

Formation of α -Hydroxycarbonyl and α -Dicarbonyl Compounds 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

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The formation of α -hydroxycarbonyl and α -dicarbonyl compounds from monosaccharides (glucose, fructose, arabinose, glyceraldehyde, and 1,3-dihydroxyacetone) was studied in three different model systems comprising an aqueous and alkaline solution of potassium peroxodisulfate ($K_2S_2O_8$), and a solution of sodium hydroxide, respectively. In total, six α -hydroxycarbonyl (in the form of *O*-ethyloximes) and six α -dicarbonyl compounds (as quinoxaline derivatives) were identified by GC/MS and quantified. Acetol, glycolaldehyde, 1,3-dihydroxyacetone, methylglyoxal, and glyoxal were the most abundant low molecular weight carbonyls. Within the model systems studied, the yield of α -hydroxycarbonyl and α -dicarbonyl compounds was 0.32–4.90% (n/n) and 0.35–9.81% (n/n), respectively. The yield of α -dicarbonyls was higher than that of α -hydroxycarbonyls only in aqueous solution of $K_2S_2O_8$ and in the other two model systems an inverse ratio of these two carbonyl types was found. For the first time, ethylglyoxal was identified as a sugar degradation product and several mechanisms explaining its formation were proposed. The achieved data indicated that low molecular weight α -hydroxycarbonyl and α -dicarbonyl compounds are predominantly formed by a direct retro-aldol reaction and α - and β -dicarbonyl cleavage. It was evident that some compounds were produced from the sugar fragmentation products. Thus, isomerisation, reduction of dicarbonyls by formaldehyde (cross-Cannizzaro reaction), and mutual disproportionation are possible reaction pathways participating in the formation of α -hydroxycarbonyl compounds. Oxidation and disproportionation of α -hydroxycarbonyl precursors as well as the aldol condensation of low molecular weight carbonyl species (followed by subsequent reactions) play an important role in the formation of several α -dicarbonyl compounds.

Keywords: monosaccharides; α -hydroxycarbonyl compounds; α -dicarbonyl compounds; retro-aldol reaction; peroxodisulfate; acetol; 1,3-dihydroxyacetone; glyceraldehyde; glycolaldehyde; acetoin; 1-hydroxybutan-2-one; lactaldehyde; glyoxal; methylglyoxal; ethylglyoxal; butane-2,3-dione; 1-hydroxybutane-2,3-dione; pentane-2,3-dione

The transformation of reducing sugars represents a complex of reactions, where many sugar-derived carbonyl intermediates (such as aliphatic aldehydes and ketones, α -hydroxycarbonyl and

α -dicarbonyl compounds, oxoacids, furan and pyran derivatives) are involved (ANGYAL 2001). Fragmentation is considered to be one of the essential transformations of sugars in foods. During

the reaction, highly reactive low molecular weight α -hydroxycarbonyl and α -dicarbonyl compounds are formed. These carbonyls undergo different subsequent reactions providing a great number of secondary products; many of them are coloured, flavour- and/or biologically-active compounds.

It is well known that reducing sugars undergo fragmentation predominantly in alkaline media, although it was observed also in weakly acidic solutions to some extent (THEANDER & WESTERLUND 1980). SHAW *et al.* (1968) identified acetol and three hydroxybutanones during a base-catalysed degradation of fructose. In addition, hexane-2,5-dione, furans, alicyclic compounds and carboxylic acids were found indicating some subsequent reactions of primary formed carbonyls. Many model studies showed that fission of sugar chain is significantly enhanced by amines (primary or secondary amines, amino acids and proteins). WEENEN and APELDOORN (1996) investigated formation of four α -dicarbonyl fragments during degradation of glucose, fructose, xylose, 3-deoxy-D-erythrohexos-2-ulose and fructosylalanine either alone or with alanine or cyclohexylamine in phosphate buffer solutions (pH 8, 100°C/1 h). The extent of α -dicarbonyls formation considerably depended on the reaction partners used and increased in the order: without amine < alanine < cyclohexylamine. The formation of α -dicarbonyl fragments (glyoxal, methylglyoxal, butane-2,3-dione) from mono- and disaccharides under caramelisation and Maillard reaction conditions was studied by HOLLNAGEL and KROH (1998). It was found that under the reaction conditions used monosaccharides form higher amount of dicarbonyls than disaccharides and glucose forms more dicarbonyl compounds than fructose. It was suggested that fructose tends to yield cyclic products rather than fragmentation products. Several ^{13}C -labelling studies with selectively enriched sugars and amino acids were performed to verify the origin of reactive C_2 , C_3 , C_4 and C_5 fragments (HUYGHUES-DESPOINTES & YAYLAYAN 1996; YAYLAYAN & KEYHANI 1999, 2000; YAYLAYAN *et al.* 2000; WEENEN 1998). For example, glucose/alanine model studies have indicated that butane-2,3-dione is formed by a single pathway involving only glucose carbon atoms, whereas pentane-2,3-dione is formed by two pathways, one involving glucose C-atoms only (10%) and the other (90%) through participation of C_1 - C_2 alanine atoms and C_3 unit of glucose (YAYLAYAN & KEYHANI 1999). These findings corroborate results

of WEENEN and APELDOORN (1996), which have been speculated that acetaldehyde (the Strecker aldehyde of alanine) is involved in the process of pentane-2,3-dione formation.

Generally, three mechanisms have been suggested to proceed during sugar fragmentation: retro-aldolisation and α - and β -dicarbonyl cleavage (WEENEN *et al.* 1993). Reducing sugars, the corresponding endiols and deoxyuloses, imines derived from amino acids and related Amadori and Heyns rearrangement products are the precursors (WEENEN 1998). Since enolisation is not restricted to any part of the molecule and water elimination is not restricted in amount, the spectrum of primary cleavage products is large. For instance, 1-deoxy-2,3-dioxo and 1-deoxy-2,4-dioxo species undergo retro-aldolisation and β -cleavage to form methylglyoxal and acetol, respectively (WEENEN 1998). Oxidative degradation *via* unstable hydroperoxides was also considered as a possible pathway leading to fission of carbohydrate skeleton (VUORINEN 1985). The cleavage is often followed by many subsequent reactions such as hydration, elimination of water, oxidation, Cannizzaro reaction, aldolisation or disproportionation giving rise to a variety of secondary products. For example, model studies indicated that hydroxyacetone and 1-hydroxybutan-2-one were the precursors of five alicyclic compounds, which were observed in fructose cleavage experiments (SHAW *et al.* 1968). HUYGHUES-DESPOINTES and YAYLAYAN (1996) found 40 compounds after heating of methylglyoxal (100°C/8 min). Propane-1,2-diol (18%), acetic acid (30%), acetol (7%), and 2,4-dimethyl-1,3-dioxolane (7%) were major products. It was found that glyceraldehyde, together with methylglyoxal, are even better precursors of 5-hydroxymethylfuran-2-carbaldehyde than monosaccharides and disaccharides (CÄMMERER & WEDZICHA 1999). The formation of various phenols and other aromatic compounds from sugar fragments was also reported. THEANDER and WESTERLUND (1980) reported five phenols identified in a slightly acidic solution of erythrose.

The presented work is focused on degradation of sugars in three different media: in an aqueous and alkaline solution (0.3M NaOH) of potassium peroxodisulfate ($\text{K}_2\text{S}_2\text{O}_8$) and in 0.05M solution of sodium hydroxide. Peroxodisulfate represents a multifunctional agent being simultaneously an acidulant, oxidising agent, and radical initiator. In aqueous solutions, it decomposes providing

disulphate ($S_2O_7^{2-}$), hydrogen sulphate (HSO_4^-), triplet oxygen, hydrogen peroxide, sulfate radical anion ($SO_4^{\bullet-}$), peroxodisulfate radical anion ($S_2O_8^{\bullet-}$), and hydroxyl radical (HO^{\bullet}). It is known that sulfate radical anion reacts with water producing hydroxyl radical and that the rate of this reaction accelerates with increasing pH (HAYON *et al.* 1972). Hence, the degradation of sugars should be initiated by free radicals more extensively in the alkaline solution of peroxodisulfate. The alkaline medium itself significantly enhances the sugar fragmentation. The objective of this study was to identify and quantify low molecular weight carbonyl fragments arising from sugars in our model systems and to elucidate the mechanisms leading to their formation.

MATERIAL AND METHODS

Chemicals. The following compounds were obtained commercially: D-glucose, D-fructose, glyoxal trimer dihydrate, methylglyoxal (Sigma, St. Louis, USA), D-arabinose, hydroxyacetone, 1,3-dihydroxyacetone dimer, glycolaldehyde dimer, acetoin, butane-2,3-dione, O-ethylhydroxylamine hydrochloride (Fluka, Buchs, Switzerland), DL-glyceraldehyde (Reanal, Budapest, Hungary), pentane-2,3-dione, guaiacol, 1,2-diaminobenzene (Aldrich, Steinheim, Germany), methanol, anhydrous sodium sulfate, sodium hydroxide, hydrochloric acid (Penta, Chrudim, Czech Republic), diethyl ether (Merck, Darmstadt, Germany), potassium peroxodisulfate (Dorapis, Praha, Czech Republic). L-Lactaldehyde was prepared by a slightly modified method (NOVOTNÝ 2004) published by ZAGALAK *et al.* (1966).

Degradation of sugars. Equimolar amounts of a sugar and potassium peroxodisulfate (5 mmol each) were dissolved in 50 ml of water and 0.3M sodium hydroxide, respectively (model 1 and model 2). The sugar (5 mmol) was 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 content of low molecular carbonyls. Three independent determinations were performed for each model system.

Determination of α -hydroxycarbonyl compounds. An aliquot of aqueous stock solution of the internal standard (acetoin or glyceraldehyde depending on their presence/absence in reaction mixtures) and 0.2 g O-ethylhydroxylamine hydro-

chloride were added to 10 ml of cooled reaction mixture. The pH value was adjusted to 7.5 with 1M and 0.1M sodium hydroxide and the mixture was held for 2 h at 40°C. The pH value was then readjusted to 6.0 with 0.1M hydrochloric acid and the mixture was extracted with three 20 ml portions of diethyl ether. Each extract was dried over anhydrous sodium sulfate. The combined extracts were concentrated to approximately 2 ml and analysed by GC/MS. Calibration solutions were prepared in water with addition of particular internal standard, derivatised by the same procedure as described above and analysed by GC/MS. Following characteristic ions were used for quantification of corresponding O-ethyloximes: m/z 131 (acetoin), m/z 103 (glycolaldehyde), m/z 117 (acetol), m/z 74 (glyceraldehyde, lactaldehyde), m/z 104 (1,3-dihydroxyacetone).

Determination of α -dicarbonyl compounds. An aliquot of the stock aqueous solutions of internal standard (guaiacol) was added to 10 ml of cooled reaction mixture. The pH value was adjusted to 6.5 with 1M and 0.1M hydrochloric acid or sodium hydroxide. Then 3 ml of a freshly prepared 1M methanolic solution of 1,2-diaminobenzene was added and the mixture was held for 2 h at 40°C. The pH value was then adjusted to 5.0 with hydrochloric acid (0.1M), the mixture was extracted with diethyl ether in the same way as described above and analysed by GC/MS and GC/FID. Standards of four α -dicarbonyl compounds (glyoxal, methylglyoxal, butane-2,3-dione and pentane-2,3-dione) were prepared in aqueous solution with addition of guaiacol (2-methoxyphenol) as internal standard, derivatised as described previously and analysed by GC/MS. For quantification of ethylglyoxal and 1-hydroxybutane-2,3-dione, the calibration curve of methylglyoxal and butane-2,3-dione was used, respectively.

Determination of parent sugars. Sugars in the reaction mixture were analysed after their conversion to silylated oximes using the method described by NOVOTNÝ (2004).

Gas chromatographic analyses (GC/FID and GC/MS). For GC/FID analyses, an Agilent Technologies 6890N chromatograph equipped with a flame ionisation detector and fused silica capillary columns CP WAX 52 CB for α -hydroxycarbonyl and α -dicarbonyl compounds and HP-5 MS for sugars (both 30 m \times 0.25 mm i.d., film thickness of 0.25 μ m) was used in this study. The GC oven was temperature programmed from 100°C (150°C

with HP-5 MS for sugar analysis) to 250°C (280°C) at a rate of 4°C (5°C), an injector and detector temperatures were held at 220°C (260°C) and 260°C (280°C), respectively. The carrier gas (He) flow rate was 0.7 ml/min. The sample (1 µl) was injected using a split ratio 1:20–1:70. For GC/MS analyses, a Hewlett-Packard G1800A apparatus equipped with the same column and operating under the conditions described above was used. 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 sample (1 µl) was injected using a split ratio 1:20.

RESULTS AND DISCUSSION

The formation of low molecular weight α -hydroxycarbonyl and α -dicarbonyl compounds arising from glucose, fructose, arabinose, glyceraldehyde, and 1,3-dihydroxyacetone was studied in three different model systems. Peroxodisulfate and alkaline solution mainly acts as an initiator and catalyst of sugar degradation, respectively. Furthermore, peroxodisulfate plays a role of oxidant; oxygen formed by its decomposition can be incorporated to sugar transformation products. Thus, the used models enabled monitoring of products arising entirely from sugars that are hardly detectable in more complex systems where they can undergo reactions with other components.

For better understanding of mechanisms of sugar decomposition, the changes of pH value were measured in all model systems. The measurements

were performed before and after boiling under reflux. Formation of a number of acids generating as products of sugar degradation significantly contributed to pH decrease. The changes of pH value in particular model mixtures are shown in Table 1. While the pH value was changed only slightly (by 1.6 units on average) in water/ $K_2S_2O_8$ /sugar systems, a considerable decrease of pH (by 3.1–6.0 units) was observed in alkaline solutions. The concentration of 0.05M NaOH in the third model was sufficient for neutralisation of the formed acids and for keeping pH in the alkaline region; pH in these models decreased by 3.6 to 5.0 units. For comparison, the changes of pH were also measured in solution of peroxodisulfate which did not contain sugar. As expected, a significant decrease of pH value (from 3.1 to 1.3) was observed in aqueous/ $K_2S_2O_8$ blank solution. In 0.3M NaOH/ $K_2S_2O_8$ blank experiment, the decrease of pH was only 0.4 units (from 12.2 to 11.8). The decrease of pH value in aqueous peroxodisulfate solution was caused by its decomposition and hydrolysis producing disulphate ($S_2O_7^{2-}$) and hydrogen sulphate (HSO_4^-), respectively.

Reactivity of parent sugars

Reactivity of the sugars in particular model systems was estimated by determination of unreacted sugar in the reaction mixture. Similar trends in a rate of sugar degradation were observed in all investigated reaction systems (Table 2). As expected, trioses were the most reactive sugars (the reacted

Table 1. Changes of pH in model systems

Model system	Reaction mixture pH (initial/final)				
	glucose	fructose	arabinose	1,3-dihydroxyacetone	glyceraldehyde
$K_2S_2O_8/H_2O$	3.0/1.3	3.1/1.3	2.9/1.3	2.8/1.3	2.9/1.3
$K_2S_2O_8/0.3M NaOH$	12.2/6.2	11.8/6.3	12.0/6.6	11.7/8.5	12.1/8.5
0.05M NaOH	11.6/6.8	11.5/6.5	11.5/6.6	11.5/7.9	11.5/7.4

Table 2. Reactivity of saccharides in model systems

Model system	Reacted amount (% n/n) including/excluding isomerisation reactions				
	glucose	fructose	arabinose	1,3-dihydroxyacetone	glyceraldehyde
$K_2S_2O_8/H_2O$	60.5/60.5	64.1/64.0	69.7/69.7	83.1/83.1	93.1/93.1
$K_2S_2O_8/0.3M NaOH$	80.7/68.3	85.4/71.2	80.3/77.4	99.7/99.7	100.0/100.0
0.05M NaOH	79.5/66.2	88.9/67.1	81.9/75.1	99.7/99.7	100.0/99.7

amount was 83.1 to 100%). The reacted amount of other sugars ranged between 60.5 and 77.4%. Reactivity of glucose and fructose was nearly identical in all systems studied. A somewhat higher reactivity was observed for arabinose in comparison with hexoses. While comparing the reactivity in alkaline reaction mixtures, it is necessary to consider that the decrease of the initial amount of saccharide is significantly caused also by isomerisation. The proportion of isomerisation to total changes is shown in Table 2.

Analysis of α -hydroxycarbonyl and α -dicarbonyl compounds

Using the modified method described by HOFMANN (1999), α -hydroxycarbonyl and α -dicarbonyl compounds were analysed in the form of stable *O*-ethyloxime derivatives (after reaction with *O*-ethylhydroxylamine) and quinoxaline derivatives (after reaction with 1,2-diaminobenzene), respectively. Pure glycolaldehyde, acetol, lactaldehyde, glyceraldehyde, 1,3-dihydroxyacetone, and acetoin were used as standards. Two peaks, which possess

identical MS spectra corresponding to *syn*- and *anti*-isomers, were observed for glycolaldehyde, acetol, and acetoin. Obviously, isomers of the other compounds were not separated under conditions used as only one peak was observed. Glyoxal, methylglyoxal, butane-2,3-dione, and pentane-2,3-dione were used as standards for the determination of α -dicarbonyl compounds. The identification was performed by GC/MS with the use of both retention time data and mass spectra.

α -Hydroxycarbonyl compounds

All α -hydroxycarbonyl compounds (Figure 1) available as standards were detected in the investigated reaction mixtures. Table 3 summarises the quantification of α -hydroxycarbonyl compounds in all reaction mixtures. Importantly, one more α -hydroxycarbonyl compound, 1-hydroxybutan-2-one, was detected in traces in diethyl ether extracts of alkaline reaction mixtures ($K_2S_2O_8/0.3M$ NaOH and $0.05M$ NaOH). The corresponding *O*-ethyloxime was not found under the conditions used.

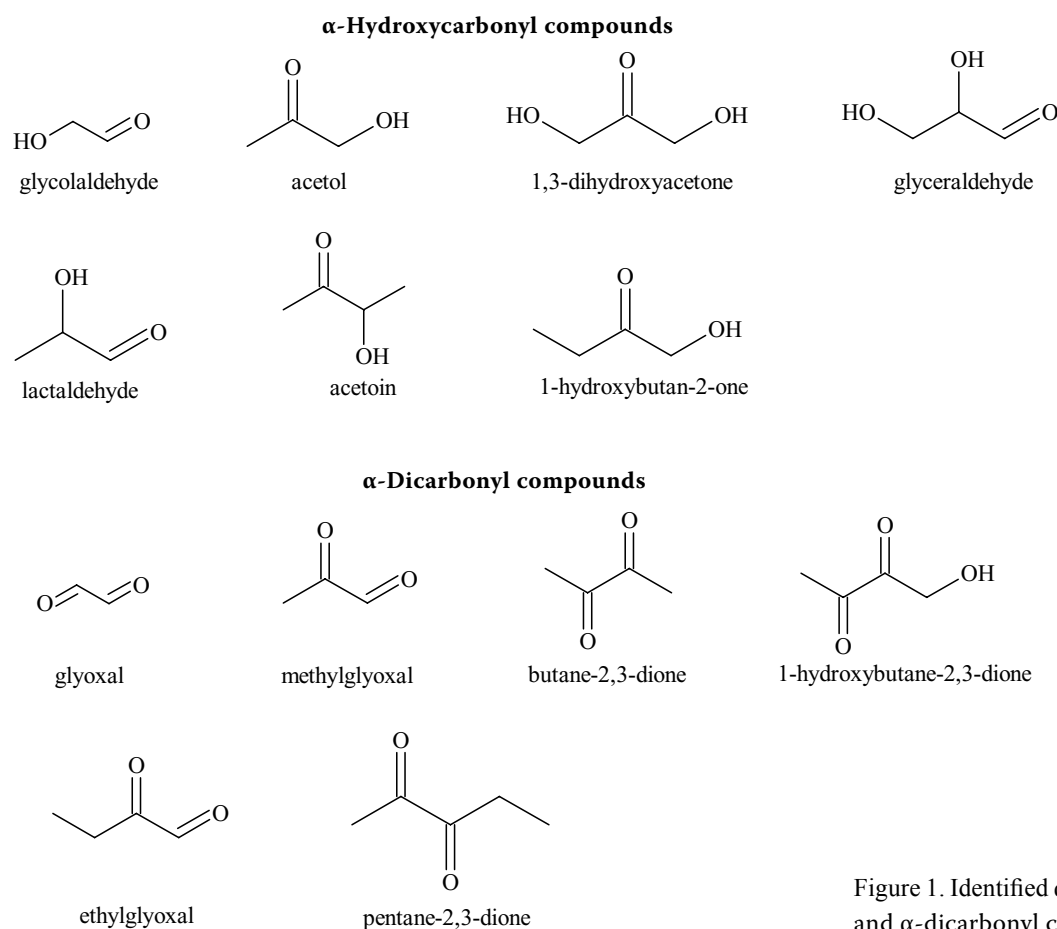


Figure 1. Identified α -hydroxycarbonyl and α -dicarbonyl compounds

Table 3. Yields of α -hydroxycarbonyl compounds in model systems* – in % (n/n)

Model system	Product	Glucose	Fructose	Arabinose	1,3-Dihydroxyacetone	Glyceraldehyde
$K_2S_2O_8/H_2O$	glycolaldehyde	0.21	0.57	0.34	4.70	4.84
	acetol	0.11	0.17	0.07	0.17	0.06
	lactaldehyde	n.d.	n.d.	n.d.	n.d.	n.d.
	glyceraldehyde	n.d.	n.d.	n.d.	n.d.	6.95
	1,3-dihydroxyacetone	n.d.	n.d.	n.d.	16.93	n.d.
	acetoin	n.d.	n.d.	n.d.	n.d.	n.d.
	total**	0.32	0.74	0.41	4.87	4.90
$K_2S_2O_8/0.3M NaOH$	glycolaldehyde	0.16	0.23	0.36	0.10	0.10
	acetol	0.47	0.54	0.49	1.59	0.85
	lactaldehyde	n.d.	n.d.	n.d.	n.d.	n.q.
	glyceraldehyde	0.06	0.25	0.05	0.02	n.d.
	1,3-dihydroxyacetone	0.41	1.52	0.31	0.32	0.06
	acetoin	n.d.	n.d.	n.q.	n.q.	n.q.
	total**	1.10	2.54	1.21	1.71	0.95
0.05M NaOH	glycolaldehyde	0.45	0.54	0.59	0.13	0.28
	acetol	2.52	2.28	2.80	2.60	3.11
	lactaldehyde	n.q.	n.q.	0.01	0.01	0.01
	glyceraldehyde	0.12	0.11	n.d.	n.d.	n.d.
	1,3-dihydroxyacetone	1.02	1.04	0.90	0.31	0.34
	acetoin	n.q.	n.q.	0.03	0.07	0.05
	total**	4.11	3.97	4.33	2.81	3.79

n.d. – not detected

n.q. – not quantified due to interference with acetoin (internal standard)

*entries are averages of three independent determinations; RSD were < 20% within the experimental series

**unreacted parent 1,3-dihydroxyacetone or glyceraldehyde are not included

The potential of the individual sugars to produce α -hydroxycarbonyl compounds depends strongly on reaction medium. Trioses gave the highest yield of α -hydroxycarbonyls (they mainly decomposed to glycolaldehyde) in $K_2S_2O_8/H_2O$. The quantified α -hydroxycarbonyl compounds in hexose and pentose systems may be lined up according to their amount formed under the given reaction conditions as follows: $K_2S_2O_8/H_2O < K_2S_2O_8/0.3M NaOH < 0.05M NaOH$. As expected, the extent of sugar fragmentation is lower in acidic media in comparison with alkaline media. In the system $K_2S_2O_8/H_2O$, the low amount of hydroxycarbonyls is obviously linked with high amount of dicarbo-

nyls (Table 4), which are apparently formed by one electron oxidation of their hydroxycarbonyl precursors (Figure 2). Acetol (hydroxyacetone, 1-hydroxypropan-2-one) was the major α -hydroxycarbonyl compound in reaction mixtures with 0.05M NaOH. The mechanism of its formation from glucose and fructose was proposed by WEENEN and APELDOORN (1996) and WEENEN (1998). It is based on β -cleavage of 1-deoxyhexo-2,4-diulose formed by isomerisation of 1-deoxy-D-erythrohexo-2,3-diulose. A pathway leading to acetol by retro-aldol reaction of 1-deoxyhexo-2,5-diulose (formed by isomerisation of 1-deoxy-D-erythrohexo-2,3-diulose) was described by YAYLAYAN

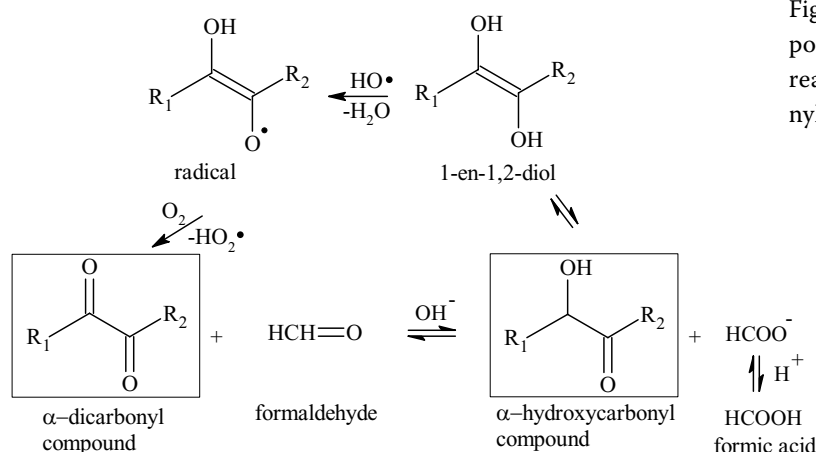


Figure 2. Reduction of α -dicarbonyl compounds by formaldehyde (cross-Cannizzaro reaction) and oxidation of α -hydroxycarbonyl compounds initiated by free radicals

et al. (2000). A significant amount of acetol was found in $\text{K}_2\text{S}_2\text{O}_8/0.3\text{M NaOH}$ reaction mixtures containing trioses. Reduction of methylglyoxal by formaldehyde (cross-Cannizzaro reaction; Figure 2) or disproportionation with α -hydroxycarbonyls may also lead to acetol (HUYGHUES-DESPOINTES & YAYLAYAN 1996).

1,3-dihydroxyacetone (1,3-dihydroxypropan-2-one) was the second most abundant α -hydroxycarbonyl compound in almost all alkaline reaction mixtures studied. A cleavage of 3-deoxy-D-erythro-hexos-2-ulose was suggested for its formation by HOMOKI-FARKAS *et al.* (1997). The other possible pathway is retro-aldolisation of fructose. Decidedly, the isomerisation of glyceraldehyde is a reaction, which takes part in formation of significant amount of 1,3-dihydroxyacetone in alkaline media.

Glyceraldehyde (2,3-dihydroxypropanal) was among the minor α -hydroxycarbonyl compounds detected in our reaction mixtures. This compound has been known as a significant retro-aldolisation product of either glucose or fructose, 1-deoxy-D-erythro-hexo-2,3-diulose (WEENEN 1998) and 3-deoxy-D-erythro-hexo-2-ulose (HOMOKI-FARKAS *et al.* 1997; WEENEN 1998). Glyceraldehyde may be also formed by aldolisation of glycolaldehyde with formaldehyde. Moreover, the isomerisation

of 1,3-dihydroxyacetone yields glyceraldehyde in alkaline media.

Glycolaldehyde (2-hydroxyethanal) was found in surprisingly high amount (nearly 5%, n/n) in triose/ $\text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{O}$ systems; in the other systems it was the minor α -hydroxycarbonyl compound (< 0.6%, n/n). THORNALLEY *et al.* (1999) described its formation from glucose by scission of erythrose and HOLLNAGEL and KROH (1998) proposed retro-aldolisation of 1-en-1,2-diol giving rise to glycolaldehyde endiol. DAVÍDEK *et al.* (2006) described its formation by cleavage of 1-deoxy-D-erythro-hexo-2,4-diulose and acetylformoin. Obviously, glycolaldehyde formation from trioses proceeds by retro-aldol reaction providing formaldehyde as the second product.

Acetoin (3-hydroxybutan-2-one) was quantified only in some reaction mixtures where it occurred in very small amounts. It was assumed (DAVÍDEK *et al.* 1990) that acetoin was formed *via* hydrolysis and cleavage of acetylformoin (1,6-dideoxy-hexo-2,4,5-triulose). Experiments with labelled glucose revealed similar label distribution in acetoin and butane-2,3-dione indicating direct reduction of butane-2,3-dione into acetoin (WNOROWSKI & YAYLAYAN 2000), possibly through disproportionation with α -hydroxycarbonyl compounds (HUYGHUES-DE-

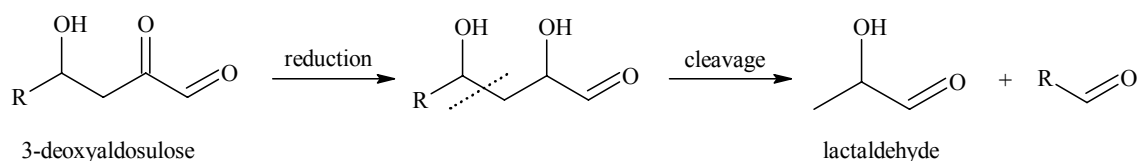


Figure 3. Formation of lactaldehyde by cleavage of reduced 3-deoxyaldosuloses

SPOINTES & YAYLAYAN 1996) or by formaldehyde in cross-Cannizzaro reaction (Figure 2).

1-Hydroxybutan-2-one was exclusively detected in diethyl ether extracts in a free form, at a very low level and only in alkaline reaction mixtures ($K_2S_2O_8/0.3M$ NaOH and $0.05M$ NaOH). Rather than a direct product of cleavage, it seems to be a reduction product of ethylglyoxal, possibly formed analogously to butane-2,3-dione/acetoin and methylglyoxal/acetol. Another possibility may be aldolisation/dehydration reaction between acetol and formaldehyde, which gives rise to 1-hydroxy-but-3-en-2-one, which would be a very good hydride acceptor, and would react swiftly with hydride donors such as glycolaldehyde to form 1-hydroxybutan-2-one (Figure 4).

Lactaldehyde (2-hydroxypropanal) was detected only in reaction mixtures with glyceraldehyde used as an internal standard. It is known that the major amount of lactaldehyde is formed by isomerisation of acetol. A notional precursor yielding by cleavage this aldehyde has to have structure motive of 3-deoxy-2,4-dihydroxy-1-oxo. Reduction of C_2 oxo group of 3-deoxyaldosuloses is assumed during the formation of such a structure (Figure 3).

α -Dicarbonyl compounds

Six α -dicarbonyl compounds (Figure 1) were identified in our reaction mixtures. Table 4 summarises quantification of α -dicarbonyl compounds in all reaction mixtures studied. According to the

Table 4. Yields of α -dicarbonyl compounds in model systems* – in % (n/n)

Model system	Product	Glucose	Fructose	Arabinose	1,3-Dihydroxy-acetone	Glyceraldehyde
$K_2S_2O_8/H_2O$	glyoxal	0.42	0.49	0.45	0.39	0.18
	methylglyoxal	0.31	1.11	0.38	8.88	1.20
	ethylglyoxal**	0.26	0.45	0.28	0.43	0.15
	butane-2,3-dione	0.04	0.05	0.05	0.06	0.04
	pentane-2,3-dione	0.01	0.02	0.01	0.02	0.01
	1-hydroxybutane-2,3-dione**	0.01	0.02	0.15	0.03	0.01
	total	1.05	2.14	1.32	9.81	1.59
$K_2S_2O_8/0.3M$ NaOH	glyoxal	0.08	0.09	0.08	0.04	0.04
	methylglyoxal	0.19	0.24	0.13	0.29	0.18
	ethylglyoxal**	0.01	0.01	0.01	<0.01	0.01
	butane-2,3-dione	0.08	0.08	0.08	0.10	0.11
	pentane-2,3-dione	0.04	0.03	0.05	0.04	0.04
	1-hydroxybutane-2,3-dione**	n.d.	n.d.	n.d.	n.d.	n.d.
	total	0.40	0.45	0.35	0.48	0.38
$0.05M$ NaOH	glyoxal	0.10	0.10	0.13	0.06	0.07
	methylglyoxal	0.63	0.54	0.87	0.89	0.97
	ethylglyoxal**	0.02	0.02	0.02	0.02	0.03
	butane-2,3-dione	0.12	0.12	0.13	0.16	0.15
	pentane-2,3-dione	0.02	0.02	0.02	0.02	0.03
	1-hydroxybutane-2,3-dione**	0.01	0.01	0.01	<0.01	<0.01
	total	0.90	0.81	1.18	1.16	1.26

n.d. – not detected

*entries are averages of three independent determinations; RSD were < 20% within an experimental series

**ethylglyoxal and 1-hydroxybutane-2,3-dione were semi-quantified as methylglyoxal and butane-2,3-dione, respectively

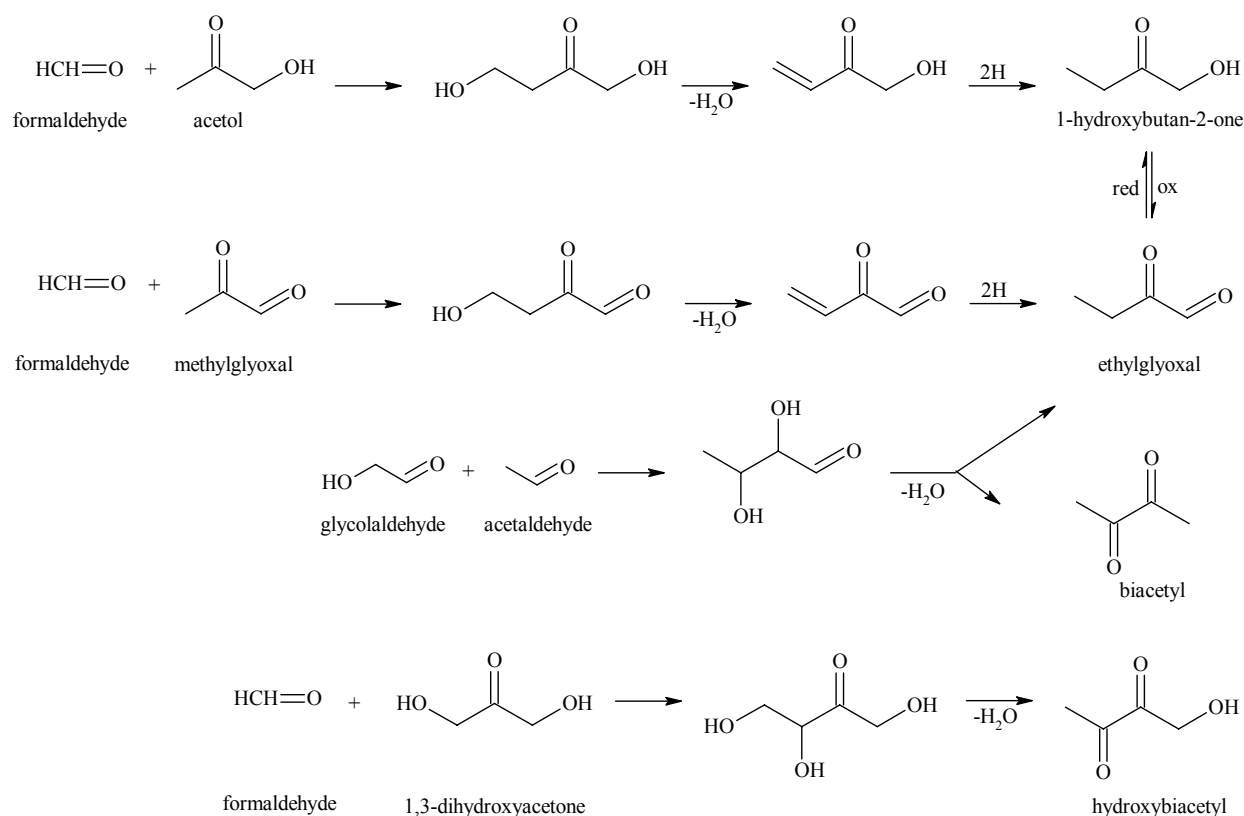


Figure 4. Formation of α -dicarbonyl and α -hydroxycarbonyl compounds from lower intermediates by aldolisation

amount of quantified α -dicarbonyl compounds, the used reaction conditions may be lined up in the following order: $\text{K}_2\text{S}_2\text{O}_8/0.3\text{M NaOH} < 0.05\text{M NaOH} < \text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{O}$. In addition to cleavage of high molecular-weight precursors, α -dicarbonyl compounds are formed also by oxidation of α -hydroxycarbonyls. Consequently, the highest amount of α -dicarbonyl compounds in $\text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{O}$ system is consistent with the lowest amount of α -hydroxycarbonyl compounds in this system. The oxidation may be accomplished by triplet oxygen (THORNALLEY & STERN 1984) and possibly initiated by free radicals which are available from peroxodisulfate decomposition or formed during autoxidation (Figure 2). Generally, the amounts of α -dicarbonyl compounds were 3–5 times lower than those of α -hydroxycarbonyl compounds in alkaline solutions; the inverse ratio was found in $\text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{O}$ systems. Evidently, α -dicarbonyl compounds undergo rapid conversion into α -hydroxy acids *via* intermolecular Cannizzaro reaction under alkaline conditions.

Glyoxal (ethane-1,2-dione) is the simplest α -dicarbonyl compound detected in large amounts in

the $\text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{O}$ model systems. THORNALLEY *et al.* (1999) assumed its direct formation by retro-aldol scission of erythritol from glucose. HOFMANN *et al.* (1999) suggested D-arabino-hexos-2-ulose as the glyoxal precursor considering its formation by the cleavage of C_2 - C_3 bond with simultaneous formation of erythrose. This aldulose formed by oxidation of glucose was also found among reaction products (NOVOTNÝ 2004). YAYLAYAN and KEYHANI (2000) proposed a mechanism based on dehydration of aldohexose (a loss of two water molecules from C_3 - C_4 and C_5 - C_6) and retro-aldol cleavage between C_2 - C_3 . Moreover, WEENEN and APELDOORN (1996) proposed two hypotheses of glyoxal formation by oxidation of glycolaldehyde: base catalysed shift of hydrogen to its acceptor (electrophile) and oxidation by means of an α -dicarbonyl compound analogously to the Strecker degradation, respectively. HOLLNAGEL and KROH (1998) and THORNALLEY *et al.* (1999) also presumed direct oxidation of glycolaldehyde to glyoxal by triplet oxygen.

Methylglyoxal (pyruvaldehyde, propane-1,2-dione) was the major α -dicarbonyl compound almost

in all reaction mixtures. The highest amount of methylglyoxal was detected in 1,3-dihydroxyacetone/ $K_2S_2O_8/H_2O$ reaction mixture (8.9%, n/n). It seems probable that one of the most important pathways of methylglyoxal formation is dehydration of trioses or oxidation of acetol, which is consistent with the largest amount observed in triose reaction mixtures. Formation of methylglyoxal in hexose systems by cleavage of C_3-C_4 bond of 1-deoxy-D-erythro-hexo-2,3-diulose was described by HOLLNAGEL and KROH (1998). Similarly, methylglyoxal can be formed by scission of glyceraldehyde from 2-en-2,3-diols of hexose (THORNALLEY *et al.* 1999). Two other authors suggested 3-deoxy-D-erythro-hexos-2-ulose as the intermediate. While WEENEN (1998) proposed its cleavage into methylglyoxal and glyceraldehyde, HOMOKI-FARKAS *et al.* (1997) assumed its cleavage into 1,3-dihydroxyacetone and glyceraldehyde, which forms methylglyoxal by dehydration. The former proposal was confirmed by YAYLAYAN and KEYHANI (2000) by the use of labelled D- $[^{13}C]$ -glucose. The predicted mechanism includes also dehydration of the formed glyceraldehyde into methylglyoxal.

Ethylglyoxal (2-oxobutanal) was one of the minor products, a somewhat higher amount was found in $K_2S_2O_8/H_2O$ systems. Up to now, there was no available reference concerning the mechanism of its formation from sugars or reporting identification as the sugar degradation product. However, ethylglyoxal was reported as one of the ascorbic acid degradation products in acidic medium (KURATA & SAKURAI 1967) and the mechanism of its formation from L-threo-pentos-2-ulose has been described (DAVÍDEK *et al.* 1990). The presence of its isomer, i.e. D-glycero-pentos-2-ulose, in our reaction systems was demonstrated (NOVOTNÝ 2004). The stepwise mechanism of ethylglyoxal formation from D-glycero-pentos-2-ulose involves two hydrations, three dehydrations and decarboxylation. Two additional mechanisms were proposed for ethylglyoxal formation from shorter intermediates (Figure 4). The first mechanism is based on aldolisation/dehydration reactions of methylglyoxal and formaldehyde. The resulting 2-oxobut-3-enal would be a very good hydride acceptor, and would react swiftly with hydride donors such as glycolaldehyde (WEENEN & APELDOORN 1996) to produce ethylglyoxal. The second mechanism is based on aldolisation of glycolaldehyde and acetaldehyde (followed by elimination of water), which directly yields ethylglyoxal. The presence of both formal-

dehyde and acetaldehyde in the reaction mixture was proved (NOVOTNÝ 2004).

Butane-2,3-dione was other minor reaction product. It was detected in 2 to 3 times higher amounts in 0.05M NaOH mixtures in comparison to models with peroxodisulfate. 1-Deoxy-D-erythro-hexo-2,3-diulose was suggested as the butane-2,3-dione precursor (HOLLNAGEL & KROH 1998; WEENEN & APELDOORN 1996). Certain amount of butane-2,3-dione was formed also from 3-deoxy-D-erythro-hexos-2-ulose. YAYLAYAN and KEYHANI (2000) proposed a mechanism of butane-2,3-dione formation based on dehydration of aldohexose (loss of two water molecules from C_3-C_4 and C_5-C_6) and retro-aldol cleavage between C_2-C_3 . Analogously to the ethylglyoxal formation, butane-2,3-dione may be generated also by aldolisation/dehydration reaction from glycolaldehyde and acetaldehyde (Figure 4; YAYLAYAN *et al.* 2000).

1-Hydroxybutane-2,3-dione was identified only in traces in our model systems. In the $K_2S_2O_8/0.3M$ NaOH model system, 1-hydroxybutane-2,3-dione was not detected at all. YAYLAYAN *et al.* (2000) demonstrated its formation by aldolisation of 1,3-dihydroxyacetone and formaldehyde (Figure 4). 1-Deoxy-D-erythro-hexo-2,4-diulose and acetylformoin were shown to be its precursors (*via* 2-hydroxy-3-oxobutanal) as well (DAVÍDEK *et al.* 2006).

Pentane-2,3-dione (ethylmethylglyoxal) was identified as the minor product in all our reaction mixtures. Its formation is difficult to explain, but it could possibly form as a result of aldolisation between either butane-2,3-dione and formaldehyde or hydroxyacetone and acetaldehyde (WEENEN & APELDOORN 1996). It is expected that the intermediate product, α,β -endione, would be a very good hydride acceptor, and would react with hydride donors such as glycolaldehyde to form pentane-2,3-dione. SHU *et al.* (1985) proposed that the aldol condensation of acetaldehyde and hydroxyacetone also leads to the formation of pentane-2,3-dione.

CONCLUSION

The obtained data confirmed that retro-aldol reactions and α - and β -dicarbonyl cleavages are the major pathways leading to the formation of low molecular weight α -hydroxycarbonyl and α -dicarbonyl compounds from sugars. Nevertheless, it is evident that some carbonyls are produced by subsequent reactions, mostly by a sequence

of reactions from shorter intermediates. Thus, isomerisation, reduction of dicarbonyls by formaldehyde (cross-Cannizzaro reaction), and their mutual disproportionation are possible reactions participating in the formation of α -hydroxycarbonyl compounds. Oxidation and disproportionation of α -hydroxycarbonyl precursors, as well as the aldol condensation of lower carbonyl intermediates followed by subsequent reactions, plays an important role in the formation of some α -dicarbonyl compounds. It is obvious that the determined amounts of low molecular weight carbonyls represent only a small part of degradation products formed under the experimental conditions used. Apparently, large portion of carbonyls swiftly reacted to give rise to a variety of secondary products.

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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: + 420 233 339 990, e-mail: jan.velisek@vscht.cz
