Formation of Carboxylic Acids during Degradation of Monosaccharides

Ondřej NOVOTNÝ, Karel CEJPEK and Jan VELÍŠEK

Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

Novotný O., Cejpek K., Velíšek J. (2008): **Formation of carboxylic acids during degradation of monosaccharides**. Czech J. Food Sci., **26**: 117–131.

The formation of low molecular carboxylic and hydroxycarboxylic acids as well as sugar and deoxysugar acids from monosaccharides (D-glucose, D-fructose, D-arabinose, DL-glyceraldehyde, and 1,3-dihydroxyacetone) was studied in three different model systems: aqueous and alkaline solutions of potassium peroxodisulfate ($K_2S_2O_8$), and sodium hydroxide solution. In total, 3 low molecular carboxylic acids (formic, acetic and propionic), 24 hydroxycarboxylic acids, and 12 corresponding lactones were identified and quantified by GC/MS. Formic, acetic, and propionic acids were isolated by extraction with diethyl ether and directly analysed by GC/MS; hydroxycarboxylic acids and their lactones were monitored as their trimethylsilylated derivatives using the same method. Formic, acetic, L-lactic, glycollic, DL-2,4-dihydroxybutanoic acids and aldonic acids derived from the parent sugars were the most abundant compounds in all model systems. Within the models investigated, the yield of carboxylic acids and hydroxycarboxylic acids (together with their lactones) ranged between 9.3–22.2% (n/n) and between 3.6–116.9% (n/n), respectively. The amount of acids was significantly lower in aqueous solutions of $K_2S_2O_8$ than in the alkaline solutions. The data obtained indicate that lower carboxylic acids are formed by both subsequent reactions (oxidation and/or intramolecular Cannizzaro reaction) of the sugar fragmentation products and direct decomposition of some intermediates such as uloses or hydroperoxides derived from the parent sugars. The acids possessing the original sugar skeleton are formed as a result of sugar oxidation or benzilic acid type rearrangement of deoxyuloses. Lower acids may also be formed by a recombination of free radicals.

Keywords: carboxylic acids; hydroxycarboxylic acids; lactones; oxidation; Cannizzaro reaction; benzilic acid type rearrangement; peroxodisulfate

Carboxylic and hydroxycarboxylic acids are an important family of products formed during oxidation of reducing sugars and/or carbonyls produced by their degradation. During thermal treatment of foods, the oxidation processes play an important role in the sugar transformations providing that either final oxidation products and/or key intermediates undergo subsequent reactions (oxidative degradation). The extent of sugar oxidation depends mainly on pH value, temperature, the type of sugar, and the presence and type of oxidising agents. Many papers reported on sugar oxidation proceeding under different conditions such as oxidation by enzymes (Geigert *et al.* 1983), free oxygen (Samuelson

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects COST 927, and MSM 6046137305).

& Thede 1968; Vuorinen 1985), hydrogen peroxide (Isbell *et al.* 1973; Isbell & Frush 1973), or ozone (Marcq *et al.* 2001), pyrolysis (Hodge 1967; Ponder & Richards 1993), γ-irradiation (Kawakishi *et al.* 1975; Drijver & Holzapfel 1986), ultraviolet radiation (Phillips *et al.* 1964), or sonolysis (Heusinger 1988).

The acids produced during the sugar transformation can be generally divided into those bearing the carbon skeleton of the parent sugar, and acids with a shorter carbon chains. Sugar-derived acids (i.e. aldonic, alduronic, aldaric, saccharinic, and ulonic acids) fall into the family of acids retaining the original carbon chain (or its part) of sugar. For example, gluconic and glucuronic acids, found in some Maillard model systems (Yaylayan & Huyghues-Despointes 1996), are well-known products formed by oxidation of aldehydic and/or primary hydroxyl group of glucose, respectively. Saccharinic acids are typical products of transformation of sugars in strong alkaline media, although their formation was observed also in acid media (Mizuno & Weiss 1974) and, to a minor extent, under high temperatures. They are formed by benzilic acid rearrangement of the corresponding deoxyuloses. Yang and Montgomery (1996b) identified several C_4, C_5, and C_6-saccharinic acids formed during the degradation of glucose and fructose in $Ca(OH)_2$ solution. Ulosonic (2-keto) acids are formed *via* aldos-2-uloses during, for example, oxidation of aldoses by $HO^{\bullet}$ radicals (generated by the Fenton reagent, H_2O_2+Fe^{2+}) (Davídek *et al.* 1990) or enzymatically (Geigert *et al.* 1983).

The acids having their carbon chain shorter than the parent sugar are formed either as direct cleavage products of sugar intermediates or by subsequent reactions of the carbonyl fragments formed. The sugar fragmentation leading directly to the acids *via* α- and β-dicarbonyl splitting was rather hypothetical for a long time. Considering the large number of citations in the literature, hydrolytic α-dicarbonyl cleavage mechanism seems to be well-established. It has been repeatedly employed to explain the formation of acetic and formic acids by splitting of 1-deoxyhexo-2,3-diuloses and 3-deoxyhexos-2-uloses, respectively (Figure 1) (Brands & van Boekel 2001). The same mechanism was proposed also for the formation of glyceric acid from 2-deoxyhexo-3,4-diulose (Yang & Montgomery 1996a). The splitting of β-dicarbonyl sugar intermediates was first proposed by Hayami (1961) and later confirmed by Weenen (1998), explaining the formation of acetol and glyceric acid from pentose and hexose sugars. The recent in-depth mechanistic study of Davídek *et al.* (2006a, b) employing labelled compounds (various ^{13}C-labeled glucose isotopomers, $^{18}O_2$, $H_2{}^{17}O$) represents a breakthrough in the understanding of the mechanisms involved in the acid formation. The experimental data obtained in this study showed coexistence of at least two reaction mechanisms leading to carboxylic acids, i.e. (*i*) hydrolytic β-dicarbonyl cleavage without intermediacy of any oxidising agent as the major pathway, and (*ii*) an alternative minor pathway *via* oxidative α-dicarbonyl cleavage induced by oxidising species. Particularly, the reaction mechanisms

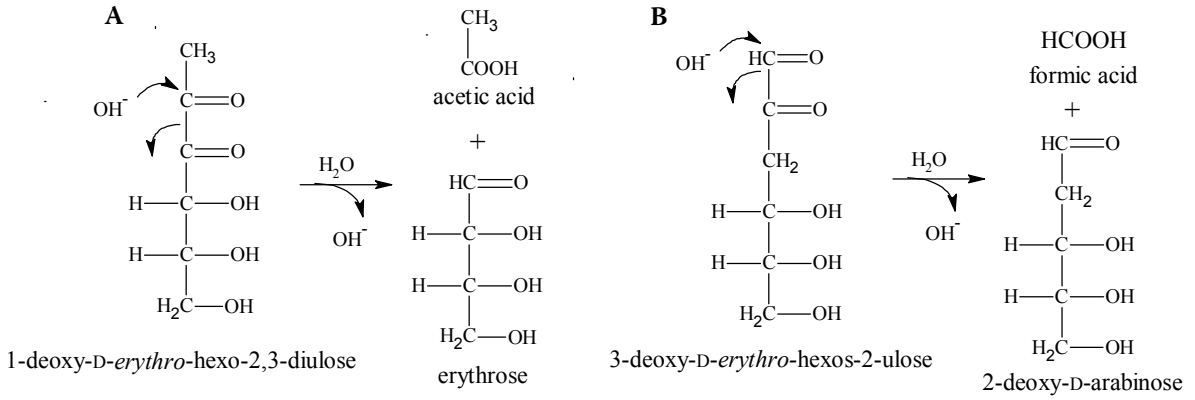

Figure 1. Hypothetical formation mechanism based on hydrolytic α-dicarbonyl cleavage of (**A**) 1-deoxy-D-*erythro*-hexo-2,3-diulose and (**B**) 3-deoxy-D-*erythro*-hexos-2-ulose leading to acetic acid and formic acid, respectively (according to Ginz *et al.* 2000)

described (Figure 2) are involved in generating acetic acid (pathway A1) and D-glyceric acid (pathway A2) from 1-deoxy-D-*erythro*-hexo-2,4-diulose by hydrolytic β-dicarbonyl cleavage. Oxidative α-dicarbonyl cleavage is employed in the formation of couples of acetic/D-erythronic acid (pathway B1) and D-glyceric/L-lactic acid (pathway B2) from 1-deoxy-D-*erythro*-hexo-2,3-diulose and 1-deoxy-D-*erythro*-hexo-3,4-diulose, respectively.

Generally, it is assumed that low molecular hydroxyacids are formed also consecutively from the corresponding hydroxycarbonyls and α-dicarbonyls by direct oxidation and intermolecular Cannizzaro reaction, respectively. For instance, the formation of glyceric acid was investigated during oxidation of glucose and fructose using ^{18}O-enriched oxygen in alkaline solutions (VUORINEN 1985). It was observed that glyceric acid is formed mainly by intramolecular Cannizzaro reaction from hydroxymethylglyoxal while only a small amount of this acid is produced by direct oxidation of glyceraldehyde.

While intermediacy of any oxidising agent is usually not required for the formation of acetic acid, the development of formic acid is often linked with the decomposition of unstable oxidation products. The decomposition of 1- or 2-hydroperoxide gives rise to formic and lower aldonic acids (VUORINEN 1985), and the adduct of hydroperoxide anion to the aldehyde form of sugar provides formic acid and lower aldose (ISBELL *et al.* 1973). It was found that aldohexoses are decomposed almost quantitatively to 6 moles of formic acid while aldopentoses provide 5 moles of formic acid (per mol aldose) in

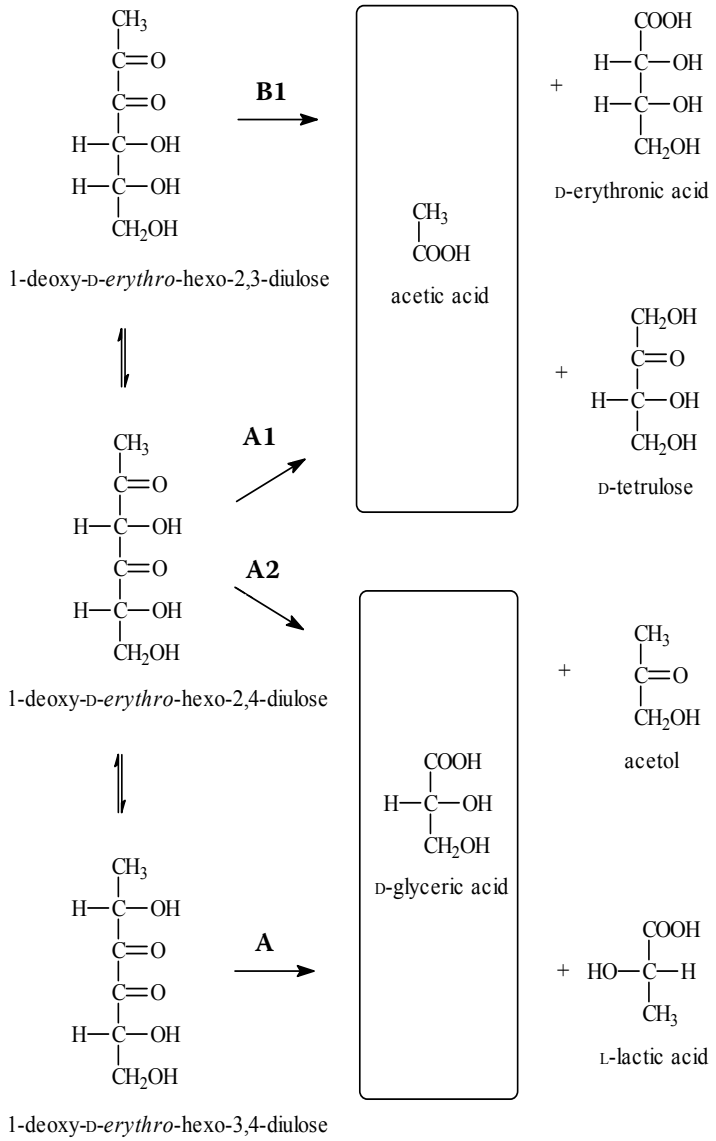

Figure 2. Scheme summarising possible pathways of sugar fragmentation *via* 1-deoxyhexodiuloses. **A1** and **A2**, hydrolytic α-dicarbonyl cleavage by nucleophilic attack of OH^- at the C-2 and C-4 carbonyl groups, respectively. **B1** and **B2**, oxidative β-dicarbonyl cleavage. Arrow thickness corresponds with the extent of reaction (adopted from DAVÍDEK *et al.* 2006b)

aqueous alkaline solutions of hydrogen peroxide at 0°C (Isbell *et al.* 1973). Another alternative mechanism based on the recombination of free radicals ($^{\bullet}CH_3$, $^{\bullet}CH_2$-COOH, $^{\bullet}$COOH) was suggested for the formation of acetic acid and other mono- and dicarboxylic acids during the degradation of amino acids in $K_2S_2O_8$ solutions (Rössner *et al.* 2001).

Recently, we reported on α-hydroxycarbonyl and α-dicarbonyl compounds formed during the degradation of selected monosaccharides in model systems (Novotný *et al.* 2007). The work presented here is a continuation of our research on sugar degradation accomplished under the same experimental conditions employed previously. The objective of this part of study was to identify and quantify carboxylic acids formed in model systems and to elucidate the mechanisms leading to their formation.

MATERIAL AND METHODS

Chemicals. All following compounds were obtained commercially: D-glucose, D-fructose (Sigma, St. Louis, USA), D-arabinose, 1,3-dihydroxyacetone dimer, glycollic acid, glyoxylic acid hydrate, heptadecane (Fluka, Buchs, Switzerland), DL-glyceraldehyde (Reanal, Budapest, H), D-glucono-5-lactone, pyruvic acid, sodium D-gluconate, *N*-trimethylsilylimidazole, *N,O*-bis(trimethylsilyl)-trifluoroacetamide (Aldrich, Steinheim, Germany), oxalic acid dihydrate, DL-lactic acid, potassium D-arabinate, L-malic acid, sodium D-glucuronate, succinic acid, sodium formate, sodium acetate trihydrate (Lachema, Brno, Czech Republic), propionic acid (Reachim, Russia), anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide (Penta, Chrudim, Czech Republic), diethyl ether, acetonitrile, *N,N*-dimethylformamide (Merck, Darmstadt, Germany), potassium peroxodisulfate (Dorapis, Prague, Czech Republic).

Degradation of sugars. Equimolar amounts of a sugar and potassium peroxodisulfate (5 mmol each) were dissolved in 50 ml of water or 0.3M sodium hydroxide, respectively (model 1 and model 2). The respective sugars (5 mmol) were also dissolved in 50 ml of 0.05M sodium hydroxide (model 3). All mixtures were heated under reflux for 1 h, then allowed to cool to room temperature and analysed for the contents of low molecular carbonyls. Three independent determinations were performed with each model system.

Quantification of formic, acetic and propionic acids. The cooled reaction mixture was adjusted to pH 3.0 (except $K_2S_2O_8/H_2O$ systems) with hydrochloric acid (1M and 0.1M) and transferred to a separatory funnel. An aliquot of the diethyl ether stock solution of the internal standard (heptadecane) was added and the mixture was extracted with four 20 ml portions of diethyl ether. Each extract was dried over anhydrous sodium sulfate. The combined extracts were concentrated to approximately 1 ml and analysed by GC/MS. The quantification was performed by the method of calibration curve (5 points). Sodium formate, sodium acetate (trihydrate), and propionic acid were used for the preparation of aqueous calibration solutions. After the addition of the internal standard, the acids were isolated from the calibration solutions by the same procedure as described above. The following ions were used for quantification: *m/z* 57 (heptadecane), *m/z* 46 (formic acid), *m/z* 43 (acetic acid), and *m/z* 74 (propionic acid).

Quantification of hydroxycarboxylic acids. An aliquot of each of the aqueous stock solution of the internal standard (L-malic acid) was added to 5 ml of cooled reaction mixture and the mixture was adjusted to pH 3.0 with hydrochloric acid (1M and 0.1M). A volume of 0.5 ml was evaporated to dryness and dissolved in 0.5 ml of the mixture of acetonitrile and *N,N*-dimethylformamide (1:1, v/v). Then 150 µl *N*-trimethylsilylimidazole and 150 µl *N,O*-bis(trimethylsilyl)trifluoroacetamide were added, the mixture was treated in ultrasonic bath for 10 s, held for 10 min at 70°C, and analysed by GC/MS. The calibration curve method (5 points) was used for quantification. The calibration curves were measured for the following compounds: oxalic acid, glyoxylic acid hydrate, glycollic acid, pyruvic acid, succinic acid, DL-lactic acid, sodium D-glucuronate, sodium D-gluconate, potassium D-arabinate, and D-glucono-1,5-lactone. The standards of the acids and lactones were prepared in water with the addition of L-malic acid as the internal standard and derivatised as described previously. Only quantification of oxalic and pyruvic acids was performed using the characteristic ions *m/z* 190 and *m/z* 217, respectively; the other acids were quantified from total ion current record. Semi-quantification of those acids and lactones for which reference standards were not available was accomplished using calibration curves obtained from structurally similar acids with the standards available.

Gas chromatographic/mass spectrometric analysis (GC/MS). For the quantification of formic,

acetic, and propionic acids, an Hewlett-Packard G1800A apparatus equipped with a fused silica capillary column CP WAX 52 CB (30 m × 0.25 mm i.d., film thickness of 0.25 μm) was used. The GC oven was temperature programmed from 60°C to 200°C (held 10 min) at the rate of 4°C, injector and detector temperatures were held at 220°C and 280°C, respectively. For the quantification of hydroxycarboxylic acids, an Agilent Technologies 6890N chromatograph with mass detector Agilent 5973N and a fused silica capillary column HP-5MS (30 m × 0.25 mm i.d., film thickness of 0.25 μm) was used. The GC oven was temperature programmed from 80°C (held 2 min) to 280°C (held 15 min) at the rate of 5°C, the injector and detector temperatures were held at 260°C and 280°C, respectively. Other parameters were the same for the analysis of both kinds of acids. The carrier gas (He) flow rate was 0.7 ml/min. The sample (1 μl) was injected using the split ratio 1:20. Mass spectra were obtained by EI ionisation at 70 eV and recorded in TIC mode. The ion source temperature was maintained at 250°C. The structural assignment of the compounds was accomplished using the Wiley275.L mass spectral computer library (Hewlett-Packard, Palo Alto, USA).

RESULTS AND DISCUSSION

The non-enzymatic degradation of sugars has been intensively studied for a long time, however, carboxylic acids formed by these reactions still lie apart from the main interest in spite of many important consequences related to their formation being obvious. We studied the formation of carboxylic and hydroxycarboxylic acids during the degradation of monosaccharides in three different model systems: aqueous solution of potassium peroxodisulfate ($K_2S_2O_8/H_2O$), alkaline solution of potassium peroxodisulfate ($K_2S_2O_8$/0.3M NaOH), and alkaline sodium hydroxide solution (0.05M NaOH), respectively. The decomposition of peroxodisulfate in aqueous solution provides several reactive species; hence peroxodisulfate represents simultaneously an oxidising agent, a radical initiator, and acidulant. It is well known that alkaline solutions cause deep changes in sugar molecule and certain similarity with the degradation of sugars under high temperatures is often considered. Recently, we identified several α-hydroxycarbonyl and α-dicarbonyl compounds formed in the systems mentioned above (Novotný *et al.* 2007). In the present work, we extended our study to another group of products, carboxylic acids, to elucidate the main reaction pathways of sugar transformations. The yields of acids identified in our reaction mixtures are given in Tables 1–3.

Formic, acetic, and propionic acids

Formic and acetic acids were the major acids identified in our reaction systems. The amounts of these acids differed considerably depending on the particular system and sugar employed (Tables 1–3).

Formic acid was present in large amounts in the reaction mixtures with $K_2S_2O_8/H_2O$ (7.6–18.0%, n/n) and $K_2S_2O_8$/0.3M NaOH (8.8–20.6%, n/n), and in a relatively small amount in 0.05M NaOH (5.3–6.7%, n/n), which implies several possible mechanisms of its formation. Formic acid is a well known by-product of many sugar degradation reactions. It is assumed that formic acid is formed by hydrolytic α-dicarbonyl cleavage of 3-deoxy-D-*erythro*-hexos-2-ulose (Figure 1B) (Brands & van Boekel 2001). Apparently, it can be formed by oxidation of formaldehyde by several oxidation reagents or in cross-Cannizzaro reaction with simultaneous reduction of co-reactants. Formic acid may be also a direct cleavage product of 1- or 2-hydroperoxides (primary products of sugar autoxidation) (Vuorinen 1985) and an aldose-hydrogen peroxide adduct (Isbell *et al.* 1973). Both intermediates appear to be formed in the systems with peroxodisulfate where both triplet oxygen and hydrogen peroxide are available. Minor pathways of formic acid formation in $K_2S_2O_8/H_2O$/hexose models would be the decomposition of 5-hydroxymethylfuran-2-carbaldehyde in an acidic solution (Davídek *et al.* 1990) and, to a much smaller extent, an electrolytic ring opening of cyclic methylglyoxal dimer dihydrate regardless the model conditions (Huyghues-Despointes & Yaylayan 1996).

Acetic acid was detected as the major acid in 0.05M NaOH model systems, in which its amount (7.8–12.45%, n/n) was much higher than in $K_2S_2O_8$/0.3M NaOH (1.2–2.6%, n/n) and $K_2S_2O_8/H_2O$ (0.8–2.6%, n/n) systems. It was observed that the amount of acetic acid correlated well with the amounts of acetol and methylglyoxal (Novotný *et al.* 2007). Both carbonyls are possible precursors of acetaldehyde (Figure 3) which seems to be the key intermediate for acetic acid forma-

Table 1. Yields of acids and lactones in $K_2S_2O_8/H_2O$ model systems

Acid or lactone	Identification[1]	Glc	Fru	Ara	Dha	Gla
		% (n/n)[2]				
Formic acid	MS, RT	9.93	7.59	18.04	10.66	11.63
Acetic acid	MS, RT	0.79	1.68	0.52	2.39	2.57
Propionic acid	MS, RT	0.01	0.01	< 0.01	0.03	0.24
DL-Lactic acid	MS, RT	0.78	1.25	0.60	0.10	0.46
Glycollic acid	MS, RT	1.09	1.35	1.94	7.45	3.04
2-Hydroxyacrylic acid	MS, RT	0.03	0.14	0.02	0.01	0.04
3-Hydroxyacrylic acid	MS	n.d.	n.d.	n.d.	0.10	0.86
Oxalic acid	MS, RT	0.85	n.d.	0.17	n.d.	n.d.
Glyoxalic (hydrate) acid	MS, RT	3.86	n.d.	n.d.	14.13	n.d.
Succinic acid	MS, RT	n.d.	n.d.	n.d.	0.02	n.d.
DL-Glyceric acid	MS	0.12	0.15	0.31	0.07	4.10
2,3-Dihydroxybutanoic acid 2[3]	MS	0.33	n.d.	n.d.	n.d.	n.d.
D-Erythrono-1,4-lactone	MS	0.18	0.07	0.14	n.d.	n.d.
2-Deoxy-D-*erythro*-pentono-1,4-lactone	MS	0.06	0.10	3.51	n.d.	n.d.
Tetronic acid 1[3]	MS	0.03	0.02	0.02	n.d.	n.d.
D-Arabinono-1,4-lactone	MS, RT	0.51	0.49	17.39	n.d.	n.d.
D-Arabinono-1,5-lactone	MS, RT	n.d.	n.d.	0.37	n.d.	n.d.
D-Ribono-1,4-lactone	MS	0.08	n.d.	n.d.	n.d.	n.d.
D-Arabinonic acid	MS, RT	n.d.	n.d.	0.65	n.d.	n.d.
2-Deoxy-D-*arabino*-hexono-1,4-lactone	MS	4.08	n.d.	n.d.	n.d.	n.d.
D-Glucurono-6,3-lactone	MS, RT	0.34	n.d.	n.d.	n.d.	n.d.
D-Glucono-5-lactone	MS, RT	8.26	n.d.	n.d.	n.d.	n.d.
D-Gluconic acid	MS, RT	2.74	n.d.	n.d.	n.d.	n.d.
D-Glucuronic acid	MS, RT	0.11	n.d.	n.d.	n.d.	n.d.
Total		**34.2**	**12.9**	**43.7**	**35.0**	**22.9**

[1]MS = identification by comparison with mass spectral library, RT = identification by comparison with retention characteristics of authentic compounds

[2]Entries are averages of three independent determinations; RSD were < 20% within the whole experimental series, Glc = D-glucose, Fru = D-fructose, Ara = D-arabinose, Dha = 1,3-dihydroxyacetone, Gla = DL-glyceraldehyde

[3]Compounds with unknown configuration of substituents; numerals correspond to diastereomers possessing identical MS spectrum

n.d. = not detected

tion. Considering the literature data, it is evident that a significant portion of acetic acid can be formed by the direct fragmentation of the sugar skeleton. Davídek *et al*. (2006a) showed recently that hydrolytic β-dicarbonyl cleavage of 1-deoxyhexo-2,4-diulose (Figure 2, pathway A1) is a major pathway of acetic acid formation, while oxidative α-dicarbonyl cleavage of 1-deoxy-D-*erythro*-hexo-2,3-diulose induced by some oxidising species is less frequent (Figure 2, pathway B1).

However, the results reported previously of kinetic studies (Brands & van Boekel 2001; Martins & van Boekel 2003) and labelling experiments (Kim & Baltes 1996; Ginz *et al.* 2000) pointed to hydrolytic α-dicarbonyl cleavage (Figure 1A) of 1-deoxy-D-*erythro*-hexo-2,3-diuloses as the predominant reaction pathway. Two papers reported on the incorporation of carbon atoms of glucose into acetic acid molecule in the binary system glucose/glycine. While Wnorowski and Yaylayan (2000) described the formation of acetic acid only from both ends of glucose in the ratio C-1/C-2 (~80%) and C-5/C-6 (~20%), Davídek *et al*. (2006a), for the first time, reported also minor

incorporation of C-3/C-4 carbon atoms. The following distribution was found out in the study by Davídek *et al.* (2006a): C-1/C-2 (~70%), C-3/C-4 (~10%), and C-5/C-6 (~20%).

Analogously to formaldehyde, acetaldehyde can undergo oxidation by the available oxidants or in cross-Cannizzaro reaction. The mechanism based on the recombination of free radicals (Figure 5) was also suggested (Rössner *et al.* 2001). This pathway would be considered especially in systems with peroxodisulfate where an extensive formation of free radicals is expected. Additionally, acetic acid may be produced also by hydrolytic dicarbonyl cleavage from the corresponding low molecular α- and/or β-dicarbonyls previously identified in the reaction mixtures (Novotný *et al.* 2007). Huyghues-Despointes and Yaylayan (1996) found acetic acid as the major (30%) product of methylglyoxal heated to 100°C. Thermal degradation (120°C, pH 6–10, up to 4 h) of pentane-2,4-dione and pentane-2,3-dione resulted in almost equimolar amounts of acetic acid and acetone as well as acetic acid and propionic acid, respectively (Davídek *et al.* 2006a).

Propionic acid was detected in negligible amounts in all the reaction mixtures investigated. Generally, larger amount of this acid were found in 0.05M NaOH models (0.05–0.20%, n/n), but the largest proportional amount (0.24%, n/n) was detected in glyceraldehyde/$K_2S_2O_8$/H_2O system. Markedly, larger amounts of propionic acid quantified in mixtures containing glyceraldehyde pointed to this aldehyde being an important precursor. We predicted a stepwise mechanism (Figure 6) considering lactic acid and lactaldehyde as the key intermediates. Lactic acid could be formed either by Cannizzaro reaction from methylglyoxal (product of glyceraldehyde dehydration) or by oxidation of lactaldehyde. Lactic acid may undergo

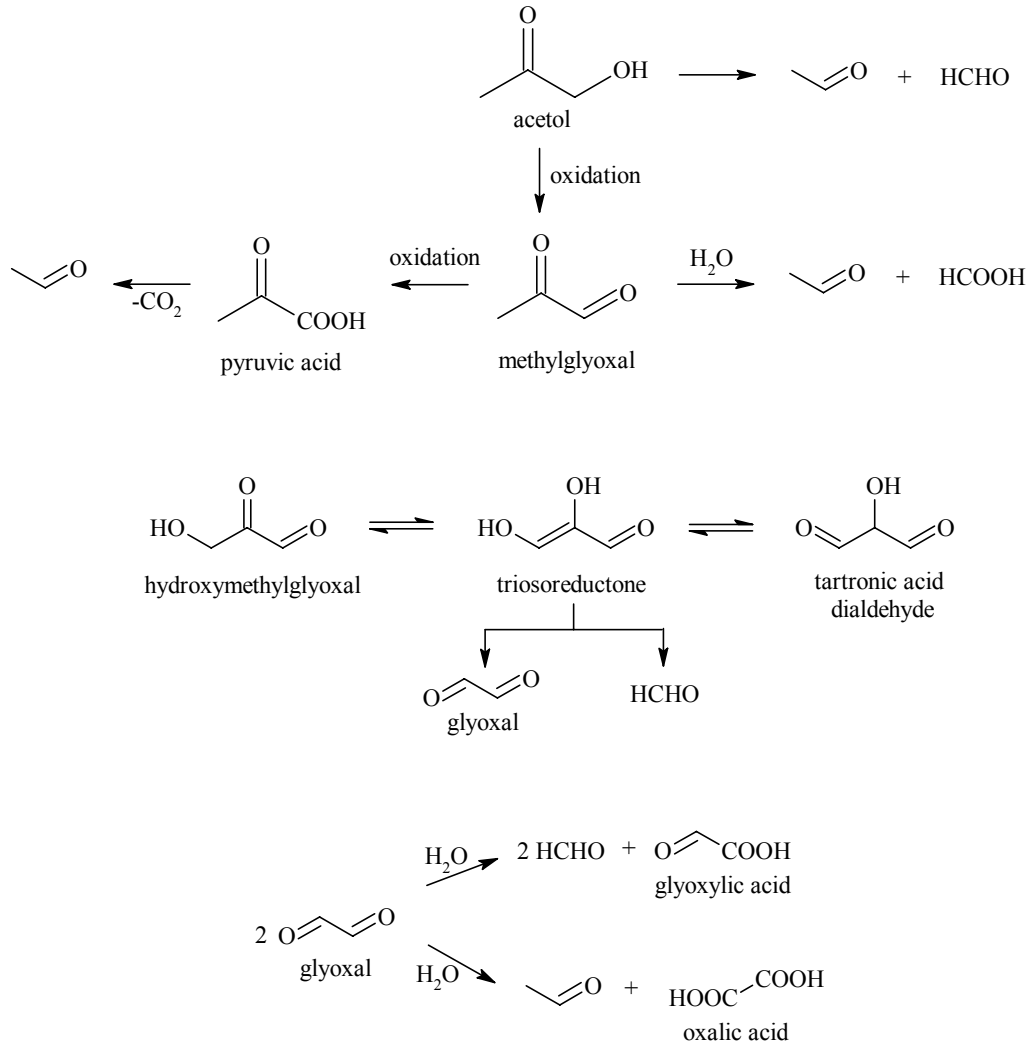

Figure 3. Formation of formaldehyde, acetaldehyde, and formic, glyoxylic, and oxalic acids

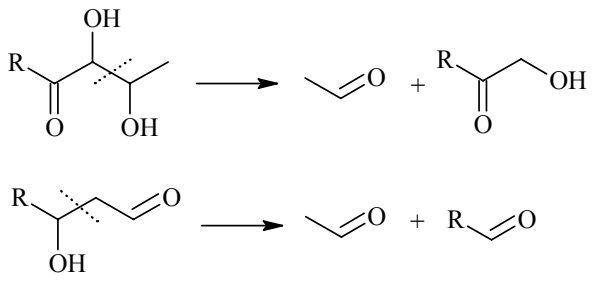

Figure 4. Acetaldehyde formation by retro-aldol cleavage

dehydration followed by the acceptance of two hydrogen atoms (by reaction with some hydride donors such as glycolaldehyde) which gives rise to propionic acid. Analogously, lactaldehyde produces propionaldehyde, which is finally oxidised to propionic acid. The formation of prop-2-enal, the key intermediate of propionaldehyde, would also proceed hypothetically by aldol condensation of acetaldehyde and formaldehyde (Figure 6). An alternative reaction pathway leading to the formation of propionic acid represents oxidative α-dicarbonyl cleavage of pentane-2,3-dione providing acetic acid as a counterpart (Davídek *et al*. 2006a). Theoretically, propionic acid may be also formed by the recombination of free radicals (Rössner *et al.* 2001) (Figure 5).

Formaldehyde and acetaldehyde

The presence of the above mentioned intermediates, formaldehyde and acetaldehyde, was observed in all reaction mixtures; nevertheless, their yields were not determined. Both aldehydes were identified as condensation products with *o*-phenylendiamine (OPDA) used for derivatisation of α-dicarbonyl compounds (Novotný *et al.* 2007). *(1H)*-Benzimidazole and 2-methyl-*(1H)*-benzimidazole were identified as the OPDA condensation products of formaldehyde and acetaldehyde, respectively. Using authentic standards, we observed that both aldehydes can subsequently undergo a second addition to the already formed benzimidazoles including also cross-addition (addition of one aldehyde to the benzimidazole arosen from the other aldehyde). Owing to these findings, we could not use this procedure for quantification.

Formaldehyde is the simplest fragment of sugars. Its formation was confirmed in many studies concerning sugar degradation. Its scission from 1-endiols yields lower aldose as the second product. Formaldehyde was also suggested as a product of cleavage of C_1–C_2 bond of the acyclic adduct of hydroperoxide anion to ketoses (Isbell *et al.* 1973). Acetaldehyde could hypothetically arise by retro-aldol cleavage of intermediates having structure motives of 1-deoxy-2,3-dihydroxy-4-oxo or 2-deoxy-3-hydroxy-1-oxo (Figure 4). In fact, no report introducing any certain six or five carbon precursor with this structure motive is available. However, both aldehydes are known to be scission products of some lower carbonyls (Figure 3). For example, cleavage of triosoreductone provides formaldehyde and glyoxal, while scission of 1-en-1,2-diol of acetol gives formaldehyde and acetaldehyde (Velíšek 2002). Rössner (2004) predicted the formation of both aldehydes during the cleavage of glyoxal and subsequent disproportionation of the fragments formed. Analogously, acetaldehyde and formaldehyde could also form from methylglyoxal with simultaneous formation of the corresponding acid. Furthermore, pyruvic acid may be formed by oxidation of methylglyoxal and can consecutively decarboxylate yielding acetaldehyde. The identification of pyruvic acid in our model systems supports this reaction pathway.

Hydroxyacids and lactones

In total, 24 hydroxycarboxylic acids and 12 corresponding lactones were identified and quantified in all model systems after derivatisation (Tables 1–3).

Basically, the acids identified in our model systems can be divided into two groups: (*i*) acids possessing the original carbon skeleton of the parent sugar and (*ii*) acids with a shorter carbon

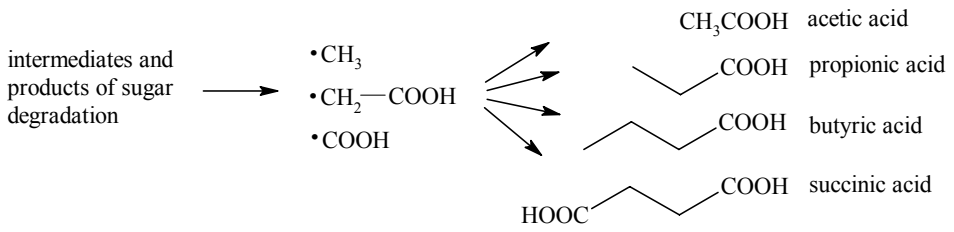

Figure 5. Formation of acids by recombination of free radicals

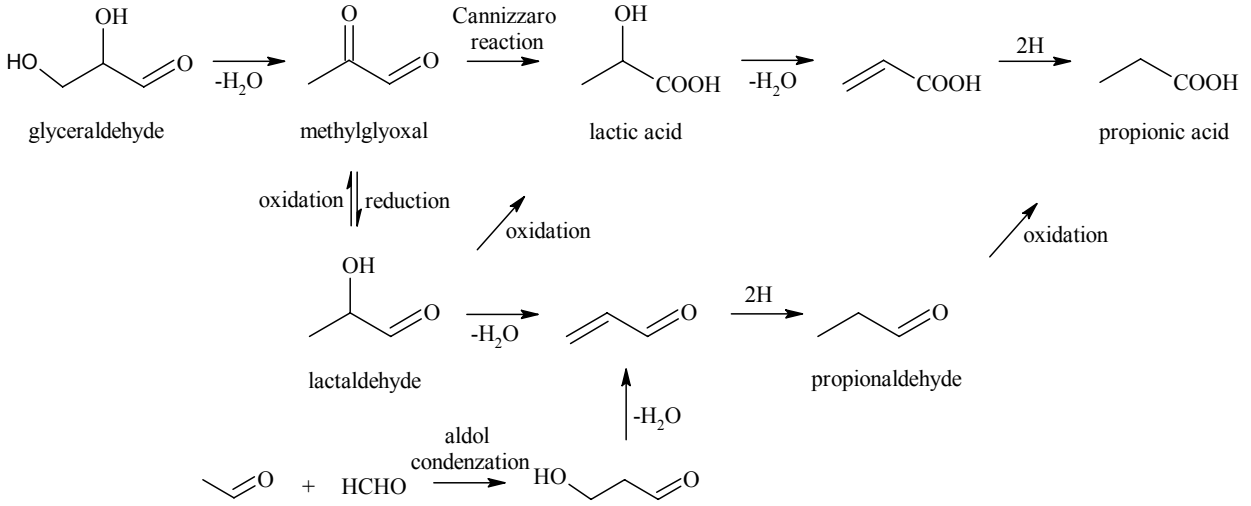

Figure 6. Formation of propionic acid

chain. Direct oxidation of sugar aldehydic group and primary hydroxyl group, respectively, and benzilic acid type rearrangement of deoxyuloses are the possible routes involved in the formation of the first group of acids, i.e. aldonic, alduronic, and saccharinic acids. The data obtained confirmed that benzilic acid type rearrangement was significantly enhanced in alkaline media, nevertheless, this rearrangement proceeded to some extent also in acidic medium ($K_2S_2O_8/H_2O$ system). Obviously, water acting as the Brønsted base remains an effective catalyst for the rearrangement reaction in this case (Antal *et al.* 1990).

Acids of the second group with a shorter carbon chains are either direct cleavage products of the corresponding intermediates or oxidation products of low-molecular carbonyls arising primarily by sugar fragmentation. Comparing, the spectra of the acids and carbonyl compounds, previously identified in the same models (Novotný *et al.* 2007), it is evident that the formation of many acids is linked with direct oxidation of α-hydroxycarbonyl compounds as well as with α-dicarbonyl compounds transformed *via* Cannizzaro reaction. Understandably, the yield of acids in particular model system increases as follows: $K_2S_2O_8/H_2O$ < 0.05M NaOH < $K_2S_2O_8$/0.3M NaOH. The extent of oxidation is significantly enhanced in alkaline media due to extensive enolisation. However, the majority of the identified acids retain the configuration of the parent sugar (*arabino-*, *erythro-*), but acids with different configurations were also found. The change of configuration is caused either by the previous isomerisation of the sugar precursor or by the formation from lower intermediates *via* aldolisation.

It is well known that sugar acids readily undergo lactonisation to form relatively stable 4-lactones and less stable 5-lactones. Thus, the free form of acid and at least one lactone were mostly identified. Both Cannizzaro reaction and benzilic acid type rearrangement result in the formation of racemic mixtures of acids. Under chromatographic conditions used, it was not possible to separate the individual acids from their diastereomeric pairs.

C_2 acids

Glycollic, oxalic, and glyoxylic acids represent two-carbon chain hydroxyacids identified in our model systems. Glycollic (hydroxyacetic) acid was the major acid almost in all mixtures studied. Glycollic acid is a direct oxidation product of glycolaldehyde but, obviously, it is predominantly formed from glyoxal *via* the intermolecular Cannizzaro reaction. The formation of glycollic acid was also observed after the cleavage of the reaction product of hydrogen peroxide with hydroxymethylglyoxal (Vuorinen 1985) and ketoses (Isbell & Frush 1973). In the arabinose mixtures, glycollic acid can be also produced either by hydrolytic β-dicarbonyl cleavage of 1-deoxypento-2,4-diulose or by oxidative α-dicarbonyl cleavage of 1-deoxypento-3,4-diulose (Davídek *et al.* 2006a, b).

Oxalic (ethanedioic) acid was present in measurable amounts only in two $K_2S_2O_8/H_2O$ reaction mixtures (glucose and fructose). In fact, oxalic acid could be formed either by oxidation of both

Table 2. Yields of acids and lactones in $K_2S_2O_8$/0.3M NaOH model systems

Acid or lactone	Identification[1]	Glc	Fru	Ara	Dha	Gla
		% (n/n)[2]				
Formic acid	MS, RT	20.64	13.50	15.64	8.76	10.11
Acetic acid	MS, RT	1.53	1.81	1.21	1.97	2.56
Propionic acid	MS, RT	0.02	0.01	< 0.01	0.02	0.04
DL-Lactic acid	MS, RT	7.35	7.40	4.97	16.19	17.45
Glycollic acid	MS, RT	28.97	32.11	32.28	34.05	44.51
DL-Glyceric acid	MS	11.72	16.30	12.78	49.47	31.25
2,3-Dihydroxybutanoic acid 1[3]	MS	0.71	n.d.	1.39	0.19	1.56
2,3-Dihydroxybutanoic acid 2[3]	MS	n.d.	n.d.	1.53	n.d.	n.d.
D-Threono-1,4-lactone	MS	n.d.	0.03	n.d.	n.d.	n.d.
Tartronic acid	MS	n.d.	0.28	n.d.	n.d.	n.d.
2,4-Dihydroxybutanoic [3]	MS	5.41	4.68	15.99	4.98	11.51
D-Erythrono-1,4-lactone	MS	n.d.	0.06	n.d.	n.d.	n.d.
3,4-Dihydroxybutanoic[3]	MS	1.49	4.55	7.45	0.33	5.14
2,3-Dihydroxyacrylic	MS	2.71	0.99	1.70	n.d.	n.d.
Tetronic acid 1[3]	MS	3.01	2.81	1.41	0.42	1.44
Tetronic acid 2[3]	MS	0.52	0.69	1.19	0.31	1.21
D-Arabinono-1,4-lactone	MS, RT	1.59	2.22	4.81	n.d.	0.92
2-Deoxy-D-*erythro*-pentonic	MS	0.32	0.13	13.28	n.d.	n.d.
D-Arabinono-1,5-lactone	MS, RT	0.42	0.51	0.40	n.d.	0.17
D-Ribono-1,4-lactone	MS	0.33	0.20	n.d.	n.d.	n.d.
3-Deoxy-D-*ribo*-hexono-1,4-lactone	MS	0.36	0.12	n.d.	n.d.	n.d.
3-Deoxy-D-*arabino*-hexono-1,4-lactone	MS	0.86	0.37	n.d.	n.d.	n.d.
D-Ribonic acid	MS	1.29	0.87	0.40	n.d.	0.69
D-Arabinonic acid	MS, RT	1.58	2.13	7.93	0.02	1.01
D-Glucono-1,5-lactone	MS, RT	2.10	0.57	n.d.	n.d.	n.d.
D-Mannono-1,4-lactone	MS	0.86	n.d.	n.d.	n.d.	n.d.
D-Mannonic acid	MS	0.06	0.04	n.d.	n.d.	n.d.
D-Gluconic acid	MS, RT	4.17	0.26	n.d.	n.d.	n.d.
D-Glucuronic acid	MS, RT	0.32	n.d.	n.d.	n.d.	n.d.
Total		**98.3**	**92.6**	**124.4**	**116.7**	**129.6**

[1]MS = identification by comparison with mass spectral library, RT = identification by comparison with retention characteristics of authentic compounds

[2]Entries are averages of three independent determinations; RSD were < 20% within the whole experimental series, Glc = D-glucose, Fru = D-fructose, Ara = D-arabinose, Dha = 1,3-dihydroxyacetone, Gla = DL-glyceraldehyde

[3]Compounds with unknown configuration of substituents; numerals correspond to diastereomers possessing identical MS spectrum

n.d. = not detected

carbonyl groups of glyoxal or by oxidation of hydroxyl group of glycollic acid. Rössner (2004) also assumed that oxalic acid is produced by the cleavage of glyoxal and subsequent disproportionation of the fragments formed (Figure 3).

Glyoxylic (oxoacetic) acid identified in the form of its hydrate was found only in the mixtures of glucose and 1,3-dihydroxyacetone in the $K_2S_2O_8$/H_2O system, but interestingly in significant amounts. Apparently, its high level (14.13%, n/n) is linked with the high content of glycollic acid (7.45%, n/n) as well as of glycolaldehyde (4.70%, n/n), as previously reported (Novotný *et al.* 2007). This fact unequivocally pointed to a stepwise mecha-

nism (Figure 7) which starts with the cleavage of 1,3-dihydroxyacetone providing glycolaldehyde and formaldehyde. The consequent oxidation of glycolaldehyde can hypothetically proceed in two ways: (*i*) oxidation of carbonyl group to form glycollic acid followed by oxidation of hydroxy group giving rise to glyoxylic acid, and/or (*ii*) direct oxidation of hydroxy group of glycolaldehyde. Considering the high content of glycollic acid, it is obvious that oxidation *via* this acid is the major reaction. The formation of glyoxylic acid is supposed to proceed also after the cleavage of glyoxal and subsequent disproportionation of the fragments formed (Figure 3) (Rössner 2004). Direct formation of glyoxylic acid hydrate is assumed to be the result of autoxidation of glyoxal dihydrate (Buxton *et al.* 1997) initiated by free radicals (Figure 7).

C_3 acids

Lactic, glyceric, pyruvic, tartronic, and 2,3-dihydroxyacrylic acids are three-carbon hydroxyacids identified in the investigated reaction mixtures.

Lactic (2-hydroxypropanoic) acid was found in large amounts in alkaline reaction mixtures indicating its predominant formation *via* intramolecular Cannizzaro reaction of methylglyoxal. Another possible way is direct oxidation of lactaldehyde. The formation of L-lactic acid in pentose and hexose mixtures can also be explained by oxidative α-dicarbonyl cleavage of 1-deoxy-3,4-diuloses providing D-glyceric acid (hexoses) and glycollic acid (pentoses) as the counterparts (for hexoses Figure 2, pathway B2) (Davídek *et al.* 2006b).

Glyceric (2,3-dihydroxypropanoic) acid was found in all mixtures studied with the largest amount quantified in $K_2S_2O_8$/0.3M NaOH systems. The highest yields of this acid were found in the reaction mixtures of glyceraldehyde; it is obvious that glyceric acid is formed mainly by direct oxidation of glyceraldehyde. Nevertheless, significant amounts of this acid can be also produced by intermolecular Cannizzaro reaction of hydroxymethylglyoxal (Vuorinen 1985). In mixtures containing hexoses, D-glyceric acid may be formed also as a direct cleavage product of the corresponding intermediates (Davídek *et al.* 2006a, b). Moreover, α- and β-splitting providing glyceric acid was previously proposed for 2-deoxyhexo-3,4-diulose (Yang & Montgomery 1996a) and 1-deoxyhexo-2,4-diulose (Weenen 1998), respectively.

Two other compounds identified, i.e. 2- and 3-hydroxyacrylic (2- and 3-hydroxyprop-2-enoic) acids are evidently isomers of pyruvic (2-oxopropionic) acid. We have found that silylation of pyruvic acid is linked with isomerisation because only 2-hydroxyacrylic acid was obtained after derivatisation of authentic pyruvic acid. Since no 3-hydroxyacrylic acid was found after silylation of pyruvic acid, it is obvious that its formation is not linked with derivatisation procedure and can be understood as a result of isomerisation proceeding during the model experiment. 2-Hydroxyacrylic and 3-hydroxyacrylic acids belonged to minor reaction products in all models. Pyruvic acid is apparently formed by oxidation of methylglyoxal, but this reaction is, in comparison with Cannizzaro reaction, markedly less frequent. Considering the origin of

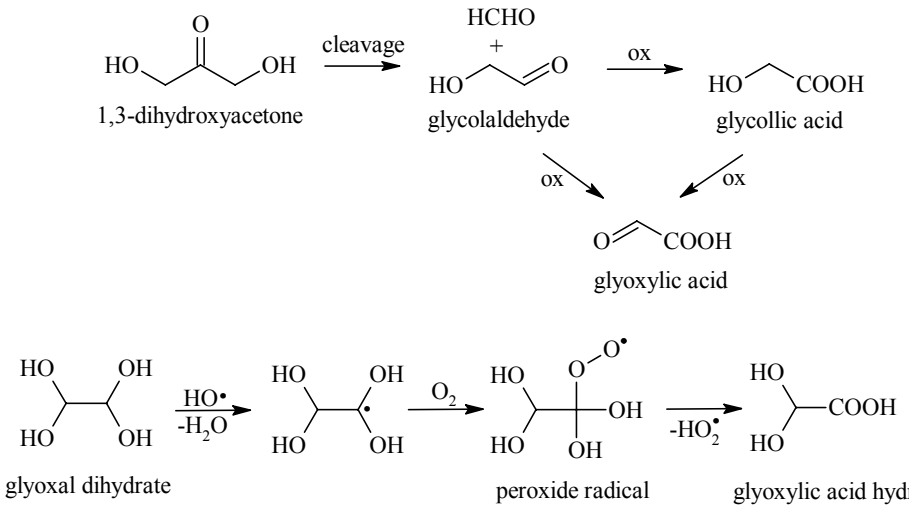

Figure 7. Formation of glyoxylic acid and glyoxylic acid hydrate

Table 3. Yields of acids and lactones in 0.05M NaOH model systems

Acid or lactone	Identification[1]	Glc	Fru	Ara	Dha	Gla
		% (n/n)[2]				
Formic acid	MS, RT	5.32	5.91	5.65	6.47	6.69
Acetic acid	MS, RT	11.48	12.06	12.45	7.81	10.64
Propionic acid	MS, RT	0.16	0.19	0.20	0.05	0.14
DL-Lactic acid	MS, RT	7.07	7.64	5.08	10.40	7.50
Glycollic acid	MS, RT	14.51	16.30	26.02	10.39	12.59
2-Hydroxyacrylic acid	MS, RT	0.33	0.39	0.25	1.97	0.65
3-Hydroxyacrylic acid	MS	0.10	0.13	0.27	0.03	0.08
DL-Glyceric acid	MS	1.11	1.38	1.61	1.13	2.42
2,4-Dihydroxybutanoic acid[3]	MS	25.53	22.25	18.87	13.53	26.99
3,4-Dihydroxybutanoic acid[3]	MS	2.15	2.61	6.35	0.84	1.94
2,3-Dihydroxyacrylic acid	MS	10.63	8.84	7.99	1.03	2.71
Tetronic acid 1[3]	MS	0.33	0.39	0.29	0.11	0.17
Tetronic acid 2[3]	MS	0.16	0.15	0.13	0.15	0.18
D-Arabinono-1,4-lactone	MS, RT	0.48	0.63	0.71	–	0.10
2-Deoxy-D-*erythro*-pentonic acid	MS	0.38	0.36	–	0.02	0.09
3-Deoxy-D-*ribo*-hexono-1,4-lactone	MS	1.80	2.40	–	0.13	0.89
3-Deoxy-D-*arabino*-hexono-1,4-lactone	MS	6.79	5.57	0.27	0.83	3.81
D-Arabinonic acid	MS, RT	0.24	0.26	0.32	–	0.20
D-Glucono-1,5-lactone	MS, RT	0.81	0.97	–	–	–
D-Gluconic acid	MS, RT	0.32	0.31	0.23	0.04	0.08
Total		**89.7**	**88.7**	**86.7**	**54.9**	**77.9**

[1]MS = identification by comparison with mass spectral library, RT = identification by comparison with retention characteristics of authentic compounds

[2]Entries are averages of three independent determinations; RSD were < 20% within the whole experimental series, Glc = D-glucose, Fru = D-fructose, Ara = D-arabinose, Dha = 1,3-dihydroxyacetone, Gla = DL-glyceraldehyde

[3]Compounds with unknown configuration of substituents; numerals correspond to diastereomers possessing identical MS spectrum

pyruvic acid *via* some C_5 or C_6 precursors, it should be an intermediate with the structure motive of 1-deoxy-hexo(pento)-2,3,4-triulose.

Tartronic (2-hydroxypropanedioic) acid was identified only in the fructose/$K_2S_2O_8$/0.3M NaOH model. Apparently, it is formed by oxidation of the corresponding aldehyde which arises by isomerisation of hydroxymethylglyoxal.

2,3-Dihydroxyacrylic (2,3-dihydroxyprop-2-enoic) acid is an uncommon acid identified only in alkaline reaction mixtures. It seems probable that the corresponding dialdehyde is its immediate precursor. In theory, the probable way of the formation of such an aldehyde is retro-aldol cleavage of 2-endiol form of 2-ulose having at least four carbon atoms. Oxidation of C_1–C_2 bond of glyceric acid possibly leads to the formation of 2,3-dihydroxyacrylic acid from trioses.

C_4 and C_5 acids

Succinic acid, aldonic acids, and different types of saccharinic acids with four- and five-carbon atoms were detected in our model systems.

Succinic (butanedioic) acid was found in negligible amounts only in the 1,3-dihydroxyacetone/$K_2S_2O_8$/H_2O system. This acid is obviously formed from lower intermediates. For example, the corresponding 2-en-dialdehyde can arise from acetaldehyde and glyoxal by aldol condensation; this intermediate can provide succinic acid in redox reactions. Succinic acid can be also formed by

recombination of free radicals (Figure 5) (Rössner *et al.* 2001).

Two isomers of D-tetronic acid having *erythro-* and *threo-*configuration were found, but they were not differentiated because they possessed identical MS spectra. In addition, the corresponding lactones, D-threono-4-lactone and D-erythrono-4-lactone, were found in some mixtures. Similarly, D-arabinonic and D-ribonic acids were found in reaction mixtures together with their 4-lactones. Basically, several mechanisms can be involved in the formation of lower aldonic acids. The first one is based on direct oxidation of lower aldose which can be formed from the parent sugar by retro-aldol scission. Ruff degradation (decarboxylation of aldonic or alduronic acids) (Isbell & Salam 1981; Marcq *et al.* 2001) or decomposition of acyclic peroxide adduct (Isbell *et al.* 1973) represent pathways providing lower aldoses as well. The direct formation of lower aldonic acids by decomposition of unstable 1- and 2-hydroperoxides with the simultaneous production of formic acid is assumed to be also involved during sugar oxidation (Davídek *et al.* 1990). Moreover, the recently described mechanism based on oxidative α-dicarbonyl cleavage of 1-deoxy-D-*erythro*-hexo-2,3-diulose (Figure 2, pathway B1) (Davídek *et al*. 2006b) may be also considered for the formation of a certain portion of C_4-aldonic acid with acetic acid as a counterpart.

In total, 3 types of saccharinic acid with four-carbon atoms (2-deoxy-, 3-deoxy- and 4-deoxytetronic) were identified. 2,3-Dihydroxybutanoic (4-deoxytetronic) acid was found in two isomeric forms possessing *erythro-* and *threo-*configuration, respectively. It was not possible to differentiate them using the mass spectra measured. This acid is probably formed *via* intramolecular Cannizzaro reaction from the corresponding 4-deoxytetros-2-ulose. 2,4-Dihydroxybutanoic (3-deoxytetronic) acid was eluted only as one peak corresponding probably to both isomeric forms. The precursor of this metasaccharinic acid is the corresponding 3-deoxytetros-2-ulose. The last deoxytetronic acid, 3,4-dihydroxybutanoic (2-deoxytetronic) acid, yielded also a single peak. It was probably formed from 3-deoxypentos-2-ulose by α-diketo cleavage with formaldehyde produced as a by-product (Yang & Montgomery 1996b). 2-Deoxy-D-*erythro*-pentonic acid (identified also as the corresponding 1,4-lactone) represents the only one five-carbon chain of deoxyacid found. Its formation is analogous to that of its lower homologue.

C_6 acids

Aldonic, alduronic, and deoxyaldonic acids and the corresponding lactones containing six carbon atoms were identified, mainly in models containing hexoses and, to some extent, also in models with pentoses and trioses. The presence of C_6 acids in the mixtures of lower sugars indicates previous aldolisation of lower carbonyl intermediates.

No free forms of deoxyhexonic acids were found; only three lactones, i.e. 3-deoxy-D-*arabino*-hexono-1,4-lactone and isomeric 3-deoxy-D-*ribo*-hexono-1,4-lactone, and 2-deoxy-D-*arabino*-hexono-1,4-lactone were identified. The formation of 3-deoxyhexonic acid corresponding to the first two lactones proceeds obviously *via* benzilic acid type rearrangement of 3-deoxy-D-*erythro*-hexos-2-ulose. We have found out that the diastereomeric pair of metasaccharinic acids (occurring as lactones) is not produced in equimolar ratio. The lactone possessing *arabino-*configuration was present usually in amounts 2–3 times larger than that possessing *ribo-*configuration. It indicates that different reaction pathways independent on benzilic acid type rearrangement are involved in their formation. Similarly, it is difficult to explain the formation of 2-deoxy-D-*arabino*-hexonic acid. It may be understood as a result of specific oxidation and cleavage of certain uloses having seven carbon atoms which can be formed by aldol condensation of lower intermediates.

D-Gluconic and D-mannonic acids with the corresponding glucono-1,5-lactone and mannono-1,4-lactone were identified as the products of oxidation of aldehydic group of the corresponding hexoses. Due to some interferences observed, it was not possible to quantify D-glucono-1,4-lactone. However, considering the stability of lactones, it can be expected that its amount is higher than that of 1,5-lactone. D-Glucuronic acid was found only in the reaction mixture of glucose/$K_2S_2O_8$/H_2O. This acid is a product of oxidation of the primary hydroxyl group of glucose. Except the linear form, glucofuranuronic acid and glucopyranuronic acid were also identified. The quantification was accomplished as the sum of all these three forms. Moreover, D-glucurono-6,3-lactone was found as well. The formation of D-glucuronic acid is not absolutely clear as some authors assume that the oxidation of primary hydroxyl group proceeds simultaneously with the protection of carbonyl group (Belitz & Grosch 1999).

CONCLUSION

The data obtained have shown that the degradation of sugars yields a wide spectrum of various carboxylic acids under the experimental conditions used. Furthermore, carboxylic acids were the major sugar transformation products. Contrary to the low molecular weight carbonyls described previously, acids can be assessed as the final reaction products. Low molecular weight acids were largely formed by oxidation and/or intermolecular Cannizzaro reaction of low molecular carbonyl intermediates, and/or by direct cleavage of 1-deoxydiuloses derived from the parent sugars. The acids possessing the original sugar chain were either the result of direct sugar oxidation (aldonic or alduronic acids) or benzilic acid type rearrangement of deoxyuloses (saccharinic acids). Obviously, the degradation proceeded *via* decomposition of unstable intermediates such as hydroperoxides or peroxide adducts which also took part in the formation of lower aldonic and formic acids. In addition, alternative mechanisms based on the recombination of free radicals should also be considered in the formation of some lower acids.

References

Antal M.J. Jr., Mok W.S.L., Richards G.N. (1990): Four-carbon model compounds for the reactions of sugars in water at high temperature. Carbohydrate Research, **199**: 111–115.

Belitz H.D., Grosch W. (1999): In: Food Chemistry, 2nd Ed. Springer-Verlag, Berlin, Heidelberg.

Brands C.M.J., van Boekel M.A.J.S. (2001): Reaction of monosaccharides during heating of sugar-casein system: Building of reaction network model. Journal of Agricultural and Food Chemistry, **49**: 4667–4675.

Buxton G.V., Malone T.N., Salmon G.A. (1997): Oxidation of glyoxal initiated by •OH in oxygenated aqueous solution. Journal of the Chemical Society, Faraday Transactions, **93**: 2889–2891.

Davídek J., Velíšek J., Pokorný J. (eds) (1990): Chemical Changes during Food Processing. Developments in Food Science Vol. 21. Elsevier Science Publishers, Amsterdam.

Davídek T., Devaud S., Robert F., Blank I. (2006a): Sugar fragmentation in the Maillard reaction cascade: isotope labelling studies on the formation of acetic acid by a hydrolytic β-dicarbonyl cleavage mechanism. Journal of Agricultural and Food Chemistry, **54**: 6667–6676.

Davídek T., Robert F., Devaud S., Vera F.A., Blank I. (2006b): Sugar fragmentation in the Maillard reaction cascade: Formation of short-chain carboxylic acids by a new oxidative α-dicarbonyl cleavage pathway. Journal of Agricultural and Food Chemistry, **54**: 6677–6684.

Drijver L. den, Holzapfel C.W. (1986): Separation and quantitative determination of the radiolysis products of D-fructose as their *O*-benzyloximes. Journal of Chromatography A, **363**: 345–352.

Geigert J., Neidleman S.L., Hirano D.S., Wolf B., Panschar B.M. (1983): Enzymic oxidation of D-*arabino*-hexos-2-ulose (D-glucosone) to D-*arabino*-2-hexulosonic acid ("2-keto-D-gluconic acid"). Carbohydrate Research, **113**: 163–165.

Ginz M., Balzer H.H., Bradbury A.G.W., Maier H.G. (2000): Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. European Food Research & Technology, **211**: 404–410.

Hayami J. (1961): Studies on the chemical decomposition of simple sugars. XII. Mechanism of acetol formation. Bulletin of the Chemical Society of Japan, **34**: 927–932.

Heusinger H. (1988): Action of ultrasound on deoxygenated aqueous solutions of D-glucose. Carbohydrate Research, **181**: 67–75.

Hodge J.E. (1967): Origin of flavor in foods. Non-enzymatic browning reactions. In: Schultz H.W., Day E.A., Libbey L.M. (eds): Chemistry and Physiology of Flavors. AVI Publishing, Westport: 465–491.

Huyghues-Despointes A., Yaylayan V.A. (1996): Retro-aldol and redox reactions of Amadori compounds: Mechanistic studies with variously labeled D-[^{13}C]glucose. Journal of Agricultural and Food Chemistry, **44**: 672–681.

Isbell H.S., Frush H.L. (1973): Reaction of carbohydrates with hydroperoxides. Part II. Oxidation of ketoses with the hydroperoxide anion. Carbohydrate Research, **28**: 295–301.

Isbell H.S., Frush H.L., Martin E.T. (1973): Reaction of carbohydrates with hydroperoxides, Part I. Oxidation of aldoses with sodium peroxide. Carbohydrydrate Research, **26**: 287–295.

Isbell H.S., Salam M.A. (1981): Degradation of calcium D-gluconate-*2-d* with alkaline hydrogen peroxide. Carbohydrate Research, **90**: 123–126.

Kawakishi S., Kito Y., Namiki M. (1975): γ-Radiolysis of D-glucose in aerated, aqueous solution. Carbohydrate Research, **39**: 263–269.

Kim M.O., Baltes W. (1996): On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one in the Maillard reaction. Journal of Agricultural and Food Chemistry, **44**: 282–289.

Marcq O., Barbe J.-M., Trichet A., Guilard R. (2001): Origin and significance of the production of carbon dioxide during the ozonization of ^{13}C-labeled D-glucose at different pH values. Carbohydrate Research, **333**: 233–240.

Martins S.I.F.S., van Boekel M.A.J.S. (2003): Kinetics modeling of Amadori *N*-(1-deoxy-D-fructos-1-yl)glycine degradation pathways. Part I – Reaction mechanism. Carbohydrate Research, **338**: 1651–1663.

Mizuno T., Weiss A.H. (1974): Synthesis and utilization of formose sugars. Advances in Carbohydrate Chemistry and Biochemistry, **29**: 173–227.

Novotný O., Cejpek K., Velíšek J. (2007): Formation of α-hydroxycarbonyl and α-dicarbonyl compounds during degradation of monosaccharides. Czech Journal of Food Sciences, **25**: 119–130.

Phillips G.O., Barber P., Rickards T. (1964): Photo-degradation of carbohydrates. Part III Sensitized photo-oxidation of D-sorbitol. Journal of the Chemical Society: 3443–3450.

Ponder G.R., Richards G.N. (1993): Pyrolysis of inulin, glucose, and fructose. Carbohydrate Research, **244**: 341–359.

Rössner J., Velíšek J., Pudil F., Davídek J. (2001): Strecker degradation products of aspartic and glutamic acids and their amides. Czech Journal of Food Sciences, **19**: 41–45.

Rössner J. (2004): Aromatické látky vznikající v Maillardově reakci. [Disertační práce.] Vysoká škola chemicko-technologická v Praze: 53–54.

Samuelson O., Thede L. (1968): Influence of oxygen upon glucose and cellobiose in strongly alkaline medium. Acta Chemica Scandinavica, **22**: 1913–1923.

Velíšek J. (2002): Chemie potravin, část I. 2. vydání. Ossis, Tábor: 270.

Vuorinen T. (1985): Cleavage of the intermediate hydroperoxides in the oxidation of D-glucose and D-fructose with oxygen. Carbohydrate Research, **141**: 319–322.

Weenen H. (1998): Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. Food Chemistry, **62**: 393–401.

Wnorowski A., Yaylayan V.A. (2000): Influence of pyrolytic and aqueous-phase reactions on the mechanism of formation of Maillard products. Journal of Agricultural and Food Chemistry, **48**: 3549–3554.

Yaylayan V.A., Huyghues-Despointes A. (1996): Identification of per-*O*-(trimethylsilyl) derivates of aldoses generated from thermal decomposition of *N*-(1-deoxy-D-fructopyranos-1-yl)proline: reversibility of the Amadori rearrangement. Carbohydrate Research, **286**: 179–187.

Yang Y.B., Montgomery R. (1996a): Alkaline degradation of glucose: effect of initial concentration of reactants. Carbohydrate Research, **280**: 27–45.

Yang Y.B., Montgomery R. (1996b): Alkaline degradation of fructofuranosides. Carbohydrate Research, **280**: 47–57.

Received for publication January 30, 2007
Accepted after corrections April 2, 2007

Corresponding author:

Prof. Ing. Jan Velíšek, DrSc., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 220 443 177, fax: + 420 220 443 185, e-mail: jan.velisek@vscht.cz