Strecker-Type Degradation of Phenylalanine Initiated by 4-Oxo-2-alkenals in Comparison to That Initiated by 2,4-Alkadienals, 4,5-Epoxy-2-alkenals, or 4-Hydroxy-2-nonenal

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ABSTRACT: The conversion of phenylalanine to phenylacetaldehyde as a consequence of its reaction with 4-oxo-2-alkenals was studied both to characterize the reaction pathway and to compare the reactivities and kinetic constants of oxoalkenals with those of other lipid oxidation products: 2,4-alkadienals, 4,5-epoxy-2-alkenals, and 4-hydroxy-2-nonenal. Oxoalkenals produced the Strecker aldehyde through imine formation, which was then decarboxylated and hydrolyzed. In the course of the reaction the lipid was converted into an unsaturated hydroxylamine that eventually cycled to 2-alkylpyrrole. The E_a of phenylacetaldehyde formation in the presence of oxoalkenals was 55−64 kJ/mol. This E_a was similar to the E_a determined for the other tertiary lipid oxidation products assayed (58−67 kJ/mol), but higher than the E_a determined for alkadienals (28−38 kJ/mol). However, this difference in E_a only correlated with the amount of phenylacetaldehyde produced at 37 °C. At higher temperatures, 4-oxo-2-nonenal was the lipid-derived carbonyl compound that produced the highest amount of the Strecker aldehyde, therefore pointing to this oxoalkenal as the most efficient Strecker aldehyde forming compound derived from lipids. For this reason, oxoalkenals should be expected to play a significant role in reactions in which Strecker aldehydes are recognized intermediates, as occurs in the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP).

KEYWORDS: carbonyl−amine reactions, lipid oxidation, Maillard reaction, oxoalkenals, Strecker aldehydes

INTRODUCTION

Strecker degradation of amino acids is an important pathway for flavor formation during the heating of foodstuffs, as well as in the preparation of process flavors.[1−3] This reaction is initiated by α-dicarbonyl or hydroxycarbonyl compounds formed by carbohydrate degradation in the Maillard reaction.[4] In addition, lipid-derived reactive carbonyls are also able to react with amino acids to produce the same Strecker aldehydes. The mechanism for this last reaction was first described in 2004 for epoxyalkenals under very mild conditions,[5] and later extended to primary and secondary products of lipid oxidation.[6,7] In the course of this reaction the lipid-derived carbonyls are modified and new lipid-derived compounds are formed, including pyridine and pyrrole derivatives.[5,8] These compounds also contribute to the changes in the sensorial properties observed in foods in which these reactions have been produced.[9]

Among the different lipid-derived reactive carbonyls, a recent study has suggested that oxoalkenals might produce the Strecker degradation of amino acids to a higher extent than other lipid oxidation products because these aldehydes are the most active compounds to produce the heterocyclic aromatic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a reaction that requires the Strecker degradation of phenylalanine.[10] However, the ability of oxoalkenals to convert phenylalanine into phenylacetaldehyde has not been studied to date.

The first objective of the present study was to investigate the Strecker degradation of amino acids produced by oxoalkenals and to characterize the products formed in these reactions. The second objective was to compare the reactivities of different lipid-derived carbonyls for this reaction so that the contribution of the different lipid oxidation products to the formation of the Strecker aldehydes can be better understood. All of these studies were carried out using phenylalanine as model amino acid. This amino acid was employed because its corresponding Strecker aldehyde phenylacetaldehyde is a very powerful odorant.[11] In addition, it has been suggested as a key intermediate in the formation of PhIP.[12]

MATERIALS AND METHODS

Chemicals. 4-Oxo-2-nonenal and 4-oxo-2-hexenal were prepared from 2-pentylfuran and 2-ethylfuran, respectively, with N-bromosuccinimide.[13,14] 4,5-Epoxy-2-heptenal and 4,5-epoxy-2-decenal were prepared by epoxidation of 2,4-heptadienal and 2,4-decadienal, respectively, with 3-chloroperoxybenzoic acid.[15,16] 4-Hydroxy-2-nonenal was synthesized according to the procedure described by Gardner et al.[17] All other chemicals were purchased from Aldrich (Milwakee, WI, USA), Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany) and were of analytical grade.

Phenylalanine/Lipid-Derived Reactive Carbonyl Reaction Mixtures. Mixtures of phenylalanine (10 μmol) and 0−10 μmol of the lipid derivative in 0.5 mL of buffer were introduced in Schott Duran test tubes (16 × 1.5 cm), which were closed and heated in a heater block at 120−190 °C for 0−1 h. The atmosphere of the test tube was air, unless otherwise indicated. The buffers employed for controlling the reaction pH were 0.3 M sodium citrate buffer, pH 2.15−5.0; 0.3 M sodium phosphate buffer, pH 6.0−8.0; and 0.3 M

sodium borate buffer, pH 9.0−10. At the end of the heating period, samples were cooled, diluted with 1 mL of acetonitrile and 50 μL of internal standard solution (54.8 mg of methyl heptanoate in 25 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 m × 0.25 i.d.; coating thickness, 0.25 μm) was used, and 1 μL of sample was injected in the pulsed splitless mode. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature programmed from 40 °C (1 min) to 240 °C at 5 °C/min and then to 300 °C at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV; ion source temperature, 230 °C; and mass range 28−550 amu.

Determination of Phenylacetaldehyde Content. Quantification of phenylacetaldehyde was carried out, as described previously,[6] by preparing standard curves of the aldehyde in the 1.55 mL of solution prepared for GC-MS injection (see above). For each curve, eight different concentration levels of the aldehyde were used. Phenylacetaldehyde content was directly proportional to the aldehyde/internal standard area ratio ($r = 0.999$, $p < 0.0001$). The coefficients of variation were <10%.

Statistical Analysis. All data given are mean values of several independent determinations. Statistical comparisons among different groups were made using analysis of variance. When significant F values were obtained, group differences were evaluated by the Tukey test.[18] Statistical comparisons were carried out using Origin v. 7.0 (OriginLab Corp., Northampton, MA, USA). The significance level is $p < 0.05$ unless otherwise indicated.

■ RESULTS

Formation of Phenylacetaldehyde in the Reaction of Phenylalanine with Oxoalkenals. When phenylalanine was

Table 1. Retention Indices of the Products Formed in Phenylalanine/Oxoalkenal Reaction Mixtures

compound	retention index
styrene	893
2-ethylpyrrole[a]	915
benzaldehyde	960
phenylacetaldehyde	1047
phenylethylamine	1097
2-pentylpyrrole[b]	1198
benzenepropionic acid	1332
cinnamic acid	1467
2-ethyl-1-phenethyl-1H-pyrrole[a]	1604
2-pentyl-1-phenethyl-1H-pyrrole[b]	1885

[a]This compound was observed only in the reaction between 4-oxo-2-hexenal and phenylalanine. [b]This compound was observed only in the reaction between 4-oxo-2-nonenal and phenylalanine.

heated alone, the formation of a small amount of phenylacetaldehyde was observed. However, when phenylalanine was heated in the presence of oxoalkenals, the Strecker degradation of the phenylalanine was produced to a much higher extent than when phenylalanine was heated in the absence of the oxidized lipid. In addition, other parallel reactions were also produced according to the different compounds produced in the reaction (Table 1). All of these compounds were identified on the basis of their retention indices and mass spectra in comparison with the retention indices and mass spectra of pure reference compounds with the exception of benzenepropionic acid. Therefore, the identification of this last compound should be considered only tentative. All of these products were either derived from the amino acid or from the lipid. Amino acid
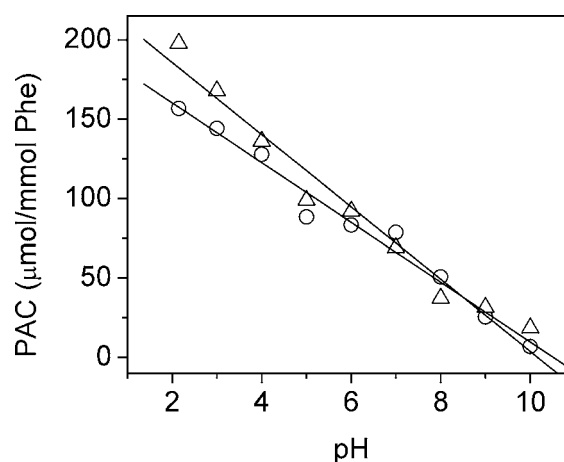

Figure 1. Effect of pH on the formation of phenylacetaldehyde (PAC) in the reaction of phenylalanine (Phe) with either 4-oxo-2-hexenal (○) or 4-oxo-2-nonenal (△) at 180 °C for 1 h. The employed buffers were sodium citrate for pH 2.15−5, sodium phosphate for pH 6−8, and sodium borate for pH 9−10.
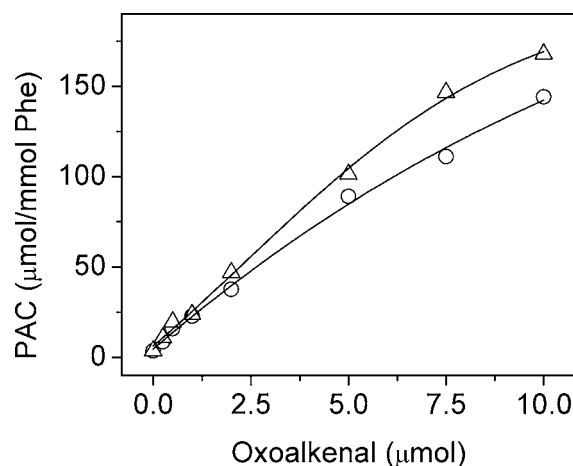

Figure 2. Effect of oxoalkenal concentration on the formation of phenylacetaldehyde (PAC) in the reaction of phenylalanine (Phe) with either 4-oxo-2-hexenal (○) or 4-oxo-2-nonenal (△) at 180 °C for 1 h and pH 3.

degradation produced six compounds: the Strecker aldehyde (phenylacetaldehyde), the aldehyde with two fewer carbon atoms than the original amino acid (benzaldehyde), the amine produced by amino acid decarboxylation (phenylethylamine), the saturated acid (benzenepropionic acid), the unsaturated acid (cinnamic acid), and the olefin produced by amine elimination (styrene). The compounds related to the oxoalkenal were different if the reaction was carried out with 4-oxo-2-hexenal (2-ethylpyrrole and 2-ethyl-1-phenethyl-1H-pyrrole) or 4-oxo-2-nonenal (2-pentylpyrrole and 2-pentyl-1-phenethyl-1H-pyrrole).

Among all of these reaction products, the compounds produced as a consequence of the Strecker degradation of the amino acid were phenylacetaldehyde (the Strecker aldehyde) and 2-alkylpyrroles (the lipid-derived products of the Strecker reaction, see below). The formation of 2-ethyl-1-phenethyl-1H-pyrrole and 2-pentyl-1-phenethyl-1H-pyrrole was the consequence of carbonyl−amine reactions between the amino group of the amino acid and the carbonyl groups of the oxoalkenal. This reaction is similar to the reaction between amino acids and
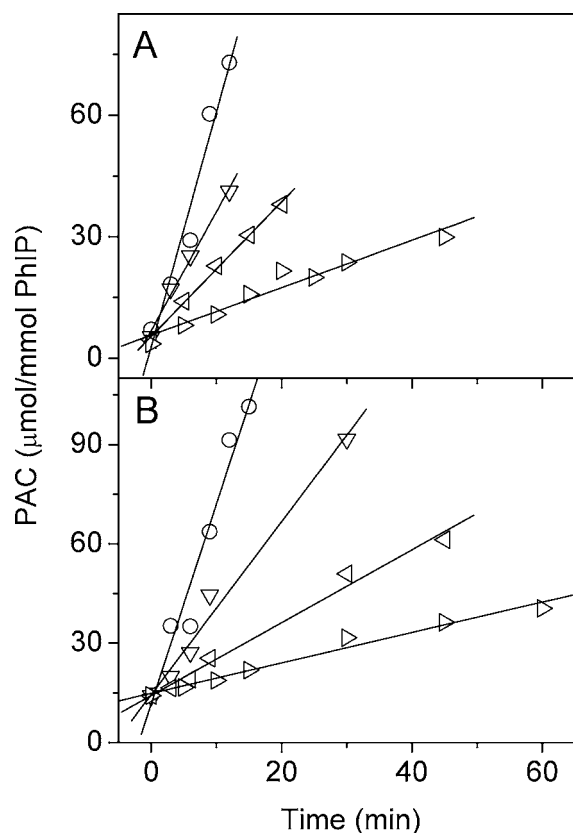
Figure 3. Effect of reaction time and temperature on the formation of phenylacetaldehyde (PAC) in the reaction of phenylalanine (Phe) with either 4-oxo-2-hexenal (A) or 4-oxo-2-nonenal (B) at pH 3. Temperatures assayed were 180 (○), 160 (▽), 140 (◁), and 120 °C (▷).

4,5-epoxy-2-alkenals described previously.[19] In addition, the formation of phenylethylamine and styrene as a consequence of amino acid/oxidized lipid reactions has been described previously,[20,21] and the formation of benzaldehyde in the course of these reactions is also very common.[6] On the other hand, to our best knowledge the formation of acids in amino acid/oxidized lipid reactions has not been yet studied in detail.

Effect of Reaction Conditions (pH, Concentration of Lipid Oxidation Product, Time, and Temperature) on the Amount of Phenylacetaldehyde Produced in the Reaction of Phenylalanine with Oxoalkenals. The amount of phenylacetaldehyde produced by phenylalanine degradation in the presence of oxoalkenals decreased linearly ($r < -0.983$, $p < 0.0001$) as a function of reaction pH (Figure 1). Because previous studies on the role of lipid oxidation products on the Strecker degradation of phenylalanine were carried out at pH 3, this pH was also employed for the rest of the experiments carried out in this study.

Phenylacetaldehyde formation was mostly a consequence of the presence of the oxoalkenals and the amount of the produced aldehyde increased as a function of oxoalkenal concentration (Figure 2). This increase was not linear and higher increases were observed at low concentrations of oxoalkenals than when high concentrations of oxoalkenals were employed.

Phenylacetaldehyde formation in the Strecker degradation of phenylalanine by oxoalkenals also depended on reaction time and temperature (Figure 3). Thus, the amount of phenyl-
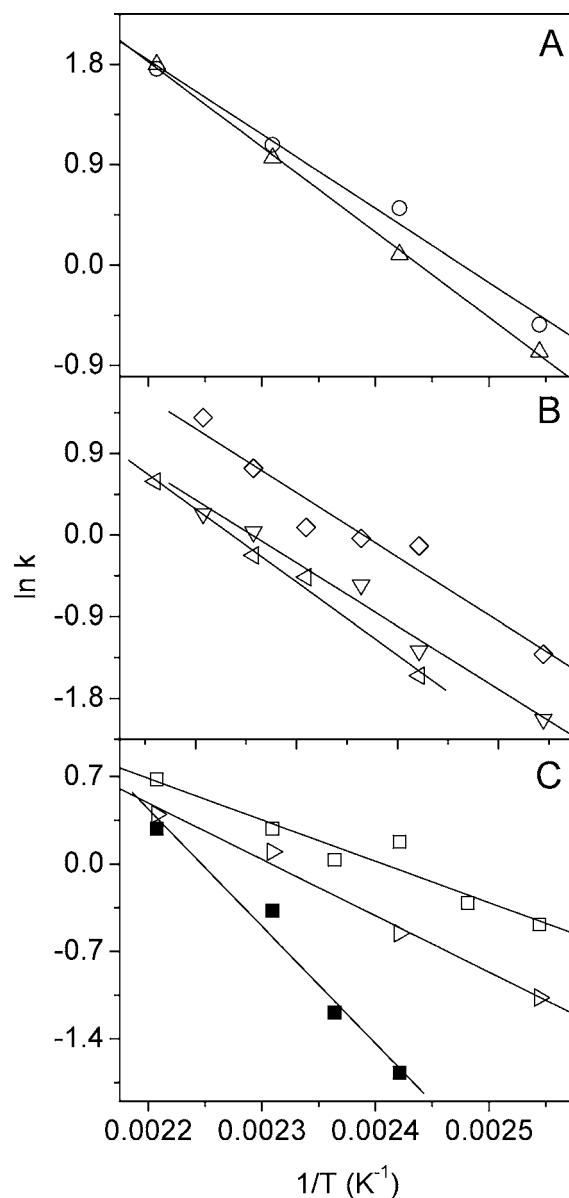

Figure 4. Arrhenius plot for phenylacetaldehyde formation in the reaction of phenylalanine with (A) 4-oxo-2-hexenal (○) or 4-oxo-2-nonenal (△); (B) 4,5-epoxy-2-heptenal (▽), 4,5-epoxy-2-decenal (◇), or 4-hydroxy-2-nonenal (◁); and (C) 2,4-heptadienal (▷), 2,4-decadienal with air (□), or 2,4-decadienal with nitrogen (■).

acetaldehyde increased linearly ($r > 0.972$, $p < 0.003$) as a function of reaction time between 120 and 180 °C when phenylalanine was heated in the presence of oxoalkenals. In addition, reaction rates increased with the temperature.

Reaction rates at the different assayed temperatures were calculated by using the equation

$$[\text{phenylacetaldehyde}] = [\text{phenylacetaldehyde}]_0 + kt$$

where $[\text{phenylacetaldehyde}]_0$ represents the intercept, k is the rate constant, and t is the reaction time. These rate constants were used in an Arrhenius plot for the calculation of the activation energy (E_a) of phenylacetaldehyde formation from phenylalanine in the presence of oxoalkenals (Figure 4A). The determined E_a values were 55.3 kJ/mol for the formation of phenylacetaldehyde in the reaction of phenylalanine with 4-

Table 2. Activation Energies (E_a) and Amount of Phenylacetaldehyde Produced in the Degradation of Phenylalanine by Lipid-Derived Reactive Carbonyls

carbonyl compound	E_a (kJ/mol)	phenylacetaldehyde (μmol/mmol of phenylalanine)[a]		
		1 h at 180 °C	2 h at 80 °C	24 h at 37 °C
none	ND[b]	8.5 ± 2.0 c	4.5 ± 0.4 c	4.8 ± 0.1 c
4-oxo-2-hexenal	55.3	140.9 ± 8.5 d	13.6 ± 1.1 de	9.5 ± 3.3 cd
4-oxo-2-nonenal	63.5	168.0 ± 9.3 e	35.5 ± 3.0 f	14.5 ± 2.7 dfg
4,5-epoxy-2-heptenal	57.9	99.8 ± 6.3 f	10.9 ± 1.1 d	10.6 ± 1.6 def
4,5-epoxy-2-decenal	58.9	92.4 ± 6.1 f	16.6 ± 2.6 e	15.8 ± 1.6 eg
4-hydroxy-2-nonenal	67.1	21.1 ± 0.9 c	5.1 ± 0.4 c	5.6 ± 0.2 cf
2,4-heptadienal	37.5	88.6 ± 7.3 f	18.1 ± 0.5 e	18.0 ± 2.2 g
2,4-decadienal	27.6	133.5 ± 2.5 d	29.3 ± 2.3 g	22.9 ± 1.6 h
2,4-decadienal (nitrogen)	78.0	ND[b]	ND[b]	ND[b]

[a]Equimolecular amounts of phenylalanine and the oxidized lipid were heated at the indicated temperature for the specified reaction time. Means in the same column with the same letters are not significantly different. [b]ND, not determined.

oxo-2-hexenal and 63.5 kJ/mol for the reaction with 4-oxo-2-nonenal (Table 2).

Comparative Reactivity of Lipid-Derived Reactive Carbonyls in the Strecker Degradation of Phenylalanine. Although the reaction between phenylalanine and lipid-derived reactive carbonyls has been the objective of previous studies,[5−8] no attempts have been undertaken to compare the reactivity of the different lipid oxidation products for this reaction. These reactivities were compared by determining both the E_a of the different reactions and the amount of phenylacetaldehyde formed by phenylalanine degradation in the presence of the lipid-derived carbonyls at several reaction times and temperatures.

Figure 5 collects the time courses of phenylacetaldehyde formation by phenylalanine degradation in the presence of 4,5-epoxy-2-heptenal (Figure 5A), 4,5-epoxy-2-decenal (Figure 5B), and 4-hydroxy-2-nonenal (Figure 5C). As can be observed, epoxyalkenals produced more phenylacetaldehyde than the hydroxynonenal. Nevertheless, for all of them the amount of phenylacetaldehyde increased linearly ($r > 0.962$, $p < 0.04$) as a function of reaction time between 120 and 190 °C. In addition, reaction rates increased with the temperature. These rate constants, calculated as described above, were used in an Arrhenius plot for the calculation of the activation energy (E_a) of phenylacetaldehyde formation from phenylalanine in the presence of either epoxyalkenals or hydroxynonenal (Figure 4B). The determined E_a are collected in Table 2. As can be observed, E_a values for epoxyalkenals were very similar among them and only slightly lower than the E_a determined for the reaction with hydroxynonenal.

Figure 6 collects the time courses of phenylacetaldehyde formation by phenylalanine degradation in the presence of 2,4-heptadienal (Figure 6A), 2,4-decadienal (Figure 6B), and 2,4-decadienal when only nitrogen was present in the reaction atmosphere (Figure 6C). The most significant difference among these three reactions was the smaller amount of phenylacetaldehyde that was produced when oxygen was absent, therefore suggesting an oxidative process for phenylalanine degradation in the presence of decadienal. As observed for other lipid oxidation products, the amount of phenylacetaldehyde also increased linearly ($r > 0.982$, $p < 0.002$) as a function of reaction time between 120 and 180 °C. In addition, the reaction rates also increased with the temperature. These rate constants, calculated as described above, were used in an Arrhenius plot for the calculation of the activation energy (E_a) of phenylacetaldehyde formation from phenylalanine in the

presence of heptadienal and decadienal using air and of decadienal using nitrogen (Figure 4C). The determined E_a values are collected in Table 2. As can be observed, the E_a determined for the reaction with alkadienals was much lower than the E_a determined for the different tertiary lipid oxidation products assayed. On the other hand, in the absence of air, the E_a for the reaction with decadienal was 2.8 times higher than when the reaction was carried out in the presence of air.

In addition to the determination of E_a for phenylalanine degradation in the presence of lipid-derived reactive carbonyls, the amount of phenylacetaldehyde produced by these lipid oxidation products at different reaction times and temperatures was also determined (Table 2). The reactivity of the different lipid oxidation products according to the amount of phenylacetaldehyde produced changed as a function of the reaction times and temperatures. For samples heated for 1 h at 180 °C, the reactivity decreased in the following order: oxononenal > oxohexenal ∼ decadienal > epoxyheptenal ∼ epoxydecenal ∼ heptadienal > hydroxynonenal ∼ control. For samples heated for 2 h at 80 °C, the reactivity decreased in the following order: oxononenal > decadienal > heptadienal ∼ epoxydecenal ∼ oxohexenal ∼ epoxyheptenal > hydroxynonenal ∼ control. For samples heated for 24 h at 37 °C, the reactivity decreased in the following order: decadienal > heptadienal ∼ epoxydecenal ∼ oxononenal ∼ epoxyheptenal ∼oxohexenal ∼ hydroxynonenal ∼ control.

■ DISCUSSION

Lipid oxidation has long been related to the loss of food attributes produced as a consequence of processing and storage.[22,23] In addition, the formed lipid-derived reactive carbonyls are able to react with the surrounding amino compounds by carbonyl−amine reactions, which have both positive and negative consequences for food quality and safety.[24,25] One of these consequences is the conversion of amino acids to Strecker aldehydes. This reaction, which was first described for epoxyalkenals,[5] seems to be produced by most of the lipid-derived reactive carbonyls produced during the lipid oxidation process. Oxoalkenals are not an exception, and the above-described results show that these lipid oxidation products are able to convert phenylalanine into phenylacetaldehyde with reaction yields of ∼20% when optimum reaction conditions are employed (Figure 1). These yields are higher than those previously described for other lipid-derived reactive carbonyls.
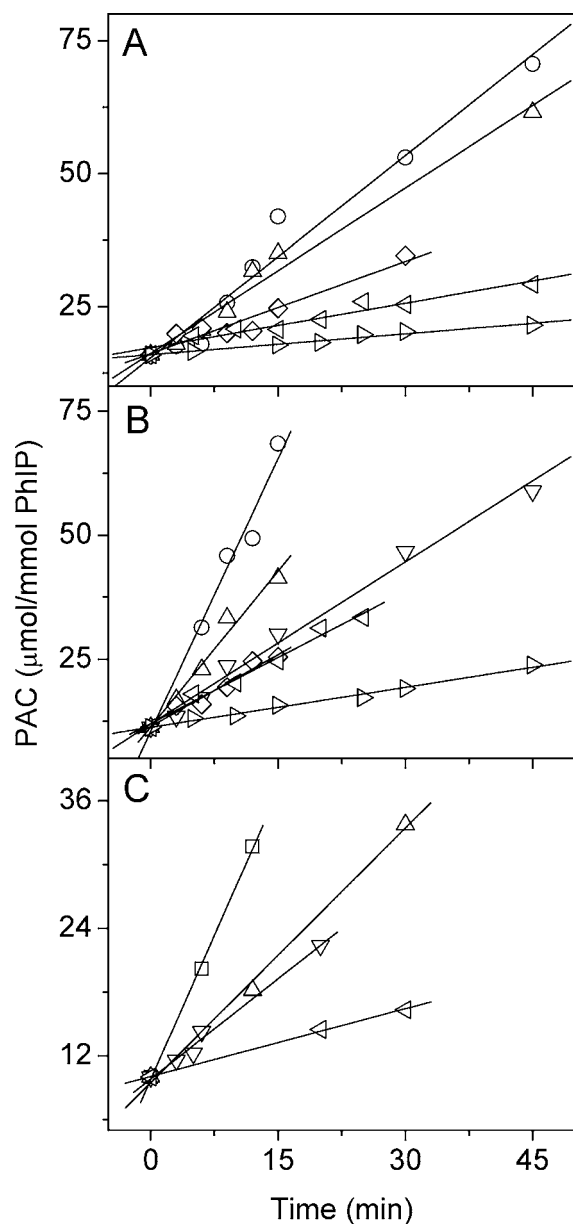
Figure 5. Effect of reaction time and temperature on the formation of phenylacetaldehyde (PAC) in the reaction of phenylalanine (Phe) with either 4,5-epoxy-2-heptenal (A), 4,5-epoxy-2-decenal (B), or 4-hydroxy-2-nonenal (C) at pH 3. Temperatures assayed were 190 (□), 180 (○), 170 (△), 160 (▽), 150 (◇), 140 (◁), and 120 °C (▷).

Figure 6. Effect of reaction time and temperature on the formation of phenylacetaldehyde (PAC) in the reaction of phenylalanine (Phe) with either 2,4-heptadienal (A), 2,4-decadienal in air (B), or 2,4-decadienal in nitrogen (C) at pH 3. Temperatures assayed were 180 (○), 160 (▽), 150 (◇), 140 (◁), 130 (☆), and 120 °C (▷).

The reaction between phenylalanine and oxoalkenals seems to follow a reaction pathway similar to that described in Figure 7. The first step of the reaction is the formation of the corresponding imine between the amino group of the amino acid and the carbonyl group of the oxoalkenal. Because the oxoalkenal has two carbonyls, two imines can be produced. The second step is the loss of carbon dioxide. This loss induces an electronic rearrangement and the formation of a new imine, which, after hydrolysis, is the origin of phenylacetaldehyde. As a consequence of the reaction, the oxoalkenal is transformed into a hydroxylamino derivative, which can be later converted into a pyrrole derivative by dehydration or be polymerized. A proof of this mechanism was obtained with the detection of this kind of pyrrole derivative in the reaction of phenylalanine with both oxohexenal and oxononenal (Table 1).
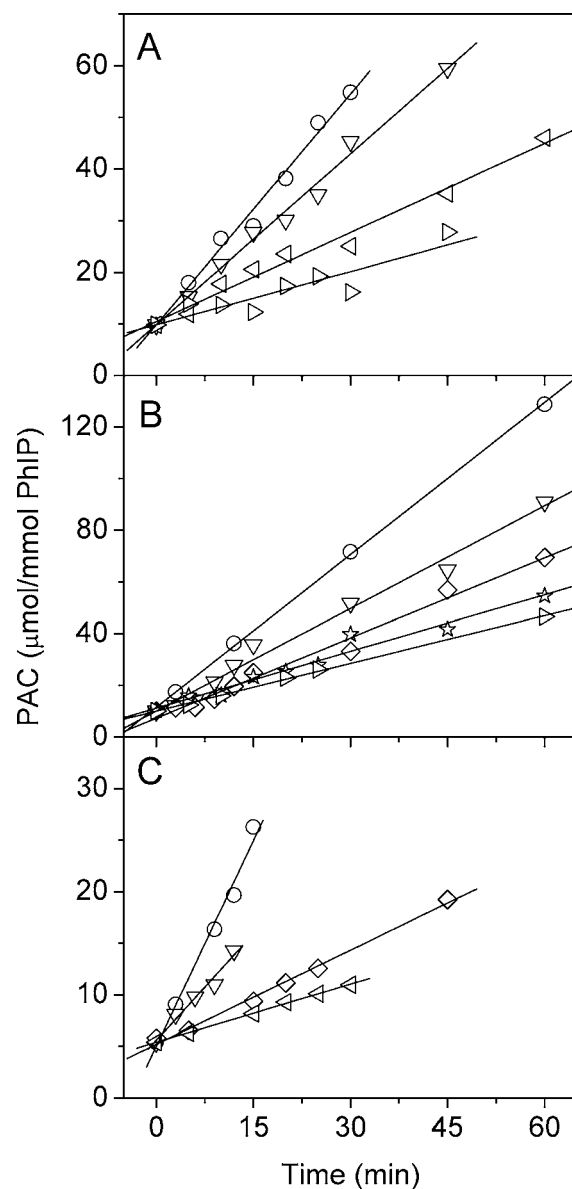
This reaction pathway is expected to be similar for the different lipid-derived reactive carbonyls. The unique difference should be the compound in which the lipid oxidation product is converted. Thus, epoxyalkenals are transformed into 2-alkylpyridines,[5] and hydroxyalkenals are converted into conjugated unsaturated amines.[8] This similarity among the proposed reaction pathways for all tertiary lipid oxidation products is in agreement with the similarity found for the E_a of the different reactions involving tertiary lipid oxidation products (55.3−67.1 kJ/mol, Table 2). On the other hand, phenylalanine degradation initiated by alkadienals had E_a that were different from the E_a found for the different tertiary lipid oxidation products assayed. The E_a determined for alkadienals was lower than those found for tertiary lipid oxidation products when the reaction was carried out in the presence of air. On the other hand, they were higher than those found for tertiary lipid
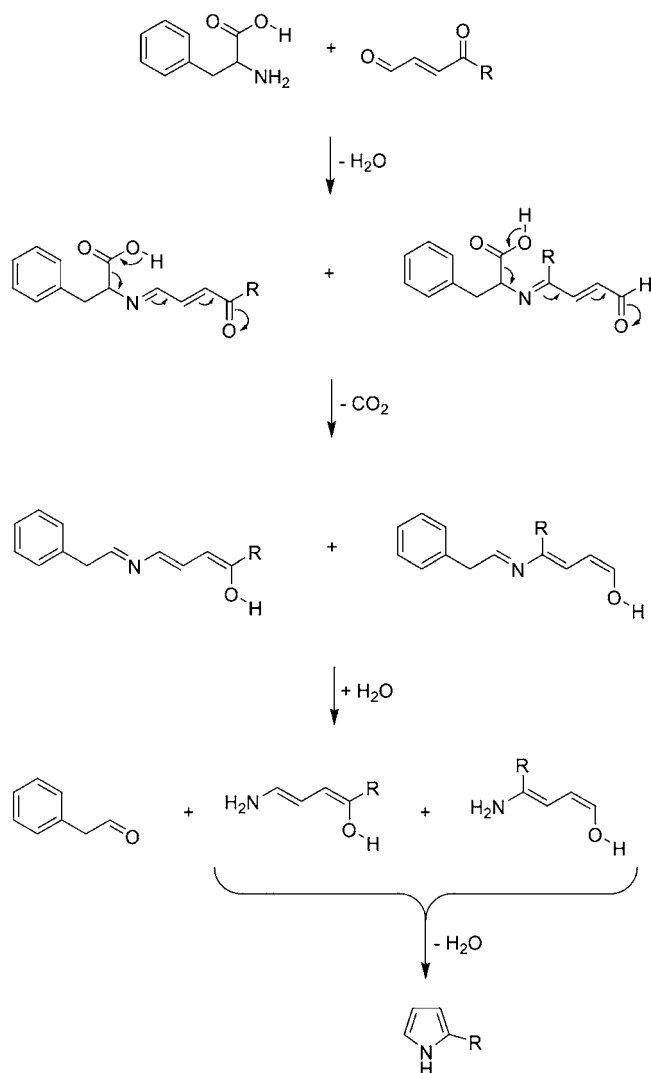
Figure 7. Proposed pathway for the conversion of phenylalanine into phenylacetaldehyde in the presence of oxoalkenals.

oxidation products when the reaction was carried out in the absence of oxygen. These results suggest that, in the presence of oxygen, alkadienals should be oxidized and the product(s) of this oxidation should be the responsible for the degradation of phenylalanine. A similar conclusion was obtained previously, and the conversion of alkadienals into epoxyalkenals was proposed.[6] The results obtained in the present study suggest that conversion of alkadienals into other more reactive intermediates should also be produced because the E_a determined for phenylalanine degradation in the presence of alkadienals was lower than the E_a determined for phenylalanine degradation in the presence of epoxyalkenals. Additional studies should be conducted to identify these intermediates.

Although E_a for phenylalanine degradation in the presence of alkadienals was lower than E_a determined for phenylalanine degradation in the presence of tertiary lipid oxidation products, phenylacetaldehyde was not produced in the reaction with alkadienals to a higher extent than in the reaction with tertiary lipid oxidation products. In fact, oxononenal was the lipid oxidation product that usually produced a higher amount of phenylacetaldehyde. Thus, the amount of phenylacetaldehyde produced by oxononenal was 25% higher than the amount of phenylacetaldehyde produced by decadienal (and 90% higher

than the amount produced by heptadienal) when the reaction was heated for 1 h at 180 °C (Table 2). This difference decreased to 21% compared to decadienal when samples were heated for 2 h at 80 °C (Table 2). Different from these results, decadienal produced more phenylacetaldehyde than oxononenal when phenylalanine/aldehyde samples were heated at 37 °C for 24 h (Table 2). These results are likely a consequence of the different competitive reactions that are taking place simultaneously. Lipid oxidation products are able to react with amino compounds in many different ways in addition to producing the Strecker degradation of the amino acids,[24] and all of these reactions require different reaction conditions and have different E_a values. At low temperatures, only reactions having very low E_a are favored. For that reason, when phenylalanine/lipid-derived carbonyl reaction mixtures were heated at 37 °C for 24 h, there was a correlation ($r = -0.83205$, $p = 0.02$) between the E_a determined for the different reactions and the amount of phenylacetaldehyde produced. On the contrary, at high temperature other reactions should be competing with the Strecker degradation of the phenylalanine, and there was not any correlation between E_a and the amount of phenylacetaldehyde produced when reactions were heated either at 80 °C for 2 h ($r = -0.37$, $p = 0.4$) or at 180 °C for 1 h ($r = -0.24$, $p = 0.6$). One of these reactions may be the conversion of phenylalanine into the corresponding biogenic amine β-phenylethylamine. Thus, alkadienals have been described as better producers of biogenic amines than oxoalkenals, a reaction that takes place a high temperature.[21,25]

All of these results demonstrate that, analogously to other lipid-derived carbonyls, oxoalkenals are able to produce the Strecker degradation of phenylalanine. Furthermore, oxoalkenals are more effective producers of phenylacetaldehyde than other lipid oxidation products when heated at high temperature. For this reason, oxoalkenals should be expected to play a significant role in reactions in which phenylacetaldehyde is required to be produced as an intermediate, such as the formation of PhIP.[10]

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