Quantitative Model Studies on the Formation of Aroma-Active Aldehydes and Acids by Strecker-Type Reactions

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Application of aroma extract dilution analysis on the volatiles formed by reacting glucose and L-phenylalanine (30 min, 100 °C) revealed the Strecker aldehyde, phenylacetaldehyde (PA), and, in addition, phenylacetic acid (PAA) as the two key odorants among the volatiles formed. Quantitative measurements on α-dicarbonyl formation revealed that the 3-deoxyosone and glyoxal were formed as the first prominent sugar degradation products, whereas 2-oxopropanal became predominant after ∼4 h at 100 °C. Among the four α-dicarbonyls analyzed, 2-oxopropanal proved to be the most effective in generating PA as well as PAA from phenylalanine, but the reaction parameters significantly influenced the ratio of both odorants; for example, at pH 3.0 the ratio of PA to PAA was 3:1, whereas at pH 9.0 the ratio was 1:5. Furthermore, in the presence of oxygen and copper ions the formation of the acid was further increased. 3-Deoxyosone and glucosone were found to be effective precursors of phenylacetaldehyde, but neither was very effective in acid generation. On the basis of the results, a new oxygen-dependent formation pathway of the Strecker reaction is proposed.

Keywords: *Aroma extract dilution analysis; Strecker reaction; phenylacetaldehyde; phenylacetic acid; 3-methylbutanal; 3-methylbutanoic acid*

INTRODUCTION

In studies on the reaction between α-amino acids and the α-tricarbonyl alloxan, Strecker (1862) had observed that an oxidative degradation of the amino acid occurs, generating, for example, acetaldehyde and carbon dioxide from L-alanine. Schönberg and Moubacher (1952) later reported that, besides alloxan, a number of other α-dicarbonyls or vinylogous dicarbonyls as well as glucose are able to degrade α-amino acids, in that way generating an aldehyde with one carbon atom fewer than the α-amino acid. In honor of Strecker, they proposed the term Strecker degradation for this reaction shown in detail for L-phenylalanine in Figure 1.

The Strecker aldehydes of certain amino acids, in particular, leucine, phenylanine, or methionine, are known to have significant odor strength, and it is well accepted in the literature (Ledl and Schleicher, 1992) that these intensely smelling aldehydes are formed in foods also from the reaction between α-amino acids and carbohydrate-derived α-dicarbonyls.

In recent years, by application of aroma extract dilution analyses (AEDA), Strecker aldehydes of these three amino acids have been confirmed as key contributors to the aromas of several thermally processed foods. For example, 3-methylbutanal has been identified in boiled beef (Kerscher and Grosch, 1997), boiled chicken (Kerler and Grosch, 1997), and yeast extract (Münch et al., 1997); phenylacetaldehyde has been identified in roasted coffee (Blank et al., 1992), and both compounds were found as important odorants in wheat and rye bread crust (Schieberle and Grosch, 1987), chocolate (Schnermann and Schieberle, 1997), and roasted sesame (Schieberle, 1996). Methional is an important odorant in rye bread crust (Schieberle and Grosch, 1992) and potato chips (Wagner and Grosch, 1997).

Besides the Strecker aldehydes, the corresponding "Strecker" acids are also often identified as key odorants in thermally processed foods. For example, 3-methylbutanoic acid has been identified in wheat bread crust (Schieberle and Grosch, 1987) and boiled chicken (Kerler and Grosch, 1997) and, together with phenylacetic acid, in milk chocolate (Schnerman and Schieberle, 1997), boiled beef (Kerscher and Grosch, 1997), yeast extract (Münch et al., 1997), and caramel (Fickert and Schieberle, 1998).

Although the occurrence of Strecker aldehydes and the corresponding odor-active acids in processed foods has been known for a long time, the effectiveness of certain dicarbonyl intermediates in generating both species has not yet been systematically studied and, in particular, no information is available on whether and how acids are formed during the Strecker reaction.

The purpose of the present investigation was, therefore, to gain insight into the effectiveness of different carbohydrate-derived α-dicarbonyls in generating the aroma-active Strecker aldehydes and acids from the corresponding α-amino acids.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: glucose, L-leucine, L-phenylalanine, and L-alanine were from Fluka (Neu-Ulm, Germany); glyoxal, 2-oxopropanal, quinoxaline, 2-methylquinoxaline, copper(II) sulfate, 1,2-diaminobenzene, [$^{13}C_2$]acetaldehyde, [$^{13}C_2$]acetic acid, and [$^{13}C_2$]-phenylacetic acid were purchased from Aldrich (Steinheim, Germany). 1,2-Diaminobenzene was recrystallized twice from methanol, and furan-2-carboxaldehyde was freshly distilled in vacuo prior to use.

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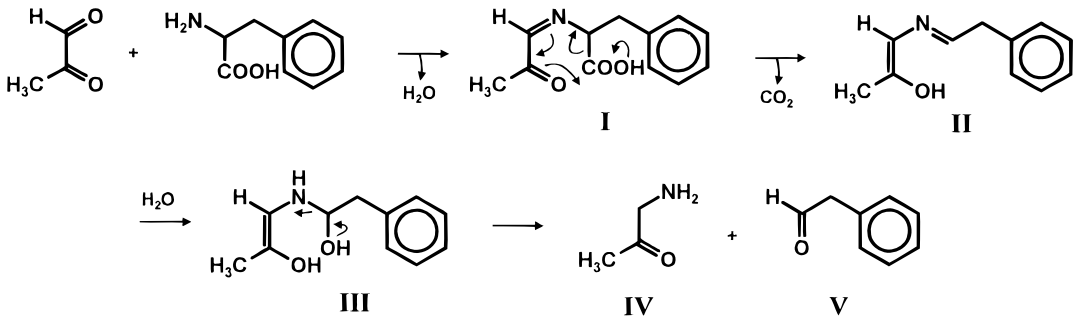

Figure 1. Mechanism of the Strecker degradation of L-phenylalanine initiated by 2-oxopropanal.

Syntheses. *3-Deoxy-2-hexosulose.* The preparation was performed following the method reported by Madson and Feather (1981) with some modifications: A stirred solution of glucose (55 mmol) and *p*-toluidine (55 mmol) in ethanol/acetic acid (236 mL; 22+1 by volume) was refluxed for 30 min. After addition of benzoyl hydrazine (120 mmol), refluxing was continued for another 7 h. The reaction mixture was cooled to room temperature and kept at −30 °C overnight. Separated 3-deoxy-2-hexosulose bis(benzoylhydrazone) was isolated by filtration over a Büchner funnel and washed with ice-cold ethanol (total volume = 100 mL). The hydrazone (24 mmol; 44% in yield) and benzaldehyde (16 mL) were then refluxed in a mixture of ethanol (300 mL), water (500 mL), and acetic acid (22 mL). After 2 h, the mixture was cooled in an ice bath, and the separated benzaldehyde−benzoylhydrazone was removed by filtration over a Büchner funnel. Mixed-bed H^+/OH^- ion-exchange resin (Serdolyt MB3; 60 g; Serva, Heidelberg, Germany) was added to the solution, and after 15 min of stirring in the dark, the suspension was filtered. The filtrate was concentrated in vacuo to ∼100 mL, then washed with diethyl ether (6 × 50 mL), and finally concentrated in vacuo to ∼5 mL. The residue was taken up in a mixture of ethanol (50 mL) and water (5 mL), mixed-bed H^+/OH^- ion-exchange resin (Serdolyt MB3; 10 g) was added, and the suspension was stirred for 15 min in the dark. After filtration, the solution was concentrated in vacuo to ∼5 mL. Freeze-drying yielded 3-deoxy-2-hexosulose as an amorphous powder (10 mmol; 42% yield). The purity was checked by HPLC after derivatization with 1,2-diaminobenzene.

The following compounds were synthesized following procedures recently reported: 2-(2,3,4-trihydroxybutyl)quinoxaline and 2-(1,2,3,4-tetrahydroxybutyl)quinoxaline (Hofmann, 1999). 2-Hexosulose was prepared according to a method described for the synthesis of 2-pentosulose (Salomon et al., 1952). The labeled standards [$^{13}C_4$]butane-2,3-dione (Schieberle and Hofmann, 1997), [$^{13}C_2$]phenylacetaldehyde (Pfnür and Schieberle, unpublished results), [$^2H_2$]-3-methylbutanal (Schieberle and Grosch, 1992), and [$^2H_2$]-3-methylbutanoic acid (Guth and Grosch, 1994) were synthesized following closely the procedures reported in the literature given in parentheses.

Model Reactions. *L-Phenylalanine/Glucose.* A solution of glucose (1.0 mmol) and L-phenylalanine (1.0 mmol) in phosphate buffer (10 mL; 0.5 mmol/L, pH 7.0) was heated at 98 °C for 30 min. After the mixture had cooled and the pH had been adjusted to 5.0, the reaction mixture was extracted with diethyl ether (3 × 15 mL) and the combined organic layers were washed with brine (5 mL), then dried over Na_2SO_4, and concentrated on a Vigreux column (60 cm × 1 cm). The volatiles were isolated by sublimation in vacuo as reported recently (Schieberle, 1995) and, finally, the distillate was concentrated to ∼200 μL. The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA (Schieberle and Grosch, 1987) by stepwise diluting the aroma extract with diethyl ether (1+1; by vol). HRGC/olfactometry was performed with aliquots (0.5 μL) of the diluted extracts.

L-Phenylalanine/α-Dicarbonyls. The α-dicarbonyl (1.0 mmol) and L-phenylalanine (1.0 mmol) dissolved in phosphate buffer (10 mL; 0.5 mmol/L, pH 7.0) were heated at 98 °C in closed vials. To remove dissolved oxygen, argon was bubbled through the solution for at least 20 min prior to thermal treatment. In further experiments, $CuSO_4$ (0.05 mmol) was added to the reaction mixture prior to heating. For further details see footnotes of the corresponding tables.

Quantification by Stable Isotope Dilution Assays (SIDAs). *Phenylacetaldehyde, Phenylacetic Acid, 3-Methylbutanal, 3-Methylbutanoic Acid, and Acetic Acid.* Depending on the amino acid reacted, the mixtures were spiked with defined amounts of the labeled internal standards [$^{13}C_2$]phenylacetaldehyde and [$^{13}C_2$]phenylacetic acid (for L-phenylalanine), [$^2H_2$]-3-methylbutanal and [$^2H_2$]-3-methylbutanoic acid (for L-leucine), or [$^{13}C_2$]acetic acid (for L-alanine) dissolved in methanol (1 mL). After equilibration (∼20 min), the pH of the mixtures was adjusted to 3.0 and the solutions were extracted with diethyl ether (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated to ∼1 mL using a Vigreux column. Quantification was performed by mass chromatography as described recently (Schieberle and Hofmann, 1997). The following molecular ions (MS-CI) of the unlabeled and labeled phenylacetaldehyde [121 (100, $[M + 1]^+$)/123 (100, $[M + 1]^+$)], phenylacetic acid [137 (100, $[M + 1]^+$)/139 (100, $[M + 1]^+$)], 3-methylbutanal [87 (100, $[M + 1]^+$)/89−90 (100, $[M + 1]^+$)], 3-methylbutanoic acid [103 (100, $[M + 1]^+$)/105−106 (100, $[M + 1]^+$)], and acetic acid [61 (100, $[M + 1]^+$)/63 (100, $[M + 1]^+$)] were used for the quantification by SIDA.

Acetaldehyde. The reaction mixture (containing L-alanine) was spiked with a defined amount of the labeled internal standard [$^{13}C_2$]acetaldehyde dissolved in water. After equilibration in a closed vial, an aliquot of the headspace was analyzed by a headspace device (TCT/PTI 4001; Chrompack, Frankfurt, Germany) coupled to a mass spectrometer operating in the CI mode. The molecular ions of labeled and unlabeled acetaldehyde [45 (100, $[M + 1]^+$)/47 (100, $[M + 1]^+$)] were monitored.

Quantification of α-Dicarbonyls. *2-Hexosulose and 3-Deoxy-2-hexosulose.* An aliquot (2−5 mL) of the reaction mixture was rapidly cooled to room temperature, then water (1 mL) and a methanolic solution of 1,2-diaminobenzene (2 mL; 1 mol/L) were added, and the mixtures were maintained for 3 h at 30 °C. An aliquot of the mixture was directly analyzed by HPLC as described recently (Hofmann, 1999). By monitoring the effluent at λ = 320 nm, 2-hexosulose and 3-deoxy-2-hexosulose were quantified as their corresponding quinoxaline derivatives 2-(1,2,3,4-tetrahydroxybutyl)quinoxaline and 2-(2,3,4-trihydroxybutyl)quinoxaline.

Glyoxal and 2-Oxopropanal. The quantification was performed following a procedure previously described by Schieberle et al. (1993) for 2,3-butanedione with some modifications. An aliquot (1 mL) of the reaction mixture was rapidly cooled to room temperature and diluted with water (5 mL). The solution was adjusted to pH 6.5 with hydrochloric acid (0.1 mol/L), and the internal standard [$^{13}C_4$]butane-2,3-dione, dissolved in methanol, and a methanolic solution of 1,2-diaminobenzene (2 mL; 1 mmol/L) were added. After 3 h at 30 °C, the pH was adjusted to 5.0 with hydrochloric acid (0.1 mol/L) and the mixture was extracted with diethyl ether (2 × 15 mL). After drying over Na_2SO_4, the organic layer was concentrated to ∼0.5 mL and the liquid phase was applied onto the top of a column (5 × 50 mm) filled with a slurry of silica gel (1 g) in *n*-pentane. Flushing the column with pentane/

Table 1. Key Odorants (FD ≥ 16) Generated from L-Phenylalanine by Thermal Treatment in the Presence of Glucose[a]

odorant	odor quality	FD factor
phenylacetaldehyde	flowery	1024
phenylacetic acid	honey-like	512
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	64

[a] A solution of glucose (1.0 mmol) and L-phenylalanine (1.0 mmol), dissolved in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0), was refluxed for 30 min.

diethyl ether (15 mL; 30:70, v/v) under a slight pressure of nitrogen yielded an eluate, which was concentrated to ~2 mL prior to analysis. An aliquot of the eluate was analyzed by mass chromatography using a GC-MS system and monitoring the molecular ions of the quinoxaline derivatives obtained in the CI mode: quinoxaline, *m/z* 131 (100, $[M + 1]^+$); 2-methylquinoxaline, *m/z* 145 (100, $[M + 1]^+$); $[^{13}C_4]$-2,3-dimethylchinoxaline, *m/z* 163 (100, $[M + 1]^+$).

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (Kontron, Eching, Germany) consisted of two pumps (type 422), a gradient mixer (M 800), and a Rheodyne injector (100 μL loop). The effluent was monitored by a diode array detector (DAD; type 440, Kontron), operating in a wavelength range between 220 and 500 nm. Separations were performed on a stainless steel column filled with RP-18 silica (ODS-Hypersil, 250 × 4.6 mm, 5 μm, Shandon, Frankfurt, Germany) using a flow rate of 0.6 mL/min.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using capillary SE-54 (30 m × 0.32 mm fused silica capillary, DB-5, 0.25 μm; J&W Scientific, Fisons, Mainz, Germany). The samples were applied by the cold on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C, held for 2 min isothermally, then raised at 6 °C/min to 230 °C, and held for 5 min. The flow of the carrier gas helium was 2.5 mL/min. MS analysis was performed with an MS 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. Mass chromatography in the chemical ionization mode (MS-CI) was performed at 115 eV with isobutane as the reactant gas.

Headspace Gas Chromatography−Mass Spectrometry. Headspace samples were injected using the purge-and-trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany) connected to a type CP-9001 gas chromatograph (Chrompack). Using capillary DB-5 (30 m × 0.32 mm fused silica capillary, 0.25 μm; J&W Scientific, Fisons), MS analysis was performed by means of an Incos XL mass spectrometer (Finnigan MAT) operating in the CI mode (115 eV) with methane as the reagent gas.

RESULTS AND DISCUSSION

Glucose/α-Amino Acids. The overall aroma formed by boiling an aqueous solution of glucose and L-phenylalanine for 30 min was judged by a sensory panel to smell intensely flowery, honey-like. Application of AEDA on an extract prepared by solvent extraction of the mixture followed by careful high-vacuum distillation revealed only three odorants. Among them, phenylacetaldehyde was identified with the highest FD factor (Table 1), closely followed by phenylacetic acid. The caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, the third odorant, exhibited a comparatively low odor impact.

Quantification of the amounts of phenylacetaldehyde and phenylacetic acid formed after 30 min at 98 °C using SIDA revealed that both odorants were formed in the same orders of magnitude of 0.042 and 0.029 mol %, respectively (cf. Table 2).

To investigate whether, besides the Strecker aldehydes, the respective acids are generally formed from

Table 2. Amounts of Strecker Aldehydes and Acids Generated from Three Different α-Amino Acids[a]

odorant	amount (μmol/mmol) generated from		
	phenylalanine	alanine	leucine
phenylacetaldehyde	0.42	na[b]	na
phenylacetic acid	0.29	na	na
acetaldehyde	na	0.48	na
acetic acid	na	0.30	na
3-methylbutanal	na	na	0.89
3-methylbutanoic acid	na	na	0.49

[a] A solution of glucose (1.0 mmol) and the respective amino acid (1.0 mmol), dissolved in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0), was refluxed for 30 min. [b] na, not analyzed.

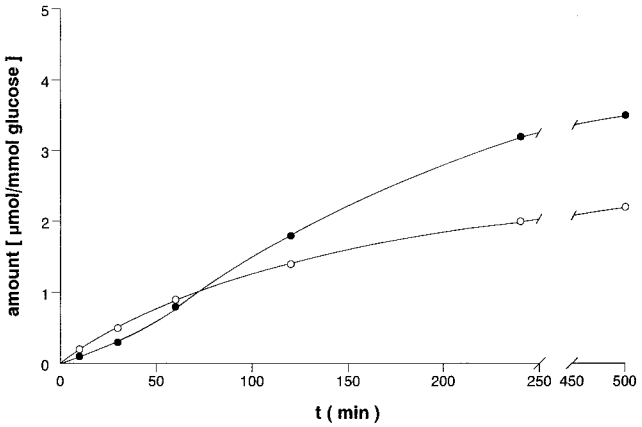

Figure 2. Time course of the formation of phenylacetaldehyde (○) and phenylacetic acid (●) from glucose and L-phenylalanine.

α-amino acids, aqueous solutions of either L-alanine or L-leucine were reacted individually in the presence of glucose, and the amounts of acetaldehyde and acetic acid or 3-methylbutanal and 3-methylbutanoic acid, respectively, were determined. The results showed (Table 2) that from these amino acids the respective acids were formed besides the aldehydes, but in somewhat lower amounts. It is interesting to note that among the amino acids analyzed, L-leucine was the most effective in generating the odorants.

To gain further insight into the time course of phenylacetaldehyde (PA) and phenylacetic acid (PAA) formation from L-phenylalanine, their generation was followed quantitatively. The results showed (Figure 2) that the formation of PA was slightly favored within the first 60 min of the thermal treatment. However, increasing the reaction time then led to a predominant generation of PAA. After ~8 h, the amounts of the acid were nearly twice as high as the concentration of the aldehyde.

L-Phenylalanine/α-Dicarbonyls. During thermal treatment of carbohydrates several α-dicarbonyl compounds, such as 2-hexosulose, 1- and 3-deoxyosone, 2-oxopropanal, and glyoxal, are known to be generated. However, it is still unknown how the structure of the α-dicarbonyl compound influences its effectiveness in volatile generation during the Strecker reaction.

Therefore, in a first experiment, the time course of the formation of four α-dicarbonyls was monitored in the glucose/L-phenylalanine mixture. The results (Figure 3) revealed a rapid increase of the concentrations of, in particular, the 3-deoxy-2-hexosulose (3-deoxyosone) and, also, glyoxal in the first stage of the reaction. Both intermediates went through a maximum after ~60 min. 2-Hexosulose was formed in much lower amounts, reaching a maximum of concentration of 0.009 mol %.

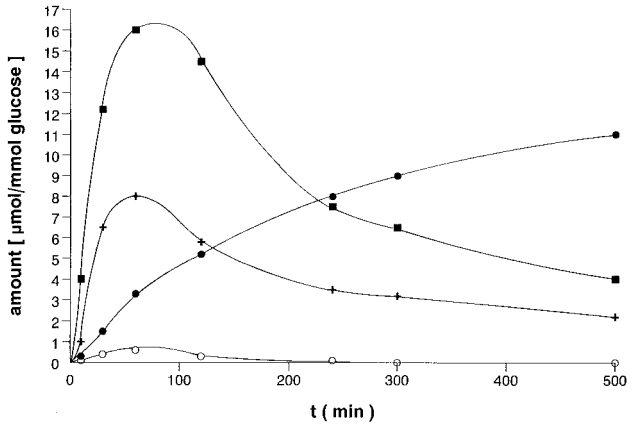

Figure 3. Time course of the formation of four α-dicarbonyls in a thermally treated glucose/L-phenylalanine solution: 3-deoxy-2-hexosulose (■); 2-hexosulose (○); glyoxal (+); 2-oxopropanal (●).

Table 3. Time Course of the Formation of Phenylacetaldehyde (PA) and Phenylacetic Acid (PAA) from L-Phenylalanine and 2-Oxopropanal[a]

reaction time (min)	PA (μmol)	PAA (μmol)	phenylalanine degraded (%)
10	6.2	10.1	9
30	9.5	17.2	18
60	10.6	20.6	36
120	12.3	23.1	49
240	13.5	24.8	58
300	13.3	25.8	61
500	12.2	27.6	69

[a] 2-Oxopropanal (1 mmol) and L-phenylalanine (1 mmol), dissolved in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0), were heated (98 °C) in a closed vial.

With increasing reaction time, the concentrations of glyoxal and both C_6-dicarbonyls decreased, whereas the concentration of 2-oxopropanal increased significantly, so that after 500 min more 2-oxopropanal was present by a factor of 3 than, for example, glyoxal.

To study the time course of the formation of PA and PAA initiated by α-dicarbonyls, first, 2-oxopropanal was reacted with phenylalanine. The results revealed (Table 3) that within 10−500 min of reaction time PAA was always predominantly formed. The amounts of both compounds increased rapidly within the first 120 min. However, increasing the reaction time to 500 min did not significantly increase the yields, although phenylalanine was continuously degraded.

It might be assumed that oxygen, the concentration of which was not controlled in the experiments displayed in Table 3, might play a role in the Strecker reaction. To study the influence of oxygen on the acid and aldehyde formation, the phenylalanine/2-oxopropanal mixture was reacted for a defined time (60 min) under an atmosphere of either argon or air oxygen, respectively. The results (Table 4, experiment 1) revealed that in the presence of oxygen (model B) more PAA was formed by a factor of nearly 4 compared to anaerobic conditions (model A). The formation of PA, however, was not much influenced by the presence or absence of oxygen. However, additions of copper(II) ions (experiment 2, Table 4) further increased the amounts of PAA when oxygen was present.

Besides 2-oxopropanal, further dicarbonyls were formed upon heating of the glucose/L-phenylalanine solution (cf. Figure 3). To study their effectiveness in aldehyde and acid formation, glyoxal, 3-deoxy-2-hexosulose, and 2-hexosulose were singly reacted with L-phenylalanine under

Table 4. Amounts of Phenylacetaldehyde (PA) and Phenylacetic Acid (PAA) Generated from L-Phenylalanine in the Presence of Different α-Dicarbonyls:[a] Influence of Oxygen and Copper Ions

		PA (μmol/mmol)		PAA (μmol/mmol)	
expt	dicarbonyl	model A[b]	model B[c]	model A[b]	model B[c]
1	2-oxopropanal	11.1	10.2	4.7	20.4
2	2-oxopropanal[d]	na	10.0	na	27.1
3	glyoxal	9.2	8.3	2.2	12.2
4	3-deoxy-2-hexosulose	8.4	7.8	1.5	3.3
5	2-hexosulose	8.1	6.9	1.3	2.1

[a] A solution of the α-dicarbonyl (1.0 mmol) and L-phenylalanine (1.0 mmol) in phosphate buffer (10 mL; 0.1 mol/L, pH 7.0) was refluxed for 60 min in a closed vial. [b] Oxygen was absent. [c] Oxygen was present. [d] The reaction was performed in the presence of oxygen and copper ions (0.05 mmol). [e] na, not analyzed.

Table 5. Amounts of $^{13}C_2$-Labeled Phenylacetic Acid Generated upon Heating of [$^{13}C_2$]Phenylacetaldehyde in the Presence of L-Phenylalanine and Glucose[a]

acid isotopomer	yield[b] (μg/mmol of phenylalanine)
phenylacetic acid	40.1
[$^{13}C_2$]phenylacetic acid	<0.5

[a] A solution of glucose (1.0 mmol), L-phenylalanine (1.0 mmol), and [$^{13}C_2$]phenylacetaldehyde (50.1 μg) in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0) was refluxed for 30 min. [b] The acid was quantified by using furan-2-carboxylic acid as the internal standard.

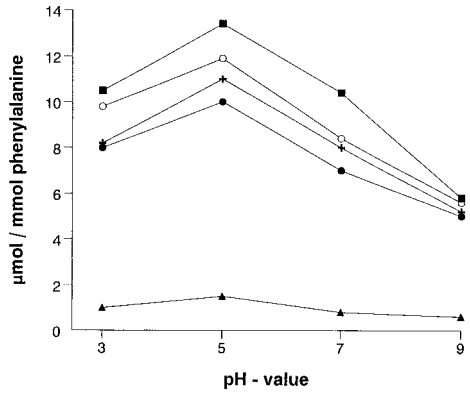

Figure 4. Influence of the pH value on the formation of phenylacetaldehyde from phenylalanine in the presence of (■) 2-oxopropanal, (○) glyoxal, (+) 3-deoxyosone, (●) glucosone, and (▲) glucose.

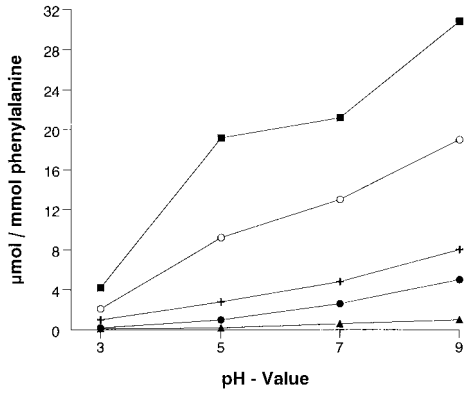

Figure 5. Influence of the pH value on the formation of phenylacetic acid from phenylalanine in the presence of (■) 2-oxopropanal, (○) glyoxal, (+) 3-deoxyosone, (●) glucosone, and (▲) glucose.

aerobic as well as anaerobic conditions. The results showed that in the absence of oxygen (model A, Table 4), and independently from the dicarbonyl reacted, phenylacetaldehyde was preferentially liberated from L-phenylalanine. Glyoxal (experiment 3, Table 4) and

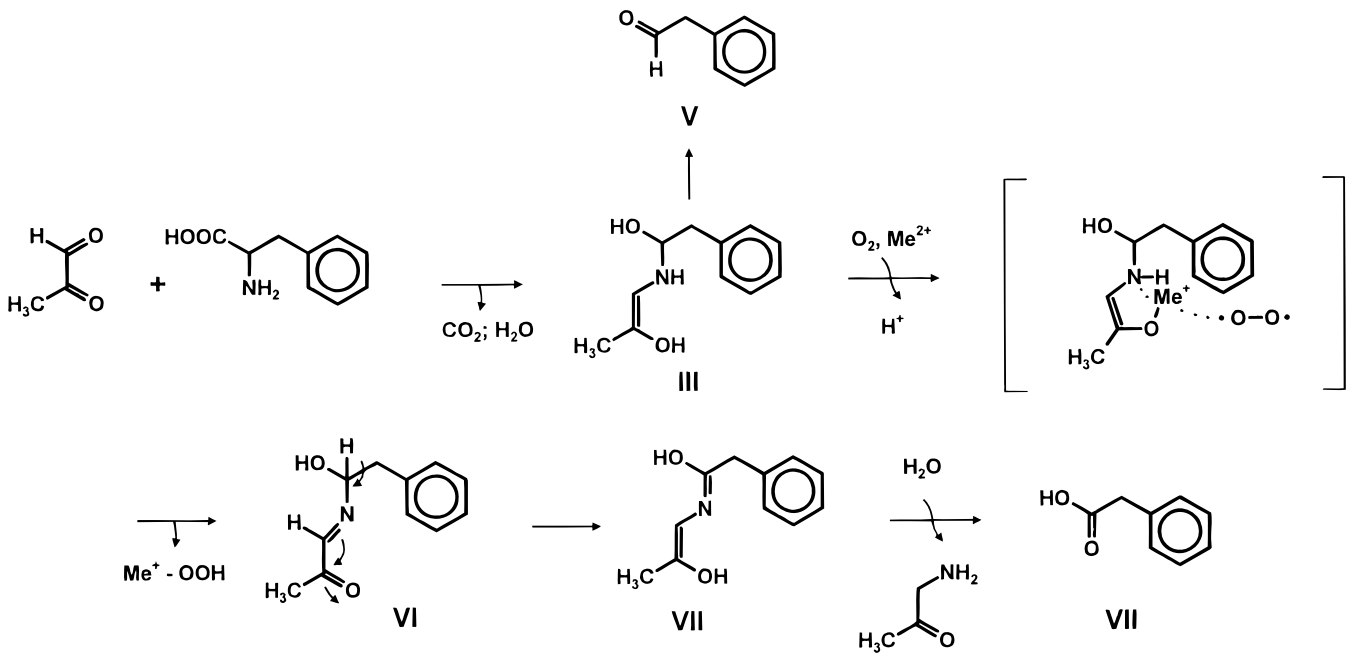

Figure 6. Proposed reaction pathway leading to the formation of phenylacetic acid from 2-oxopropanal and L-phenylalanine.

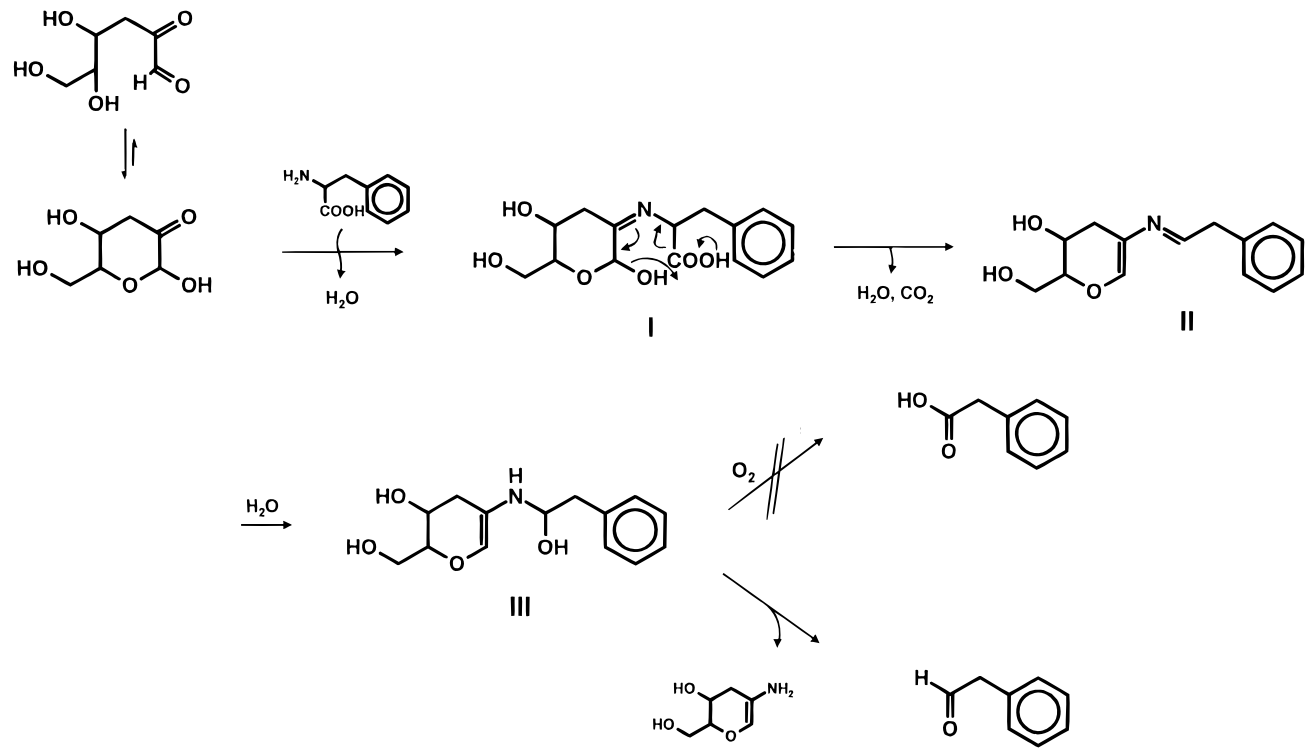

Figure 7. Proposed mechanism for the formation of phenylacetaldehyde from 3-deoxyosone and L-phenylalanine.

2-oxopropanal were somewhat more effective in generating the Strecker aldehyde compared to the C-6 α-dicarbonyls (cf. experiments 1 and 3 to experiments 4 and 5). However, as already found for 2-oxopropanal (experiment 1), also from glyoxal, in the presence of air oxygen, a drastic increase in the formation of phenylacetic acid occurred (model B).

Interestingly, the C_6-dicarbonyls did not form increased amounts of phenylacetic acid; independently from the oxygen content, the formation of phenylacetaldehyde was always significantly favored (experiments 4 and 5, Table 4). These data are well in line with the time course of the formation of phenylacetaldehyde and phenylacetic acid from L-phenylalanine in the presence of glucose (Figure 2). As 3-deoxyosone is among the first degradation products of glucose (Figure 3), and because this intermediate forms predominantly phenylacetaldehyde (experiment 4, Table 4), its predominance in the first hour of the glucose/phenylalanine reaction can easily be explained (Figure 2).

To gain insights into further factors governing the formation of phenylacetaldehyde and phenylacetic acid in Strecker-type reactions, glucose and each dicarbonyl were individually reacted in the presence of L-phenylalanine at different pH values. Independently from the carbonyl moiety used, the formation of phenylacetalde-

hyde was significantly favored at pH 5.0 (Figure 4). Thus, double the amount of phenylacetaldehyde was formed from 2-oxopropanal/phenylalanine at pH 5.0 compared to the amount formed at pH 9.0. In general, all α-dicarbonyls were more effective in generating the Strecker aldehyde from L-phenylalanine than glucose.

In contrast to the aldehyde formation, the formation of phenylacetic acid was drastically increased with increasing the pH from 3 to 9. For example, 7 times higher amounts of the acid were formed from 2-oxopropanal/phenylalanine at pH 9.0 compared to the amounts formed at pH 3.0 (Figure 5).

Formation of the Strecker Acids. The results have shown that the presence of oxygen generally favors the formation of the respective Strecker acid. It might, therefore, be speculated that the acids are formed simply by an oxidation of the aldehyde during the thermal treatment. To check this assumption, the following experiment was performed: Isotopically labeled [$^{13}C_2$]phenylacetaldehyde was added to a mixture of glucose and L-phenylalanine prior to the thermal treatment. The reaction was started and, after 30 min at reflux, the amounts of unlabeled phenylacetic acid, which is expected to be formed from glucose/L-phenylalanine, as well as [$^{13}C_2$]phenylacetic acid, probably formed by oxidation of [$^{13}C_2$]phenylacetaldehyde, were quantified. As shown in Table 5, phenylacetic acid was formed in high amounts, whereas the labeled acid was not detectable. This result clearly indicates that the acids are not formed to a significant extent by oxidation of the aldehyde during the thermal treatment.

Our very recent investigations have revealed that cyclic as well as open-chain enaminol intermediates (Hofmann and Schieberle, 1996; Hofmann and Schieberle, 1999, unpublished results) generated by Maillard-type reactions are easily oxidized into the corresponding imino ketones. Such compounds containing an enaminol group are also suggested as intermediates in the Strecker reaction (**III** in Figure 1).

Assuming the oxidation of such an enaminol during the Strecker reaction, a reaction route for the formation of phenylacetic acid can be proposed (Figure 6). Following the proposal for the Strecker reaction, Schiff base formation, followed by decarboxylation and hydrolysis, leads to the hemi aminal (**III**). This intermediate may either liberate phenylacetaldehyde (**V**) or be oxidized by a transition metal catalyzed reaction with air oxygen to form an iminoketone (**VI**). Upon enolization an imino acid results (**VII**), which may easily be hydrolyzed to yield phenylacetic acid (**VIII**).

This scheme is also corroborated by the quantitative data obtained at different pH values. Intermediate **III** should be very susceptible to acid hydrolysis and, therefore, the aldehyde (**V**) is preferentially formed at pH 5.0 (cf. Figure 4). The fact that the acid formation is favored at higher pH values can also be explained by the higher stability of **III** under alkaline conditions, thereby favoring its oxidation into **VI** (Figure 6).

The formation of the Strecker acids was significantly decreased when the C-6 α-dicarbonyls were reacted with phenylalanine (Table 4). 3-Deoxy-2-hexosulose and other C-6 dicarbonyls are stabilized as the corresponding cyclic hemi acetals and do not likely exist as open-chain compounds (Ledl and Schleicher, 1992). This might the reason these α-dicarbonyls were not very effective in acid generation: As a first reaction step the amino acid reacts with these cyclic forms (Figure 7). After formation of the Schiff base (**I**, Figure 7), especially in slightly acidic media, water and carbon dioxide can be eliminated yielding an imine (**II**). Hydration of **II** yields the aminal **III**, which is then easily cleaved into phenylacetaldehyde and an aminopyranone. Because **III** does not have an enaminol substructure, it cannot be oxidized to form phenylacetic acid as discussed in Figure 6. Therefore, predominantly phenylacetaldehyde is formed in the presence of, for example, the 3-deoxyosone (cf. Table 4).

Conclusion. The results have shown that in the Strecker reaction, besides the well-known Strecker aldehydes, the respective acid is always formed when α-amino acids are reacted in the presence of glucose or α-dicarbonyls generated therefrom. The ratio of the formation of aldehyde and acid, however, was found to be significantly influenced by the reaction parameters, in particular, the presence of oxygen, the structure of the α-dicarbonyl reaction with the amino acid, and the pH of the reaction mixture. The data can now be used to selectively generate either Strecker aldehydes or Strecker acids, for example, in the production of tailor-made reaction flavors.

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