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Amine Degradation by 4,5-Epoxy-2-decenal in Model Systems

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The reactions of 4,5-epoxy-2-decenal with octylamine, benzylamine, and 2-phenylglycine methyl ester were studied to investigate if amines may suffer a Strecker type degradation by epoxyalkenals analogously to amino acids. In addition to other reactions, the studied amines were converted into their corresponding Strecker aldehydes (octanal, benzaldehyde, and methyl 2-oxo-2-phenylacetate, respectively) to an extent that depended on the pH, the temperature, the amount of epoxyalkenal, and the amine involved. Each amine exhibited an optimum pH for the reaction, but the corresponding Strecker aldehydes were produced to a significant extent within a broad pH range. In addition, the temperature mostly influenced the reaction rate, which was increased between 6.5 and 9.5 times when the reaction was carried out at 60 °C than when it took place at 37 °C. Furthermore, Strecker aldehyde formation was linearly correlated with the amount of the epoxyalkenal present in the reaction mixture. Nevertheless, the reaction yield mostly depended on the amine involved. Thus, octylamine only produced trace amounts of octanal, benzylamine was converted into benzaldehyde with a yield of 4.3%, and 2-phenylglycine methyl ester was converted into methyl 2-oxo-2-phenylacetate with a reaction yield of 49%. All of these results suggest that suitable amines can be degraded by epoxyalkenals to their corresponding Strecker aldehydes to a significant extent.

KEYWORDS: Alkylpyridines; amines; carbonyl-amine reactions; epoxyalkenals; flavors; lipid oxidation; Maillard reaction; Strecker aldehydes

INTRODUCTION

In contrast to the well-documented role of Strecker degradation of amino acids in flavor formation via Maillard reaction (*1*, *2*), Strecker degradation of amines is still an open question (*3*). However, it is an important subject because the conversion of amines into carbonyl derivatives may be an alternative route to the observed increase of carbonyl content in foods as a consequence of processing and/or storage (*4*, *5*). In an attempt to clarify the conditions needed to produce the Strecker degradation of amines, this study describes the reaction of 4,5-epoxy-2-decenal (**1**) with different amines at several pH and temperature conditions.

4,5-Epoxy-2-alkenals are secondary products of lipid peroxidation. They are produced in the decomposition of intermediate epoxyhydroperoxy fatty acids by a mechanism that is common for the different polyunsaturated fatty acids. Thus, when starting from *n*-6 polyunsaturated fatty acids, the epoxyalkenal obtained is 4,5-(*E*)-epoxy-2-(*E*)-decenal (**1**) by decomposition of an intermediate 12,13-(*E*)-epoxy-9-hydroperoxy-10-octadecenoic acid (*6*). Analogously, the 4,5-(*E*)-epoxy-2-(*E*)-heptenal is the product of oxidation of the *n*-3 polyunsaturated fatty acids (*7*). These epoxyalkenals have been detected in many different food systems (*8*).

4,5-Epoxy-2-alkenals have been shown to degrade amino acids by a Strecker type mechanism (*9*). This reaction, which has also been described for epoxyoxoene fatty esters (*10*) and 4-hydroxy-2-alkenals (*11*), is produced through imine formation, which is then decarboxylated and hydrolyzed. This ability to degrade amino acids points them out as potential candidates for degrading amines. The amines selected for this study were octylamine (**2**), as a representative aliphatic amine; benzylamine (**3**), as an amine with a phenyl group at the α-position; and 2-phenylglycine methyl ester (**4**), as an amine with both a phenyl group and a methoxycarbonyl group at the α-position.

EXPERIMENTAL PROCEDURES

Materials. 2,4-Decadienal, octylamine (**2**), benzylamine (**3**), (*S*)-+-2-phenylglycine methyl ester hydrochloride (**4**), 1-benzyl-1*H*-pyrrole (**9**), hexanal (**11**), octanal (**16**), benzaldehyde (**17**), methyl 2-oxo-2-phenylacetate (**18**), and 2-pentylpyridin (**20**) were purchased from Aldrich (Milwaukee, WI). All other chemicals were analytical grade and were purchased from reliable commercial sources.

4,5-Epoxy-2-decenal (**1**) was prepared from 2,4-decadienal as described previously (*12*). Briefly, 3-chloroperoxybenzoic acid (25 mmol) was dissolved in chloroform (175 mL) and 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

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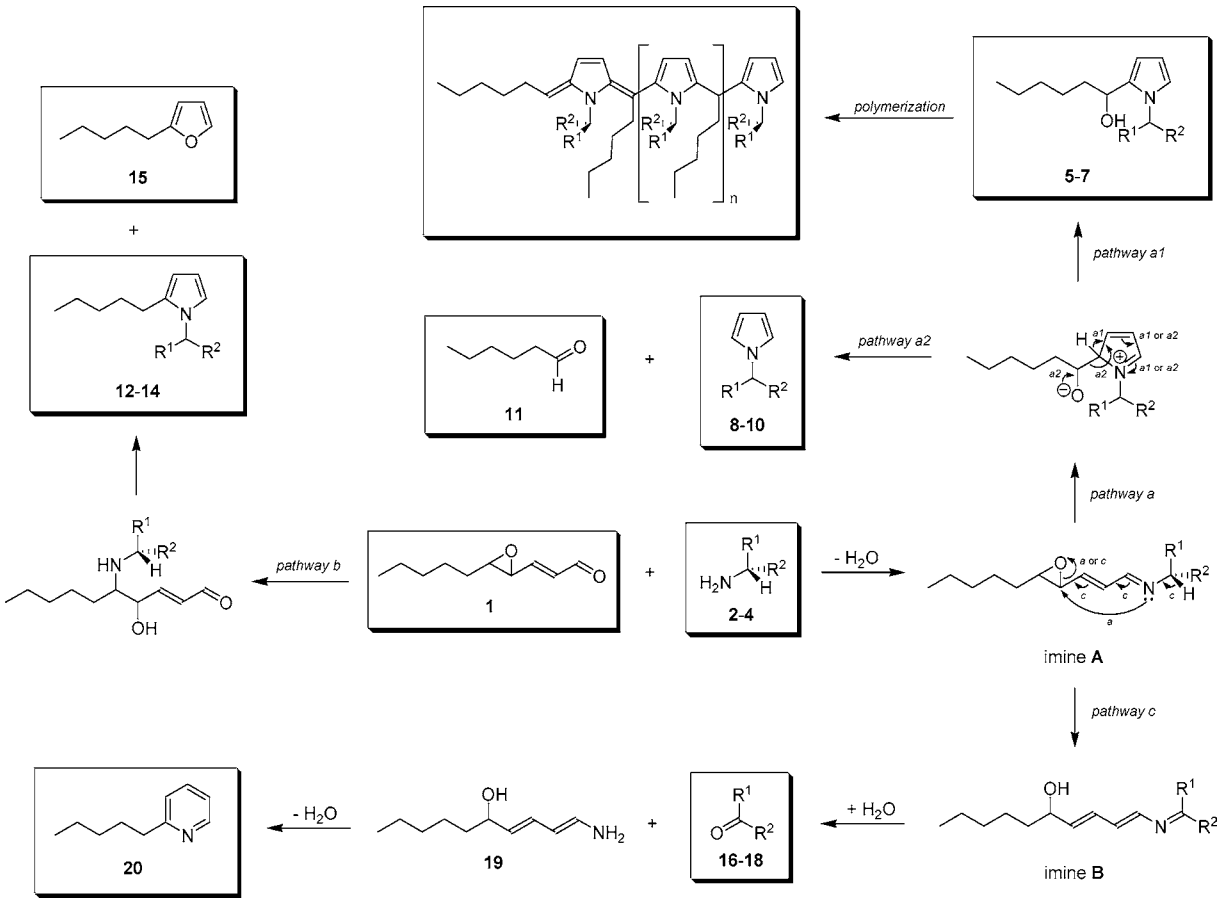
Scheme 1. Reaction of 4,5-Epoxy-2-decenal with Primary Amines[a]



[a] For **2**, **5**, **8**, **12**, and **16**: $R^1 = CH_3(CH_2)_7$, and $R^2 = H$. For **3**, **6**, **9**, **13**, and **17**: R^1 = phenyl, and $R^2 = H$. For **4**, **7**, **10**, **14**, and **18**: $R^1 = CO_2CH_3$, and R^2 = phenyl.

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 the 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 gas chromatography−mass spectrometry (GC-MS).

4,5-Epoxy-2-decenal/Amine Reaction Mixtures. A solution of 0−5 μmol of 4,5-epoxy-2-decenal (**1**) and 5 μmol of the amine (**2**−**4**) in 100 μL of acetonitrile-sodium citrate or sodium phosphate buffer (2:1) was heated at 37 or 60 °C and studied for either GC-MS or the content of the produced Strecker aldehyde (**17** and **18**) determined by GC−flame ionization detection (FID). Samples were diluted with chloroform (550 μL) for GC-MS analyses. For Strecker aldehyde content determination, samples were diluted with 500 μL of chloroform, and 50 μL of internal standard solution [337 μg of 3-(*Z*)-nonenol in 1 mL of methanol] was added. Either 50 mM sodium citrate buffer, pH 2.15−6.0, or 50 mM sodium phosphate buffer, pH 6.0−8.0, was employed to control the reaction pH.

The products of 4,5-epoxy-2-decenal/amine reactions are formed by different competing reactions. Therefore, other products, in addition to Strecker aldehydes (**16**−**18**), were also produced (**Scheme 1**). These compounds were *N*-substituted 1*H*-pyrroles (**8**−**10**), *N*-substituted 2-(1-hydroxyhexyl)-1*H*-pyrroles (**5**−**7**), *N*-substituted 2-pentyl-1*H*-pyrroles (**12**−**14**), hexanal (**11**), 2-pentylfuran (**15**), and 2-pentylpyridine (**20**). Some of these compounds were commercial, and their formation in the reaction mixtures was confirmed by comparison of their retention indices and mass spectra with those of authentic compounds. This occurred with 1-benzyl-1*H*-pyrrole (**9**), hexanal (**11**), 2-pentylfuran (**15**), and 2-pentylpyridine (**20**). Other reaction products could be identified by comparison with described compounds. Thus, 1-octyl-1*H*-pyrrole (**8**) and 1-benzyl-2-pentyl-1*H*-pyrrole (**13**) were characterized previously (*13*, *14*). Finally, other compounds were only tentatively identified in this study on the basis of their mass spectra. Their spectra exhibited the typical fragmentations of *N*-substituted 1*H*-pyrroles, *N*-substituted 2-(1-hydroxyhexyl)-1*H*-pyrroles, or *N*-substituted 2-pentyl-1*H*-pyrroles (see, for example, ref *15* for a summary of mass spectroscopic characteristics of these types of pyrrole derivatives). These compounds were 1-(1-octyl-1*H*-pyrrol-2-yl)hexan-1-ol (**5**), 1-octyl-2-pentyl-1*H*-pyrrole (**12**), 1-(1-benzyl-1*H*-pyrrol-2-yl)hexan-1-ol (**6**), methyl 2-(2-(1-hydroxyhexyl)-1*H*-pyrrol-1-yl)-2-phenylacetate (**7**), methyl 2-phenyl-2-(1*H*-pyrrol-1-yl)acetate (**10**), and methyl 2-(2-pentyl-1*H*-pyrrol-1-yl)-2-phenylacetate (**14**).

The GC-MS *m*/*z* (relative intensity, ion structure) of tentatively assigned compound **5** in the intact form was 279 (7, M^+), 261 (13, $M^+ - H_2O$), 250 (2, M^+ − ethyl), 246 (1, $M^+ - H_2O$ − methyl), 232 (5, $M^+ - H_2O$ − ethyl), 218 (44, $M^+ - H_2O$ − propyl), 209 (16, M^+ − pentyl + 1), 208 (100, M^+ − pentyl), 204 (7, $M^+ - H_2O$ − butyl), 190 (4, $M^+ - H_2O$ − pentyl), 178 (19, M^+ − hydroxyhexyl), 162 (5, $M^+ - H_2O$ − heptyl), 148 (5), 134 (17), 120 (17), 106 (22), 96 (15), 80 (18), 68 (10), 57 (15), 55 (16), 43 (30), and 41 (34). This compound also appeared in the dehydrated form in the chromatogram. Its GC-MS (relative intensity, ion structure) was 261 (37, M^+), 246 (3, M^+ − methyl), 232 (11, M^+ − ethyl), 219 (19, M^+ − propyl + 1), 218 (100, M^+ − propyl), 204 (15, M^+ − butyl), 190 (8, M^+ − pentyl), 178 (9, M^+ − hexenyl), 176 (8, M^+ − hexyl), 162 (9, M^+ − heptyl), 148 (10), 134 (29), 132 (15), 120 (32), 118 (24), 106 (45), 91 (8), 80 (13), 79 (13), 55 (10), 43 (25), and 41 (32).

The GC-MS *m*/*z* (relative intensity, ion structure) of tentatively assigned compound **12** was 249 (26, M^+), 234 (1, M^+ − methyl), 220 (4, M^+ − ethyl), 206 (26, M^+ − propyl), 193 (17, M^+ − butyl + 1), 192 (100, M^+ − butyl), 178 (49, M^+ − pentyl), 164 (10, M^+ − hexyl), 150 (21, M^+ − heptyl), 136 (18), 122 (24), 108 (25), 95 (33), 94 (60), 80 (27), 69 (9), 55 (15), 43 (22), and 41 (32).

The GC-MS (relative intensity, ion structure) of tentatively assigned compound **6** was 257 (6, M^+), 239 (5, $M^+ - H_2O$), 228 (1, $M^+ -$ ethyl), 210 (1, $M^+ - H_2O -$ ethyl), 196 (11, $M^+ - H_2O -$ propyl), 186 (36, $M^+ -$ butyl), 170 (5), 108 (9), 92 (9), 91 (100, benzyl), and 65 (9). This compound also appeared in the dehydrated form in the chromatogram. Its GC-MS (relative intensity, ion structure) was 239 (21, M^+), 210 (3, $M^+ -$ ethyl), 197 (9, $M^+ -$ propyl + 1), 196 (43, $M^+ -$ propyl), 118 (5), 104 (4), 92 (7), 91 (100, benzyl), and 65 (12).

Tentatively assigned compound **7** only appeared in the chromatogram in its dehydrated form. Its GC-MS (relative intensity, ion structure) was 297 (71, M^+), 254 (48, $M^+ -$ propyl), 238 (28, $M^+ - CO_2CH_3$), 194 (41), 149 (37), 121 (100), 118 (32), 105 (53), 91 (52), and 77 (34).

The GC-MS (relative intensity, ion structure) of tentatively assigned compound **10** was 215 (19, M^+), 157 (13, $M^+ - CO_2CH_3 + 1$), 156 (100, $M^+ - CO_2CH_3$), 153 (9), 128 (13), 121 (12), 77 (10), and 51 (6).

The GC-MS (relative intensity, ion structure) of tentatively assigned compound **14** was 285 (23, M^+), 228 (18, $M^+ -$ butyl), 226 (24, $M^+ - CO_2CH_3$), 168 (100), 156 (14), 149 (18), 136 (16), 121 (60), 91 (28), 80 (19), and 77 (15).

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 mm × 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, 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; and ionization EI, 70 eV.

Determination of Benzaldehyde (17) and Methyl 2-Oxo-2-phenylacetate (18) Content by GC-FID. GC-FID analyses were conducted with an Aligent 6890 GC Plus. Column and working conditions were analogous to the above-described for the GC-MS analyses, and compounds were detected with a FID. Quantification of benzaldehyde (**17**) and methyl 2-oxo-2-phenylacetate (**18**) was carried out by preparing standard curves over a concentration range of 15−175 nmol of benzaldehyde (**17**) or 50−1800 nmol of methyl 2-oxo-2-phenylacetate (**18**) in the 650 μL of solution prepared for GC-FID injection (see above). For each curve, five different concentration levels of the aldehyde were used. Benzaldehyde (**17**) and methyl 2-oxo-2-phenylacetate (**18**) 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%.

RESULTS

Reaction between 4,5-Epoxy-2-decenal (1) and Octylamine (2). The reaction between 4,5-epoxy-2-alkenals and primary amines is very complex, and it has been the objective of different studies. In fact, it seems to be the result of different competing reactions. For this reason, when the reaction between 4,5-epoxy-2-decenal (**1**) and octylamine (**2**) was studied by GC-MS, different compounds were detected (**Table 1**).

The major reaction product, at the three assayed pH values, was the *N*-substituted pyrrole **8**. According to previous studies (*16*, *17*), this compound should be produced by the pathway a2 shown in **Scheme 1**. The reaction between the epoxyalkenal **1** and the amine **2** produces in the first step the imine A that it is the precursor of a cyclic intermediate (pathway a). Depending on the electronic rearrangement produced in this intermediate, either the *N*-substituted pyrrole **8** (pathway a2) or the *N*-substituted 2-(1-hydroxyhexyl)pyrrole **5** (pathway a1) may evolve. Formation of pyrrole **8** should be accompanied by production of hexanal (**11**), which was also identified in the chromatogram.

The product of pathway a1 (pyrrole **5**) was tentatively identified in the chromatogram on the basis of its mass spectrum. Like other hydroxyalkylpyrroles, compound **5** should be unstable and contribute by polymerization to the brown color and fluorescence development in these reactions (*18*).

Table 1. Retention Indices of Compounds Described in This Study[a]

compd no.	compd name	retention index
1	4,5-epoxy-2-decenal	1366
5	1-(1-octyl-1*H*-pyrrol-2-yl)hexan-1-ol[b]	1976[c]/2069
6	1-(1-benzyl-1*H*-pyrrol-2-yl)hexan-1-ol[b]	1932[c]/2030
7	methyl 2-[2-(1-hydroxyhexyl)-1*H*-pyrrol-1-yl]-2-phenylacetate[b]	2167[c]
8	1-octyl-1*H*-pyrrole	1388
9	1-benzyl-1*H*-pyrrole	1341
10	methyl 2-phenyl-2-(1*H*-pyrrol-1-yl)acetate[b]	1627
11	hexanal	801
12	1-octyl-2-pentyl-1*H*-pyrrole[b]	1837
13	1-benzyl-2-pentyl-1*H*-pyrrole	1783
14	methyl 2-(2-pentyl-1*H*-pyrrol-1-yl)-2-phenylacetate[b]	2028
15	2-pentylfuran	993
16	octanal	1006
17	benzaldehyde	960
18	methyl 2-oxo-2-phenylacetate	1285
20	2-pentylpyridin	1191

[a] Structures for these compounds are given in **Scheme 1**. [b] These compounds were tentatively assigned. [c] This retention index corresponds to the dehydrated compound.

In addition to the formation of compounds **5** and **8**, the reaction between 4,5-epoxy-2-decenal (**1**) and octylamine (**2**) may follow an alternative pathway to produce pyrroles and furans with nine carbons (pathway b in **Scheme 1**) (*15*). The mechanism of this reaction is not yet fully understood, but it may be hypothesized to take place through the addition of the amine to the epoxide producing an intermediate aminocarbonyl derivative. This last compound should be the origin of 1-octyl-2-pentyl-1*H*-pyrrol (**12**) and 2-pentylfuran (**15**). Compound **15** appeared in the chromatogram, and a compound tentatively assigned to the *N*-substituted 2-pentyl-1*H*-pyrrole **12** was also present.

Other compounds, like 2-octenal and 2,4-decadienal, were also produced in the reaction, and others could not be identified. However, the detection of trace amounts of octanal (**16**) was significant (see below). In addition, by increasing the pH from 4 to 8, the epoxyalkenal **1** disappeared completely and some changes in the relative proportions of the different products were observed (data not shown).

Reaction between 4,5-Epoxy-2-decenal (1) and Benzylamine (3). When the reaction was carried out between the epoxyalkenal **1** and benzylamine (**3**), the formation of analogous products to the above-described for the reaction between 4,5-epoxy-2-decenal (**1**) and octylamine (**2**) was also observed. As expected, pathway a produced 1-benzyl-1*H*-pyrrole (**9**) and hexanal (**11**) as major products, and a compound tentatively assigned as 1-(1-benzyl-1*H*-pyrrol-2-yl)hexan-1-ol (**6**) was also produced to a lesser extent. In addition, the corresponding products of pathway b were also produced. Thus, 1-benzyl-2-pentyl-1*H*-pyrrole (**13**) and 2-pentylfuran (**15**) could be easily identified in the chromatogram.

Nevertheless, the reaction between 4,5-epoxy-2-decenal (**1**) and benzylamine (**3**) also produced other products that were not so clearly observed in the above-described reaction between the epoxyalkenal **1** and the octylamine (**2**). These compounds were benzaldehyde (**17**) and 2-pentylpyridine (**20**). Previous studies have shown that 2-pentylpyridine is produced when 4,5-epoxy-2-decenal degraded amino acids (*9*). Analogously, the Strecker type degradation of amines produced by epoxyalkenals

should also produce this pyridine in addition to the corresponding Strecker aldehyde. Pathway c in **Scheme 1** shows the proposed mechanism for the Strecker type degradation of amines produced by 4,5-epoxy-2-decenal (**1**). Thus, the imine A should first be converted into the imine B, which after hydrolysis would produce the hydroxyl amino derivative **19** and the aldehyde **17**. The internal cyclation reaction and aromatization of compound **19** should be the origin of the pyridine **20**. This last reaction was produced in more or less extension depending on the reaction conditions.

Differently to pyrrole formation produced by pathways a and b, the Strecker degradation of amines produced by pathway c seemed to be very dependent on the reaction conditions, and benzaldehyde (**17**) content seemed to decrease when the pH was increased (see below).

Reaction between 4,5-Epoxy-2-decenal (1) and 2-Phenylglycine Methyl Ester (4). The introduction of a methoxycarbonyl group at the α-position of the amino group favored the Strecker type degradation of the amine. Therefore, the proportions among the products of pathways a, b, and c in the reaction of 4,5-epoxy-2-decenal (**1**) with 2-phenylglycine methyl ester (**4**) were very different to the above-described reactions between 4,5-epoxy-2-decenal (**1**) and octylamine (**2**) or benzylamine (**3**). Thus, although the products of pathway a [the corresponding *N*-substituted 1*H*-pyrrole (**10**), the corresponding *N*-substituted 2-(1-hydroxyhexyl)-1*H*-pyrrole (**7**), and hexanal (**11**)] or pathway b [the corresponding *N*-substituted 2-pentyl-1*H*-pyrrole (**14**) and 2-pentylfuran (**15**)] could be easily identified in the chromatogram, the Strecker aldehyde **18** produced by pathway c was the major product of the reaction.

Analogously to the above-described for the reaction between the 4,5-epoxy-2-decenal (**1**) and the benzylamine (**3**), the proportion among the different products of pathways a, b, and c in the reaction between 4,5-epoxy-2-decenal (**1**) with 2-phenylglycine methyl ester (**4**) seemed also to depend on the reaction pH.

Effect of pH on Strecker Aldehyde Formation in the Reaction of 4,5-Epoxy-2-decenal (1) and Primary Amines. The reaction of 4,5-epoxy-2-decenal (**1**) with primary amines produced the Strecker type degradation of the amine to an extent that depended on the involved amine and the reaction pH. **Figure 1** shows the amount of Strecker aldehyde produced as a function of pH when starting from benzylamine (**3**) (**Figure 1A**) or 2-phenylglycine methyl ester (**4**) (**Figure 1B**) at 37 °C. Benzaldehyde (**17**), which is the Strecker aldehyde derived from benzylamine (**3**), was produced to a higher extent at pH 2.5−4 (**Figure 1A**). A higher pH decreased linearly ($r = -0.97$, $p = 2.38 \times 10^{-4}$) the amount of aldehyde produced, and benzaldehyde was produced similarly in both citrate and phosphate buffers.

The effect of pH on methyl 2-oxo-2-phenylacetate (**18**) formation (**Figure 1B**) was different to the above-described for the formation of benzaldehyde (**17**). Thus, degradation of 2-phenylglycine methyl ester (**4**) increased from pH 2.15 to 6.0 when using sodium citrate buffer. However, when sodium citrate was changed by sodium phosphate, methyl 2-oxo-2-phenylacetate (**18**) was produced to a much lesser extent and continued increasing from pH 6 to 7.

These results suggested that pH played an important role in the Strecker type degradation of amines. In addition, the optimum pH seemed to be specific for each amine. The pH values selected for studying Strecker aldehyde production in the rest of this study were pH 4 for the reaction with benzylamine (**3**) and pH 6 (sodium citrate buffer) for the reaction with 2-phenylglycine methyl ester (**4**). These pH values produced the highest amounts of benzaldehyde (**17**) and methyl 2-oxo-2-phenylacetate (**18**), respectively.

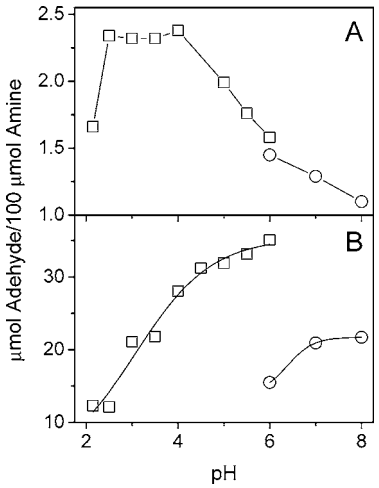


Figure 1. Effect of pH on Strecker aldehyde formation in the reaction of 4,5-epoxy-2-decenal (**1**) with (**A**) benzylamine (**3**) and (**B**) 2-phenylglycine methyl ester (**4**), in acetonitrile/buffer (2:1) after 21 h at 37 °C. The employed buffers were 50 mM sodium citrate buffer for pH 2.15–6 (□) and 50 mM sodium phosphate buffer for pH 6–8 (○). The Strecker aldehydes determined were benzaldehyde (**17**) (**A**) and methyl 2-oxo-2-phenylacetate (**18**) (**B**).

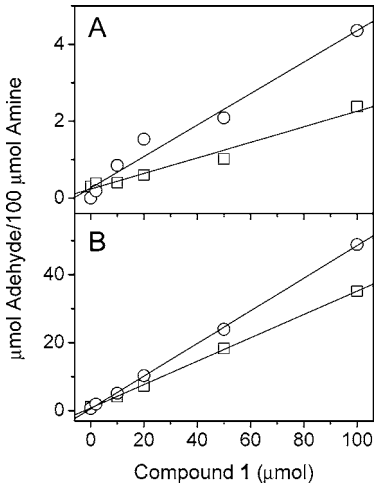


Figure 2. Effect of epoxyalkenal concentration on Strecker aldehyde formation in the reaction of 4,5-epoxy-2-decenal (**1**) with (**A**) benzylamine (**3**) and (**B**) 2-phenylglycine methyl ester (**4**), in acetonitrile/50 mM sodium citrate buffer (2:1) after 21 h at 37 (□) or 60 °C (○). The reaction pH values were 4 (**A**) and 6 (**B**). The Strecker aldehydes determined were benzaldehyde (**17**) (**A**) and methyl 2-oxo-2-phenylacetate (**18**) (**B**).

Effect of 4,5-Epoxy-2-decenal (1) Concentration on Strecker Aldehyde Production. The effect of the concentration of the epoxyalkenal on the Strecker type degradation of primary amines is shown in **Figure 2**. The amount of Strecker aldehyde produced increased linearly with the amount of epoxyalkenal present in the reaction mixture for the two tested amines at the two assayed temperatures. Thus, the amount of benzaldehyde (**17**) produced correlated linearly with the amount of 4,5-epoxy-2-decenal (**1**) added at both 37 ($r = 0.99$, $p = 2.86 \times 10^{-4}$) and 60 °C ($r = 0.99$, $p = 3.17 \times 10^{-4}$) (**Figure 2A**). In addition, the amount of methyl 2-oxo-2-phenylacetate (**18**) produced was also linearly correlated with the amount of 4,5-epoxy-2-decenal (**1**) present in the reaction mixture at both 37 ($r = 0.9997$, $p < 0.0001$) and 60 °C ($r = 0.9998$, $p < 0.0001$) (**Figure 2B**).

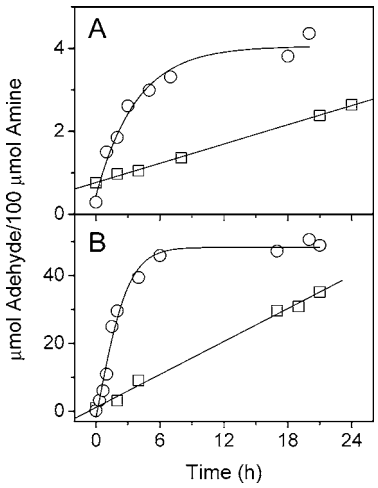


Figure 3. Time-course of Strecker aldehyde formation in the reaction of 4,5-epoxy-2-decenal (**1**) with (**A**) benzylamine (**3**) and (**B**) 2-phenylglycine methyl ester (**4**), in acetonitrile/50 mM sodium citrate buffer (2:1) at 37 (□) or 60 °C (○). The reaction pH values were 4 (**A**) and 6 (**B**). The Strecker aldehydes determined were benzaldehyde (**17**) (**A**) and methyl 2-oxo-2-phenylacetate (**18**) (**B**).

For both amines, their corresponding Strecker aldehydes were produced to a higher extent at 60 than at 37 °C. Thus, when the epoxyalkenal/amine ratio was 1:1, the 2.4 or 4.3% of benzylamine (**3**) was converted into benzaldehyde (**17**) after 21 h of reaction at 37 or 60 °C, respectively. The conversion of 2-phenylglycine methyl ester (**4**) into methyl 2-oxo-2-phenylacetate (**18**) was much higher, and 35.1 or 48.9% of the amine was converted into its Strecker aldehyde after 21 h at 37 or 60 °C, respectively.

Effect of Incubation Time on Strecker Aldehyde Production in the Reaction of 4,5-Epoxy-2-decenal (1) with Primary Amines. Strecker aldehydes were produced in the reaction of 4,5-epoxy-2-decenal (**1**) with the assayed amines at a reaction rate that depended on the amine employed and the reaction temperature (**Figure 3**). Thus, benzaldehyde (**17**) formation at 37 °C followed zero-order reaction kinetics ($r = 0.999$, $p < 0.0001$) and was described by eq 1:

$$BA = BA_0 + kt \quad (1)$$

where BA_0 represents the concentration of benzaldehyde in the intercept, k is the rate constant, and t is the time. The values for BA_0 and k were 0.77 μmol benzaldehyde/100 μmol benzylamine and 0.077 μmol benzaldehyde/100 μmol benzylamine h, respectively.

Benzaldehyde (**17**) formation at 60 °C also followed zero-order reaction kinetics ($r = 0.98$, $p = 0.02$) during the first 3 h and was also described by eq 1. After that, benzaldehyde concentration increased much more slowly until a maximum value was achieved. The values for BA_0 and k at 60 °C were 0.47 μmol benzaldehyde/100 μmol benzylamine and 0.731 μmol benzaldehyde/100 μmol benzylamine h, respectively.

Methyl 2-oxo-2-phenylacetate (**18**) formation also followed zero-order reaction kinetics ($r = 0.998$, $p < 0.0001$) at 37 °C and was described by eq 2:

$$MO = MO_0 + kt \quad (2)$$

where MO_0 represents the concentration of methyl 2-oxo-2-phenylacetate in the intercept, k is the rate constant, and t is the time. The values for MO_0 and k were 1.15 μmol methyl 2-oxo-2-phenylacetate/100 μmol 2-phenylglycine methyl ester and 1.62 μmol methyl 2-oxo-2-phenylacetate/100 μmol 2-phenylglycine methyl ester h, respectively.

Methyl 2-oxo-2-phenylacetate (**18**) formation at 60 °C also followed zero-order reaction kinetics ($r = 0.999$, $p < 0.0001$) during the first 4 h and was also described by eq 2. After that, methyl 2-oxo-2-phenylacetate concentration increased much more slowly until a maximum value was achieved. The values for MO_0 and k at 60 °C were 2.03 μmol methyl 2-oxo-2-phenylacetate/100 μmol 2-phenylglycine methyl ester and 10.53 μmol methyl 2-oxo-2-phenylacetate/100 μmol 2-phenylglycine methyl ester h, respectively.

DISCUSSION

Strecker degradation of amino acids is one of the most important reactions leading to final aroma compounds in the Maillard reaction (*1*, *2*). However, the results obtained in this study suggest that amines can also be degraded in a similar way, in accordance with previous studies showing that ninhydrin reacts with amines via a Strecker type transamination reaction (*19*, *20*). According to the obtained results, amine degradation produced by epoxyalkenals depended on the pH, the temperature, the amount of epoxyalkenal, and, mainly, the amine involved.

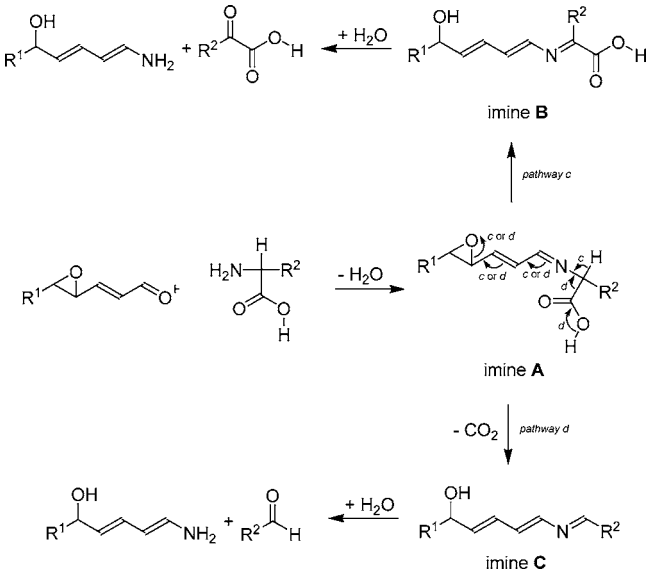
The reaction yield depended on the reaction pH, and different optimum pH values were obtained for the two assayed amines. However, although the reaction yield was reduced to the third part when the reaction was carried out at a pH far from the optimum, the Strecker degradation of the amine was always observed within the pH range studied (pH 2−8). Therefore, this reaction should be produced to some extent with independence of the pH if epoxyalkenals and suitable amines are in close contact.

The temperature of the reaction also influenced the reaction yield to some extent, but it was determinant for the reaction rate. Thus, the rate constant for benzaldehyde (**17**) formation at 37 °C was 0.077 μmol benzaldehyde/100 μmol benzylamine h, and it increased 9.5 times at 60 °C. In addition, the rate constant for methyl 2-oxo-2-phenylacetate (**18**) formation at 37 °C was 1.62 μmol methyl 2-oxo-2-phenylacetate/100 μmol 2-phenylglycine methyl ester h, and it increased 6.5 times at 60 °C. Nevertheless, both assayed amines were degraded at 37 °C. Because lipid oxidation is produced at low temperatures (*21*) and the reaction of lipid oxidation products with amines, amino phospholipids, amino acids, and proteins is the last step of the lipid peroxidation process (*22*−*25*), the Strecker degradation of suitable amines should be produced at low temperatures as a common process within the lipid peroxidation pathway.

The amount of the epoxyalkenal determined the amount of Strecker aldehyde produced because a linear relationship was observed between 4,5-epoxy-2-decenal (**1**) concentration in the reaction mixture and the amount of the produced aldehyde. Therefore, even small amounts of epoxyalkenals should be expected to degrade suitable amines to some extent.

Although pH, temperature, and the amount of the aldehyde played a significant role in the Strecker degradation of amines, the yield of the Strecker aldehyde produced was mostly a consequence of the structure of the amine involved. Thus, octylamine, which has only one alkyl chain at the α-position of the amino group, only produced trace amounts of octanal. However, the change of an alkyl chain by a phenyl ring increased considerably the amount of the Strecker aldehyde produced. Thus, benzylamine (**3**) was converted into benzaldehyde (**17**) with a yield of almost 5%. This increase in the reaction yield should be related to the easiness of conversion

Scheme 2. Alternative Pathways for the Strecker Type Degradation of Amino Acids Produced by Epoxyalkenals[a]



[a] For 4,5-epoxy-2-heptenal: $R^1 = CH_3CH_2$. For 4,5-epoxy-2-decenal: $R^1 = CH_3(CH_2)_4$. R^2 is the polar or nonpolar group of amino acids.

of imine A into imine B by pathway c (**Scheme 1**). The presence of an aromatic ring in R^1 extends the conjugation and stabilizes the imine B, therefore, favoring the conversion of imine A into imine B. For this reason, the introduction of a new group that also contributes to extend the conjugation should increase to a higher extent the reaction yield. As an example, 2-phenylglycine methyl ester (**4**) was converted into methyl 2-oxo-2-phenylacetate (**18**) with a reaction yield of almost 50%.

This essential role of amine structure is likely to determine what food amines are susceptible to suffer Strecker degradation. Thus, for example, the ϵ-amino group of lysine residues in proteins does not seem to be a candidate for being degraded significantly as a consequence of this reaction (it should have a behavior similar to the above-described for octylamine). However, amino acids, which have a carboxyl group at the α-position of the amino group, are likely to suffer to some extent the described degradation. This points to the existence of two alternative pathways for the Strecker type degradation of amino acids. As shown in **Scheme 2**, amino acids react with epoxyalkenals producing the imine A, among other products, in a first step (*9*). According to the results described in a previous study (*9*), this imine A is then converted into imine C (pathway d), which is the origin of the corresponding Strecker aldehyde. However, according to the results obtained in this study, imine A might also be converted into imine B (pathway c) as an intermediate in the formation of an α-oxo acid.

Conversion of α-amino acids into α-oxo acids is commonly produced in foods, such as fermented foods. It is generally believed to be produced enzymatically (*26*). However, the results obtained in this study suggest a potential alternative chemical route for the formation of these flavor-significant food components (*26*, *27*). Additional studies are needed to determine the relative importance of pathways c and d in the Strecker type degradation of amino acids. These studies are currently in progress in this laboratory.

Recent studies from this and other laboratories have shown that lipid oxidation and Maillard reaction produce analogous products by the same mechanisms (*28*, *29*). This was previously found in the Strecker type degradation of amino acids produced by lipid oxidation products (*9*−*11*). In addition, and although this study has been carried out with epoxyalkenals, it may be hypothesized that α-dicarbonyl compounds produced in the Maillard pathway will also be able to degrade suitable amines in a similar way. In fact, when ribose and methyl 2-phenylglycine (**4**) were incubated overnight at 60 °C under the same conditions that are above-described for the epoxyalkenal **1**, methyl 2-oxo-2-phenylacetate (**18**) was produced as a major reaction product (data not shown). Therefore, this reaction may constitute a new alternative pathway for flavor formation in the Maillard reaction.

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