Formation of Aroma-Active Strecker-Aldehydes by a Direct Oxidative Degradation of Amadori Compounds

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α-Dicarbonyls, generated by sugar degradation, catalyze the formation of the so-called Strecker aldehydes from α-amino acids. To check the effectiveness of Amadori compounds (suggested as important intermediates in α-dicarbonyl formation from carbohydrates) in Strecker aldehyde formation, the amounts of phenylacetaldehyde (PA) formed from either an aqueous solution of L-phenylalanine/glucose or the corresponding Amadori compound N-(1-deoxy-D-fructosyl-1-yl)-L-phenylalanine (ARP-Phe) were compared. The results revealed the ARP-Phe as a much more effective precursor in PA generation. On the contrary, a binary mixture of glucose/phenylalanine yielded preferentially phenylacetic acid, in particular, when reacted in the presence of oxygen and copper ions. Further model experiments gave evidence that a transition-metal-catalyzed oxidation of the ARP-Phe by air oxygen into the 2-hexosulose-(phenylalanine) imine is the key step responsible for the favored formation of phenylacetaldehyde from the Amadori compound. This mechanism might explain differences in the ratios of Strecker aldehydes and the corresponding acids depending on the structures of carbohydrate degradation products involved.

Keywords: *Strecker reaction; flavor precursor; phenylacetaldehyde; phenylacetic acid; Amadori compound*

INTRODUCTION

The Strecker reaction (Strecker, 1862) is known as an efficient source of aroma-active aldehydes, such as 3-methylbutanal (malty) or phenylacetaldehyde (honey-like) from the corresponding amino acids leucine and phenylalanine, respectively, when reacted in the presence of α-dicarbonyl compounds. Besides these aldehydes, the corresponding odor-active acids also were recently shown to be formed in the course of Strecker-type reactions (Hofmann et al., 1999). Systematic model experiments have pointed out that carbohydrate degradation products, such as glyoxal and 2-oxopropanal, preferentially generated the acid, whereas α-dicarbonyls with an intact carbohydrate skeleton, such as 3-deoxy-2-hexosulose or 2-hexosulose, favored the formation of the Strecker aldehydes (Hofmann et al., 1999).

It is suggested in the literature that prior to α-dicarbonyl formation, the Amadori compounds are formed as the first stable products when carbohydrates are reacted with amino acids. The extent of Strecker aldehyde formation from Amadori compounds is, however, not yet clear.

The purpose of the present investigation was, therefore, to compare the yields of the Strecker aldehydes from the synthesized Amadori compound to those from the corresponding binary amino acid−carbohydrate mixture. Furthermore, model experiments were undertaken to elucidate the importance of the Amadori compound in sugar degradation reactions.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: D-glucose and L-phenylalanine were purchased from Fluka (Neu-Ulm, Germany); and [$^{13}C_2$]-phenylacetic acid, copper(II) sulfate, and 1,2-diamino benzene were purchased from Aldrich (Steinheim, Germany). 1,2-Diamino benzene was recrystallized twice from methanol prior to use. The labeled standard [$^{13}C_2$]-phenylacetaldehyde, was synthesized as recently reported (Pfnuer and Schieberle, 2000).

Synthesis of N-(1-Deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe). A mixture of powdered anhydrous D-glucose (300 mmol) and L-phenylalanine (400 mmol) was refluxed in a mixture of anhydrous methanol and dimethyl formamide (400 mL; 1:1 v/v) with stirring. After 3 h, malonic acid (90 mmol) was added and, after the mixture was refluxed for another 2 h, it was cooled to room temperature and concentrated to about 200 mL under vacuum. Unreacted L-phenylalanine was filtered off and the filtrate was then cooled to −20 °C. Dropwise addition of acetone yielded the target compound as a hygroscopic solid. Concentration of the mother liquor, recooling, and addition of acetone yielded additional raw product. The two crops were combined, recrystallized from hot methanol−acetone, and freeze-dried, yielding N-(1-deoxy-D-fructosyl-1-yl)-L-phenylalanine as a white powder with a purity of about 90% (24 mmol; yield 8%).

LC/MS: 328 (100, $[M+1]^+$), 310 (29, $[M+1\text{-}H_2O]^+$).

^{1}H NMR (360 MHz in D_2O; DQF−COSY): δ 3.11 (d, 1H, ^2J = 10.0 Hz, $-CH_aH_b-N$), 3.13 (d, 1H, ^3J = 7.9 Hz, $-CH_aH_b-$Phe), 3.15 (d, 1H, ^2J = 10.0 Hz, $-CH_aH_b-N$), 3.25 (d, 1H, ^3J = 7.9 Hz, $-CH_aH_b-$Phe), 3.70−4.20 (m, 5H, 3 × $-CH(OH)-$, $-CH_2-O$), 3.95 (d, 1H,^3J = 7.9 Hz, $-CH(COOH)-N$).

Reaction Mixtures. Either a mixture of D-glucose and L-phenylalanine (0.1 mmol each) or N-(1-deoxy-D-fructosyl-1-yl)-L-phenylalanine (0.1 mmol) dissolved in phosphate buffer (1 mL; 0.5 mmol/L, pH 7.0) was thermally treated under different conditions as detailed in the footnotes of the tables.

Aroma Extract Dilution Analysis. The analysis was performed as described previously (Schieberle, 1991).

Quantitation of Phenylacetaldehyde and Phenylacetic Acid by Stable Isotope Dilution Assays (Hofmann et al., 2000). The reaction mixture was diluted with water (5 mL) and then spiked with defined amounts of the labeled internal standards [$^{13}C_2$]-phenylacetaldehyde and [$^{13}C_2$]-phenylacetic

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Table 1. Key Odorants Formed by Refluxing Solutions of N-(1-Deoxy-D-fructosyl)-L-phenylalanine (I) or Glucose/L-Phenylalanine (II)

odorant	odor quality	FD factor[a] I[b]	FD factor[a] II[c]
phenylacetaldehyde	flowery	8192	1024
phenylacetic acid	honey-like	1024	512
4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	256	64

[a] The flavor dilution (FD) factors were determined by the aroma extract dilution analysis. [b] A solution of N-(1-deoxy-D-fructosyl)-L-phenylalanine (1.0 mmol) in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0) was refluxed for 30 min. [c] A solution of glucose (1.0 mmol) and L-phenylalanine (1.0 mmol) in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0) was refluxed for 30 min.

acid dissolved in methanol (0.5 mL). After equilibration (20 min), the pH of the mixture was adjusted to 3.0 and the solution was extracted three times with diethyl ether (total volume, 15 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated to about 1 mL by using a Vigreux column. Quantification was performed by mass chromatography as described recently (Hofmann et al., 1999). The following molecular ions (MS/CI) of the unlabeled and labeled phenylacetaldehyde [*m/z* 121 (100, $[M+1]^+$)/*m/z* 123 (*m/z* 100, $[M+1]^+$)] or phenylacetic acid [*m/z* 137 (100, $[M+1]^+$)/*m/z* 139 (100, $[M+1]^+$)] were used.

Quantification of L-Phenylalanine and N-(1-Deoxy-D-fructosyl)-L-phenylalanine. The reaction mixtures were diluted with water, filtered over a membrane (0.45 μm), and then analyzed by means of an amino acid analyzer Type 3000 (Biotronic, Maintal, Germany). After postcolumn derivatization with ninhydrine, the reaction products were determined photometrically at 570 or 440 nm, respectively.

Quantification of 2-Hexosulose, 1-Deoxy-2,3-hexodiulose, and 3-Deoxy-2-hexosulose. A mixture of N-(1-deoxy-D-fructosyl-1-yl)-L-phenylalanine (ARP-Phe 1.0 mmol) and 1,2-diaminobenzene (1.2 mmol) was thermally treated at 85 °C in phosphate buffer (2 mL; 0.5 mmol/L, pH 7.0) under an atmosphere of argon or air oxygen in the presence of copper-(II) ions (0.05 mmol), respectively. After 20, 40, 80, 160, and 320 min, aliquots (10−100 μL) were withdrawn and the quinoxaline derivatives formed were analyzed by RP−HPLC as recently reported (Hofmann, 1999).

High-Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS). HRGC was performed by means of a Type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) and using capillary DB-5 (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 and 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 HRGC. Mass chromatography in the chemical ionization mode (MS/CI) was performed at 115 eV using isobutane as the reactant gas.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^1H and DQF−COSY experiments were performed on a Bruker AC-200 or a Bruker AM-360 spectrometer (Bruker, Rheinstetten, Germany) using the acquisition parameters described previously (Hofmann, 1997).

RESULTS AND DISCUSSION

Aroma Extract Dilution Analysis. To gain first insights into its role as aroma precursor, synthesized N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) was refluxed for 30 min in phosphate buffer (pH 7.0); the volatiles were isolated by solvent extraction and high vacuum distillation, and finally, were ranked in their odor impact by means of an aroma extract dilution analysis. Three odorants were detected at the sniffing port, among which phenylacetaldehyde was identified with by far the highest flavor dilution (FD) factor (Table 1, column I). With an 8-times-lower FD factor, phenylacetic acid was detected, whereas the caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2H)-furanone showed a much lower odor impact. Compared to the odor-active volatiles formed by reacting glucose and phenylalanine under the same conditions (Table 1, column II), the ARP-Phe proved to be more effective in the generation of the three odorants.

Table 2. Concentrations (μmol/mmol) of Phenylacetaldehyde (PA) and Phenylacetic Acid (PAA) Generated upon Thermal Treatment of Either N-(1-Deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) or D-Glucose/Phenylalanine (Glc/Phe) under Various Conditions

	ARP-Phe		Glc/Phe	
expt	PA	PAA	PA	PAA
I[a]	0.6	0.3	0.4	0.2
II[b]	5.5	3.0	1.4	1.8
III[c]	13.8	7.6	2.6	3.9

[a] The precursors (1 mmol each) were heated (100 °C) in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0) for 120 min in a closed vial under an atmosphere of argon. [b] Argon was replaced by air oxygen. [c] Heating was performed in an air atmosphere and in the presence of copper (II) ions (0.05 mmol $CuSO_4$).

Table 3. Formation of N-(1-Deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) from Glucose and L-Phenylalanine[a]

reaction time (min)	ARP-Phe (μmol)	phenylalanine unreacted (μmol)	yield of ARP-Phe (%)[b]
10	42	920	52.5
30	48	825	27.4
60	32	640	9.0
120	21	510	4.3
300	9	398	1.5

[a] A solution of glucose (1.0 mmol) and L-phenylalanine (1.0 mmol) was refluxed in phosphate buffer (10 mL; 0.5 mol/L, pH 7.0). [b] The yield of ARP-Phe was calculated based on the amounts of L-phenylalanine reacted.

Quantification of Odorants. In further experiments the exact amounts of phenylacetaldehyde (PA) and phenylacetic acid (PAA) formed in both reaction systems were determined by means of stable isotope dilution assays. The results, summarized in Figure 1, revealed that the ARP-Phe preferentially gave the aldehyde independently from the reaction time (circles, Figure 1). On the contrary, in the glucose/phenylalanine solution the formation of PA (empty squares, Figure 1) was favored only within the first 60 min of thermal treatment. Increasing the reaction time led to the predominant formation of PAA (full squares, Figure 1), e.g., by a factor of 1.7 higher amounts of the acid were formed after 500 min.

To clarify the reasons for the different yields of PA and PAA from glucose/phenylalanine or the ARP-Phe, respectively, aqueous solutions of both precursors were individually reacted under an atmosphere of pure argon, and the amounts of both odorants formed were determined (Table 2, expt I). Under these conditions, the yields of both odorants were quite low from either ARP-Phe or Glc/Phe and the aldehyde was formed in higher amounts compared to the acid. Flushing of the models with oxygen (expt II in Table 2) increased the yields of PA and PAA from both precursors, however, from the ARP-Phe a favored formation of the aldehyde was observed. From the glucose/phenylalanine mixture, PAA

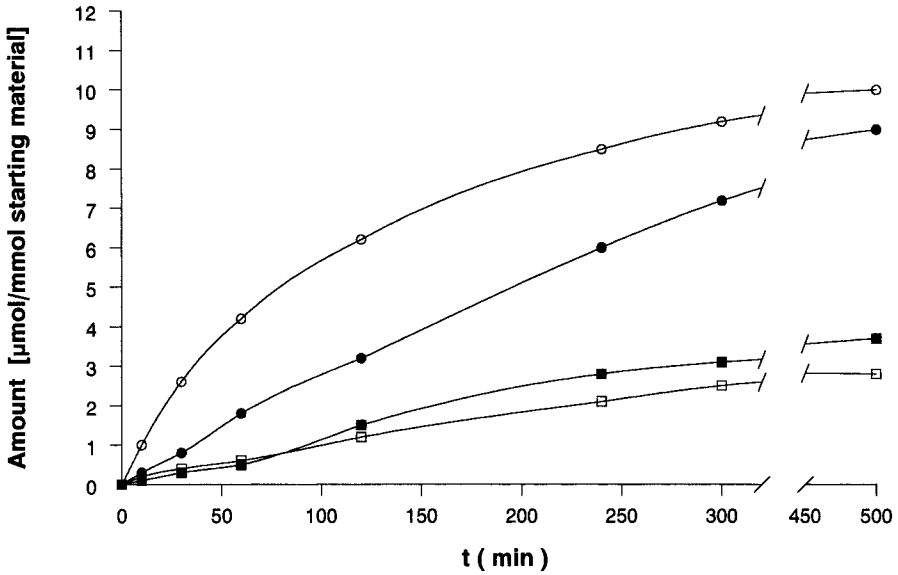

Figure 1. Formation of phenylacetaldehyde (PA) and phenylacetic acid (PAA) from glucose/phenylalanine (■, PAA; □, PA) or N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) (●, PAA; ○, PA).

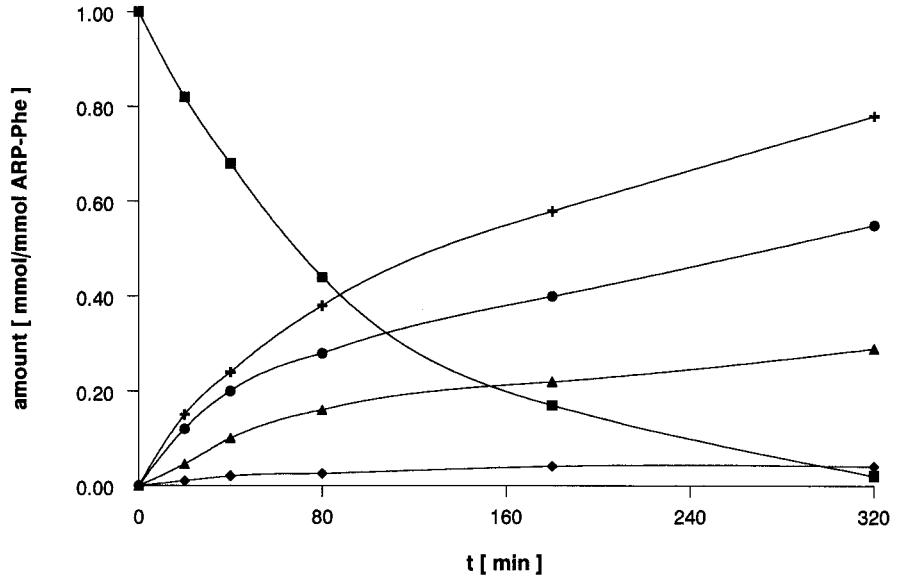

Figure 2. Formation of α-dicarbonyls and L-phenylalanine during degradation of N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) under an atmosphere of argon (■, ARP-Phe; ●, L-phenylalanine; +, 1-deoxy-2,3-hexodiulose; ▲, 3-deoxy-1,2-hexodiulose; ♦, 1,2-hexodiulose).

formation was slightly favored. Addition of copper ions to the aerobic system (exp. III, Table 2) led to a further increase of the aldehyde and the acid from both precursor systems, but again the aldehyde was preferably formed from the ARP-Phe, whereas the reverse was true for the binary precursor mixture Glc/Phe. It should be stressed that in any experiment the yields of both aroma compounds were higher from the ARP-Phe.

Formation of Strecker Aldehydes from the Amadori Compound. The differences in aldehyde and acid formation suggest the potential existence of a different pathway in Strecker aldehyde formation from the binary sugar/amino acid mixture compared to that from the corresponding Amadori compound .

To study its formation in more detail, first, the time course of ARP-Phe generation from glucose/phenylalanine was measured. In parallel, the degradation of L-phenylalanine was followed. Compared to the amount of L-phenylalanine reacted, a quite high concentration of the ARP-Phe was already present after 10 minutes (52.5%; Table 3). After 30 min of thermal treatment, the Amadori compound reached a maximum concentration of 48 μmol. However, compared to the amount of L-phenylalanine degraded, the "yield" was lower compared to the yield after 10 min. A further increase in the reaction time led to a drastic decrease of the amounts of the ARP-Phe formed. After 300 min, about 60% of the L-phenylalanine was degraded, but only 1.5% of the ARP-Phe was left.

Based on the results one might speculate that in the first stages of carbohydrate/amino acid reactions, the Amadori compound is the most favored intermediate, whereas in later stages, undoubtedly due to the formation of α-dicarbonyls, the amino acid is more and more involved in other reactions, such as the Strecker degradation. This speculation is strengthened by our recent results demonstrating that short-chain dicarbonyls, such as glyoxal and 2-oxopropanal, are quite rapidly formed from glucose/phenylalanine, in particular, in the presence of oxygen (Hofmann et al., 1999). It has to be

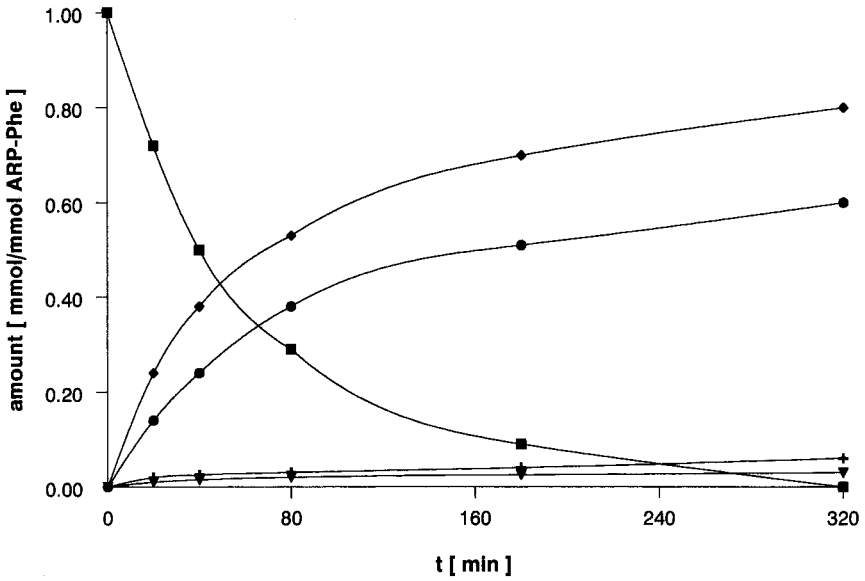

Figure 3. Formation of α-dicarbonyls and L-phenylalanine by a degradation of N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) in the presence of air oxygen and copper II ions (■, ARP-Phe; ●, L-phenylalanine; +, 1-deoxy-2,3-hexodiulose; ▲, 3-deoxy-2-hexosulose; ♦, 1,2-hexodiulose).

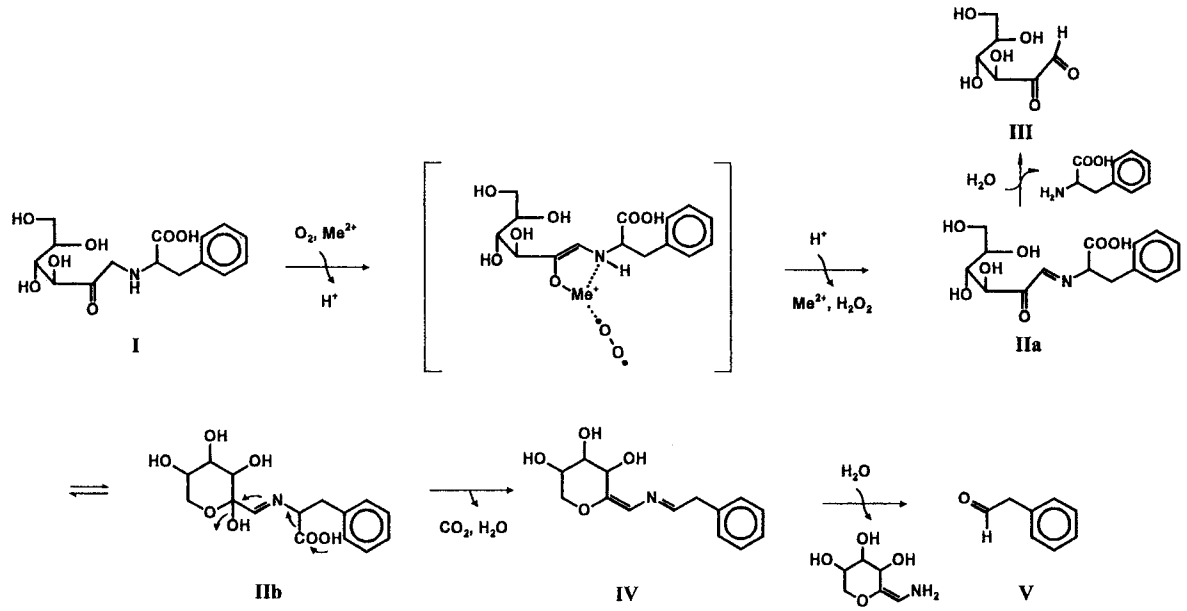

Figure 4. Formation of phenylacetaldehyde via an oxidative degradation of N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe).

stated, however, that labeling experiments are necessary to determine the role of ARP in the whole reaction cascade.

To gain some insight into the mechanism of the oxygen-dependent formation of phenylacetaldehyde, the thermal degradation of pure ARP-Phe was studied under aerobic and anaerobic conditions. In these experiments, the α-dicarbonyls formed were trapped in situ by a reaction with 1,2-diamino benzene. Heating the ARP-Phe in an atmosphere of argon generated high amounts of the 1-deoxyosone (+ in Figure 2), in particular after longer reaction times. In contrast, 1,2-hexodiulose (glucosone) was formed in very low amounts (♦ in Figure 2). In parallel with the degradation of the ARP-Phe (■ in Figure 2), phenylalanine was liberated during the thermal process reaching a yield of about 55% after 320 min (● in Figure 2).

On the contrary, in a second experiment performed in the presence of air oxygen and trace amounts of copper(II) ions (Figure 3), 1,2-hexodiulose (♦ in Figure 3) was the main α-dicarbonyl formed from the Amadori compound, whereas both the 1- and the 3-deoxyosone (+ and ▲ in Figure 3) were generated in only low yields. The liberation of phenylalanine from the ARP-Phe (● in Figure 3) was in the same order of magnitude as that from the reaction under anaerobic conditions.

These data allow proposal of the following new mechanism for the formation of the Strecker aldehyde phenylacetaldehyde via a direct degradation of the respective Amadori compound without α-dicarbonyls present: Catalyzed by oxygen and metal ions the aminoketone function of the open chain ARP-Phe (I in Figure 4) may be oxidized into the iminoketone IIa, which, upon subsequent hydrolysis, might then liberate the 2-hexosulose (III in Figure 4). This reaction is obviously suppressed in the absence of oxygen (cf. Figure 3), and the data are well in line with results reported earlier by Kawakishi et al. (1991), Hofmann and Schie-

berle (1996), and Hofmann (1998) for similar compounds having an α-amino ketone function.

However, if IIa is stabilized by formation of the cyclic hemi ketal (IIb in Figure 4), a decarboxylation of this aza vinylogous β-keto acid and subsequent dehydration forms the enamine imine (IV), which upon hydrolysis generates phenylacetaldehyde (V).

In the absence of oxygen, the ARP-Phe (I in Figure 4) should preferably eliminate L-phenylalanine, thereby forming the 1-desoxyosone as the main degradation product. This is corroborated by the results given in Figure 2.

These data clearly suggest that, in particular under aerobic conditions, an alternative mechanism leading from α-amino acids to Strecker aldehydes does exist.

CONCLUSION

The results have shown that, besides the well-established Strecker reaction of dicarbonyls and amino acids, aroma-active Strecker aldehydes can also be formed by an alternative reaction route via an oxidative degradation of Amadori compounds. This novel information opens further possibilities in industrial processes, e.g., to manipulate the formation of desired odorants during reaction flavor manufacturing.

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