Z Lebensm Unters Forsch (1992) 194:222–228

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Original paper

Detection of 5-hydroxymethyl-2-methyl-3(2H)-furanone and of α-dicarbonyl compounds in reaction mixtures of hexoses and pentoses with different amines

Wolfgang Nedvidek [1], Franz Ledl [1], and Peter Fischer [2]

[1] Institut für Lebensmittelchemie und Analytische Chemie und [2] Institut für Organische Chemie der Universität Stuttgart, Pfaffenwaldring 55, W-7000 Stuttgart 80, Federal Republic of Germany

Received September 5, 1991

Nachweis von 5-Hydroxymethyl-2-methyl-3(2H)-furanon und von α-Dicarbonylverbindungen in Reaktionsgemischen von Hexosen und Pentosen mit verschiedenen Aminen

Zusammenfassung. In Reaktionsgemischen von Hexosen mit primären Aminen, die bei pH 5 und pH 7 erhitzt wurden, findet man im höheren pH-Bereich wesentlich größere Mengen an Fragmentierungsprodukten. Zur Abschätzung des Fragmentierungsanteils werden alle α-Dicarbonylverbindungen herangezogen, die mit *o*-Phenylendiamin im Umsetzungsgemisch zu Chinoxalinen reagieren. Erstmals werden die α-Dicarbonyl-Zwischenstufen in Pentose-Amin-Reaktionsansätzen untersucht und die Bildung des 2,3,4-Pentantrions nachgewiesen. Interessant ist, daß bei Hexosen und Pentosen das 1,4-Dideoxyoson in wesentlich größeren Mengen entsteht, wenn α-Aminosäuren an der Umsetzung beteiligt sind, d.h. wenn ein Strecker-Abbau stattfinden kann. 5-Hydroxymethyl-2-methyl-3(2H)-furanon wird als neues Hexoseprodukt des Strecker-Abbaus identifiziert. Die Absicherung der Struktur erfolgt durch Synthese und NMR-Untersuchungen.

Summary. In reaction mixtures of hexoses with primary amines, heated at pH 5 and 7, distinctly higher amounts of fragmentation products are formed in the upper pH range. To estimate the amount of fragmentation, all α-dicarbonyl compounds which react with *o*-phenylenediamine to quinoxaline derivatives were recorded. For the first time the α-dicarbonyl intermediates in a pentose amine reaction mixture were determined and the formation of 2,3,4-pentantrione was detected. The 1,4-dideoxyosone was formed from hexoses and pentoses in higher yields in the presence of α-amino acids, i.e. when Strecker degradation could take place. 5-Hydroxymethyl-2-methyl-3(2*H*)-furanone was identified as a new hexose product of Strecker degradation, and its structure established unequivocally by independent synthesis as well as by ^{1}H and ^{13}C NMR.

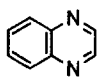
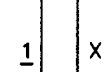
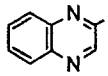
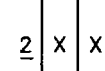
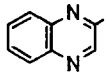
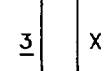
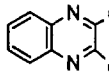
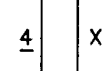
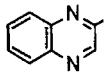
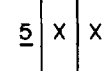
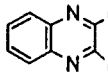
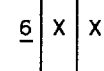
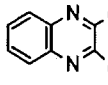
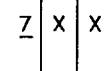
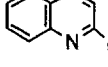
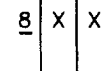
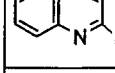
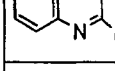
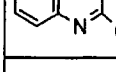
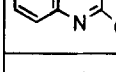
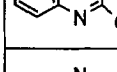
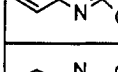
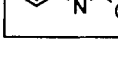
Offprint requests to: W. Nedvidek

Introduction

If aqueous solutions of reducing sugars and amines are heated or even stored at ambient temperatures, a multitude of products is formed (Maillard reaction). The Maillard reaction is important in food chemistry and can also be observed in biological systems, e.g. in human metabolism [1, 20]. Significant aspects of the Maillard reaction in food are the formation of flavor and color and, in vivo, its contribution to complications during aging especially in diabetics. The compounds formed at an early stage of the Maillard reaction, as well as the respective reactions, have been studied extensively during several decades. In the last few years, volatile and less volatile Maillard products have been detected by GC/MS and HPLC, respectively. Up to now, however, only a small fraction of such Maillard products has been isolated and identified. In particular, the structures of colored and high-molecular-mass substances (melanoidines) have remained quite unknown. It is reasonable to suppose that highly reactive intermediates of the Maillard reaction are involved in the formation of these compounds.

Thus, we have recently trapped reactive α-dicarbonyl compounds in Maillard reaction mixtures from mono- and disaccharides with *o*-phenylenediamine as stable quinoxaline derivatives [2, 21]. We now report further experiments with sugars and amines in the presence of *o*-phenylenediamine. We have studied the α-dicarbonyl pattern of hexose degradation at different pH values and identified the α-dicarbonyl compounds formed from pentoses. Furthermore, remarkably higher amounts of 1,4-dideoxyosones were found when the sugars had been heated with α-amino acids. Finally, we describe the detection of a new Strecker degradation product, its independent synthesis, and the elucidation of its structure by NMR.

Table 1. Quinoxalines: (A) detected in heated alkaline solutions of reducing sugars, (B) identified in glucose/β-alanine reaction mixtures at different pH values; (C) found in xylose/α-amino acid reaction mixtures, (D) corresponding α-dicarbonyl compounds

	A	B	C	D
 1		X	X	16 
 2	X	X	X	17 
 3		X	X	18 
 4		X	X	19 
 5	X	X	X	20 
 6	X	X	X	21 
 7	X	X	X	22 
 8	X	X	X	23 
 9			X	24 
 10			X	25 
 11			X	26 
 12			X	27 
 13		X		28 
 14	X	X		29 
 15		X		30 

Experimental procedures

General methods. Melting points were determined on a Electrothermal apparatus and are uncorrected. Infrared spectra (KBr discs and solutions in methylene chloride) were measured with a Pye Unicam SP 1100 instrument, Ultraviolet spectra with a Pye Unicam SP 8-100 apparatus. NMR spectra (external standard, substances usually dissolved in $CDCl_3$) were recorded with Bruker 250 MHz and 300 MHz instruments. GC(1) was performed with a Carlo-Erba 5160 Mega series equipped with a flame-ionisation detector and a capillary column (30 m, ID 0.32 mm, Fth 0.25 µm, DB-1701, hydrogen, 40 kPa, 40 cm·s^{-1}, injection and detection ports were at 270° C and the temperature programme was 100° C→200° C at 3° C/min and 200° C→270° C at 15° C/min and 30 min isothermal at 270° C). GC(2) was performed with a Perkin-Elmer 8600 instrument equipped with a flame-ionisation detector and a capillary column (25 m, ID 0.32 mm, Fth 1 µm, PVMS 54, helium, 100 kPa, 36 cm·s^{-1}, the injection and detection ports were at 270° C and the temperature program was 100° C→200° C at 5° C/min and 200° C→270° C at 15° C/min and 15 min isothermal at 270° C). GC-MS was performed with a Perkin-Elmer 8420 (quartz capillary column: 25 m, ID 0.25 mm, Fth 0.3 µm, PVMS 54, helium, 80 kPa, 27 cm·s^{-1}, injection port at 270° C, the temperature program was as described under GC(2)) connected to a Finnigan MAT Iontrap 800, EI and CI (positive methane) mode. Silica gel 60 F_{254} (Merck, 5554 and 5744) was used for TLC and silica gel (Baker, 0253) for column chromatography. Solvents were distilled and generally removed under diminished pressure.

Acetylation (general method). Up to 500 mg sample was dissolved or suspended in 5 ml chloroform; 3 ml acetic anhydride and 200 mg anhydrous sodium acetate were added. The mixture was heated under reflux for 2 h, poured into 30 ml water, stirred for 2 h, extracted with methylene chloride, and 1 µl of the organic layer injected into the GC.

Silylation (general method). Up to 10 mg of the sample was dissolved or suspended in 200 µl pyridine, and 200 µl *N,O*-Bis (trimethylsilyl) acetamide was added. After 1 h at room temperature, the silylation was completed; 1 µl of the solution was injected into the GC.

Materials. (Al = Aldrich, Fl = Fluka). Glucose (Fl 49139); xylose (Fl 95729); phenylalanine (Fl 78019); sodium tetrahydridoborate (Fl 71320); sodium hydride dispersion (Fl 71620); ethyl-L-lactate (Fl 69799); palladium on activated charcoal (10% Pd, Fl 75990); hydroxyacetone (Fl 54142); 3,4-dihydro-2*H*-pyrane (Fl 37350); pyridinium-(toluene-4-sulfonate) polymer bound (Fl 82817); propylamine (Fl 82100); *o*-phenylenediamine (Fl 78410); *N,O*-bis-(trimethylsilyl)-acetamide (Fl 15241); benzyl bromide (Fl 13250); methyl magnesium bromide (3 mol in diethyl ether, Al 18,989-8) for use, diluted to 0.2 mol with dry tetrahydrofuran); quinoxaline 1: (Al Q160-3); 2-methylquinoxaline 2: (Al M8,020-2); 2,3-dimethylquinoxaline 4: (Al D18,497-7); 2-hydroxymethyl-3-methylquinoxaline 6: reduction of 31 with sodium tetrahydridoborate (largely according to [3]); 2-(1′,2′,3′-Trihydroxypropyl)-3-methylquinoxaline 13 was reference material from J. Beck (University of Munich); 2-(2′,3′-dihydroxypropyl)-3-methylquinoxaline 15 was reference material from B. Huber (University of Munich); hydrolyzed wheat protein (about 4 M) was used as a source of α-amino acids.

2-(2′,3′-Dihydroxypropyl) quinoxaline 8. A solution of 100 mg (0.75 mmol) 3-deoxypentulose 23 (synthesized largely according to [4]) and 100 mg (0.92 mmol) *o*-phenylenediamine in 3 ml water was kept under nitrogen at room temperature for 24 h, then extracted with methylene chloride. The residue of the organic layer was separated on TLC (2 mm) with a mixture of acetonitrile/water (19+1). From a band with an R_f-value between 0.4 and 0.5, compound 8 was eluted with methanol. 8: crystals, 50 mg, 33%, mp. 96.5–99.1° C, GC(1) (acet.) t_R = 38.7 min. ^{13}C NMR [ppm]: 154.9, 146.3, 141.4, 141.3, 130.4, 129.6, 129.3, 128.6, 71.0, 66.2 and 38.2 ppm. In

order to obtain ^{1}H-NMR spectral data with greater validity, 20 mg 8 was acetylated. – 8 (acetylated): ^{1}H NMR: δ/ppm = 1.98 (s, 3H), 2.06 (s, 3H), 3.33 (q, 2H), 4.19 (q, 1H), 4.42 (q, 1H), 5.60 (m, 1H), 7.72 (m, 2H), 8.04 (m, 2H), 8.74 (s, 1H).

2-Formyl-3-methylquinoxaline 31. 430 mg (1.8 mmol) 13 was dissolved in 25 ml ethanol. A solution of 650 mg $NaIO_4$ (3.0 mmol) and 900 mg (11.9 mmol) $NaHCO_3$ in 40 ml water was added. After stirring for 1 h, the ethanol was removed and the reaction mixture was extracted with methylene chloride. The residue of the organic layer was compound 31 [needles, mp. 145.8–146.2° C, 275 mg, 74.5%, GC(1) t_R = 16.6 min]. Infrared: 1715, 1555, 1490, 1380, 1180, 910, 820, 770, 760 cm^{-1}. MS(CI) *m/z:* 173 (M^+ + 1, 100%), 144 (4).

2-(1'-Hydroxyethyl)-3-methylquinoxaline 32. To a cooled solution (−78° C) of 100 mg (0.58 mmol) 31 in 5 ml dry tetrahydrofuran, 4 ml methyl magnesium bromide was added slowly. The mixture was stirred for 30 min and then allowed to reach room temperature. The reaction mixture was poured into a solution of 3 g NH_4Cl in 10 ml water and stirred until two phases were visible. The water phase was extracted with methylene chloride. The residue of the organic layer was separated on a silica gel column with hexane/ethyl acetate (8 + 2). The fractions with fluorescence-absorbing spots of R_f = 0.4 (TLC control with hexane/ethyl acetate, 5 + 5) were collected and after removal of the solvent compound 32 remained as an oil [60 mg, 55%, GC(1) (silyl) t_R = 23.5 min]. MS(CI) (silyl.) *m/z:* 262 (M^+ + 2, 41%), 261 (M^+ + 1, 100), 246 (26), 245 (20), 216 (4).

2-Acetyl-3-methylquinoxaline 11. 60 mg 32 (0.32 mmol) was dissolved in 3 ml methylene chloride and to this solution was added 0.5 ml of an oxidation mixture (to a solution of 10 g $Na_2Cr_2O_7$ in 30 ml water, 7.4 ml conc. H_2SO_4 was added, the mixture was then diluted with water to 50 ml). The two phases were shaken for 30 min. After separation, the aqueous phase was extracted with methylene chloride, the organic phase washed with water [5]. The residue of the collected organic layer was separated on a silica gel column with hexane/ethyl acetate 8 + 2. The fractions with fluorescence-absorbing spots of R_f = 0.4 (TLC control) were collected and after evaporation compound 11 remained as crystals [mp. 87° C, 5 mg, 8.5%, GC(1) t_R = 19.3 min]. ^{1}H NMR: δ/ppm = 2.79 (s, 3H), 2.91 (s, 3H), 7.7 (m, 2H), 8.05 (m, 2H). The NMR spectral data were identical to those described in the literature [6].

2-(2',3',4'-Trihydroxybutyl)-quinoxaline 14. As in the preparation of 8, a mixture of 140 mg (0.86 mmol) 3-deoxyhexulose 29 [4] and 115 mg (1.06 mmol) *o*-phenylenediamine were dissolved in 3 ml water. The mixture was kept at room temperature under nitrogen for 24 h, then extracted with methylene chloride. The residue of the organic layer was separated on TLC (2 mm) with a mixture of acetonitrile/water (19 + 1); from the band with an R_f-value between 0.3 and 0.4 compound 14 was eluted with methanol. 14: crystals, mp 120° C with decomposition, 80 mg, 40%, GC(1) (acet.) t_R = 42.8 min. ^{1}H-NMR δ/ppm = 3.14 (q, 1H, ^2J = 13.9 Hz, ^3J = 9.5 Hz), 3.43 (q, 1H, ^2J = 13.9 Hz, ^3J = 2.9 Hz), 3.61 (m, 1H, ^3J = 3.7, 5.9, 6.6 Hz), 3.65 (q, 1H, ^2J = 11.0 Hz, ^3J = 5.9 Hz), 3.80 (q, 1H, ^2J = 11.0 Hz, ^3J = 3.7 Hz), 4.09 (m, 1H, ^3J = 2.9, 6.6, 9.5 Hz), 7.80 (m, 2H), 8.05 (m, 2H), 8.85 (s, 1H).

Isolation of quinoxalines (7, 8, 9, 10, 11) from a xylose/α-amino acid reaction mixture. 24 g (148 mmol) xylose, 3 g (28 mmol) anhydrous Na_2CO_3, 8 g (74 mmol) *o*-phenylenediamine and 400 ml hydrolyzed wheat protein were heated for 12 h at pH 6.5. After extraction with methylene chloride, the residue of the organic layer was divided into two parts.

One part was acetylated and fractionated on a silica gel column with hexane/ethyl acetate (7 + 3) and increasing amount of ethyl acetate up to hexane/ethyl acetate (3 + 7). Three fractions were collected (TLC control with hexane/ethyl acetate, 8 + 2) containing, amongst others, the following substances in significant amounts: (I) R_f = 0.4–0.55 (10, 11); (II) R_f = 0.15 (7 acet.); (III) R_f = 0.1 (8 acet.).

The residue of the fraction (I) was separated on TLC (2 mm) with hexane/ethyl acetate (8 + 2). Two bands were extracted with methanol, containing substance 10 and 11; respectively (a) R_f = 0.4 (11), the t_R and the mass spectrum were identical to those obtained from synthesized 11 (b) R_f = 0.3 (10), syrupy oil, 5 mg, GC(1) t_R = 17.2 min. ^{1}H NMR: δ/ppm 2.74 (s, 3H), 5.67 (q, 1H, ^2J = 1.9 Hz, ^3J = 10.7 Hz), 6.54 (q, 1H, ^2J = 1.9 Hz, ^3J = 16.9 Hz), 7.09 (q, 1H, ^3J = 10.7, 16.9 Hz), 7.60 (m, 2H), 7.93 (m, 2H).

After removal of the solvent, fraction (II) contained acetylated 7, syrupy oil, 450 mg, GC(1) t_R = 37.3 min. ^{1}H-NMR δ/ppm = 1.99 (s, 3H), 2.08 (s, 3H), 2.79 (s, 3H), 4.55 (q, 1H, ^2J = 11.9 Hz, ^3J = 7.4 Hz), 4.61 (q, 1H, ^2J = 11.9 Hz, ^3J = 4.9 Hz), 6.29 (q, 1H, ^3J = 4.9, 7.4 Hz), 7.65 (m, 2H), 7.95 (m, 2H). ^{13}C NMR: 170.7, 170.3, 152.6, 150.1, 141.8, 140.7, 130.4, 129.3, 129.2, 128.5, 70.4, 64.3, 22.3, 20.9, 20.8 ppm.

The second part of the residue mentioned above (not acetylated) was used to isolate 9. The residue was fractionated on a silica gel column with ethyl acetate. Two fractions were collected (TLC control) containing the following substances: (I) R_f = 0.3 (7) and (II) R_f = 0.25–0.3 (7, 9). The residue of fraction (I) was compound 7, [needles, 150 mg, mp 130.1–130.4° C]. Infrared: 3350, 1495, 1090, 1030, 1010, 920, 885, 860, 765, 680 cm^{-1}. (For ^{1}H and ^{13}C NMR data see acetylated 7). To separate 9 from 7 in fraction (II) a periodate cleavage was performed (described under synthesis of 31). 7 was cleaved to 31, 9 was stable under the conditions. The separation of 9 and 31 was performed on a silica gel column ethyl acetate (TLC control). Compound 31 had an R_f value of 0.8, while that of 9 was 0.3. The corresponding fraction which contained 9 was collected and after evaporation compound 9 remained as a crystalline substance [200 mg, mp. 76.5–78.2° C, GC(1) (acet.) t_R = 32.5 min]. ^{1}H NMR δ/ppm = 2.65 (s, 3H), 3.11 (t, 2H, ^3J = 5.3 Hz), 4.17 (t, 2H, ^3J = 5.3 Hz), 7.61 (m, 2H), 7.91 (m, 2H). Infrared: 3410, 2970, 1495, 1405, 1380, 1055, 1040, 770 cm^{-1}.

Preparation of sugar/amine reaction mixtures in the presence of o-phenylenediamine. The following mixtures were heated in 2 ml phosphate buffer (pH 7, 1.13 mol) for 12 h, extracted with methylene chloride and the residues were acetylated. Mixture 1: 60 mg (0.4 mmol) xylose, 20 mg $Na_2CO_3 \cdot 10\ H_2O$ (0.07 mmol), 20 mg (0.18 mmol) *o*-phenylenediamine and 350 mg (4 mmol) β-alanine; mixture 2: 60 mg (0.4 mmol) xylose, 20 mg $Na_2CO_3 \cdot 10\ H_2O$ (0.07 mmol), 20 mg (0.18 mmol) *o*-phenylenediamine and 1 ml hydrolyzed wheat protein; mixture 3: 60 mg (0.33 mmol) glucose, 20 mg $Na_2CO_3 \cdot 10\ H_2O$ (0.07 mmol), 20 mg (0.18 mmol) *o*-phenylenediamine and 350 mg (4 mmol) β-alanine; mixture 4: 60 mg (0.33 mmol) glucose, 20 mg $Na_2CO_3 \cdot 10\ H_2O$ (0.07 mmol), 20 mg (0.18 mmol) *o*-phenylenediamine and 1 ml hydrolyzed wheat protein.

The quinoxalines were identified by comparison of their GC(1) retention times (t_R of acetylated samples) and of their mass spectral data with those obtained of purchased, synthesized or reference compounds.

1: t_R = 8.30 min; MS(EI) *m/z*: 130 ($M^{+\cdot}$, 100%), 103 (91), 76 (69), 50 (48). – 2: t_R = 11.37 min; MS(EI) *m/z*: 144 ($M^{+\cdot}$, 92%), 117 (100), 103 (8), 90 (22), 77 (42), 76 (51), 63 (11), 50 (51). – 4: t_R = 14.90 min; MS(EI) *m/z*: 158 ($M^{+\cdot}$, 61%), 143 (3), 117 (100), 102 (5), 90 (18), 77 (37), 76 (36), 63 (10), 50 (40). – 6: t_R = 29.08 min; MS(EI) (acet.) *m/z*: 216 ($M^{+\cdot}$, 8%), 174 (100), 156 (43), 143 (40), 117 (12), 116 (11), 102 (30), 89 (19), 77 (22), 76 (35), 75 (29), 63 (15), 50 (38). – 7: t_R = 37.30 min; MS(CI) (acet.) *m/z*: 289 (M^+ + 1, 100%), 229 (81), 187 (31), 169 (9). – 8: t_R = 38.70 min; MS(CI) (acet.) *m/z*: 289 (M^+ + 1, 100%), 229 (4), 169 (69). – 9: t_R = 32.50 min; MS(CI) (acet.) *m/z*: 231 (M^+ + 1, 67%), 199 (11), 172 (42), 171 (100). – 10: t_R = 17.16 min; MS(CI) *m/z*: 199 (M^+ + 29, 22%), 172 (43), 171 (M^+ + 1, 100). – 11: t_R = 19.27 min; MS(CI) *m/z*: 187 (M^+ + 1, 100%), 158 (6). – 13: t_R = 40.30 min; MS(CI) (acet.) *m/z*: 361 (M^+ + 1, 100%), 301 (19), 241 (7). – 14: t_R = 42.80 min; MS(CI) (acet.) *m/z*: 361 (M^+ + 1, 100%), 301 (3), 241 (6), 181 (17). – 15: t_R = 39.00 min; MS(CI) (acet.) *m/z*: 303 (M^+ + 1, 100%), 183 (57).

In the acetylated mixtures separated by GC we detected some peaks which did not correspond to any quinoxaline listed above.

Through interpretation of their mass spectral data, the following structures were tentatively assigned:

3: $t_R = 26.20$ min; MS(EI) (acet.) *m/z*: 160 ($M^+ - 42$, 100%), 143 (6), 131 (17), 129 (20), 102 (30), 89 (3), 77 (9), 76 (22), 75 (19), 63 (7). – 5: $t_R = 30.17$ min; MS(EI) (acet.) *m/z*: 217 ($M^+ + 1$, 4%), 173 (30), 156 (100), 145 (24), 144 (20), 129 (31), 117 (7), 102 (33), 89 (9), 77 (13), 76 (33), 75 (17), 63 (9), 50 (39). – 12: $t_R = 40.90$ min; MS(CI) (acet.) *m/z*: 347 ($M^+ + 1$, 52%), 305 (6), 287 (100), 227 (46), 185 (12).

Formation of 11 from 32. To a mixture of xylose with α-amino acids and *o*-phenylenediamine (described above) were added 13 mg of 32 and 2,3-diphenylquinoxaline (synthesized from benzil and *o*-phenylenediamine, according to the synthesis of substance 8 or 14) as an internal standard. The mixture was treated as described above and compared to the same experiment without addition of 32. Neither a significant increase of 11, nor a decrease in the amount of 32 was observed.

Formation of 11 from 7. 11 mg 7 was heated in 0.5 ml water (adjusted to pH 6 with 0.1 mol acetic acid) for 12 h. After that time compound 11 was not detectable.

1-(Tetrahydro-2-pyranyloxy)-5-benzyloxy-2,4-hexanedione 37. To a cooled suspension (0° C) of 2.4 g (60 mmol, 60%) sodium hydride in 10 ml dry tetrahydrofuran successively 5 g (31 mmol) (tetrahydro-2-pyranyloxy)-acetone 35 [7] and 6.24 g (30 mmol) *O*-benzyl-ethyl-L-lactate 36 [8] were slowly added. The mixture was then heated under reflux for 3 h and, after cooling to room temperature, the reaction mixture was poured into a mixture of 50 ml ethyl acetate and 50 ml water. After shaking, the organic phase was separated and extracted twice with 40 ml 0.5 M sodium hydroxide. The water phases were collected, adjusted to pH 6.5 with 4 M acetic acid and extracted with ethyl acetate. The residue of the organic layer was purified on a silica gel column with hexane/ethyl acetate (9+1). The fractions with fluorescence absorbing spots with $R_f = 0.4$ (TLC control with hexane/ethyl acetate, 8+2) were collected. After evaporation of the solvent, compound 37 remained as an oil [1 g, 10% after purification, GC(2) $t_R = 29.2$ min]. MS(CI): *m/z* 237 ($M^+ - 83$, 46%), 220 (69), 219 (100), 201 (31), 129 (19).

2-Methyl-5-(tetrahydro-2-pyranyloxymethyl)-3(2H)-furanone 38. To a solution of 1 g (3 mmol) 37 in 50 ml methanol, 0.5 g palladium (10%) on activated charcoal was added. The mixture was hydrogenated (room temperature, pH 7) for 15–60 min (various reaction times, TLC control with hexane/ethyl acetate (8+2), the R_f value of 37 was 0.45 and that of 38 0.15). The mixture was filtered and the methanol removed. Compound 38 was obtained as two diastereomers a and b [syrupy oil,. 600 mg, 94%, GC(2) $t_R = 19.3$ min (38a) and $t_R = 19.9$ min (38b)] with different mass spectral data. MS(EI) 38a: *m/z* 213 ($M^+ + 1$, 89%), 129 (7), 113 (42), 112 (69), 111 (100), 101 (9), 85 (51), 84 (49), 69 (29), 67 (67), 57 (24), 56 (25), 55 (44). MS(EI) 38b: *m/z* 129 ($M^+ - 83$, 2%), 115 (7), 101 (9), 85 (100), 69 (7), 67 (27), 57 (18), 55 (27).

5-Hydroxymethyl-2-methyl-3(2H)-furanone 34. 600 mg (2.8 mmol) 38 was dissolved in 50 ml methanol and 10–20 mg pyridinium-(toluene-4-sulfonate) polymere bound was added. The suspension was stirred at 40° C for 15–60 min (TLC-control with ethyl acetate, the R_f-value of 38 was 0.75 and that of 34 was 0.5). After filtration and evaporation, the residue was purified on a silica gel column with methylene chloride/ethyl acetate (1+1). The fraction containing substances with $R_f = 0.5$ (TLC control with ethyl acetate) was collected. After removal of the solvent, compound 34 remained as a syrupy oil (150 mg, 42%). The t_R in the GC/MS system for compound 34 was 6.3 min; for silylated compound 34, $t_R = 8.23$ min. MS(EI): *m/z* 128 ($M^{+\cdot}$, 26%), 84 (31), 69 (17), 55 (100). Infrared: 3630, 3450, 2975, 1765, 1715, 1615, 1080, 965, 800 cm^{-1}. Ultraviolet (methanol): λ_{max}/nm (lg ε) = 259 (3.79). For NMR spectral data see Table 3.

Detection of 34 in glucose/α-amino acid mixtures. 11 g phenylalanine (37 mmol) and 12 g (67 mmol) glucose were pulverized with 13 g sea-shore sand. The mixture was heated to 220° C for 10 min [9]. The volatile compounds were removed under diminished pressure (3 Pa) and condensed in an ice trap. The condensation products were examined with the GC/MS system. A peak was found with retention time and mass spectral data identical to those of synthesized compound 34. In the silylated product mixture a peak with datas identical to those of silylated 34 was obtained, as well.

Results and discussion

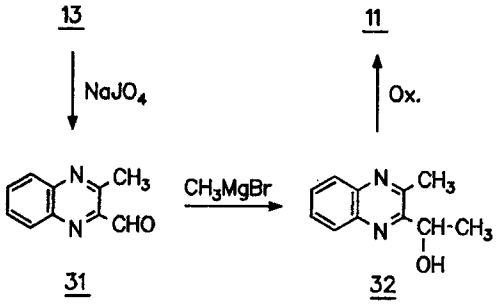
It is well known that reducing sugars undergo retro-aldol reactions in the pH range above 8 and that highly reactive, low-molecular mass compounds are formed, e.g. dihydroxyacetone, glyceraldehyde, tetroses, and glycolaldehyde [10]. The retro-aldol cleavage is often followed by elimination of water, or by oxidation to an α-dicarbonyl compound.

Morita et al. have studied the degradation of reducing sugars in alkaline solution; in the presence of *o*-phenylenediamine, they obtained some of the quinoxalines listed in Table 1 [11]. Because of the high pH value, these results are not representative of either food or biological systems. However, amines are known to catalyze retro-aldol cleavage of the carbohydrate carbon skeleton under conditions which are normally encountered in food and in living organisms (pH 4–7.4). Amines, like amino acids or proteins, are present in all foods and in the human body.

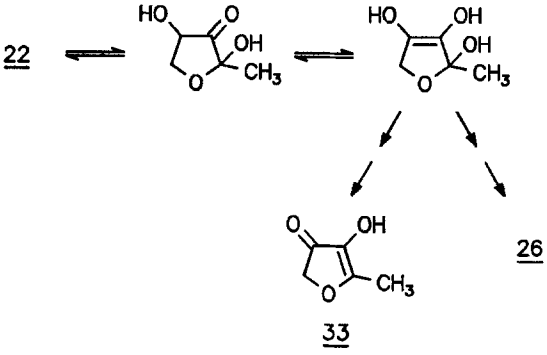
Recently, we studied the formation of α-dicarbonyl compounds in reaction mixtures of mono- and disaccharides with primary and secondary amines, in the presence of *o*-phenylendiamine [2, 21]. At first, we were interested primarily in the amount of deoxyosones formed, i.e. in those α-dicarbonyl compounds with the original carbon backbone. We have now extended our investigations to the fragmentation products. The quinoxalines which could be separated by GC/MS were identified by comparison with authentic material (either commercially available or synthesized independently) or on the basis of the MS data. The amount of some quinoxalines, detected in glucose/β-alanine reaction mixtures with *o*-phenylendiamine at pH 5 and 7 is listed for direct comparison in Table 2. The intensities were calculated from GC peak areas. This semi-quantitative evaluation is restricted to those fragmentation products which have an α-dicarbo-

Table 2. Peak area of quinoxalines in glucose/β-alanine reaction mixture of pH 5 and pH 7 Peak area obtained at

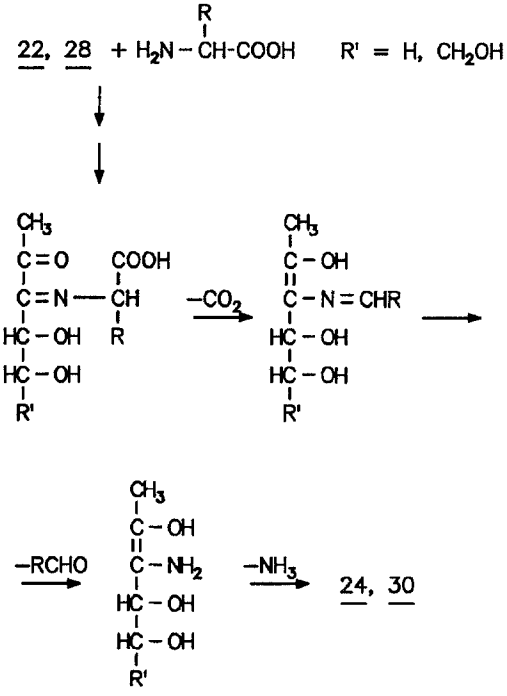
Quinoxaline	pH 5	pH 7
1	34.700	49.300
2	270.000	282.000
3	3.100	16.200
4	38.800	49.600
5	1.200	16.600
6	1.400	17.800
13	479.000	60.700
14	114.500	2.900



Scheme 1. Synthesis of quinoxaline 11



Scheme 2. Formation of triketone 26 from the 1-deoxyosone 22



Scheme 3. Strecker degradation of 1-deoxyosones leading to 1,4-dideoxyosones

nyl partial structure, or which were transformed into this type of compound in the course of the heating period. It is remarkable that the fragmentation-product/deoxyosone ratio changes from 2:3 at pH 5 to 7:1 at pH 7. When we transfer this in vitro result to the human body, with the physiological pH of 7.4, one may assume that reactions between glucose and proteins form many reactive fragmentation products which are, for example, active in the cross-linking of proteins.

In meat and meat products, pentoses are involved in the Maillard reaction. In Table 1, the quinoxalines and corresponding α-dicarbonyl compounds are listed which may be formed during the degradation of these sugars. The individual compounds were identified as described above.

As expected, the 1- and 3-deoxyosone 7 and 8 were isolated from xylose/α-amino acid mixtures. The quinoxaline 8 had already been detected some years ago, in the form of the bis(dinitrophenyl)hydrazone derivative, in Maillard reaction mixtures [12]; the isomeric compound 7 is established here for the first time as an intermediate in pentose degradation in the presence of amines. Remarkably, the quinoxaline 11 was detected which must have been formed from the triketone 26; the structure of 11 was established by independent synthesis (see Scheme 1). Furthermore, we have ensured that 11 is not formed from either 7 or 32 by elimination of water, or by oxidation during the heating period. We propose that formation of the triketone 26 proceeds from 22. The 1-deoxyosone 22 undergoes cyclization and enolization and, finally, cleavage of the C5-O bond (see Scheme 2). Alternative abstraction of the hydroxy group in position 2 yields the well known furanone 33 [13]. The triketone 26 may be involved in the formation of flavor and color in foods which contain significant amounts of pentoses.

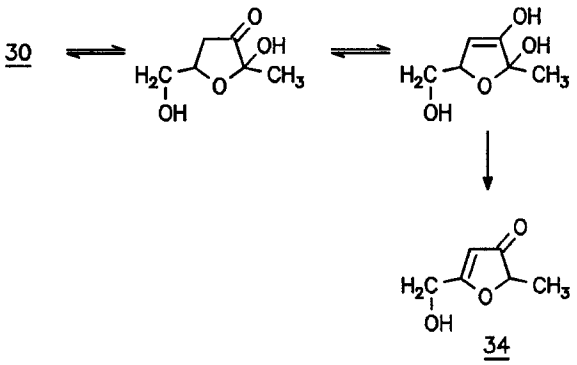
The analogous triketo compound, formed from hexoses, is unstable: spontaneous β-diketo cleavage leads to the formation of the ester of β-propionic acid with lactic acid [14]. The analogous product from disaccharides is also known: it exists in at least two cyclic forms [15, 18].

The amount of quinoxaline 9 formed decreases significantly when the Maillard reaction is carried out with β-alanine instead of α-amino acids. The same effect is observed for the quinoxaline 15 when glucose is heated with α- and β-amino acids. The higher amount of 9 and 15, formed with α-amino acids may be attributed to Strecker degradation and the concomitant reduction of the 1-deoxyosones 22 and 28 to the 1,4-dideoxyosones 24 and 30 (see Scheme 3).

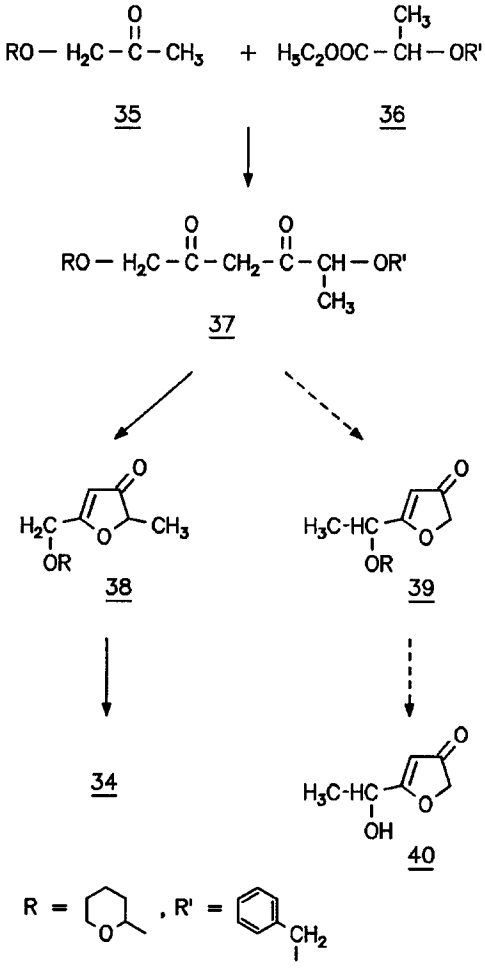
The small amount of 1,4-dideoxyosones found in reaction mixtures without α-amino acids may be derived from the corresponding 1-deoxyosones by reaction with reductones [1, 16, 19, 20].

From the structure of the 1,4-dideoxyosone 30, the furanone 34 can be postulated as a transformation product (see Scheme 4). It was synthesized independently to facilitate detection of 34 in reaction mixtures of hexoses and α-amino acids (see Scheme 5). The structure of the synthetic compound was unequivocally established as 34 by ^{1}H and ^{13}C NMR; thus, it was confirmed that the tetrahydropyranyl group did not migrate after elimination of the benzyl group from 37 to form the furanones 39 and 40, respectively, instead of 38 and 34.

The ^{13}C-NMR spectrum of 34 shows the expected six resonances (see Table 3). In the fully coupled spectrum, the resonance at lowest field ($\delta = 205.83$ ppm) is split into a straightforward sextet. The respective carbon atom



Scheme 4. Formation of furanone 34 from the 1,4-dideoxyosone 30



Scheme 5. Synthesis of furanone 34

must thus be coupled to five neighbouring protons with virtually identical coupling constants. As the compilation in Table 3 shows, the respective couplings are ^2J(4-H, C-3), ^2J(2-H,C-3), and three times ^3J(7-H,C-3). This splitting is possible only for the proposed structure 34; the methyl protons of the alternative structure 40 would not result in any visible coupling at C-3.

For all other carbon atoms, the multiplicity, due to both ^1J and long-range C,H coupling, would appear more or less the same as in 40. The numerical values,

Table 3. NMR data of 5-hydroxymethyl-2-methyl-3(2*H*)-furanone 34, synthesized independently (see Scheme 5)

^{13}C	δ (ppm)	J (Hz)	
C–3	205.83	^2J(C–3, 4–H) ^2J(C–3, 2–H) ^3J(C–3, 7–H$_3$)	3.4 (Σ 17.1)
C–5	193.20	^2J(C–5, 4–H) ^2J(C–5, 6–H^{A,B}) ^3J(C–5, 2–H)	8.8 5.3 3.1
C–4	101.80	^1J(C–4, 4–H) ^3J(C–4, 2–H) ^3J(C–4, 6–H^A)	171.1 2.2 2.5
C–2	83.07	^1J(C–2, 2–H) ^3J(C–2, 4–H) ^2J(C–2, 7–H$_3$)	151.1 5.2 4.6
C–6	59.44	^1J(C–6, 6–H^{A,B}) ^3J(C–6, 4–H)	143.7 1.0
C–7	16.15	^1J(C–7, 7–H$_3$) ^2J(C–7, 2–H)	130.1 4.1
^{1}H			
4–H	5.71	^4J(4–H, 6–H^{A,B})	1.0
2–H	4.57	^3J(2–H, 1–H$_3$) ^5J(2–H, 6–H^{A,B})	7.2 1.0
7–H	1.46	^3J(7–H$_3$, 2–H)	7.2
6–H^{A,B}	—[a]		

Values of δ are given relative to tetramethylsilane; C–6 corresponds to the hydroxymethyl group, C–7 to the methyl group

[a] Unresolved signal due to intermediate OH exchange rate

though, all favor the proposed structure 34 (see Table 3), especially in comparison with the respective C,H coupling values for 2,5-dimethyl-4-hydroxy-3(2H)-furanone [17].

The analysis of the high-resolution ^{1}H NMR spectrum (see Table 3) provides additional independent proof of structure 34. The olefinic proton 4-H shows a well-defined allylic coupling ($\approx$1.0 Hz) to the two methylene protons at C-6.

The new Strecker product 34 was indeed detected in reaction mixtures of α-amino acids, e.g. phenylalanine, with glucose in up to 0.1% yield. Further experiments are necessary to obtain more information about the properties of 34.

References

1. Ledl F, Schleicher E (1990) Angew Chem 102:597
2. Beck J, Ledl F, Severin T (1988) Carbohydr Res 177:240
3. Knapp D (1979) Handbook of analytical derivatization reactions. John Wiley, New York, p 545
4. Feather M, Madson M (1981) Carbohydr Res 94:183
5. Gattermann L, Wieland H (1982) Die Praxis des organischen Chemikers, 43rd edn. W. de Gruyter, Berlin, p 481
6. Haddadin MJ, Zahr GE, Rawdah TN, Chelot NC, Issidorides CH (1974) Tetrahedron 30:659
7. Manninger G (1976) Thesis, University of Munich
8. Czernecki S, Georgoulis C, Provelenghiou C (1976) Tetrahedron Lett 39:3535
9. Baltes W, Mevissen L (1988) Z Lebensm Unters Forsch 187:209

10. Anet EFLJ (1980) In: Pigman W, Horton D (eds) The carbohydrates: Chemistry and biochemistry, vol 1 A. Academic Press, New York, p 175
11. Morita N, Takagi M (1986) In: Fujimaki M, Namiki M, Kato H (eds) Development in food science: Amino-carbonyl reactions in food and biological systems. Kodansha, Tokyo 13:59
12. Kato H (1960) Bull Agric Chem Soc Jpn 24:1
13. Severin T, Seilmeier W (1967) Z Lebensm Unters Forsch 134:230
14. Beck J, Ledl F, Sengl M, Severin T (1990) Z Lebensm Unters Forsch 190:212
15. Ledl F, Ellrich G, Klostermeyer H (1986) Z Lebensm Unters Forsch 182:12
16. Huber B (1990) Thesis, University of Munich
17. Hodge JE, Fisher BE, Nelson EC (1963) Proc Am Soc Brew Chem p 84
18. Estendorfer S, Ledl F, Severin T (1990) Tetrahedron 46:5617
19. Noll P (1992) Thesis, University of Stuttgart (in preparation)
20. Ledl F, Schleicher E (1990) Angew Chem Int Ed Engl 29:565
21. Beck J, Ledl F, Severin T (1989) Z Lebensm Unters Forsch 188:118