Some formaldehyde reaction products in non-enzymatic browning reactions

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Einige Reaktionsprodukte von Formaldehyd in nicht-enzymatischen Bräunungsreaktionen

Zusammenfassung. Es wurden aus einem Modellsystem Glycin-Glyoxal flüchtige Stoffe isoliert und zwei Verbindungen, abgeleitet vom Formaldehyd (der durch die Streckersche Reaktion der Degradierung von Glycin entsteht) identifiziert. Die Struktur dieser zwei Verbindungen 2,4,6-Trioxaheptan und 2,4,6,8-Tetraoxanonan wurde durch die Analyse der Referenzverbindungen aus der Lösung von Formaldehyd des Handels bestätigt. Die Analyse wurde mit der Methode MGC/MS und ^{1}H-NMR Spektrometrie vorgenommen. Die oben angeführten Verbindungen (Reaktionsprodukte von Oligomeren von Formaldehyd mit Methanol) wurden zum erstenmal als flüchtige Stoffe in den Reaktionen der nicht-enzymatischen Bräunung beschrieben.

Summary. The volatiles isolated from the glycine-glyoxal model system were analysed and two compounds derived from formaldehyde (arising from the Strecker degradation of glycine) were identified. The structure of the two compounds, i.e., 2,4,6-trioxaheptane and 2,4,6,8-tetraoxanonane, was confirmed by the analysis of the reference compounds isolated from the commercial formaldehyde solution and analysed by gas chromatography/mass spectrometry and ^{1}H-NMR spectrometry. The above compounds (the reaction products of formaldehyde oligomers with methanol) are described, for the first time, as minor volatiles in non-enzymatic browning reactions.

Introduction

The breakdown of α-amino acids by carbonyl compounds and some other oxidizing agents, resulting in the evolution of carbon dioxide, aldehydes, ammonia and other compounds, is known as the Strecker degra-

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dation and is one of the most important reactions of amino acids occur during food processing and storage [1].

Formaldehyde, arising from glycine, is a very reactive compound. In aqueous solutions, at a concentration equal to or lower than 1 mol l^{-1}, it occurs mainly in the monomeric state in the form of the hydrate (methylene glycol). Concentrated aqueous solutions of formaldehyde contain not only methylene glycol but also a series of low-molecular-weight polymeric hydrates $H-(O-CH_2)_n-OH$ [2]. Under neutral and acidic conditions, formaldehyde forms open-chain and ring compounds, such as paraformaldehyde and 1,3,5-trioxane. On heating, these compounds spontaneously decompose back to formaldehyde. The reaction of formaldehyde in alkaline aqueous solutions leads to irreversible $-(CH-OH-)_n$ bond formation. This reaction yields a variety of monosaccharides and is called the formose reaction. Simultaneously with the formose reaction (the autocatalytic self-condensation reaction) the Cannizzaro reaction occurs, leading to the formation of methanol and formic acid [3]. In the presence of alcohols, methylene glycol forms unstable hemiacetals and acetals which are stable under neutral and alkaline conditions. Under acidic conditions they decompose to the parent compounds. Thus, methanol and formaldehyde yield dimethoxymethane (formaldehyde dimethylacetal) [4]. In foods, dimethoxymethane has been found as constitutent of wine volatiles [5].

Experimental

Materials

Isolation of volatile compounds from the glyoxal-glycine system

Three litres of an aqueous solution containing 3 mol glyoxal and 6 mol glycine was refluxed for 24 h. After cooling to room tempera-

ture, the pH of the solutions was adjusted to 11 and the solution was extracted with diethyl ether (1 × 500 ml and 3 × 200 ml). The extract was then re-extracted with water (3 × 200 ml), the organic layer was dried over anhydrous sodium sulphate, concentrated to 0.1 ml and analysed by gas chromatography (GC) and mass spectrometry (MS) (GC-MS).

Isolation of reference compounds

An aqueous solution of formaldehyde (30%) was extracted with diethyl ether and the dried, concentrated extract analysed by GC and GC-MS and also used in preparative GC.

Gas-liquid chromatography

GC analysis was performed on a Hewlett-Packard 5880A instrument equipped with a flame-ionization detector and a Supelcowax 10 fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 μm). Splitless injection was performed with the injector heated at 200 °C. The temperature program was 50°–220 °C at 2 °C min^{-1} with an initial hold of 10 min. The nitrogen carrier-gas flow rate was 1 ml min^{-1}. – A Chrom 5 gas chromatography (Laboratorní přístroje, Prague) equipped with a flame-ionization detector and a 2700 × 7 mm glass column packed with 15% Carbowax 20M on Chromaton N-AW (0.125–0.16 mm) and operated under the above conditions (nitrogen flow rate was 60 ml min^{-1}) was used for preparative purposes.

Gas chromatography-mass spectrometry

For the GC-MS measurements, a Shimadzu QP-1000 quadrupole mass spectrometer was used under the following conditions: a Supelcowax 10 fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 μm) with an injector temperature of 250 °C was temperature programmed from 50° to 270 °C at 10 °C/min with 5 min initial hold, separator temperature was 270 °C, ion source temperature 275 °C, electron energy 70 eV, helium carrier-gas flow rate was 1 ml min^{-1}, the injection port was a grob type with split ratio 1:100.

^1H-NMR spectrometry

^{1}H-NMR spectra were measured on a Bruker AM400 (400 MHz), CDCl$_3$ was used as a solvent and tetramethylsilane was used as the standard.

Results and discussion

GC analysis of the concentrated diethyl ether extract obtained from the glycine-glyoxal model system revealed the presence of a number of volatile reaction products (Fig. 1). Mass spectra of the individual compounds showed that the majority of products were N-containing heterocyclic compounds (mainly pyrazines and pyrroles and furans) [6]. Two of the volatiles, compounds A and B, having retention times of 12.9 and 30.1 min, respectively, were aliphatic compounds, the mass spectra of which were similar to the spectra of formaldehyde acetals (Table 1).

Aqueous formaldehyde solutions are often stabilized by the addition of methanol the residue of which (including the raw material for formaldehyde synthesis) is always present in technical-grade formaldehyde solutions. Therefore, dimethoxymethane and possibly
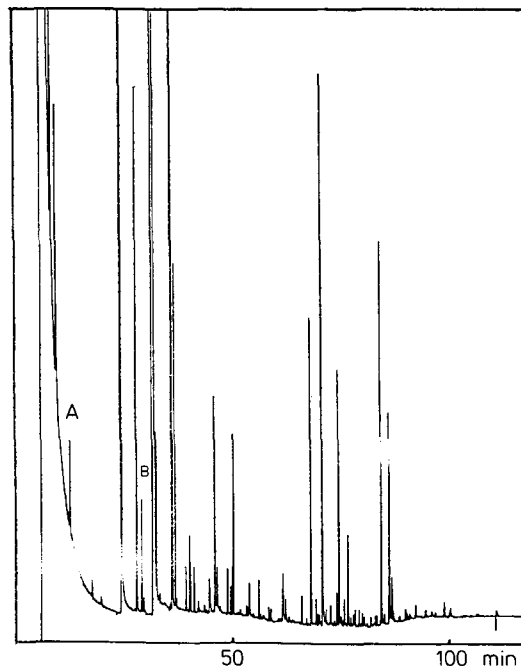

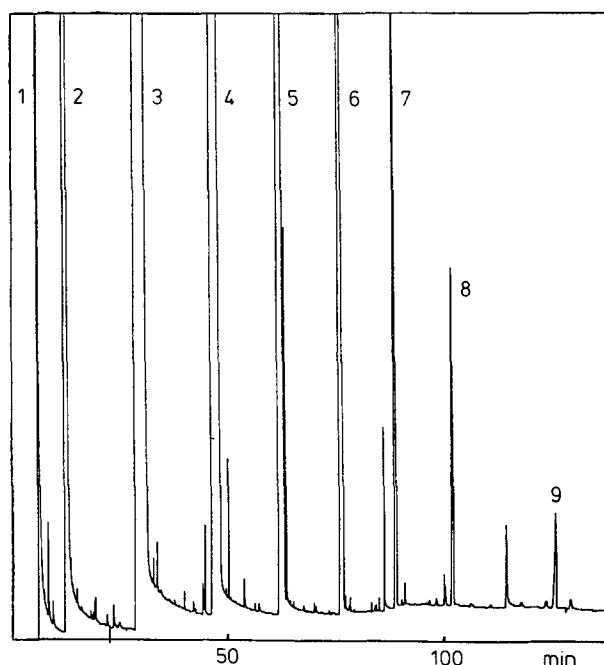**Fig. 1.** Volatile reaction products of glycine-glyoxal

Fig. 2. Polyoxa-alkanes in the formaldehyde solution

Table 1. Mass spectra of dimethoxymethane and compounds A and B (see Fig. 1)

Compound	Mass spectral data, m/e (%)
Dimethoxy- methane [7]	45 (100), 75 (40), 31 (10)
Compound A	45 (100), 75 (60), 31 (13), 105 (3), 61 (3)
Compound B	45 (100), 75 (60), 31 (15), 61 (10), 105 (8), 135 (1)

Table 2. Mass spectra and ^{1}H-NMR spectra of the analysed compounds

| Compound no.	Structure	Mass spectral, m/e (%) and ^{1}H-NMR spectral data (ppm)
1	$CH_3-O-CH_2-O-CH_3$	MS: 45 (100), 75 (40), 31 (10)
2	$CH_3-O(-CH_2-O)_2-CH_3$	MS: 45 (100), 75 (60), 31 (13), 105 (3), 61 (3) NMR: s (CH$_3$):3.39, s (CH$_2$):4.74
3	$CH_3-O(-CH_2-O)_3-CH_3$	MS: 45 (100), 75 (60), 31 (15), 61 (10), 105 (8), 121 (1.4), 135 (1), 91 (0.7) NMR: s (CH$_3$):3.39, s (CH$_2$):4.74, s (CH$_2$):4.87
4	$CH_3-O(-CH_2-O)_4-CH_3$	MS: 45 (100), 75 (24), 31 (7), 105 (6), 91 (2), 61 (1), 135 (1) NMR: s (CH$_3$):3.39, s (CH$_2$):4.74, s (CH$_2$):4.89
5	$CH_3-O(-CH_2-O)_5-CH_3$	MS: 45 (100), 75 (45), 31 (25), 105 (17), 61 (10), 91 (7), 135 (5), 121 (1), 151 (0.3) NMR: s (CH$_3$):3.39, s (CH$_2$):4.74, s (CH$_2$):4.91
6	$CH_3-O(-CH_2-O)_6-CH_3$	MS: 45 (100), 75 (42), 31 (31), 105 (20), 61 (9), 135 (8), 91 (7), 121 (2), 165 (0.5)
7	$CH_3-O(-CH_2-O)_7-CH_3$	MS: 45 (100), 75 (23), 31 (23), 105 (12), 135 (6), 61 (6), 121 (2), 91 (1), 165 (1)
8	$CH_3-O(-CH_2-O)_8-CH_3$	MS: 45 (100), 75 (24), 31 (40), 105 (14), 61 (8), 91 (3), 121 (3), 135 (3), 165 (2)
9	$CH_3-O(-CH_2-O)_9-CH_3$	MS: 45 (100), 75 (27), 105 (17), 135 (11), 61 (12), 91 (4), 61 (3), 165 (4)
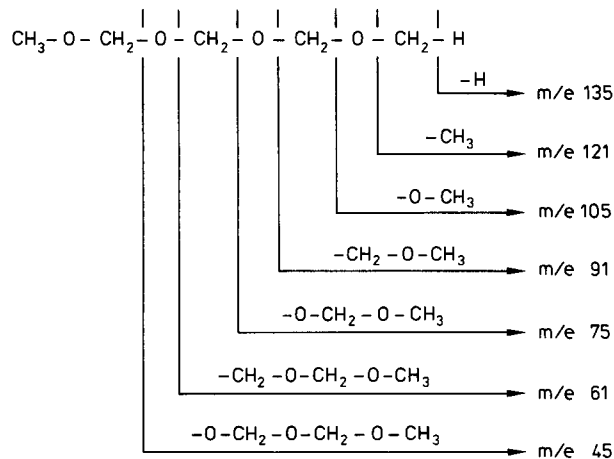
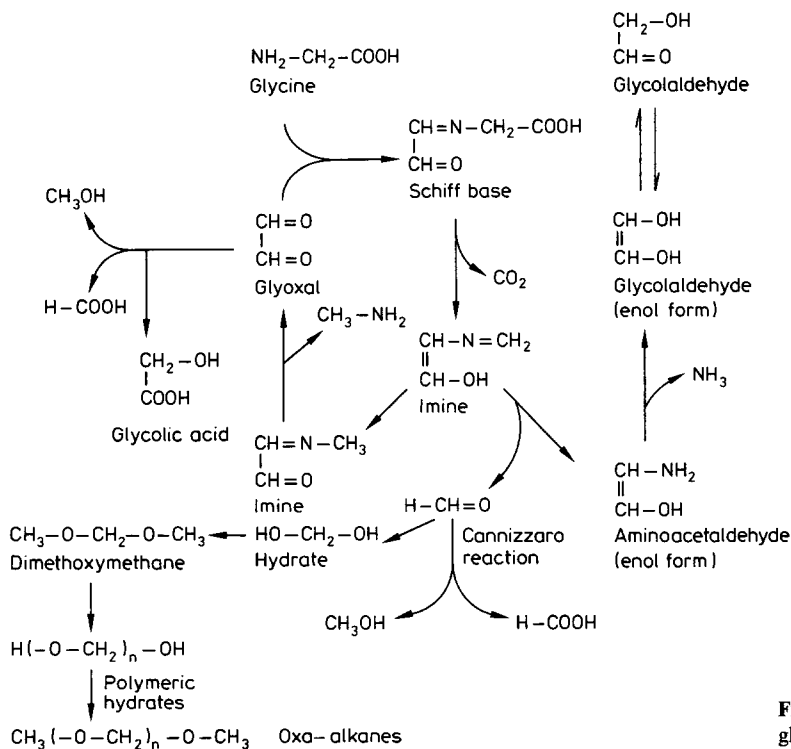**Fig. 3.** Fragmentation scheme of 2,4,6,8-tetraoxanonane

Fig. 4. Strecker degradation of glycine in the presence of glyoxal and the reactions of formaldehyde
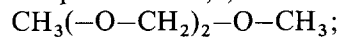
compounds A and B could be expected to occur in aqueous formaldehyde solutions as minor components.

The formaldehyde solution (30%) was therefore extracted with diethyl ether and the concentrated extract analysed by GC and GC-MS (Fig. 2). Com-
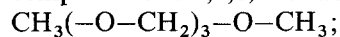
pounds 2 and 3 had retention times identical with those of compounds A and B from the glycine-glyoxal model system. Retention indices were 1010 and 1300, respectively. Retention indices of the other compounds, i.e. compounds 4–9 differed by about 290 units. Mass spectra of the individual components are

summarized in Table 2. It can be seen that compound 1 is dimethoxymethane eluted together with diethyl ether and the mass spectra of compounds 2 and 3 are identical with those of compounds A and B, respectively.
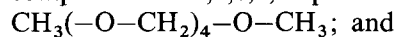
The obtained mass spectra, however, enabled only the tentative identification of the compounds. Therefore, the main components (nos. 2–5) were isolated by preparative GC and their ^{1}H-NMR spectra were measured (Table 2). The obtained 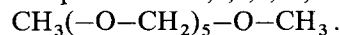spectral data showed that compound 2 is 2,4,6-trioxaheptane, $CH_3(-O-CH_2)_2-O-CH_3$; compound 3 is 2,4,6,8-tetraoxanonane, $CH_3(-O-CH_2)_3-O-CH_3$; compound 4 is 2,4,6,8,10-pentaoxaundecane, $CH_3(-O-CH_2)_4-O-CH_3$; and compound 5 is 2,4,6,8,10,12-hexaoxatridecane, $CH_3(-O-CH_2)_5-O-CH_3$.

The formaldehyde solution, therefore, contained a series of homologues of the general formula $CH_3(-O-CH_2)_n-O-CH_3$, where $n \in \langle 1,9 \rangle$; the main component was compound 3.

2,4,6-Trioxaheptane and 2,4,6,8-tetraoxanonane were found in the glycine-glyoxal model system for the first time. They were present in concentrations amounting to 9 and 12 μg, respectively (i.e., 14 nmol of these compounds arise per mole of glycine). It is possible that dimethoxymethane and the higher homologues of the identified compounds may also arise in the described model system. Their occurrence, however, was not proved due to the presence of many interfering volatile compounds.

It is obvious that in the above non-enyzmatic browning system, methanol formed by the Cannizzaro reaction of formaldehyde reacts either directly with formaldehyde hydrate or with formaldehyde oligomers (low-molecular-weight polyoxmethylenes, $n = 2$–9) forming linear oxa-alkanes. Formaldehyde arises mainly as a product of the Strecker degradation of glycine. According to Grimmet and Richards [8], low quantities of formaldehyde may be formed directly from glyoxal via the splitting of the molecule and disproportionation of the fragments. The reactions scheme is given in Fig. 4.

References

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