Ester (propylene glycol alginate, EA/LH, 60% esterified, Alginate Industries Ltd.) (0.1 g) were derivatised, as described for xylan (Table I).

DISCUSSION

The conditions presently used for the generation of cyclic carbonate groups in polysaccharides were those which had been shown to give the highest degree of substitution for cellulose, together with the lowest ratio of acyclic to cyclic carbonate groups[4]. The i.r. spectrum of xylan carbonate was similar to that of cellulose carbonate[4], showing bands at 1840, 1810, and 1750 cm^{-1}. The content of cyclic carbonate was quite high, and the ratio of acyclic to cyclic carbonate (0.32, Table I) was almost the same as for cellulose carbonate (0.36). The higher content of acyclic carbonate for dextrin carbonate, compared with that for dextran carbonate, has been explained in terms of the availability of HO-6 in the former carbohydrate[3]. However, it is clear from the results for 6-*O*-tritylamylose[1] and for xylan that acyclic carbonate formation can occur equally well when there is no primary hydroxyl group. This appears to be in agreement with our earlier claim[4] that the ratio of acyclic to cyclic groups is dependent upon the reaction conditions. Attempts to prepare cellulose 2,3-carbonate (containing exclusively cyclic carbonate groups) from cellulose xanthate, in a way similar to that used for 6-*O*-tritylamylose, were unsuccessful (unpublished observations). Whereas 6-*O*-tritylamylose xanthide was soluble in pyridine, giving 6-*O*-tritylamylose thionocarbonate and thence the 2,3-carbonate, cellulose xanthide was hardly soluble in pyridine and treatment without dissolution gave only a very minor conversion of xanthide into thionocarbonate groups.

Strong absorption bands were observed for nigeran carbonate at 1820 and 1750 cm^{-1}, but no absorption band in the region of 1840 cm^{-1} was observed although vicinal hydroxyl groups in the polysaccharide are suitably disposed for formation of *trans*-2,3-carbonate. The relative substitution of nigeran (Table I, 2 × 39) approaches that of cellulose in terms of the D-glucopyranose residues capable of forming *trans*-cyclic carbonate groups.

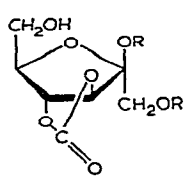
Inulin is of interest since the dihedral angle for HO-3 and HO-4 is 120°, and thus it can be argued that they are not suitably disposed for *trans*-cyclic carbonate formation. The particular inulin used in the present work was obtained from *Cichorium intybus*, contained 97% of D-fructose and 3% of D-glucose arranged as shown in **1**, and had a molecular weight of 5,500. Thus, for each chain of approximately thirty D-fructofuranose residues, there exists one D-glucose residue. The i.r. peak at

$$\beta\text{-D-Fru}f\text{-}(2\rightarrow[1)\text{-}\beta\text{-D-Fru}f\text{-}(2\rightarrow]_n1)\text{-}\beta\text{-D-Fru}f\text{-}(2\rightarrow1)\text{-}\alpha\text{-D-Glc}p$$

1

1820 cm^{-1} can be due only partially to *trans*-2,3-cyclic carbonate groups on the terminal D-glucose residues. Even if all the D-glucose residues were converted, this would correspond to a degree of substitution of 0.03, whereas the observed value is 0.43 (material precipitated before filtration; Table I). An additional or alternative explanation for the absorption at 1820 cm^{-1} is therefore required.

Carbonate structures bridging vicinal-*trans* oxygen atoms of a furanoid ring have been tentatively reported[9] but were not confirmed. In the case of inulin, structure **2** would be involved if such a very highly strained ring could be formed. A more likely possibility is a 4,6-carbonate group on the D-fructofuranose residues. The formation of the 3,5-carbonate (**3**) of 1,2-*O*-isopropylidene-α-D-xylofuranose[10] and the 4,6-carbonate (**4**) of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside[2] have been reported. Molecular models show that the cyclic carbonate groups in **3** and **4** involve little or no torsional strain of the furanoid and pyranoid rings. The hydroxyl and hydroxymethyl groups involved in carbonate ring formation in **3** are *cis*-related and are conveniently situated for the formation of a cyclic carbonate. A similar situation holds for **4**, and the *trans*-cyclic carbonate has an i.r. carbonyl absorption at 1764–1770 cm^{-1}. However, the formation of the 4,6-carbonate **5** of the D-fructofuranose residues within the inulin chain would involve torsional strain. Since the higher wavenumber of the carbonyl group i.r. absorption of cyclic carbonates can be attributed to



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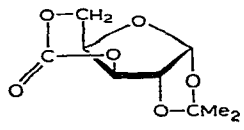

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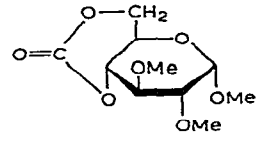

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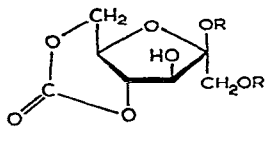

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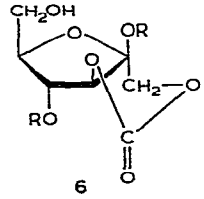

6

R = adjacent carbohydrate residues

strain in the ring, it is possible that the absorption at 1820 cm^{-1} for inulin carbonate arises from a *trans*-4,6-carbonate group. Clearly, in addition to formation of such a ring on the terminal D-fructofuranose residue, the *trans*-1,3-carbonate (**6**) could also be formed. This ring has the same stereochemistry as the *trans*-4,6-carbonate.

From the results obtained, it appears that the higher the carbonate substitution of inulin, the more soluble it is in the reaction medium. This is no doubt aided by the small molecular weight of inulin itself. Had cross-linking occurred to any great extent, the product would be expected to be largely insoluble.

Although a carbonate derivative of dextran has been reported[3], attempted derivatisation of Sephadex G-25 and G-100 was unsuccessful. This failure can be attributed to the involvement of HO-2 and HO-3 in the cross-links. However, Sephadex on activation with cyanogen bromide, has been reported to give a product containing iminocarbonate groups derived from HO-2 and HO-3 in the cross-linked dextran[11].

Since cyclohexa-amylose and cyclohepta-amylose are oligosaccharides containing α-(1→4)-linked D-glucopyranose residues, their structures are appropriate for the formation of *trans*-2,3-carbonate derivatives. Although the cycloamylose carbonates were largely insoluble in the reaction mixture used for cyclic carbonate formation, special isolation procedures were necessary because the carbonates were soluble in ethanol which is used for removal of triethylamine hydrochloride in the standard procedure. The products* both showed i.r. absorption bands at 1825 and 1750 cm^{-1}, and the relative contents of acyclic carbonate were high. However, the cyclic carbonate contents and cyclic–acyclic carbonate ratios could be considerably improved, but with loss in yield, by more-extensive washing of the cycloamylose–triethylamine hydrochloride mixture with cold 1,4-dioxane prior to extraction with hot solvent. It thus appeared that the molecules in which higher proportions of cyclic carbonate were formed during the heterogeneous reaction were less soluble in 1,4-dioxane.

The i.r. spectrum of diethylaminoethylcellulose carbonate showed bands at 1840 and 1810 cm^{-1}, corresponding to *trans*-cyclic carbonate groups. Only a small absorption maximum occurred in the region of 1760 cm^{-1}. The preferential formation of *trans*-cyclic carbonates is attributed to the participation of the basic diethylaminoethyl groups, which are regarded as occurring predominantly at C-6 of the D-glucopyranose residues, during derivatisation. The i.r. spectrum of carboxymethylcellulose carbonate showed the presence of only small amounts of cyclic and acylic carbonate groups. The low content of cyclic carbonate may be due to prevention of derivatisation of the polysaccharide by the carboxyl groups. Although the susceptibility of the cyclic carbonate group to acid-catalysed hydrolysis is much less than its susceptibility to alkaline hydrolysis, acid hydrolysis does occur. However, a theory of intermolecular or intramolecular hydrolysis of cyclic carbonate groups formed by the carboxyl

*Obtained *via* extraction with hot solvent; attempted precipitation of cyclohexa-amylose and cyclohepta-amylose carbonates by complex formation with carbon disulphide and bromobenzene, respectively, was unsuccessful, and losses occurred on dialysis.

groups cannot be invoked, since the acyclic carbonate group is stable and, being formed at the same time as cyclic carbonate, would be expected to give an i.r. band.

The interference by a carboxyl group in the carbohydrate structure in the formation of both cyclic and acyclic carbonate groups was further demonstrated by the failure to derivatise alginic acid. The carboxyl-group effect in this case was not overcome by salt formation, and it was not until it was esterified with propylene glycol that carbonate-group formation was possible. For alginic acid, account must also be taken of the fact that the structure involves α-(1→4)-linked D-mannuronic and, to a lesser extent, L-guluronic acid residues in which *trans*-carbonates cannot be formed. The i.r. spectrum of the alginate ester carbonate showed absorption maxima at 1810 and 1750 cm^{-1} but none in the region of 1840 cm^{-1}.

The reactivity of the cyclic carbonate group in insoluble compounds has already been demonstrated. Thus, cellulose *trans*-2,3-carbonate[6,7] (five-membered ring) and poly(allyl carbonate)[12,13] (eight-membered ring) have been employed in the preparation of active, water-insoluble derivatives of enzymes and immunoglobulins. Of the presently reported polysaccharide carbonates, xylan, nigeran and, possibly, diethylaminoethylcellulose carbonates would be expected to be similarly useful. The *trans*-4,6-cyclic carbonate group of inulin carbonate could well fill a similar role since it could be highly reactive on account of ring strain; the less-strained carbonate ring of **4** was reported to be more reactive than a *trans*-2,3-carbonate[2]. The successful derivatisation of xylan and cellulose pointed to the possibility that woody materials could be used in large-scale industrial applications. Sawdust from soft and hard woods has been successfully treated to yield significant contents of cyclic carbonate (unpublished results).

The cycloamylose carbonates are also of particular interest, since, on coupling with enzymes, they would provide species in which the macromolecules radiated from a centre. Such a situation could present a lower degree of crowding of the active sites in the macromolecules than in a linear polysaccharide having the same degree of substitution.

Currently, there is considerable interest in the attachment of biologically active molecules to sheets for subsequent use as specific membranes and as antibody carriers in solid-phase radio-immunoassays. From the results obtained for cellulose in sheet form (Table I), it is clear that the initial, fine division of the polysaccharide is essential for a good, overall yield of cyclic carbonate substitution. However, this does not rule out the possibility of formation of a high density of the reactive carbonate groups on the surface of the sheet.

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