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D-Galacturonic Acid as a Highly Reactive Compound in Nonenzymatic Browning. 1. Formation of Browning Active Degradation Products

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ABSTRACT: Thermal treatment of an aqueous solution of D-galacturonic acid at pH 3, 5, and 8 led to rapid browning of the solution and to the formation of carbocyclic compounds such as reductic acid (2,3-dihydroxy-2-cyclopenten-1-one), DHCP (4,5-dihydroxy-2-cyclopenten-1-one), and furan-2-carbaldehyde, as degradation products in weak acidic solution. Studies on their formation revealed 2-ketoglutaraldehyde as their common key intermediate. Norfuraneol (4-hydroxy-5-methyl-3-(2*H*)-furanone) is a typical alkaline degradation product and formed after isomerization. Further model studies revealed reductic acid as an important and more browning active compound than furan-2-carbaldehyde, which led to a red color of the model solution. This red-brown color is also characteristic of thermally treated uronic acid solutions.

KEYWORDS: *D-galacturonic acid, colored compounds, nonenzymatic browning, furan-2-carbaldehyde, reductic acid*

INTRODUCTION

Thermal treatment of food and food products causes changes mostly by nonenzymatic browning of reducing carbohydrates, amino acids, and proteins. Those complex and multiple reaction pathways are well known, and a lot of intermediates and reaction products have been postulated, identified, and characterized in the last decades.[1,2] Uronic acids have the same structure as reducing sugars with an additional carboxylic group on the C-6. They can also take part in nonenzymatic browning reactions, but reaction pathways have not been studied in a systematical way so far.[3−7]

It is known that in model studies uronic acids especially D-galacturonic acid, the main monomer of pectin, produces more brown color than reducing sugars, but surprisingly little is known about the structures of the compounds responsible for this typical reddish-brown color. Model studies already revealed that the kinetics of the formation of browning is different in reducing sugars and uronic acids.[8] In comparison to pentoses and hexoses, less information is yet available regarding degradation products and intermediates of uronic acids. Furanoic compounds such as furan-2-carbaldehyde, 2-furanoic acid, and 5-formyl-2-furanoic acid as well as reductic acid (2,3-dihydroxycyclopent-2-en-1-on), an *aci*-reductone and structure analogue of L-ascorbic acid, are postulated as degradation products in acidic aqueous solutions. Decarboxylation of D-galacturonic acid to L-arabinose as the first step of reaction followed by dehydration and cyclization yielding typical degradation products of pentoses has been assumed as the main degradation pathway.[4] Stutz and Deuel found out that only small amounts of L-arabinose are formed during thermal treatment of D-galacturonic acid and that there have to be other and more important degradation pathways.[9] Our studies also revealed that decarboxylation is not the only degradation pathway leading to the known degradation products because of their imbalanced concentrations in model studies of L-arabinose and D-galacturonic acid under the same reaction conditions.

The objectives of the present investigations were therefore to characterize key intermediates and quantitate degradation products formed in aqueous unbuffered solutions of D-galacturonic acid at pH 3.0, 5.0, and 8.0, respectively, and to evaluate the influence of certain reaction intermediates as precursors in colorant formation to obtain more detailed information on the chemical reactions involved in the degradation. The so-formed caramel-like structures and the resulting color were further investigated by gel permeation chromatography (GPC) with refractive index and diode array detection.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: D-galacturonic acid monohydrate (Chemodex), barium carbonate, furan-2-carbaldehyde, norfuraneol, Amberlite 4400 OH^-, 2-ketoglutaric acid, 5-formyl-2-furanoic acid, 2-furanoic acid (Sigma), tetrabutylammonium hydrogen sulfate (Acros), sulfuric acid, succinic acid, malic acid (Fluka). Furan-2-carbaldehyde was freshly distilled at 300 °C in high vacuum prior to use. Solvents were of HPLC grade (Merck).

Synthesis of Reductic Acid [2,3-Dihydroxycyclopent-2-en-1-one]. Following a procedure of Reichstein and Oppenauer with modifications of Goldstein,[7,10] D-galacturonic acid (3 g) was dissolved in 15 mL of 1 N sulfuric acid in a sealed Pyrex reaction tube and heated for 90 min at 150 °C. After cooling the solution to room temperature and filtration of the residue the sulfuric acid was precipitated with barium carbonate. After filtration of the precipitate the solution was filtrated and percolated through a strong cation exchange column (Dowex 50x8, H^+ form, 50−100 mesh) and after that percolated on a strong anion exchange column (Amberlite 4400 OH^-). Nonionic compounds are not adsorbed on the anionic material; anions such as reductic acid are adsorbed and can be displaced from the resin with 0.1 N formic acid. Control of the

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displaced fractions was done on silica plates (silica gel 60 F254) with *n*-butanol/glacial acid/water (4:2:1) as an eluent. Detection of reductic acid was done under UV light, and detection of partly co-eluating, unreacted D-galacturonic acid was done by dipping the plate into a methanolic 10% sulfuric acid solution and heating it at 120 °C for 10 min. The effluents from the resin containing only reductic acid were pooled and concentrated to dryness under reduced pressure at 35 °C. The light brown syrup was washed with cold acetone to obtain crude crystals, which were recrystallized from a mixture of ethyl acetate and ethyl alcohol or sublimed.

Model Reactions. *D-Galacturonic Acid.* To investigate the degradation behavior and the formation of key intermediates and degradation products, D-galacturonic acid monohydrate (0.5 M) was dissolved in water, and the pH adjusted with sodium hydroxide and hydrochloric acid solutions to 3.0, 5.0, or 8.0. Aliquots of the solution were filled into glass ampules and incubated at 100 °C for 2 h in a heating block. The reaction was stopped at various time points and worked up as follows. Browning was measured as the absorption at 420 nm with a spectrophotometer (UV-1650 PC, Shimadzu, Duisburg, Germany), and the CIELab color with a reflectance attachment with a Spectralon integrating sphere (Specord 40, Analytik Jena, Jena, Germany). pH values were measured with a pH electrode (SenTix Mic, WTW, Weilheim, Germany; pH meter CG 820, Schott, Mainz, Germany) after cooling the sample to room temperature. For organic acids and *aci*-reductones, samples were diluted (1:10) with 1% *m*-phosphoric acid and 10 mM dithiothreitol and measured by HPLC-DAD. For furans and furanones, samples were diluted (1:100; v/v) with 10 mM phosphate buffer (pH 6.0) and subjected to HPLC-DAD. For quinoxalines, *o*-phenylenediamine hydrochloride (OPD; 20 mM) was added (1:1; v/v) and the samples were reincubated for at least 3 h at room temperature and subsequently analyzed by HPLC-DAD. For aldehydes, 2,4-dinitrophenylhydrazine (DNPH) and toluene were added to the sample (1:2:1; v/v/v) and reincubated for 12 h at room temperature and in the dark. The samples were vigorously shaken, and the organic layer was taken and dried under a stream of nitrogen. The residue was dissolved in acetonitrile and subjected to LC-MS. For unknown carboxylic acids, dicarboxylic acids, and sugar derivatives, an aliquot of the solution was dried under a stream of nitrogen and derivatized with 200 mL of *N*,*O*-bis(trimethylsilyl)acetamide with 5% trimethylchlorosilane in anhydrous pyridine (1:1; v/v) and subjected to GC-MS.

Reductic Acid and Furan-2-carbaldehyde. To investigate the browning behavior of degradation products of D-galacturonic acid, 0.05 M reductic acid or furan-2-carbaldehyde with and without L-alanine (0.005 M) was dissolved in water and the pH adjusted to 5.0 or 8.0. The solutions were further treated and analyzed like the model solutions of D-galacturonic acid. All experiments were conducted in three independent replications, and the arithmetical mean of all quantified values was calculated.

Characterization and Quantitation. *Organic Acids and aci-Reductones.* The diluted samples were analyzed by HPLC-DAD using a phosphate buffer (20 mM, pH 2.8) as an isocratic eluent and monitoring the effluent at wavelengths of 214 nm for organic acids (formic acid (3.9 min), acetic acid (6.3 min), succinic acid (11.4 min), 2-ketoglutaric acid (6.9 min), malic acid (4.2 min)) and 254 nm for reductic acid (7.5 min) detection. Identification of the organic acids was accomplished by comparing their retention time with authentic known acids and by comparing with the results of GC-MS analysis after silylation of the sample. Standard curves were prepared for each compound, and the peak areas of the compounds in the samples were measured and the concentrations calculated from the standard curves.

Furans and Furanones. The diluted samples were analyzed by HPLC-DAD using a solvent gradient starting with a mixture (99/1; v/v) of phosphate buffer (5 mM, pH 6.0) and methanol and increasing the methanol content after 10 min to 20% within 5 min and after another 5 min to 99% within 5 min. By monitoring the effluent at a wavelength of 280 nm, furan-2-carbaldehyde (6.7 min), 5-formyl-2-furanoic acid (16.5 min), and norfuraneol (3.6 min) were detected, and by monitoring at a wavelength of 254 nm 2-furanoic acid (8.5 min) was detected. Quantitation was performed by external calibration with pure reference compounds.

α-Dicarbonyl Compounds as Quinoxalines. The derivatized samples were analyzed by HPLC-DAD using a flow rate of 0.5 mL/min, an oven temperature of 40 °C, and a solvent gradient starting with a mixture (95/5; v/v) of water and acetonitrile and increasing the acetonitrile content after 5 min to 10% within 3 min. After another 5 min the content was increased to 20% within 7 min and after 5 min increased to 50% within 10 min. Quantitation was performed by comparing the peak areas with those of a standard solution at 317 nm containing known amounts of each pure reference or pure authentic quinoxaline isolated and elucidated from reaction mixtures in our working group.

Aldehydes as 2,4-Dinitrophenylhydrazones. Identification of reaction products was achieved by LC-MS measurements in the total ion mode. HPLC separation (Thermo Fischer Scientific Accela pump, CTC PAL autosampler) was carried out by injecting the derivatized samples using a solvent gradient starting with a mixture (40/60; v/v) of water and acetonitrile and increasing the acetonitrile content after 7 min to 100% within 13 min. Ionization (TSQ Vantage System with Ion Max Source, H-ESI II probe) in the ESI negative mode followed, using a spray voltage of 3 kV, a vaporizer temperature of 450 °C, a sheath gas pressure of 60 psi, and a capillary temperature of 270 °C. Spectra were obtained by setting the scan at 50 to 1000 *m*/*z*. Thermo Excalibur 2.1.0.1139 software was used for the identification of the substances.

Gas Chromatography–Mass Spectrometry (GC-MS). GC was performed on a Shimadzu GC-2010 by using a capillary DP-5 column (30 m × 0.25 mm, 0.25 mm, Supelco SLB-5MS, Bellefonte, PA, USA). The samples were applied by split injection (1:10) at an injection temperature of 250 °C and an oven temperature of 100 °C. After 4 min, the temperature of the oven was raised at a rate of 8 °C/min to 200 °C and held for 10 min, then raised again at a rate of 40 to 275 °C and held for 2 min. The flow of the carrier gas, helium, was 2 mL/min. MS analysis was performed with a GCMS-QP2010plus (Shimadzu) in tandem with the GC.

High-Performance Liquid Chromatography (HPLC)-DAD. The HPLC apparatus consisted of one pump (LC-9A, Shimadzu), a gradient mixer (FCV-9 AL, Shimadzu), a column oven (VDS Optilab, Berlin, Germany), and an autosampler (Gina50, Dionex, Germering, Germany). The effluent was monitored by a diode array detector (DAD, Gynkotek UVD 340S, Gynkotek, Germering, Germany) operating in a wavelength range between 200 and 500 nm. Separations of organic acids and *aci*-reductones were performed on a stainless steel column (150 × 4.6 mm), packed with ODS-AQ material (3 mm, YMC, Kyoto, Japan), with a flow rate of 0.6 mL/min at a column temperature of 25 °C. Separations of furans and furanones were performed on a stainless steel column (125 × 4.6 mm, Macherey-Nagel, DÃ1/4 ren, Germany), packed with an RP-18 material (Nucleosil, 3 mm), with a flow rate of 0.5 mL/min at a column temperature of 40 °C. For the separation of α-dicarbonyl compounds as quinoxalines the same column, but a column temperature of 35 °C, was used.

Gel Permeation Chromatography–Refractive Index Detection (GPC-RI). For the characterization of the molecular weight distribution of the thermally treated samples at 100 °C for 2 h, the samples were diluted with water and analyzed by GPC-RI. The molecular distribution of the reacted D-galacturonic acid was compared to standard solutions of D-glucose, a pullulan standard of 40 kDa, and a reaction mixture consisting of D-glucose and L-alanine.

RESULTS AND DISCUSSION

Thermal treatment of unbuffered solutions of D-galacturonic acid started at pH 3.0, 5.0, or 8.0 at 100 °C led to 10 times higher browning after 2 h compared to L-arabinose and D-glucose or D-galactose.

The browning was measured as the absorption at 420 nm. It was highest at pH 5.0 and a little lower at pH 3.0 and 8.0 at the beginning of the reaction. The pH was not controlled during

further reaction. The color formed was the same for all three starting pH values and more reddish compared to model solutions of reducing sugars treated in the same way. CIELab measurements revealed that the lightness is much lower and the $+a$ value (red) is much higher in the thermally treated D-galacturonic acid solutions (L = 60, $+a$ = 30) compared to reducing sugars (L = 90, $+a$ = 0).

Unlike the decreasing pH value of the reducing sugars, the pH value of D-galacturonic acid solutions increased until it remained steady at about 6.5. Only at pH 3.0 did the value remain stable during the whole reaction time. During the degradation of reducing sugars higher amounts of short-chain organic acids were formed due to oxidative cleavage reactions of the α-dicarbonyl compounds, leading to the decrease in pH and a deceleration in reaction speed.[11] Thermal treatment of D-galacturonic acid tended to result in less acidic heterocyclic or carbocyclic compounds, which are responsible for the increase in the pH value and an acceleration of the reaction.

Table 1 gives an overview of the concentrations of the formed products of thermally treated D-galacturonic acid

Table 1. Concentration of the Main Degradation Products Reductic Acid (RA), DHCP, Furan-2-carbaldehyde (FF), and Norfuraneol (Norf) of D-Galacturonic Acid after Thermal Treatment at 100 °C for 2 h with Different pH Values at the Start of the Reaction and the Resulting Absorption at 420 nm (Abs_{420})

	pH	Abs_{420}	GalA [mM]	RA [mM]	DHCP[a] [mM]	FF [mM]	Norf [mM]
GalA	3	2.3	182	1.9	61.5	1.8	n.d.
	5	4.0	153	0.2	39.8	n.d.	0.3
	8	3.3	160	0.4	12.5	n.d.	0.2

[a]Quantified as malic acid equivalent.

solutions after 2 h. In model solutions started at pH 5.0 about 31% of the unreacted uronic acid could be detected, but the sum of the main degradation products is only around 8%. Therefore we suppose that the other 61% of the degraded D-galacturonic acid is mostly incorporated into colored polymer structures.

Teleman et al. postulated that at very low pH values the hemiacetal ring of the D-galacturonic acid opens and dehydration on the C-3 proceeds. This leads to the postulated but unstable 3-deoxyhexosulosuronic acid, which readily forms a furanoic intermediate that is transformed to the stable degradation products 5-formyl-2-furanoic acid and 2-furanoic acid.[12] These stable furanoic acids could not be detected in any of the investigated model reactions of D-galacturonic acid in this work. A pH of 3.0 and a temperature of 100 °C seemed to be inadequate for this reaction pathway. Instead the highest concentrations of reductic acid and furan-2-carbaldehyde were formed. 4,5-Dihydroxy-2-cyclopenten-1-one (DHCP), an isomer of reductic acid, was also formed during thermal degradation of D-galacturonic acid. Isbell proposed DHCP as the main precursor in the formation of reductic acid,[13] but Ahmad et al. determined that DHCP is only an isomer but not a tautomer of reductic acid, and therefore there have to be different formation pathways.[14] We observed that DHCP is preferably formed at pH 3, but according to Ahmad et al.[5] we also suppose that it is not the precursor or a tautomer of reductic acid but an alternative degradation product of D-galacturonic acid.

Feather postulated the existence of a unique mechanism for the formation of reductic acid from uronic acid based on their labeling experiments,[15] but they could not verify this mechanism any further. The other known mechanism responsible for the formation of reductic acid is the same as in pentoses assuming furan-2-carbaldehyde as precursor.[13,16,17]

Our experiments indicated different isomers of 2-ketoglutaraldehyde (**4**) as the common intermediate in the formation of reductic acid (**5**), DHCP (**6**), and furan-2-carbaldehyde (**7**) from D-galacturonic acid. One of these isomers, **4c**, can also be formed by dehydration of pentoses **2**. Decarboxylation of D-galacturonic acid (**1**) to L-arabinose (**2**) takes place only in concentrated mineral acids.[18] Thus, decarboxylation of **1** is not a major degradation pathway of D-galacturonic acid under the slightly acidic conditions investigated in this work. Eliminative decarboxylation of D-galacturonic acid led to the formation of a very reactive 4,5-unsaturated 4-deoxy-L-arabinose (**3**), which after ring-opening and dehydration yielded the enolic form of 2-ketoglutaraldehyde (**4a**) and further cyclized to reductic acid (**5**). Figure 1 shows the postulated degradation pathway of D-galacturonic acid leading to the postulated precursor and key intermediates of the known degradation products under slightly acidic to alkaline reaction conditions.

So far, 2-ketoglutaraldehyde was only supposed as the key intermediate,[13,16,19] but could not be identified and characterized in heat-treated solutions of D-galacturonic acid. It is susceptible to secondary reactions and therefore has a decisive influence on the product spectrum. Such a reactive intermediate may be transformed into a stable derivative with trapping reagents and thus be protected from further reactions. While our postderivatization with OPD was not successful, postderivatization with DNPH led to a stable tris(2,4-dinitrophenylhydrazone) derivative, which we could then analyze by LC-MS. We identified 2-ketoglutaraldehyde as a direct intermediate of the D-galacturonic acid degradation for the first time. The total ion chromatogram of the model solution derivatized with DNPH is shown in Figure 3. The peak at 11.07 min represents the tris(2,4-dinitrophenylhydrazone) derivative of 2-ketoglutaraldehyde, as the mass spectrum in Figure 4 shows.

The confirmation of 2-ketoglutaraldehyde as a key intermediate of D-galacturonic acid also gives further indication for eliminative decarboxylation as the main degradation pathway under slightly acidic to neutral conditions. Due to the trans position of the substituents on C-4 and C-5 of D-galacturonic acid in its lowest energy $^1C_4$ chair conformation, a hydrogen bond can form a six-center intermediate stage. In a concerted mechanism dehydratization of C-4 and decarboxylation at C-5 proceed simultaneously and a 4,5-unsaturated 4-deoxypentose (**3a**), a labile precursor in the formation of 2-ketoglutaraldehyde, is formed.

We suppose that after further dehydratization the postulated 4,5-unsaturated 4-deoxypentose **3b** can yield the enolic form of 2-ketoglutaraldehyde (**4a**), which leads directly to the formation of reductic acid (**5**) and after further isomerization also to DHCP (**6**) and furan-2-carbaldehyde (**7**). The formation of the enolic form of 2-ketoglutaraldehyde (**4a**) instead of **4c** also gives a reasonable explanation for the high concentration of reductic acid (**5**) and the relatively low concentration of furan-2-carboxyldehyde (**7**) compared to similar model reactions with L-arabinose (**2**). L-Arabinose was relatively stable under the same reaction conditions, and we recovered 64% of the intact pentose and only 60 mM furan-2-

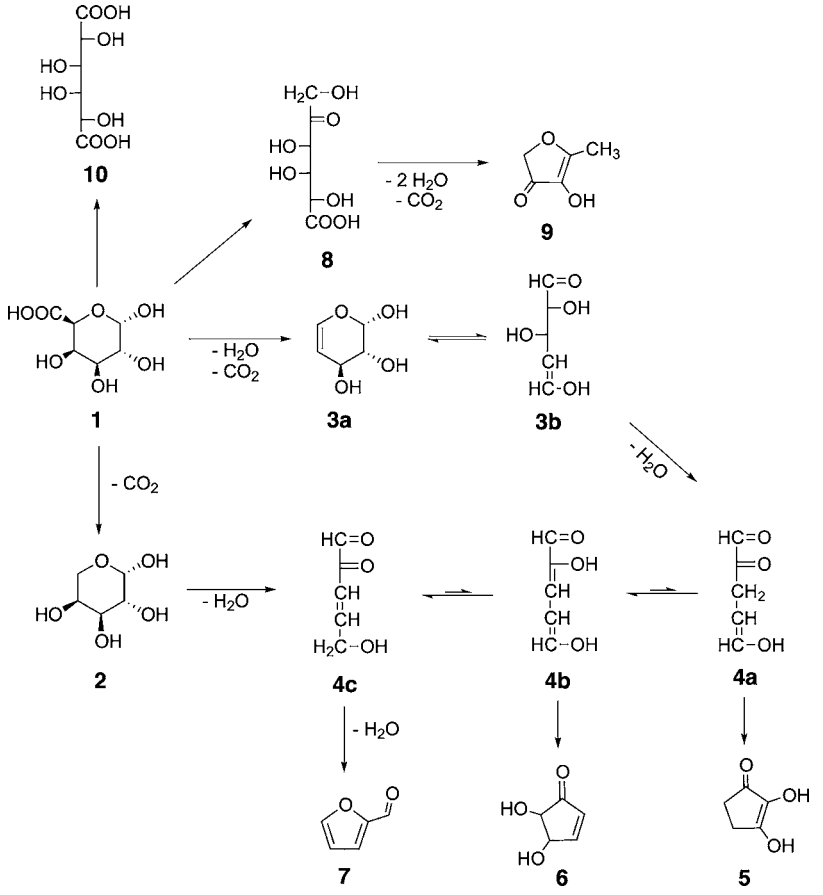

Figure 1. Degradation pathways of D-galacturonic acid in slightly acidic to alkaline media. Decarboxylation to L-arabinose (**2**) or eliminative decarboxylation to 4,5-unsaturated 4-deoxy-L-arabinose (**3**) to 2-ketoglutaraldehyde (**4**) leading to reductic acid (**5**), DHCP (**6**), and furan-2-carbaldehyde (**7**). Isomerization to D-tagaturonic acid (**8**) and formation of norfuraneol (**9**) or oxidation to galactaric acid (**10**).

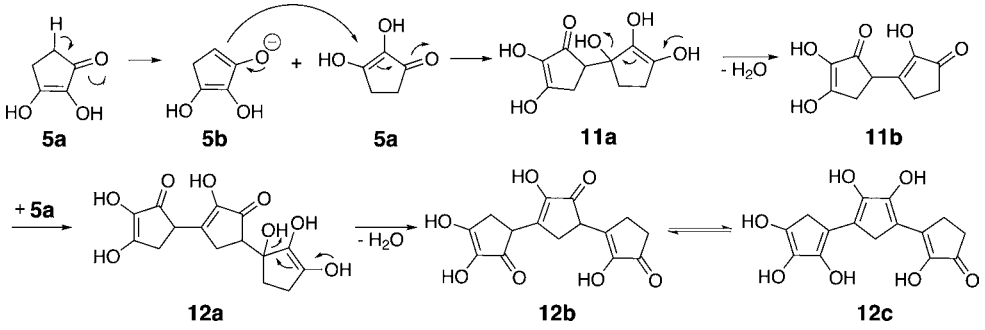

Figure 2. Polymerization of reductic acid due to aldol condensation. Because of the highly conjugated structure, the color of the formed polymer appears red.

carbaldehyde as well as 0.2 mM 3-deoxypentosulose as the main degradation products.

We also observed that at higher pH values at the beginning of the reaction isomerization of the D-galacturonic to D-tagaturonic acid (**8**) was favored over other reaction pathways. The highly reactive norfuraneol (**9**), a methylene active aroma impact compound, was formed to a higher degree at pH 5 and 8 compared to pH 3. The formation pathway of norfureneol was postulated by Kraehenbuehl et al.[20] Besides isomerization, also oxidation of D-galacturonic acid to galactaric acid (**10**) occurred to a higher extent compared to model reactions started at lower pH values. Compared to pH 3 at pH 8 reductic acid and DHCP were formed only in small amounts, but no furan-2-carboxyaldehyde could be detected at all (see Table 1).

Investigations of the molecular size distribution by GPC showed that compared to reducing sugars not only is the browning more intense and the color different but also the polymer structure seemed to be different.[21] Yet, due to the strong interaction of the carboxylic groups with the column material of the GPC and the totally different behavior compared to pullulans, an estimation of the molecular size was not possible.

To gain more insight into precursors of colored products, it might be promising to identify the chromophoric structures formed by reacting certain intermediates known as uronic acid degradation products. For example, furan-2-carbaldehyde is also known as one of the main reaction products formed from pentoses during thermal treatment. It was reported that this

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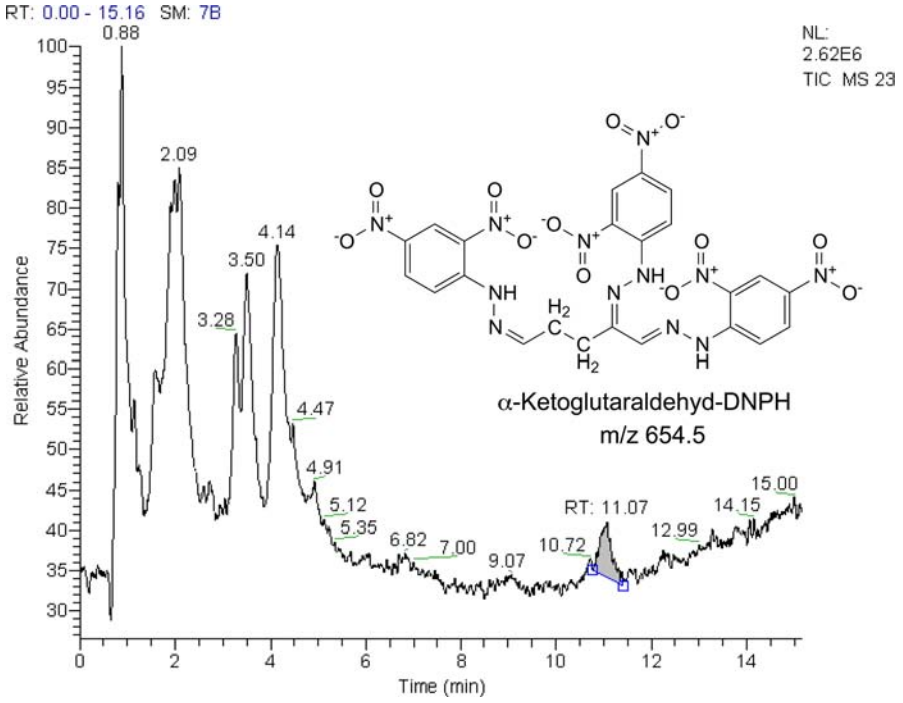

Figure 3. Confirmation of 2-ketoglutaraldehyde as a key intermediate in the degradation of D-galacturonic acid by LC-MS after postderivatization with DNPH. Total ion chromatogram of the tris(2,4-dinitrophenylhydrazine) derivative (t_R 11.07 min).

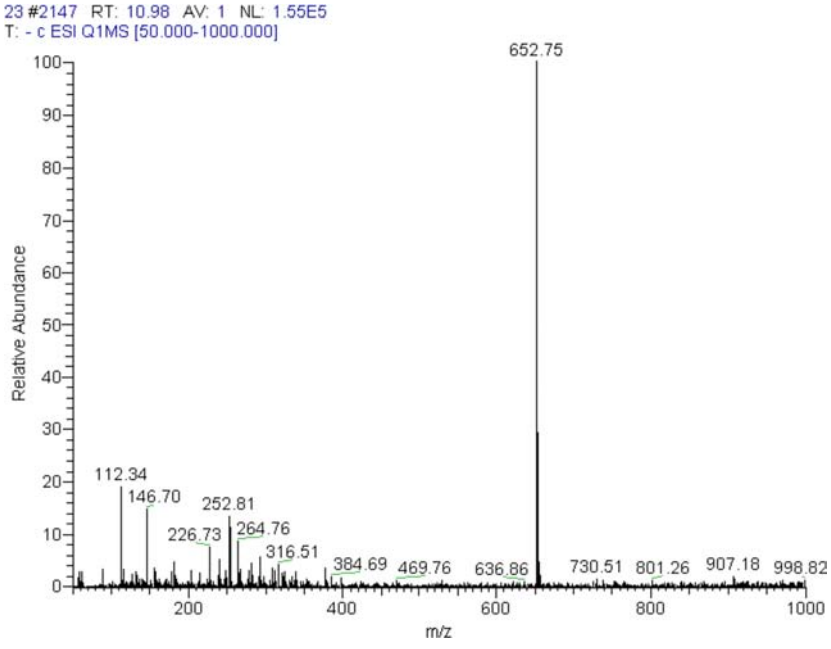

Figure 4. Mass spectrum of 2-ketoglutaraldehyde as the tris(2,4-dinitrophenylhydrazine) derivative at m/z 653 [M − 1].

aldehyde easily reacts with other carbohydrate degradation products containing an activated methylene group, such as norfuraneol, giving rise to yellow reaction products.

The browning activity of neither reductic acid nor DHCP has been investigated so far. We suppose that both can form colored polymeric resins, and this may be one of the reasons for the higher browning activity of D-galacturonic acid compared to reducing sugars. We suggest a Michael addition as the polymerization reaction for DHCP and an aldol condensation for the polymerization of reductic acid (see Figure 2). The colored condensation products in turn could further react with primary amines or carboxylic acids to form more intense and more highly cross-linked structures. Our observations provided more evidence that reductic acid is the most reactive degradation product of D-galacturonic acid and that DHCP is more stable and accumulates in the solution, as shown in Table 1. In contrast to the literature, the cyclopentenone ring of the reductic acid in our experiments was still intact.[22] The furan ring of the furan-2-carbaldehyde can be reopened, for example, in an amine-catalyzed reaction.[23] After this ring-opening several condensation reactions between methylene active intermediates

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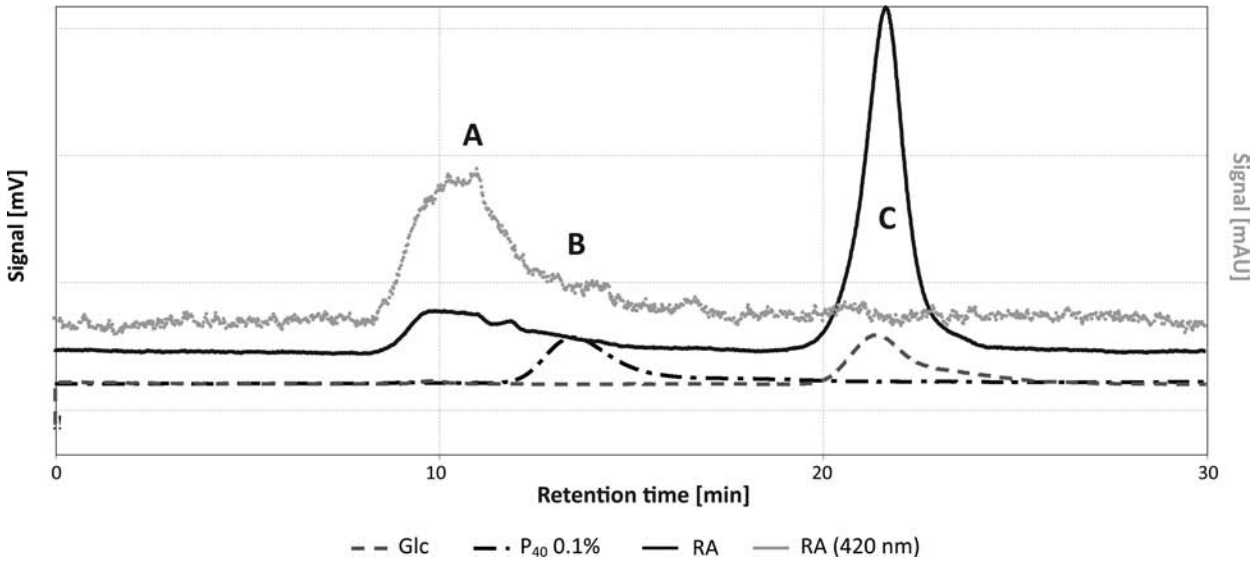

Figure 5. GPC chromatogram with refractive index and UV detection at 420 nm of the aqueous solution of reductic acid after thermal treatment for 120 min at 120 °C. A pullulan standard represents the high molecular weight fraction of 40 kDa (domain B), and a D-glucose solution represents the low molecular weight fraction, <1 kDa (domain C). Domain A is the newly formed polymer with a molecular weight of >40 kDa and a relatively strong absorption at 420 nm.

and carbonyl compounds can take place, which lead to the formation of several chromophoric compounds.[23]

We therefore reacted reductic acid, as well as furan-2-carbaldehyde, with L-alanine, respectively, to obtain colored condensation products. The solution of reductic acid already changed its appearance after 5 min and led to an intense red-colored solution after a reaction time of 2 h. Amino acids additionally increased the red color formation, but there was no acceleration of the reaction. Reductic acid was degraded rapidly, but no Dulkin and Friedemann degradation products such as 2-ketoglutaric acid or succinic acid could be detected.[22] GPC measurements revealed that high molecular weight compounds were formed (domain A), which accounted for the absorption at 420 nm and the red color (see Figure 5). We suppose that these polymer structures are formed in an autocatalytic reaction and directly from reductic acid or its dehydro form due to an aldol condensation. We suppose that a highly conjungated, high molecular weight structure is the reason for the red color of the reductic acid model solutions.

Freshly distilled furan-2-carbaldehyde treated in the same way as reductic acid did not give rise to color formation or polymeric structures. Only in combination with L-alanine, pyrrol-2-carbaldehyde, or norfuraneol did the solution turn slightly yellow after 2 h. Thus, reductic acid surely contributes to color formation in the course of thermally treating D-galacturonic acid, whereas furan-2-carbaldehyde contributes to browning only in the presence of amines and/or methylene active compounds and polymer formation is much slower.

In summary, the browning activity of D-galacturonic acid is up to 10 times higher compared to pentoses and hexoses. The reason for the higher browning activity and the different color is a divergence in the reaction pathways because of the carboxylic group on C-6 of the uronic acid leading to different key intermediates and degradation products. We suggest the main key intermediate of D-galacturonic acid to be 2-ketoglutaraldehyde and its isomers, which are formed after eliminative decarboxylation at elevated temperatures in slightly acidic to neutral solutions. 2-Ketoglutaraldehyde is the main precursor in the formation of reductic acid, DHCP, and furan-2-carbaldehyde.

Our investigations showed that at pH 5 typical degradation products of acidic D-galacturonic acid solution such as reductic acid and DHCP are formed as well as products of alkaline solution such as norfuraneol and galactaric acid. All these compounds possess reactive groups that would allow their further involvement in nonenzymatic browning, possibly including the formation of colored materials. Because of the large diversity of degradation products, which can contribute to browning due to synergistic effects on the formation of chromophoric and/or polymer structures, browning activity is highest under slightly acidic conditions. Reductic acid is unequivocally established as a precursor of chromophoric structures, and therefore we suppose that it contributes to color formation in the course of nonenzymatic browning of D-galacturonic acid as well. Furan-2-carbaldehyde is also a color precursor but only in association with other reactive degradation compounds of D-galacturonic acid such as norfuraneol and reductic acid or other nitrogen-containing compounds, for example, amines or pyrrols, present in the heat-treated solution.

We also investigated the influence of amino acids on the browning behavior of D-galacturonic and identified some new and specific degradation products, which we will present in our next paper.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Ara, L-arabinose; DAD, diode array detector; DHCP, 4,5-dihydroxy-2-cyclopenten-1-one; DNPH, 2,4-dinitrophenylhydrazine; FF, furan-2-carbaldehyde; GalA, D-galacturonic acid;

GC, gas chromatography; GPC, gel permeation chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; OPD, *o*-phenylenediamine; Norf, norfuraneol; RA, reductic acid

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