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Chemical Conversion of α-Amino Acids into α-Keto Acids by 4,5-Epoxy-2-decenal

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The comparative formation of phenylalanine and phenylpyruvic acid in the reaction of 4,5-epoxy-2-decenal with phenylalanine was studied to determine whether epoyalkenals may also degrade amino acids without producing their decarboxylation. Both compounds were produced in the reaction to an extent that depended on the reaction pH, the amount of lipid oxidation product, and the reaction time and temperature. The optimum pH was 3 for producing both carbonyl derivatives, and the amount of both compounds increased linearly with the amount of epoxyalkenal present in the reaction mixture. In addition, phenylpyruvic acid was produced to a higher extent than phenylacetaldehyde at 37 °C. However, at 60 °C the degradation of phenylpyruvic acid was observed and phenylacetaldehyde was usually found to a higher extent than the α-keto acid in the overnight-incubated reaction mixtures. The degradation of phenylpyruvic acid produced benzaldehyde and phenylacetaldehyde. All these results suggest that epoxyalkenals can not only degrade amino acids by a Strecker-type mechanism but convert them into their corresponding α-keto acids. This new reaction may be an alternative chemical route for the formation in foods of α-keto acids, which can later participate in the generation of important amino acid-derived flavor compounds.

KEYWORDS: Carbonyl−amine reactions; epoxyalkenals; flavors; lipid oxidation; Maillard reaction; phenylacetaldehyde; phenylalanine; phenylpyruvic acid; Strecker aldehydes

INTRODUCTION

Analogously to carbohydrates, appropriate oxidized lipids having two oxygenated functions (one of them being a carbonyl group and the other either an epoxy or a hydroxy group) are able to degrade amino acids by a Strecker-type mechanism (*1*−*3*). The reaction is believed to be produced through imine formation, which is then decarboxylated and hydrolyzed. The reaction products are the corresponding Strecker aldehydes of the amino acids and an unsaturated amine, which is structurally related to the starting oxidized lipid and later suffers a cyclization reaction to produce significant food flavors. Thus, epoxyalkenals have been suggested to be one of the origins of 2-pentylpyridine, when starting from $n - 6$ fatty acids, and 2-ethylpyridine, when starting from $n - 3$ fatty acids. These pyridine derivatives have been found in processed foods (*4*−*7*). In addition, 4-hydroxy-2-nonenal has been related to the origin of 2-pentyl-1*H*-pyrrole, which has also been found in processed foods (*8*−*10*).

In addition to amino acids, amines can also be degraded by an analogous mechanism to their corresponding aldehydes. Thus, octylamine, benzylamine, and 2-phenylglycine methyl ester were converted into their corresponding Strecker aldehydes (octanal, benzaldehyde, and methyl 2-oxo-2-phenylacetate, respectively) when incubated in the presence of 4,5-epoxy-2-decenal (*11*). Therefore, decarboxylation might not be an essential step in the Strecker-type degradation of amino acids, and two different pathways might be competing for amino acid degradation by oxidized lipids: the previously described pathway that involves decarboxylation to produce the corresponding Strecker aldehydes and an alternative pathway that would produce α-keto acids.

This investigation was undertaken to clarify whether lipid oxidation products may also degrade amino acids without producing their decarboxylation and compare the relative contributions of both mechanisms. 4,5-Epoxy-2-decenal was selected as model oxidized lipid because it is a common secondary product of lipid peroxidation that has been detected in many different food systems (*12*) and has been shown to degrade both amino acids and amines (*1, 11*). Phenylalanine was selected as a model amino acid because both its Strecker aldehyde is a very powerful odorant (*13*) and its α-keto acid is the origin of the amino acid-derived flavor benzaldehyde in fermented foods (*14*).

MATERIALS AND METHODS

Materials. 2,4-Decadienal, phenylalanine, phenylpyruvic acid, phenylacetaldehyde, benzaldehyde, 2-pentylpyridine, *O*-(2,3,4,5,6-pen-

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tafluorobenzyl)hydroxylamine hydrochloride, and *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide were purchased from Aldrich (Milwaukee, WI). All other chemicals were analytical grade and purchased from reliable commercial sources.

4,5-Epoxy-2-decenal was prepared from 2,4-decadienal as described previously (*15*). Briefly, 3-chloroperoxybenzoic acid (25 mmol) was dissolved in chloroform (175 mL), washed with three 100 mL portions of buffer (0.2 M $Na_2HPO_4 \cdot 12H_2O$ adjusted to pH 7.5 with 0.1 M citric acid monohydrate) followed by three 100 mL portions of water, and dried with anhydrous sodium sulfate. This solution was added slowly (25 mL every 10 min) to a solution of 2,4-decadienal (3.0 g, 19.7 mmol) in chloroform (29 mL), which was stirred at room temperature. The reaction mixture was then stirred overnight and, finally, washed with three 100 mL portions of buffer (0.2 M $Na_2HPO_4 \cdot 12H_2O$ adjusted to pH 7.5 with 0.1 M citric acid monohydrate) followed by three 100 mL portions of water to remove 3-chlorobenzoic acid. The organic solution was dried with anhydrous sodium sulfate and concentrated under vacuum. The residue was fractionated by column chromatography using hexane/acetone (95:5) as eluent. 4,5-Epoxy-2-decenal was obtained chromatographically pure. Additional confirmations of identity and purity were obtained by ^1H and ^{13}C NMR and GC-MS.

4,5-Epoxy-2-decenal/Phenylalanine Reaction Mixtures. A solution of 0−31 μmol of 4,5-epoxy-2-decenal and 25 μmol of phenylalanine in 500 μL of acetonitrile−sodium citrate or sodium phosphate buffer (2:1) was heated at 37 or 60 °C. Incubated mixtures were analyzed for phenylacetaldehyde and phenylpyruvic contents. For phenylacetaldehyde analysis, incubated samples (75 μL) were diluted with 125 μL of acetonitrile−water (2:1) and 25 μL of internal standard solution [337 μg of 3-(*Z*)-nonenol in 1 mL of methanol] and analyzed by GC-MS. Phenylpyruvic acid was determined according to Lee et al. (*16*) using a slightly modified procedure. Briefly, incubated samples (100 μL) were diluted with 200 μL of acetonitrile−water (2:1) and 50 μL of internal standard solution [386 μg of stearic acid in 1 mL of methanol] and treated with 4.6 mg of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride. This reaction mixture was maintained for 2 h at 37 °C and then taken to dryness. The obtained residue was derivatized with 150 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide for 30 min at 60 °C and analyzed by GC-MS.

Phenylpyruvic Acid/Phenylalanine Reaction Mixtures. A solution of 0.5 μmol of phenylpyruvic acid and 25 μmol of phenylalanine in 500 μL of acetonitrile−sodium citrate buffer (2:1), pH 3.0, was heated at 60 °C. Incubated mixtures were analyzed for phenylacetaldehyde and benzaldehyde contents. Incubated samples (75 μL) were diluted with 125 μL of acetonitrile−water (2:1) and 25 μL of internal standard solution [337 μg of 3-(*Z*)-nonenol in 1 mL of methanol] and analyzed by GC-MS.

GC−MS Analyses. GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (Mass Selective Detector-Quadrupole type). A fused silica HP5-MS capillary column (30 × 0.25 mm i.d.; coating thickness 0.25 μm) was used. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector temperature, 250 °C; oven temperature, from 70 (1 min) to 240 °C at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV.

Determination of Phenylacetaldehyde, Phenylpyruvic acid, and Benzaldehyde Contents. Quantification of phenylacetaldehyde and benzaldehyde was carried out by preparing standard curves over a concentration range of 2−60 nmol of phenylacetaldehyde or 5−60 nmol of benzaldehyde in the 225 μL of solution prepared for GC-MS injection (see above). For each curve five different concentration levels of the aldehyde were used. Phenylacetaldehyde and benzaldehyde contents were directly proportional to the aldehyde/internal standard area ratio ($r > 0.99$, $p < 0.0001$). The coefficients of variation within this range were lower than 5%.

Quantification of phenylpyruvic acid was carried by preparing standard curves over a concentration of 15−400 nmol of phenylpyruvic acid in the 100 μL of sample used in the derivatization reaction (see above). For each curve five different concentration levels of the aldehyde were used. Phenylacetaldehyde content was directly proportional to the aldehyde/internal standard area ratio ($r > 0.99$, $p < 0.0001$). The coefficients of variation within this range were lower than 5%.

Table 1. Retention Indices and Mass Spectra of Compounds Determined in This Study[a]

compound	retention index	mass spectrum
benzaldehyde	960	106 (100), 105 (98), 77 (89), 51 (37)
phenylacetaldehyde	1049	120 (23), 92 (22), 91 (100), 65 (16)
phenylpyruvic acid (derivatized)	2015	431 (3), 416 (5), 189 (14), 181 (46), 116 (34), 91 (23), 73 (100)

[a] Structures for these compounds are given in **Scheme 1**.

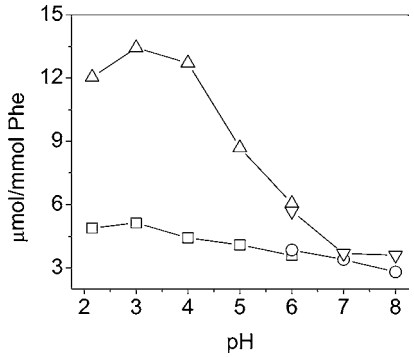


Figure 1. Effect of pH on phenylacetaldehyde (□ and ○) and phenylpyruvic acid (△ and ▽) formation in the reaction of 4,5-epoxy-2-decenal with phenylalanine (Phe) in acetonitrile/buffer (2:1) after 21 h at 37 °C. The employed buffers were 50 mM sodium citrate buffer for pH 2.15–6 (□ and △) and 50 mM sodium phosphate buffer for pH 6–8 (○ and ▽).

RESULTS

Effect of pH in the Formation of Both Phenylacetaldehyde and Phenylpyruvic Acid in 4,5-Epoxy-2-decenal/Phenylalanine Reaction Mixtures. As described previously (*1*), direct injection of epoxydecenal/phenylalanine reaction mixtures allowed detection of phenylacetaldehyde by GC-MS. However, when the incubated reaction mixtures were derivatized, the presence of phenylpyruvic acid was also observed. Both compounds were identified unambiguously by comparison with the retention indices and mass spectra of authentic compounds. Retention indices and mass spectra of compounds determined in this study are collected in **Table 1**.

Both carbonyl derivatives of the amino acid were produced simultaneously in the reaction mixtures to an extent that depended on the pH. **Figure 1** shows the effect of pH in the production of both compounds after 21 h at 37 °C. Both phenylpyruvic acid and phenylacetaldehyde were produced to a higher extent at pH 2−4, and the amount of phenylpyruvic acid was ∼2.5 times the amount of phenylacetaldehyde. This preference for the formation of phenylpyruvic acid was reduced at a higher pH. The rest of this study was carried out at pH 3 because the highest amounts of both compounds were produced at this pH.

Effect of 4,5-Epoxy-2-decenal Concentration in the Formation of Both Phenylacetaldehyde and Phenylpyruvic Acid. The amount of phenylacetaldehyde and phenylpyruvic acid produced depended on the concentration of the epoxyalkenal added, therefore confirming the role of this lipid oxidation product in the degradation of the amino acid. **Figure 2** shows the formation of both phenylacetaldehyde and phenylpyruvic acid after 21 h at pH 3 and 37 °C as a function of epoxyalkenal concentration. The concentration of both carbonyl derivatives increased linearly ($r > 0.995$, $p < 0.0001$) with the concentration of epoxyalkenal, but both lines were not parallel, and a higher amount of epoxyalkenal favored the production of phenylpyruvic

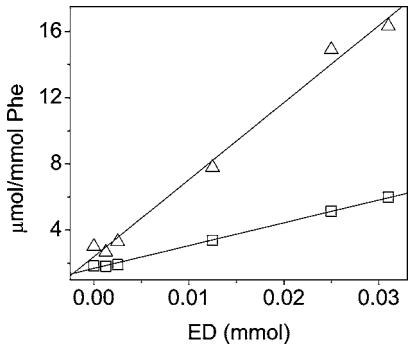


Figure 2. Effect of epoxyalkenal (ED) concentration on phenylacetaldehyde (□) and phenylpyruvic acid (△) formation in the reaction of 4,5-epoxy-2-decenal with phenylalanine (Phe) in acetonitrile/sodium citrate (2:1), pH 3, after 21 h at 37 °C.

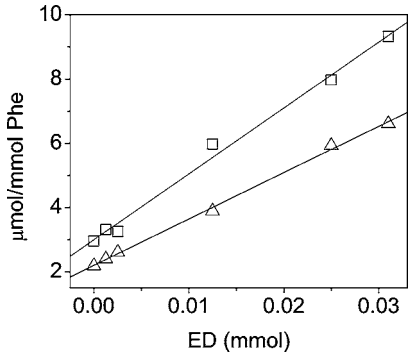


Figure 3. Effect of epoxyalkenal (ED) concentration on phenylacetaldehyde (□) and phenylpyruvic acid (△) formation in the reaction of 4,5-epoxy-2-decenal with phenylalanine (Phe) in acetonitrile/sodium citrate (2:1), pH 3, after 21 h at 60 °C.

acid more than the production of phenylacetaldehyde. The ratio between the slopes of both lines was 3.4.

When the effect of epoxyalkenal concentration was studied after 21 h at 60 °C (**Figure 3**), the formation of both phenylpyruvic acid and phenylacetaldehyde also increased linearly as a function of epoxydecenal concentration ($r > 0.996$, $p < 0.0001$). However, the determined phenylacetaldehyde content was higher than the phenylpyruvic content. This was a consequence of both the amount of phenylacetaldehyde produced being higher at 60 °C than at 37 °C and the amount of phenylpyruvic acid determined after 21 h at 60 °C being smaller than that determined at 37 °C (see below).

Effect of Incubation Time in the Formation of Both Phenylacetaldehyde and Phenylpyruvic Acid in 4,5-Epoxy-2-decenal/Phenylalanine Reaction Mixtures. The formation of both phenylacetaldehyde and phenylpyruvic acid as a function of incubation time at pH 3 and 37 °C is shown in **Figure 4**. As observed, phenylpyruvic content increased linearly during the whole studied period ($r = 0.992$, $p < 0.0001$). The slope of the obtained line was 0.530. On the contrary, phenylacetaldehyde content increased linearly during the first 3 h, producing an amount of aldehyde very similar to that of phenylpyruvic acid (the slope of the obtained line was 0.541), and then it increased much more slowly for the next 21 h (the slope of this new line was 0.075).

Something similar also occurred at 60 °C (**Figure 5**). The formation of phenylacetaldehyde also increased very rapidly during the first hour and then increased much more slowly for the next 24 h. The amount of phenylacetaldehyde produced at the end of the studied period was higher at 60 °C than at 37 °C. Phenylpyruvic acid content also increased linearly for

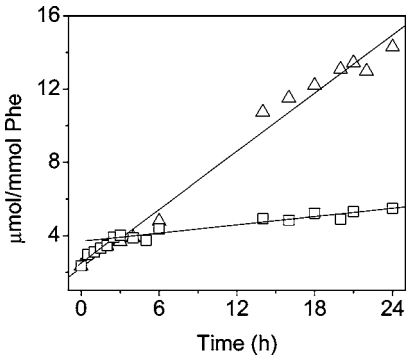


Figure 4. Time course of phenylacetaldehyde (□) and phenylpyruvic acid (△) formation in the reaction of 4,5-epoxy-2-decenal with phenylalanine (Phe) in acetonitrile/sodium citrate (2:1), pH 3, at 37 °C. The reaction was carried out with 25 µmol of 4,5-epoxy-2-decenal and 25 µmol of phenylalanine in 500 µL of solvent.

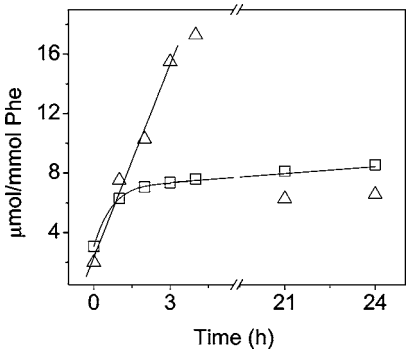


Figure 5. Time course of phenylacetaldehyde (□) and phenylpyruvic acid (△) formation in the reaction of 4,5-epoxy-2-decenal with phenylalanine (Phe) in acetonitrile/sodium citrate (2:1), pH 3, at 60 °C. The reaction was carried out with 25 µmol of 4,5-epoxy-2-decenal and 25 µmol of phenylalanine in 500 µL of solvent.

the first 3 h ($r = 0.993$, $p = 0.007$), and the value achieved after this time was higher than the value achieved after 24 h at 37 °C. However, at 60 °C phenylpyruvic acid content decreased after achieving a maximum and the amount of phenylpyruvic acid determined after 21−24 h was lower than that of phenylacetaldehyde after the same time at 37 °C.

Degradation of Phenylpyruvic Acid in Phenylpyruvic Acid/Phenylalanine Reaction Mixtures. The decrease observed in the concentration of phenylpyruvic acid after incubation at 60 °C was a consequence of its degradation. Phenylpyruvic acid has been shown to be chemically degraded to flavor substances (*14*). This also happened when a phenylpyruvic acid/phenylalanine mixture, similar to that produced in the epoxyalkenal/phenylalanine reaction mixture, was incubated at 60 °C. Benzaldehyde and phenylacetaldehyde were produced in the degradation of phenylpyruvic acid and unambiguously identified on the basis of their retention indices and mass spectra (**Table 1**). **Figure 6** shows that both aldehydes were produced by degradation of phenylpyruvic acid and their concentration increased linearly as a function of incubation time ($r > 0.992$, $p < 0.008$). Benzaldehyde was produced to a slightly higher extent than phenylaceldehyde, and the slopes of both lines were very similar.

DISCUSSION

Many flavors in fermented products are derived from amino acids. Benzaldehyde, for example, is mostly produced enzymatically during cheese fermentation. Thus, protein degradation is

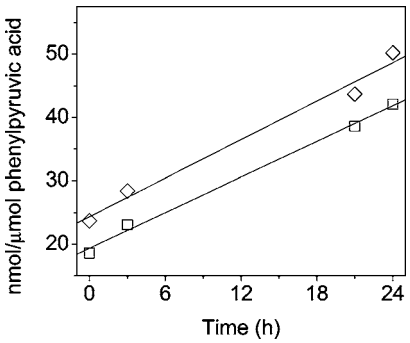
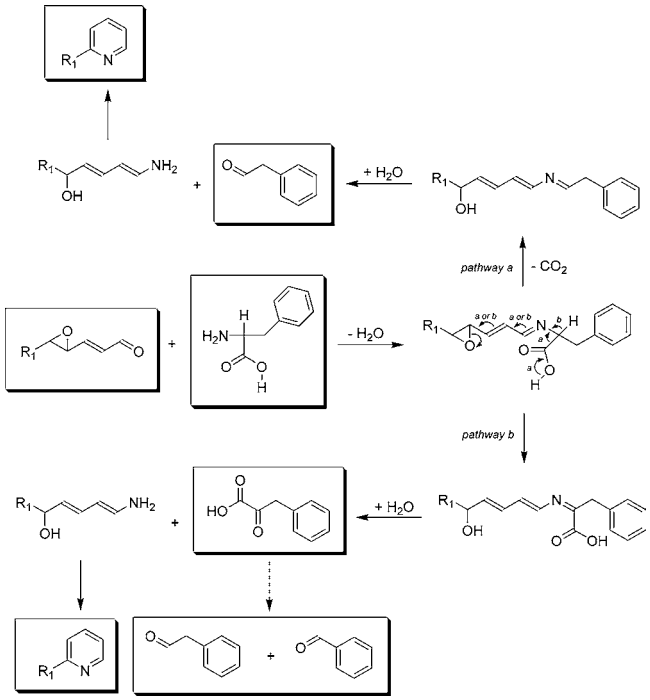


Figure 6. Time course of phenylacetaldehyde (□) and benzaldehyde (◇) formation in the reaction of phenylpyruvic acid with phenylalanine in acetonitrile/sodium citrate (2:1), pH 3, at 60 °C. The reaction was carried out with 0.5 μmol of 4,5-epoxy-2-decenal and 25 μmol of phenylalanine in 500 μL of solvent.

Scheme 1. Formation of Flavor Compounds in the Reaction of 4,5-Epoxy-2-alkenals with Phenylalanine[a]



[a] R_1 = Pentyl for 4,5-epoxy-2-decenal.

initiated by proteolysis and peptidolysis, leading to free amino acids (*17*−*19*). The produced phenylalanine is then transaminated in the bacterial cell to the corresponding phenylpyruvic acid (*20*), and this compound is finally converted to various metabolites, including benzaldehyde, both enzymatically and chemically (*14*, *21*, *22*).

The results obtained in this study suggest that benzaldehyde, and other significant flavor compounds, can also be produced chemically as a consequence of lipid oxidation. Thus, some of the reactive carbonyls produced during polyunsaturated fatty acid oxidation are able to degrade amino acids. Among them, epoxyalkenals have been found to convert phenylalanine into phenylpyruvic acid and phenylacetaldehyde. The proposed mechanism for these reactions is shown in **Scheme 1**.

The reaction of the epoxyalkenal and the amino acid produces in a first step the corresponding imine, which may suffer then two alternative rearrangements. The loss of carbon dioxide (pathway a) is the origin of a new decarboxylated imine that, after hydrolysis, produces the corresponding Strecker aldehyde and an unsaturated hydroxylamine. The cyclization reaction of this hydroxylamine produces the 2-pentylpyridine identified in these reactions. An alternative pathway (pathway b), which does not need the decarboxylation of the imine, is the origin of phenylpyruvic acid. It also produces the same unsaturated hydroxylamine that, after a cyclization reaction, also produces the corresponding 2-pentylpyridine.

According to the results obtained in this study, pathway b seems to be produced to a higher extent than pathway a. However, depending on the reaction conditions, phenylpyruvic acid may be found in a smaller concentration than phenylacetaldehyde. This is related to the relative instability of phenylpyruvic acid. This α-keto acid has been shown to be easily oxidized to produce benzaldehyde, among other compounds (*23*, *24*). Although conversion of phenylpyruvic acid to benzaldehyde has also been described to be accompanied with the formation of oxalic acid (*14*), this acid was not found in the present study (data not shown). In addition, in the present study, conversion of phenylpyruvic acid into phenylacetaldehyde was also observed.

All these results suggest that epoxyalkenals can not only degrade amino acids by a Strecker-type mechanism but convert them into their corresponding α-keto acids. This new reaction may be an alternative chemical route for the formation in foods of α-keto acids, which can later participate in the generation of important amino acid-derived flavor compounds.

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