High-performance liquid chromatography of reaction mixtures from the oxidation and degradation of lactose

L. A. Th. VERHAAR, H. E. J. HENDRIKS, W. P. Th. GROENLAND and B. F. M. KUSTER*
Laboratorium voor Chemische Technologie, Technische Universiteit Eindhoven, P.O. Box 513, 5600 MB
Eindhoven (Netherlands)
(First received December 4th, 1990; revised manuscript received February 28th, 1991)

ABSTRACT

A method is described for the high-performance liquid chromatographic analysis of reaction mixtures from the heterogeneously catalysed oxidation of lactose to lactobionate and the homogeneous alkaline oxidative degradation of lactose to β-O-D-galactopyranosyl-(1→3)-D-arabinonate. Two columns in series, a resin-based anion-exchanger in the acetate form and a resin-based cation exchanger in the H⁺ form with water as the eluent, allow the quantification of the sugars without interference from the oxidation and degradation products. Occasionally an RP-18 reversed-phase column was used for the determination of lactulose in the reaction samples. A resin-based anion-exchange column in the Cl⁻ or SO₄²⁻ form allows the determination of the anionic oxidation and degradation products without interference from the sugars present in the reaction samples. In the analyses of the anionic oxidation and degradation products a method was developed to determine some of these products without their pure standards. The method is based on the regular behaviour in refractive index detection of members of a homologous series of aldonic acids.

INTRODUCTION

Lactose or milk sugar is produced from the liquid residue that remains in the process of cheese or milk protein production. This by-product can be upgraded by selective oxidations such as the heterogeneous catalytic oxidation of lactose to lactobionate [1] or the homogeneous oxidative degradation of lactose to β-O-D-galactopyranosyl-(1→3)-D-arabinonate [2]. The main reaction paths of both processes are depicted in Fig. 1 by routes 1 and 2, respectively. In both instances mixtures of sugars and alkali metal salts of oxidation and degradation products are obtained.

Sugars and their oxidation and degradation products have been analyzed by gas chromatography (GC) after derivation to their trimethylsilyl ethers [3–5]. Because the silylating agent also reacts with water, water has to be evaporated almost quantitatively before silylation can be carried out. Another complicating factor in GC can be the appearance of separate peaks for anomeric forms and ring structures.

Aqueous solutions of sugars and their ionic oxidation and degradation products have been analyzed without derivatization using liquid chromatography.

Non-ionic sugars have been chromatographed by (i) amine-modified silica gel
Fig. 1. Simplified reaction scheme for the heterogeneous catalytic oxidation of lactose (route 1) and homogeneous oxidative degradation of lactose (route 2). LAC = lactose; LBA = lactobionate; GARA = β-D-galactopyranosyl-(1→3)-D-arabinonate; LYXA = D-lyxonate; 2-deTA = 2-deoxy-D-tetronate; FA = formate; GA = glycolate.

columns [6–9], (ii) octadecyl-modified silica gel columns [10–13], (iii) anion-exchange resin columns [14–17] and (iv) cation-exchange resin columns [18–21]. In amine-modified silica gel chromatography, using acetonitrile–water mixtures as the eluent, good separations have been achieved between sugars which differ in molecular weight or structure; much lower selectivity exists between the stereoisomers. The chromatographic separation of deionized lactose–lactulose–galactose mixtures using amine-modified silica gel stationary phase was described by Parrish et al. [9]. However, the time-consuming deionization step, necessary to prevent coelution of the organic anions and the sugars, and the use of the toxic acetonitrile are drawbacks of this method. Chromatography on RP-18 columns with water as the eluent has been reported for sugar oligomers. The retention increases with increasing molecular weight and separate elution has been obtained for the anomeric forms. The selectivity for monosaccharides is very low and for retention of the disaccharides a subambient column temperature is necessary.

Anion-exchange chromatography based on borate complexation in combination with chemical detection methods has frequently been used in the past. Although a high selectivity for mono- and disaccharide separation can be obtained, the relatively long analysis time and the loss of resolution by the chemical detection methods
are serious drawbacks of this method. Moreover, the high pH of the borate buffer used as the eluent gives rise to isomerization of the sugars during their elution, as reported for lactose-lactulose by Carubelli [14].

Sugars have also been analysed using resin-based cation exchangers in the Ca$^{2+}$ [18,19], Pb$^{2+}$ [20] or H$^+$ form [21]. If a high column temperature was chosen or mutarotation catalysts [22,23] were used, separate peaks for the anomic furanose and pyranose forms could be avoided. However, the usability of cation-exchange resin columns is limited by their low retention for sugars. This is caused by molecular exclusion, which is in competition with complexation forces between the polyhydroxy compounds and the immobilized cation on the resin. As a result, capacity factors lower than 2 are obtained for sugars, as can be seen from the results of Pecina et al. [24]. Moreover, the anionic oxidation and degradation products present in the reaction mixtures will also have a low retention [25], hampering simple and accurate sugar determinations.

The organic anions formed in the oxidation and degradation reactions are polar compounds without any apolar functionality, so a separation mechanism based on hydrophobic interactions (reversed-phase chromatography) can be excluded. However, differences in the strengths of the acids opens up the possibility of using anion-exchange chromatography [26-30]. For polyhydroxycarboxylic acids, retention increases with decreasing molecular size and, moreover, dibasic acids elute after monobasic acids of the same carbon number. The retention can be influenced by the addition of a complexing cation to the eluent. For example, the elution of oxalate and tartrionate can be accelerated drastically by the addition of Mg$^{2+}$ ions to the eluent [30]. Complexation of these organic anions by Mg$^{2+}$ ions reduces the interaction between the organic anion and the counter ion of the resin. For all the anion-exchange systems mentioned above, non-ionic sugars were not retarded.

This paper describes a high performance liquid chromatographic (HPLC) method for the analysis of mixtures of sugars and their oxidation and degradation products, shown in Fig. 1.

The analysis of the non-ionic sugars in the presence of oxidation and degradation products is mainly performed on an anion-exchange column in the acetate (Ac$^-$) form in series with a cation-exchange column in the H$^+$ form, using water as the eluent and refractive index (RI) detection. Occasionally an RP-18 reversed phase column was used to verify the occurrence of lactulose under the reaction conditions applied.

Two independent anion-exchange columns allow the analysis of the anionic oxidation and degradation products, using serial UV and RI detection. The oxidation products could be chromatographed without interference from the sugars and vice versa. In the analysis of the oxidation and degradation products a method was developed to determine some products without the pure standards. The method used is based on the regular behaviour in refractive index detection for a homologous series of aldonates.

EXPERIMENTAL

Apparatus

A Spectra-Physics Type SP 8800 ternary HPLC pump in combination with an
SP 8775 autosampler, a Rheodyne Model 7010 injection valve with a 20-µl sample loop and a SP 8790 column heater were used. A Millipore Waters Type R410 refractive index detector in series with a Spectra-Physics SP 8450 variable-wavelength UV–VIS detector operated at 212 nm and connected to a SP 4290 two-channel digital integrator allowed dual detection and peak integration for the various compounds.

Columns

Before packing a column, the resin material was washed twice with 20 equiv. of a 0.5 M solution of the ionic form needed. After washing, the resin was dispersed for 30 min in an ultrasonic bath and packed in the analytical column with the eluent as the liquid phase at a pressure of about 100 atm.

Five columns were used, as follows.

**BA-X8 (Ac⁻) column.** This was a 70 × 4.6 mm I.D. Lichroma stainless-steel tube slurry packed with Benson Type BA-X8 anion-exchange resin, having quaternary ammonium functional groups attached to the polystyrene–divinylbenzene (8%) lattice and with a particle diameter of 7–10 µm, and during the packing washed overnight with 1 dm³ of a 0.5 M solution of sodium acetate in water to obtain the AC⁻ form.

**BA-X8 (Cl⁻) column.** This was the same as the BA-X8 (AC⁻) column, but 280 mm in length, and during the packing it was washed overnight with 1 dm³ of a 0.2 M solution of sodium chloride in water to obtain the Cl⁻ form.

**BA-X8 (SO₄²⁻) column.** This was as for the BA-X8 (Cl⁻) column, but during the packing it was washed with 1 dm³ of a 0.5 M solution of ammonium sulphate in water to obtain the SO₄²⁻ form.

**K-X8 (H⁺) column.** This was the same as the BA-X8 (Cl⁻) column but slurry packed with Benson Type BC-X8 cation-exchange resin having sulphonic acid groups and during the packing washed overnight with 1 dm³ of a 0.05 M solution of sulphuric acid in water to obtain the H⁺ form.

**RP-18 column.** This was a 250 × 4.6 mm I.D. stainless-steel column obtained from Chrompack, prepacked with CP Spher C₁₈, consisting of octadecylsilane-modified silica with 5-µm spherical particles.

Pretreatment. Before use, the BA-X8 (Ac⁻) and BC-X8 (H⁺) columns were rinsed with water to obtain an effluent free from sodium acetate or sulphuric acid, respectively. All liquids used for preparing the columns and all eluents were filtered over a Millipore HAWP filter (0.45 µm) and degassed with helium.

Sampling

If necessary, the reaction samples were acidified with 2 M hydrochloric acid to pH ≈ 9 and diluted with water to give a maximum concentration per solute of about 0.05 M, and stored at ca. 278 K. Before analysis, the samples were filtered over a Schleicher and Schüll GC 92/RC 55 filter combination.

For kinetic measurements, the composition of the reaction mixtures was determined as a function of time; therefore, the concentrations found were corrected for all dilutions and for the effects of sampling [1,2].
RESULTS AND DISCUSSION

Analysis of sugars

Injection of a reaction sample from the oxidation of lactose to lactobionate and also from the alkaline oxidative degradation reaction of lactose to β-O-D-galactopyranosyl-(1→3)-D-arabinonate (GARA) on the BC-X8 (H⁺) column with a 0.005 M sulphuric acid as the eluent, or the same resin in the Ca²⁺ or Pb²⁺ form with water as the eluent, resulted in complex chromatograms using refractive index detection. For all three columns, co-elution of the non-ionic sugars and the anionic reaction products took place, so quantification of the chromatograms is difficult. However, on connecting a BA-X8 (Ac⁻) column upstream of the BX-X8 (H⁺) column and using water as the eluent, the chromatograms shown in Fig. 2 and 3, respectively, were obtained.

The anionic products present in the samples exchange with the acetate of the BA-X8 (Ac⁻) column and adsorb strongly. The exchanged acetate behaves like an injected compound whose retention depends on the properties of both columns. Hydrophobic interactions between the apolar part of the acetate molecule and the apolar resin matrix give just enough retention to the acetate for elution after the non-ionic sugars. The liberated acetate is equimolar to the amount of anions injected, so by integrating the size of the acetate peak the total amount of anions present in the reaction sample could be determined.

Although the amount of exchangeable acetate is limited, the capacity of the short BA-X8 (Ac⁻) column was high enough for the injection of more than 250 samples containing 0.15 anion equivalents per dm³ each. On the verge of exhaustion of the acetate capacity, the second weakest anion will also be liberated and influence

![Fig. 2. Chromatogram of a sample from a reaction mixture at ca. 50% conversion in the heterogeneous catalytic oxidation of lactose. Analytical conditions: BA-X8 (Ac⁻) column in series with a BC-X8 (H⁺) column, both at 85°C; eluent, distilled water; flow-rate, 0.4 ml min⁻¹; refractive index detection.](image1)

![Fig. 3. Chromatogram of a sample from a reaction mixture at ca. 70% conversion in the alkaline oxidative degradation reactions of lactose. Analytical conditions as in Fig. 2.](image2)
the chromatogram. Therefore, in a series of reaction samples, a few samples containing potassium chloride were included; if only the acetate peak was obtained, the system could be used for sugar determinations. If regeneration had to be carried out, both columns were disconnected and the BA-X8 (Ac⁻) and BC-X8 (H⁺) columns were flushed overnight with 1 dm³ of a 0.5 M solution of sodium acetate in water and with 1 dm³ of 0.05 M sulphuric acid, respectively. After separate conditioning by flushing with pure water, the column combination could be used again. The regeneration procedure used had no significant influence on the effectiveness of the separation.

In the heterogeneous catalytic oxidation of lactose (Fig. 1, route 1), the sugar part of the chromatogram given in Fig. 2 consists of the lactose peak only. However, in the alkaline oxidative degradation reaction of lactose (Fig. 1, route 2), the lactose isomer lactulose and the monosaccharide D-galactose will also be present, giving a more complex chromatogram. The first peak of the chromatogram in Fig. 3 belongs to the co-elution of lactose and lactulose, and the second peak belongs to D-galactose.

Because almost identical molar responses were found for lactose and lactulose with RI detection, the total concentration of lactose and lactulose could be measured. However, in experiments to study the kinetics of the oxidation and degradation reactions it is important to know their individual concentrations. The lactose–lactulose separation could not be achieved using a combination of an Ac⁻ column with either a Pb²⁺ or a Ca²⁺ column. Owing to the use of the Ac⁻ precolumn, injection broadening occurred, resulting in an unacceptable decrease in resolution.

Based on the results of Rajakylä [13] for maltose and sucrose, a solution to the problem was found by using an RP-18 column with water as the eluent. Injection of an aqueous solution of pure lactose, pure lactulose and a reaction sample on to an

![Fig. 4. Chromatograms of (a) a 32 mM aqueous lactose solution, (b) a 22 mM aqueous lactulose solution and (c) a sample from a reaction mixture at ca. 65% conversion in the oxidative degradation reaction of lactose. Analytical conditions: RP-18 column, ice cooled; eluent, distilled water; flow-rate, 0.5 ml min⁻¹; refractive index detection.](image-url)
ice-cooled RP-18 column resulted in the chromatograms shown in Fig. 4a, b and c, respectively. In Fig. 4a two peaks are obtained for lactose with a 60:40 peak-area ratio, representing the β- and α-anomeric pyranose forms, respectively. In Fig. 4b three peaks are obtained for lactulose with a peak-area ratio of 25:7:68, probably representing the β-furanose, α-furanose and β-pyranose tautomers present in the aqueous lactulose solutions. These ratios for aqueous lactulose solutions are in good agreement with the results of Pfeffer and Hicks [31,32] obtained by 13C NMR spectroscopy. The chromatograms given in Fig. 4a and b indicate co-elution of the small furanose peaks of lactulose with both the anomeric lactose peaks but a separate elution of the pyranose form of lactulose.

Attempts to activate the mutarotation reaction under analytical conditions to obtain one-peak chromatograms for both lactose and lactulose were unsuccessful; a higher column temperature resulted in a complete loss of retention and addition of sulphuric acid to the eluent did not influence the chromatograms markedly. Under all conditions applied, d-galactose and the anionic products gave no retention and did not influence the determination of lactose and lactulose.

As can be seen in Fig. 4c, the furanose forms of lactulose co-elute with both lactose anomers and separate elution of the β-pyranose form of lactulose is obtained. Because the lactose and lactulose mutarotation equilibria mentioned above appeared to be independent of variations in pH between 5 and 9 and addition of potassium chloride at concentrations up to 150 mM as occurs in the reaction samples, the lactulose concentration could be determined using the peak-area ratios and the lactulose β-pyranose peak area only. Under the reaction conditions applied, the lactulose concentration was very low during the entire course of reaction, so the lactose–lactulose determination with the RP-18 column was occasionally carried out. Determination of the non-ionic sugars was mainly carried out using the BC-X8 (H+) column connected in series with the BA-X8 (Ac-) column, as mentioned before. In serial analysis only standard solutions of lactose and d-galactose were used for determination of the lactose–lactulose and d-galactose contents in the reaction samples.

**Analysis of the anionic oxidation and degradation products**

From the scheme given in Fig. 1, it can be seen that the alkaline oxidative degradation of lactose (route 2) leads to a relatively complex reaction mixture. The oxidative degradation of lactose will lead to β-O-d-galactopyranosyl-(1→3)-d-arabinonate (GARA) and an equal amount of formate; non-oxidative degradation of lactose will lead via a β-elimination to the formation of a reactive 4-deoxy-2,3-hexodulose fragment and the more stable d-galactose [2]. Just like the lactose to GARA mechanism, d-galactose will give d-lyxonate and formate in a parallel oxidative degradation reaction whereas the hexodulose can be oxidized easily to 2-deoxy-d-tetroxyl (2-deTA) and glycolate. Moreover, β-O-d-galactopyranosyl-(1→2)-d-erythro-nate and glycolate may be formed when enolization and oxidation occur between the C-2 and -3 of the glucose moiety in lactose.

As has been described, chromatography based on anion-exchange mechanisms can be used for the separation of the anionic oxidation and degradation products. Preliminary qualitative experiments were carried out using the BA-X8 (Ac-) column with 0.2 M sodium acetate solution as the eluent. It was found for aldonic acids that the retention and resolution increase with a decrease in molecular size [33]. On the
BA-X8 (Ac\(^-\)) column a clear distance was found between the peaks of lactobionate and GARA. Corresponding to the rules described above, lactobionate eluted in front of GARA.

Following these rules, \(\beta\)-O-\(\alpha\)-galactopyranosyl-(1\(\rightarrow\)2)-\(\alpha\)-erythronate was expected to elute at a clear distance after the GARA peak, but no peak was observed that can be ascribed to a significant production of \(\beta\)-O-\(\alpha\)-galactopyranosyl-(1\(\rightarrow\)2)-\(\alpha\)-erythronate under the reaction conditions applied. However, owing to a strong UV-adsorption at 212 nm, aqueous acetate was not a suitable eluent for quantitative low-wavelength UV detection. Therefore, in spite of a decrease in resolution, a low UV-absorbing eluent such as aqueous chloride or sulphate was chosen. Analyses were carried out with the BA-X8 (Cl\(^-\)) column and an aqueous solution of 0.16 \(M\) NaCl and 0.02 \(M\) MgCl\(_2\) as the eluent or the BA-X8 (SO\(_4^{2-}\)) column and an aqueous 0.2 \(M\) \((\text{NH}_4)_2\text{SO}_4\) solution as the eluent. In the latter instance, the aqueous \((\text{NH}_4)_2\text{SO}_4\) solution was made alkaline to pH \(\approx 7.5\) with a few drops aqueous ammonia. Dual detection was applied by placing the RI detector in series with the UV (212 nm) detector.

From the reactions of lactose to lactobionate and to GARA, chromatograms for the anionic products were obtained with the BA-X8 (Cl\(^-\)) system as shown in Figs. 5 and 6, respectively. The heterogeneous catalytic oxidation of lactose with a bismuth-modified palladium-on-carbon catalyst leads to an almost quantitative production of lactobionate [1] and therefore the chromatogram in Fig. 5 is simple. Be-

![Fig. 5. Chromatograms of a sample as described for Fig. 2. Analytical conditions: BA-X8 (Cl\(^-\)) column; column temperature, 85°C; eluent, 0.02 \(M\) MgCl\(_2\) + 0.16 \(M\) NaCl in water; flow-rate, 1.1 ml min\(^{-1}\); (a) refractive index detection, (b) in series with UV (212 nm) detection.](image-url)
cause lactobionate was available as a pure compound and its peak areas could be integrated well, accurate quantification of this reaction product was possible.

As can be seen in Fig. 6, the chromatogram obtained from the alkaline degradation of lactose to GARA is more complex. Separate elution was obtained for GARA, glycolate and formate. The broad peak appearing in front of the peak of glycolate can be ascribed to co-elution of D-lyxonate and 2-deTA. The retention of D-lyxonate could be verified by carrying out an alkaline oxidative degradation reaction of D-galactose, wherein D-lyxonate was expected to be the main product. According to the retention rules described above, co-elution of the byproduct 2-deTA in this peak is plausible.

When the BA-X8 (SO$_4^{2-}$) system was used for the same reaction sample, the chromatogram shown in Fig. 7 was obtained. Separate peaks were obtained of D-lyxonate, the expected 2-deTA, glycolate and formate, but the main reaction product GARA eluted too close to the injection peak, hampering an accurate GARA peak integration. Because an accurate determination of the main product GARA was preferred, standard analyses were carried out using the BA-X8 (Cl$^-$) system. Occasionally analyses with the BA-X8 (SO$_4^{2-}$) system were carried out for byproduct determination. Comparison of the RI and UV (212 nm) detection methods leads to the conclusion that for the low-molecular-weight products UV detection is far superior in sensitivity to RI detection.

In liquid chromatography, loop injection of aqueous samples can be done with high reproducibility. Therefore, when the reaction sample components are available as pure compounds, an external standardization method can be used for quantifica-
Fig. 7. Chromatogram of a sample from a reaction mixture at ca. 85% conversion in the oxidative degradation reaction of lactose. Analytical conditions: BA-X8 (SO₄²⁻) column; column temperature, 85°C; eluent, 0.2 M (NH₄)₂SO₄ in water; flow-rate, 1.1 ml min⁻¹; (a) refractive index detection, (b) in series with UV (212 nm) detection.

Fig. 8. (●) Refractive index and (○) UV (212 nm) responses of equimolar amounts of polyhydroxy monocarboxylic acids as a function of the number of carbon atoms in the compounds. Abscissa: 1 = formate; 2 = glycolate; 3 = D-glycerate; 4 = D-erythronate; 5 = D-arabinonate; 6 = D-glyconate; 12 = lactobionate.
tion. However, from the products formed during the alkaline oxidative degradation of lactose, GARA, D-lyxonate and 2-deTA were not available in a specified quality, so their determination had to be verified using an alternative method.

It was found in other sugar oxidation experiments that in RI detection a linear relationship is obtained for aldohexoses when their relative molar responses are plotted against the number of carbon atoms in a homologous series. Moreover, for stereoisomers almost identical RI responses were obtained. Such regularities were not found with UV (212 nm) detection, as can be seen in Fig. 8. It is to be expected that the RI sensitivity of GARA and D-lyxonate will conform to the same regular behaviour. Based on this regularity of the molar RI response in a homologous series, the molar RI response of GARA was defined as being equal to the lactobionate response minus one sixth of difference between the responses of lactobionate and glucuronate. For D-lyxonate the relative molar response for RI detection was defined as being equal to that of its commercially available stereoisomer D-arabinonate.

Owing to its deoxy-group, 2-deTA differs in molecular structure from the aldohexoses, so an irregular behaviour of the relative molar RI response is to be expected. The relative molar RI responses of D-glycerate and D-lactate indicated that replacement of a hydroxy group by a deoxy group results in a substantial decrease in molar response. An almost similar difference in molecular structure exist between D-erythronate and 2-deTA. The relative molar response for 2-deTA was therefore taken as the response value for D-erythronate minus the difference between the values for D-glycerate and D-lactate.

From the above-defined relative molar RI response values for GARA, D-lyxonate and 2-deTA and the UV (212 nm)/RI ratios obtained from the “one-injection chromatograms” using dual detection, the relative molar UV (212 nm) responses could be calculated. For both RI and UV (212 nm) detection the results are summarized in Table I, including some values for relevant compounds. With these values the concentrations of the compounds present in the reaction samples for the reactions of

<table>
<thead>
<tr>
<th>Compound</th>
<th>BA-X8 (SO₄²⁻) system</th>
<th>BA-X8 (Cl⁻) system</th>
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<tr>
<td></td>
<td>UV</td>
<td>RI</td>
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<tr>
<td><strong>Experimental values</strong></td>
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<tr>
<td>D-Gluconate</td>
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<td>100</td>
</tr>
<tr>
<td>D-Lactate</td>
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<td>26</td>
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<tr>
<td>D-Glycerate</td>
<td>91</td>
<td>38</td>
</tr>
<tr>
<td>D-Arabinonate</td>
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<td>81</td>
</tr>
<tr>
<td>Lactobionate</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Defined values</strong></td>
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<tr>
<td>D-Lyxonate</td>
<td>85</td>
<td>81</td>
</tr>
<tr>
<td>2-deTA</td>
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<td>56</td>
</tr>
<tr>
<td>GARA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
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TABLE I
RELATIVE MOLAR UV (212 nm) RESPONSES, RI RESPONSES AND UV/RI RATIOS FOR SEVERAL COMPOUNDS OBTAINED USING THE BA-X8 (SO₄²⁻) SYSTEM AND THE BA-X8 (Cl⁻) SYSTEM.
TABLE II
COMPOSITION OF A REACTION MIXTURE FROM THE ALKALINE OXIDATIVE DEGRADATION OF LACTOSE, GIVEN AS CONCENTRATIONS AND % OF INITIAL MOLES OF CARBON
\[ [\text{LAC}]_0 = 250.0 \text{ mmol dm}^{-3}. \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mmol dm(^{-3}))</th>
<th>% of initial moles of carbon</th>
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<tr>
<td>Sugars</td>
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<tr>
<td>Lactose–Lactulose</td>
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<td>14.4</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>8.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Sugar acids</td>
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<td></td>
</tr>
<tr>
<td>GARA</td>
<td>188.7</td>
<td>69.2</td>
</tr>
<tr>
<td>d-Lyxonate</td>
<td>5.1</td>
<td>0.9</td>
</tr>
<tr>
<td>2-deTA</td>
<td>14.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Glycolate</td>
<td>10.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Formate</td>
<td>209.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Total</td>
<td>95.8</td>
<td></td>
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</table>

lactose to lactobionate and of lactose to GARA could be calculated. For both reactions satisfactory carbon mass balances were obtained up to almost complete conversion. As an example, the product distribution of a reaction sample of the oxidative degradation of lactose to GARA is given in Table II. It clearly shows that GARA and formate are the main reaction products and, moreover, that the reaction pathway given in Fig. 1 (route 2) covers over 96% of the product mass.

ACKNOWLEDGEMENT

This investigation was carried out with support of the Dutch National Innovation Oriented Program Carbohydrates (IOP-k).

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