# Synthesis and reactions of uronic acid derivatives XXI.* Controlled esterification of oligo-D-galactosiduronic acids with diazomethane

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A method of preparing partial methyl esters of oligo-D-galactosiduronic acids (DP 2—9) having defined, predetermined degree of esterification, based on controlled reaction of the corresponding acids with diazomethane, has been elaborated.

Был разработан метод приготовления частичных метиловых эфиров олиго-D-галактозидуроновых кислот со степенью полимеризации DP 2—9 с определенной, предварительно заданной, степенью этерификации при помощи этерификации с диазометаном.

Methyl oligo-D-galactosiduronates (DP 2—9, degree of esterification 50—70%) are suitable substrates for studying the action and, particularly, the size of the binding site of the enzyme pectinesterase (pectin pectyl-hydrolase, EC 3.1.1.11). Compounds of this type are difficult to obtain and preparation of partially esterified oligo-D-galactosiduronic acids having defined, predetermined degree of esterification has not been described in the literature.

Previously developed procedures for preparing partially esterified oligo-D-galactosiduronic acids and D-galacturonans are based on treatment for several hours of an oligo-D-galactosiduronic acid with methanolic hydrogen chloride at various temperatures, and periodical determination of the esterification degree achieved; when the desired degree of esterification has been achieved the reaction is terminated by neutralization, and the product is isolated [2—4]. Alternatively, the substrate is esterified completely and partially deesterified with an alkali hydroxide [5].

The disadvantage of partial esterification of oligo-D-galactosiduronic acids and related polymeric materials (DP > 10) with methanolic hydrogen chloride lies in the fact that the final degree of esterification cannot be predetermined. Moreover, formation of glycosides occurs simultaneously with esterification under these conditions to a not neglectable extent, especially when the reaction is conducted at temperatures above 0°C [2]. Another drawback of the esterification methods based

*For Part XX see Ref. [1].

on treatment of substrates with methanol under acidic conditions is in the accompanying losses of precious substances, resulting from periodical determination of the degree of esterification. Also, when partially esterified oligo-D-galactosiduronic acids are prepared by partial deesterification of fully esterified products under alkaline conditions partial degradation by $\beta$ elimination may occur [6].

Following the original work of *Morell* and *Link* [7] who were the first to prepare methyl D-galactopyranuronate by treatment of D-galacturonic acid with diazomethane, pectic substances have been treated with the same reagent. Here, the reaction is conducted under heterogeneous conditions and the achieved degree of esterification is periodically determined. It was recommended [8] that the reaction be carried out at temperatures below $-25$°C, since it had been found [9] that treatment of pectin with diazomethane at 0°C resulted in its partial depolymerization. *Smit* and *Bryant* [10], after having partially neutralized free carboxyl groups, treated pectin with excess of diazomethane under heterogeneous conditions and obtained partially esterified products (degree of esterification $>60\%$); the reagent has not, however, been used to obtain partially esterified oligo-D-galactosiduronic acids.

The present work describes a method of controlled esterification of oligo-D-galactosiduronic acids (DP 2—9) with diazomethane. The procedure makes partially esterified substances of this class, with defined, predetermined esterification degree, readily available. The reaction, conducted under homogeneous conditions, comprises neutralization of a portion of carboxyl groups present in oligo-D-galactosiduronic acids by addition of a defined amount of sodium hydroxide, followed by addition of excess of diazomethane to convert the remaining free carboxyl groups to the corresponding methyl esters. The thus obtained sodium salts of partial esters are then converted to the desired partially esterified free acids having defined, predetermined degree of esterification by percolating their solutions through a column of cation-exchange resin ($H^+$ form). No cleavage of glycosidic linkages of the used substrates has been observed during these processes (t.l.c.). The products were analyzed for methoxyl and carboxyl groups and the obtained results agreed with the expected, predetermined degree of esterification indicating that the formation of glycosides had not occurred to a significant extent. This was confirmed by $^1$H-n.m.r. spectra of the products showing that the intensity of the signals corresponding to the glycosidic methoxyl groups was negligible, as compared to that of the signals corresponding to the ester methoxyl groups.

The described esterification procedure can be recommended particularly for small-scale work with substances that are difficult to obtain. The yields of desired products are virtually theoretical; the produced partial esters can be stored conveniently in the form of salts for at least five months. The distribution of ester groups along the oligosaccharide chain, as that in products obtained by applying a different method of preparation, is assumed to be random.

*Table 1*

Partially esterified oligo-D-galactosiduronic acids obtained by controlled esterification with diazomethane

| Oligo-D-galactosid-uronic acid DP | Desired degree of esterification | Esterification degree achieved | | Yield % |
|---|---|---|---|---|
| | | according to the content of methoxyl groups | according to the content of carboxyl groups | |
| 2 | 0.50 | 0.47 | 0.48 | 93.5 |
| 3 | 0.66 | 0.65 | 0.67 | 92.0 |
| 4 | 0.50 | 0.50 | 0.49 | 95.3 |
| 5 | 0.60 | 0.58 | 0.59 | 91.7 |
| 5 | 0.40 | 0.39 | 0.41 | 89.1 |
| 6 | 0.50 | 0.48 | 0.49 | 92.7 |
| 7 | 0.57 | 0.55 | 0.56 | 94.0 |
| 8 | 0.50 | 0.50 | 0.48 | 90.3 |
| 9 | 0.70 | 0.67 | 0.68 | 89.5 |

## Experimental

Oligo-D-galactosiduronic acids (DP 2—9) were prepared according to *Rexová-Benková* [11], and their purity was verified by determination of reducing [12] and carboxyl groups (by titration), and by chromatography (DP *vs.* log $R_f/1 - R_f$ [13]). They were stored in the form of potassium salts.

The degree of esterification of the final products was determined by titration of decationized and lyophilized partial esters, and by determination of methoxyl groups [14]. Since the formation of glycosides, as a result of treatment of the substrates with diazomethane, could not be a priori excluded, the extent of glycosidation was checked by $^1$H-n.m.r. spectroscopy: the intensity of signals corresponding to OMe groups (at $\delta \sim 3.4$) was compared with that of COOMe groups (at $\delta \sim 3.8$), and the spectra were compared also with those of the starting substrates.

$^1$H-N.m.r. spectra (80 MHz) for solutions in $D_2O$ (internal standard sodium 3-(trimethylsilyl)(2,2,3,3-$^2H_4$)propionate) were obtained at 25°C using a Tesla BS 487 B spectrometer.

Ethereal diazomethane was prepared as follows: aqueous potassium hydroxide (50%, 5 ml) was added at 0°C to a stirred mixture of *N*-nitroso-*N*-methylurea (10 g) in ether (100 ml). When the urea derivative had reacted completely (~15 min) the mixture was dried with potassium hydroxide pellets, the ethereal solution was decanted into a dry flask and stored over solid potassium hydroxide at −20°C. Prior to the use, the solution was diluted with an equal volume of cold methanol.

### *Partially esterified oligo-D-galactosiduronic acids*

A sample of sodium oligo-D-galactosiduronate (DP 2—9, 100 mg) was dissolved in deionized water (20 ml) and converted to a free acid by percolating the solution through a column of Dowex 50 W X8 cation-exchange resin ($H^+$ form). According to the desired degree of esterification, a calculated amount of 0.1 M sodium hydroxide was added (determining the amount of remaining free carboxyl groups), followed by methanol (to final concentration of methanol, 20%). The mixture was cooled (~30°C) and, with stirring, ethereal diazomethane (see above) was added portionwise until yellow colour persisted (for ~1 min), while the reaction vessel was immersed in an acetone—dry ice cooling bath. Organic solvents were removed at reduced pressure without heating and water at 30°C, and the residue was freeze-dried to give sodium salts of partially esterified oligo-D-galactosiduronic acids in the form of amorphous solids. For analyses and/or enzymic studies the partially esterified, free acids were obtained by percolating aqueous solutions of the afore-mentioned products through a column of Dowex 50 W X8 cation-exchange resin ($H^+$ form). Pertinent data for the prepared products are collected in Table 1.

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