JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Strecker-type Degradation Produced by the Lipid Oxidation Products 4,5-Epoxy-2-Alkenals

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Strecker degradation is one of the most important reactions leading to final aroma compounds in the Maillard reaction. In an attempt to clarify whether lipid oxidation products may be contributing to the Strecker degradation of amino acids, this study analyzes the reaction of 4,5-epoxy-2-alkenals with phenylalanine. In addition to N-substituted 2-(1-hydroxyalkyl)pyrroles and N-substituted pyrroles, which are major products of the reaction, the formation of both the Strecker aldehyde phenylacetaldehyde and 2-alkylpyridines was also observed. The aldehyde, which was produced at 37 °C—as could be determined by forming its corresponding thiazolidine with cysteamine—and pH 6−7, was not produced when the amino acid was esterified. This aldehyde is suggested to be produced through imine formation, which is then decarboxylated and hydrolyzed. This reaction also produces a hydroxyl amino derivative, which is the origin of the 2-alkylpyridines identified. All these data indicate that Strecker-type degradation of amino acids is produced at 37 °C by some lipid oxidation products. This is a new proof of the interrelations between lipid oxidation and Maillard reaction, which are able to produce common products by analogue mechanisms.

**KEYWORDS: 2-Alkylpyridines; carbonyl-amine reactions; flavor production; lipid oxidation; Maillard reaction; nonenzymatic browning; pyrroles; Strecker aldehydes**

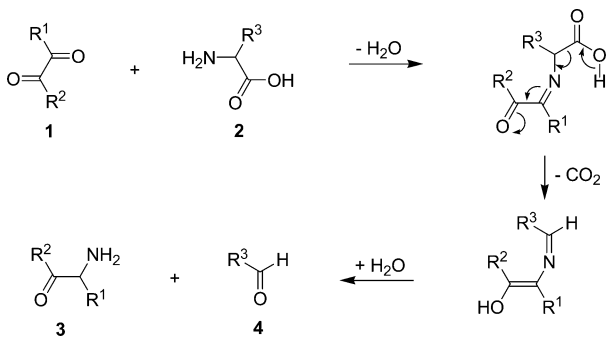
## INTRODUCTION

Strecker degradation is one of the most important reactions leading to final aroma compounds in the Maillard reaction (*1*−*4*). It involves the initial Schiff base formation of an α-dicarbonyl compound (**1**) with an amino acid (**2**). After rearrangement, decarboxylation, and hydrolysis, an α-amino carbonyl compound (**3**) and the corresponding Strecker aldehyde (**4**) are generated (**Scheme 1**). α-Amino carbonyl compounds (**3**) are precursors of pyrazines (*5*−*7*), and many Strecker aldehydes (**4**) are significant flavor compounds (*8*).

Strecker degradation is supposed to be produced from carbohydrate degradation products, despite the well-known contribution of lipids to flavor formation in cooked foods (*9, 10*). This is likely a consequence of the lack of lipids to produce significant amounts of α-dicarbonyl derivatives upon oxidation. However, compounds that have a certain analogy to α-dicarbonyl compounds are common products of lipid oxidation, and they might also produce Strecker-type degradation of amino acids. This is the case of 4,5-epoxy-2-alkenals (**5**), which have two conjugated oxygenated functions.

4,5-Epoxy-2-alkenals (**5**) 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 (**5b**) by decomposition of an intermediate 12,13(*E*)-epoxy-9-hydroperoxy-10-octadecenoic acid (*11*). Analogously, the 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) is the product of oxidation of the $n - 3$ polyunsaturated fatty acids (*12*). These epoxyalkenals have been detected in many different food systems (*13*).

**Scheme 1.** Strecker Degradation of Amino Acids Produced by α-Dicarbonyl Compounds




In an attempt to clarify whether lipid oxidation products may be contributing to the Strecker degradation of amino acids, this study analyzes the reaction of 4,5-epoxy-2-alkenals (**5**) with phenylalanine (**6**). Phenylalanine (**6**) was selected because its aldehyde derivative phenylacetaldehyde (**14**) has a high boiling point (195 °C), can be easily determined by gas chromatogra-

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10.1021/jf048883r CCC: $27.50 
Published on Web 10/15/2004

phy−mass spectrometry (GC-MS), and is a very powerful odorant (*8*). 4,5-Epoxy-2-alkenals (**5**) were previously shown to contribute to flavor production in oxidized lipid/amino acid and oxidized lipid/protein reaction mixtures, although Strecker aldehydes were not detected in those reactions because those studies were carried out with lysine (*14*).

## EXPERIMENTAL PROCEDURES

**Materials.** 4,5(*E*)-Epoxy-2(*E*)-heptenal (**5a**) and 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) were prepared from heptadienal and decadienal, respectively, as described previously (*15*). L-Phenylalanine (**6**), l-phenylalanine methyl ester hydrochloride (**6M**), phenylacetaldehyde (**14**), 2-ethylpyridine (**15a**), and 2-pentylpyridine (**15b**) were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from reliable commercial sources.

**Epoxyalkenal/Amino Acid Reaction Mixtures.** Mixtures of 0.1 mmol of the epoxyalkenal [4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) or 4,5-(*E*)-epoxy-2(*E*)-decenal (**5b)]** and 0.1 mmol of the amino acid [phenylalanine (**6**) or phenylalanine methyl ester (**6M**)] were incubated in 1 mL of acetonitrile−water (2:1) or in 1 mL of buffer at 37 °C and analyzed by GC-MS at different incubation times. The following buffers were employed: 0.3 M sodium citrate at pH 4, 5, 5.5, and 6; 0.3 M sodium phosphate at pH 6, 6.5, 7, 7.4, and 8; and 0.3 M sodium borate at pH 8, 9, and 10. Reaction mixtures also included 50 $\mu$L of 2 N KOH in methanol for phenylalanine methyl ester incubated in acetonitrile−water (2:1). Additionally, major compounds of the reaction were isolated by column chromatography and their structures were determined by $^1$H and $^{13}$C NMR and MS.

Samples for GC-MS analyses were prepared differently if reactions were done in acetonitrile−water (2:1) or in buffer. Samples incubated in acetonitrile−water (2:1) (100 $\mu$L) were diluted with 100 $\mu$L of ether−2-propanol (1:1) and 25 $\mu$L of the internal standard solution [1.35 mg of 3(*Z*)-nonenol in 1 mL of methanol] and injected in the chromatograph. Samples incubated in buffer (200 $\mu$L) were treated with 25 $\mu$L of the internal standard solution and extracted with 200 $\mu$L of chloroform. Phases were separated by centrifugation at 2000*g* for 10 min and the organic extracts were injected in the chromatograph.

Compounds isolated by column chromatography and characterized by $^1$H and $^{13}$C NMR and MS were obtained from overnight incubations in 0.3 M sodium phosphate buffer, pH 7, which were taken to pH 3 with HCl and extracted with chloroform. This extract was fractionated on silica gel with hexane−acetone (2:1) as solvent.

**Thiazolidine Derivatives of Aldehydes Produced in Epoxyalkenal/Amino Acid Reaction Mixtures.** In addition to their direct determination by GC-MS, aldehydes produced in epoxyalkenal/amino acid reaction mixtures were derivatized with cysteamine according to the method of Yasuhara et al. (*16*), which was slightly modified, and the thiazolidines produced were analyzed by GC-MS. Briefly, the incubated epoxyalkenal/amino acid reaction (400 $\mu$L) was diluted with 200 $\mu$L of methanol−water (1:1), treated with 45 mg of cysteamine hydrochloride, and the pH of the solution was immediately adjusted to 8 with 2 N NaOH. The resulting solution was then stirred for 3 h at room temperature. After that time, samples were diluted with 1 mL of water and extracted with 1 mL of ethyl acetate. The resulting organic layer was dried over sodium sulfate and injected in the gas chromatograph under the conditions described below. Thiazolidine derived from propanal (**11aT**): $R_t$ 8.54 min; mass spectrum *m*/*z* (%) 117 (18), 88 (100), 71 (12), 70 (24), 61 (11), and 56 (16). Thiazolidine derived from hexanal (**11bT**): $R_t$ 17.85 min; mass spectrum *m*/*z* (%) 159 (12), 88 (100), 70 (11), 61 (10), and 56 (25). Thiazolidine derived from phenylacetaldehyde (**14T**): $R_t$ 25.24 min; mass spectrum *m*/*z* (%) 179 (1), 150 (1), 132 (5), 117 (3), 103 (3), 92 (4), 91 (20), 90 (5), 89 (7), 88 (100), 77 (4), 65 (6), and 61 (8).

**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 $\mu$m) 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; ionization (EI), 70 eV.

**$^1$H and $^{13}$C NMR.** $^1$H and $^{13}$C NMR spectra at 300 and 75.4 MHz, respectively, were determined in a Bruker AC-300P (Karlsruhe, Germany), with $Me_4Si$ as internal standard. Two-dimensional NMR was used to assign the $^{13}$C NMR spectra.

**Browning.** Samples incubated in buffer (200 $\mu$L) were extracted with 200 $\mu$L of chloroform, and 180-$\mu$L aliquots of the resulting organic and aqueous extracts were diluted to 1 mL with chloroform or water, respectively. Their colors were determined spectrophotometrically on a Shimadzu UV-2401 PC UV−vis spectrophotometer. Color differences ($\Delta E$) at the different periods of time were calculated from the determined Cielab $L^*$, $a^*$, and $b^*$ values according to Hunter (*17*):

$$\Delta E = [(L^* - 100)^2 + (a^*)^2 + (b^*)^2]^{1/2}$$

by referring the determined values to an ideal colorless solution of $L^* = 100$, and $a^* = b^* = 0$ (*18*).

## RESULTS

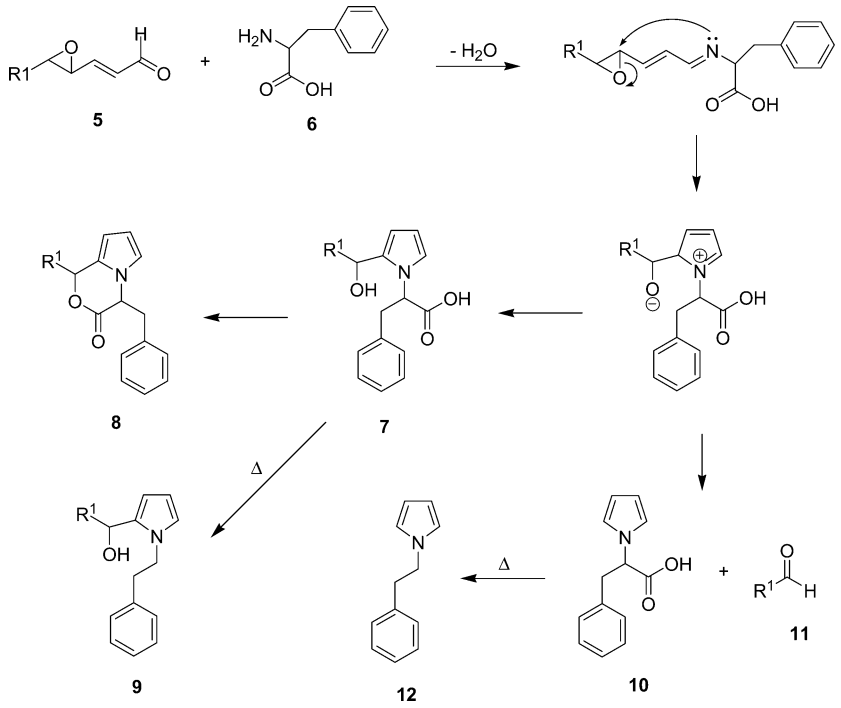
**Isolation and Characterization of the Major Products of 4,5(*E*)-Epoxy-2(*E*)-heptenal/Phenylalanine Reaction.** To identify by GC-MS the different compounds produced in the reaction between epoxyalkenals and phenylalanine, the major products of reaction between 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**) were isolated by column chromatography and characterized by $^1$H and $^{13}$C NMR and MS.

Analogously to previous studies of the reaction between epoxyalkenals and amines and amino acids (*19*−*21*), this reaction produced the corresponding N-substituted 2-(1-hydroxyalkyl)pyrrole (**7**) and the N-substituted pyrrole (**10**) as main reaction products. The reaction scheme, including the different compounds produced, is shown in **Scheme 2**. This reaction scheme implies in a first step the transformation of the imine trans carbon−carbon double bond into a cis double bond, which can occur by conjugate addition−elimination of amine to the C═C, well-known for $\alpha,\beta$-unsaturated carbonyl compounds. This cis isomer of imine would then convert to the indicated cyclic intermediate, the transformation of which into products **7** and **10** is likely to occur by electronic arrangement. This electronic arrangement implies the exit of either an aldehyde (**11**), when compound **10** is produced, or the proton at position 5 of the ring of the intermediate, producing compound **7**. In addition, the close presence of the carboxylic group and the hydroxyl group in **7** may facilitate the formation of lactone **8** by intramolecular dehydration under favorable conditions. Finally, the thermal treatment of compounds **7** and **10** may produce their decarboxylation to compounds **9** and **12**, respectively. This heating may occur, for example, in the injection port of the chromatograph.

When the reaction was carried out between 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**), the major isolated compounds were the lactone 4-benzyl-1-ethyl-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-3-one (**8a**) and the N-substituted pyrrole 3-phenyl-2-pyrrol-1-ylpropionic acid (**10**), which were characterized by $^1$H and $^{13}$C NMR and MS.

4-Benzyl-1-ethyl-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-3-one (**8a**): $^1$H NMR ($CDCl_3$) $\delta$ 0.96 t (3H, $J$ = 7.3 Hz, C*H*$_3$CH$_2$), 1.90 m (2H, CH$_3$C*H*$_2$CH), 3.35 m (2H, PhCH$_2$), 4.10 m (1H, CH$_3$-CH$_2$C*H*), 5.07 t (1H, $J$ = 5.2 Hz, PhCH$_2$C*H*), 5.88 m (1H, H3 of pyrrole), 6.20 dd (1H, $J$ = 2.7 and 3.5 Hz, H4 of pyrrole), 6.51 m (1H, H5 of pyrrole), 6.86 m (2H, H2 and H6 of phenyl), and 7.23 m (3H, H3, H4, and H5 of phenyl); $^{13}$C NMR ($CDCl_3$) $\delta$ 8.86 q (CH$_3$), 25.99 t (CH$_3$*C*H$_2$), 40.86 t (Ph*C*H$_2$), 59.65 d (PhCH$_2$*C*H), 76.80 d (CH$_3$CH$_2$*C*H), 103.10 d (C3 of pyrrole), 109.73 d (C4 of pyrrole), 118.37 d (C5 of pyrrole), 125.83 s

**Scheme 2.** Formation of Pyrrole Derivatives between 4,5-Epoxy-2-alkenals and Phenylalanine[a]




[a] For **5a**, **7a**, **8a**, **9a**, and **11a**, $R^1 = CH_3CH_2$; for **5b**, **7b**, **8b**, **9b**, and **11b**, $R^1 = CH_3(CH_2)_4$. **M** is employed for the corresponding methyl esters; **T** is employed for the thiazolidines of the corresponding aldehydes.

(C2 of pyrrole), 127.76 d (C4 of phenyl), 128.69 d (C3 and C5 of phenyl), 129.59 d (C2 and C6 of phenyl), 134.64 s (C1 of phenyl), and 168.85 s (CO); MS *m/z* (%, ion structure) 255 (36, $M^+$), 226 (17, $M^+$ − ethyl), 198 (100, $M^+ - C_2H_5CO$), 164 (28, $M^+ - PhCH_2$), 136 (23), 105 (24), 91 (76, $PhCH_2^+$), 77 (24), and 65 (23).

3-Phenyl-2-pyrrol-1-ylpropionic acid (**10**): $^1$H NMR ($CDCl_3$) $\delta$ 3.30 m (2H, C*H*$_2$CH), 4.60 m (1H, CH$_2$C*H*), 6.02 m (2H, H3 and H4 of pyrrole), 6.60 m (2H, H2 and H5 of pyrrole), 6.96 m (2H, H2 and H6 of phenyl), and 7.15 m (3H, H3, H4, and H5 of phenyl); $^{13}$C NMR ($CDCl_3$) $\delta$ 39.20 t (*C*H$_2$CH), 65.57 d (CH$_2$*C*H), 107.93 d (C3 and C4 of pyrrole), 120.34 d (C2 and C5 of pyrrole), 126.58 d (C4 of phenyl), 128.34 d (C3 and C5 of phenyl), 128.65 d (C2 and C6 of phenyl), 137.67 s (C1 of phenyl), and 177.92 s (COOH); MS *m/z* (%, ion structure): 215 (61, $M^+$), 170 (34, $M^+$ − COOH), 147 (11), 124 (88, $M^+ - PhCH_2$), 103 (11), 91 (100, $PhCH_2^+$), 78 (21), 65 (16), and 51 (16).

In addition, the decarboxylated products 1-(1-phenethyl-1*H*-pyrrol-2-yl)propan-1-ol (**9a**) and 1-phenethyl-1*H*-pyrrole (**12**) were characterized by GC-MS.

1-(1-Phenethyl-1*H*-pyrrol-2-yl)propan-1-ol (**9a**): MS *m/z* (%, ion structure) 229 (22, $M^+$), 211 (23, $M^+ - H_2O$), 200 (46, $M^+$ − ethyl), 182 (10), 172 (38), 120 (57, $M^+ - H_2O - PhCH_2$), 105 (100, $PhCH_2CH_2^+$), 104 (36), 103 (24), 91 (48, $PhCH_2^+$), 80 (21), 77 (28), and 65 (20).

1-Phenethyl-1*H*-pyrrole (**12**): MS *m/z* (%, ion structure) 171 (45, $M^+$), 104 (17), 91 (24, $PhCH_2^+$), 80 (100, $M^+ - PhCH_2$), 77 (10), 65 (10), 53 (19), and 51 (14).

**GC-MS Analysis of Epoxyalkenal/Phenylalanine Reaction Mixtures. Figure 1A** shows the total ion chromatogram of GC-MS analysis for the reaction mixture of 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**) after overnight incubation in acetonitrile−water (2:1) at 37 °C. In addition to the original aldehyde, the corresponding pyrrole derivatives **8a**, **9a**, and **12** were among the major reaction products and could be easily identified by their mass spectra and retention times. Other major products were a pyrrole derivative, which has been tentatively identified as 2-ethyl-1-phenethyl-1*H*-pyrrole (**EP**), the Strecker

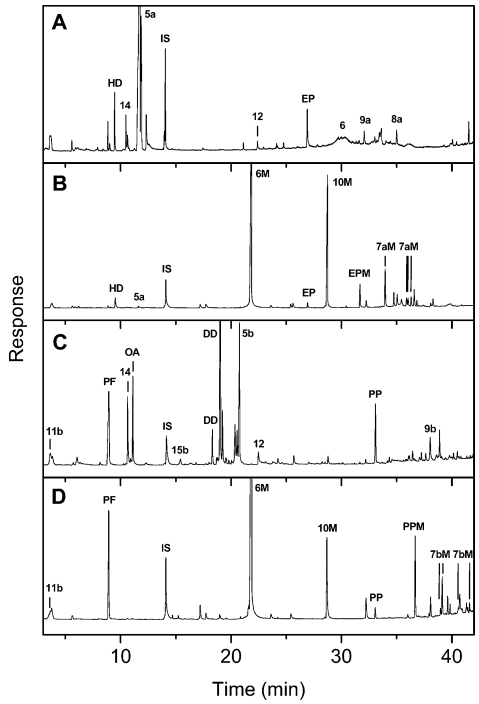



**Figure 1.** Total ion chromatograms of GC-MS analysis for the reactions of (**A**) 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) with phenylalanine (**6**), (**B**) 4,5-(*E*)-epoxy-2(*E*)-heptenal (**5a**) with phenylalanine methyl ester (**6M**), (**C**) 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) with phenylalanine (**6**), and (**D**) 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) with phenylalanine methyl ester (**6M**) after overnight incubation at 37 °C in acetonitrile–water (2:1). Structure for the identified compounds are given either in Schemes 2 and 3 or in the text. The internal standard [3(*Z*)-nonenol] is marked IS.

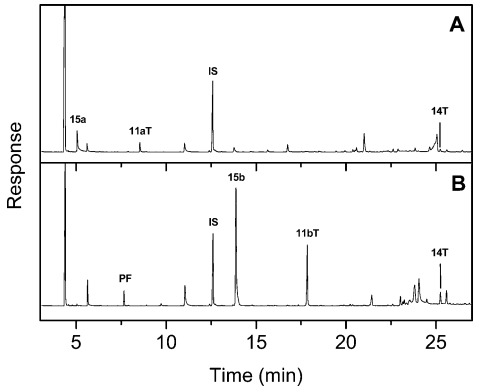



**Figure 2.** Total ion chromatogram of GC-MS analysis for the reactions of (**A**) 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) with phenylalanine (**6**) and (**B**) 4,5-(*E*)-epoxy-2(*E*)-decenal (**5b**) with phenylalanine (**6**) after overnight incubation at 37 °C in acetonitrile–water (2:1) and derivatization with cysteamine. Thiazolidines of propanal, hexanal, and phenylalanine are marked **11aT**, **11bT**, and **14T**, respectively. The internal standard [3(*Z*)-nonenol] is marked IS. Structures for other identified compounds are given either in Schemes 2 and 3 or in the text.

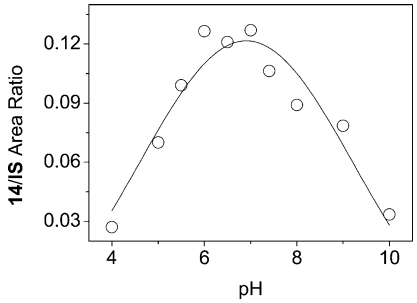



**Figure 3.** Effect of pH on phenylacetaldehyde (**14**) formation in the reaction between 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**). Samples were incubated overnight in 0.3 M sodium citrate (pH 4–6), sodium phosphate (pH 6–8), or sodium borate (pH 8–10) buffer and then extracted with chloroform, and phenylacetaldehyde (**14**) was determined by GC-MS. Phenylacetaldehyde/internal standard (**14/IS**) area ratios are given. The IS was 3(*Z*)-nonenol.

aldehyde phenylacetaldehyde (**14**), and 2,4-heptadienal (**HD**). These last two compounds were identified by comparison of mass spectra and retention times with authentic standards.

Analogous products were also formed in 4,5(*E*)-epoxy-2(*E*)-decenal/phenylalanine reaction mixtures (**Figure 1C**). Thus, compound **12** was identical to the one obtained in the 4,5(*E*)-epoxy-2(*E*)-heptenal/phenylalanine reaction mixtures, and compound **9b** could be identified by analogy of its mass spectrum to that of **9a**. The mass spectrum of 1-(1-phenethyl-1*H*-pyrrol-2-yl)hexan-1-ol (**9b**) was *m*/*z* (%, ion structure) 271 (13, $M^+$), 253 (31, $M^+ - H_2O$), 210 (56, $M^+ - H_2O -$ propyl), 200 (56, $M^+ -$ pentyl), 172 (26), 162 (14, $M^+ - H_2O - PhCH_2$), 118 (20), 106 (17), 105 (100, $PhCH_2CH_2^+$), 104 (27), 103 (16), 91 (31, $PhCH_2^+$), 80 (19), and 77 (21). In addition, the formation of hexanal (**11b**), 2-pentylfuran (**PF**), the Strecker aldehyde phenylacetaldehyde (**14**), 2-octenal (**OA**), 2-pentylpyridine (**15b**), 2,4-decadienal (**DD**), and a pyrrole derivative, which has been tentatively identified as 2-pentyl-1-phenethyl-1*H*-pyrrole (**PP**), could be identified.

The identification of compounds **8**, **9**, **11**, and **12** in both reactions confirmed that the reaction scheme indicated in **Scheme 2** was being produced. However, the presence of Strecker aldehyde phenylacetaldehyde (**14**) suggested that pyrrole formation was competing with other reaction(s) that produced this aldehyde.

**GC-MS Analysis of Epoxyalkenal/Phenylalanine Methyl Ester Reaction Mixtures.** To confirm that formation of compound **14** was not an artifact, the reactions of epoxyalkenals **5a** and **5b** with phenylalanine methyl ester (**6M**) were studied. **Figure 1B** shows the total ion chromatogram of GC-MS analysis for the reaction mixture of 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine methyl ester (**6M**) after overnight incubation in acetonitrile−water (2:1) at 37 °C. Because methyl esters are not easily decarboxylated, the corresponding **9a** and **12** were absent. However, the expected N-substituted 2-(1-hydroxyalkyl)-pyrrole (**7**) and *N*-substituted pyrrole (**10**) were present, and they could be easily identified by their mass spectra.

2-[2-(1-Hydroxypropyl)pyrrol-1-yl]-3-phenylpropionic acid methyl ester (**7aM**): MS *m*/*z* (%, ion structure) 287 ($M^+$, 29), 269 (49, $M^+ - H_2O$), 258 (24, $M^+ -$ ethyl), 230 (75, $M^+ - C_3H_5O$), 210 (22), 198 (75, 230 − methanol), 170 (79), 168 (24), 131 (29), 121 (23), 118 (45), 108 (32), 104 (33), 91 (100, Ph $CH_2^+$), 80 (26), 77 (35), and 65 (26).

3-Phenyl-2-pyrrol-1-ylpropionic acid methyl ester (**10M**): MS *m*/*z* (%, ion structure) 229 (67, $M^+$), 170 (60, $M^+ - CO_2CH_3$), 168 (29), 162 (18), 138 (100, $M^+ - PhCH_2$), 131 (13), 110 (12), 103 (14), 91 (59, $PhCH_2^+$), 78 (15), 77 (14), 65 (14), 59 (16), and 51 (12).

In addition, the pyrrole derivative **EP** and its analogous ester (**EPM**) were also present. However, the phenylacetaldehyde (**14**) was absent, therefore suggesting that the acid group is necessary for its formation.

Analogous results were also obtained when the reaction of 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) with phenylacetaldehyde methyl ester (**6**) was studied. **Figure 1D** shows the total ion chromatogram of GC-MS analysis obtained for this reaction in acetonitrile−water (2:1) after overnight incubation at 37 °C. The corresponding N-substituted 2-(1-hydroxyalkyl)pyrrole (**7**) and N-substituted pyrrole (**10**) were also major products of the reaction and could be easily identified by their mass spectra. The mass spectrum of 2-[2-(1-hydroxyhexyl)pyrrol-1-yl]-3-phenylpropionic acid methyl ester (**7bM**) was *m*/*z* (%, ion structure) 329 (17, $M^+$), 311 (41, $M^+ - H_2O$), 268 (51, $M^+ - H_2O -$ propyl), 258 (33, $M^+ -$ pentyl), 242 (17), 232 (17), 230 (100, $M^+ - C_6H_{11}O$), 226 (48), 203 (22), 198 (64, 230 − methanol), 170 (58), 162 (31), 151 (29), 131 (35), 121 (43), 104 (29), 103 (23), 91 (43, $PhCH_2^+$), 80 (29), and 68 (35).

Other compounds formed were hexanal (**11b**), pentylfuran (**PF**), and the pyrrole (**PP**) and its analogous ester (**PPM**). However, neither phenylacetaldehyde (**14**) nor 2-ethylpyridine (**15**) was present, therefore confirming that the acid group is necessary for the formation of these compounds.

**Thiazolidine Derivatives of Aldehydes Produced in Epoxyalkenal/Amino Acid Reaction Mixtures.** To confirm that phenylacetaldehyde (**14**) was produced at 37 °C and not as a consequence of the heating at the injection port of the chromatograph, aldehydes produced in epoxyalkenal/phenylalanine reaction mixtures were derivatized with cysteamine, and the produced thiazolidines were determined by GC-MS. **Figure 2A** shows the total ion chromatogram of GC-MS analysis for the reaction mixture of 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**) after overnight incubation in acetonitrile−water (2:1) at 37 °C and derivatization with cysteamine. Two aldehydes were detected: propanal (**11aT**) and phenylacetaldehyde (**14T**). The former is produced as a byproduct of N-substituted pyrrole **10** formation (**Scheme 2**), and the ap-

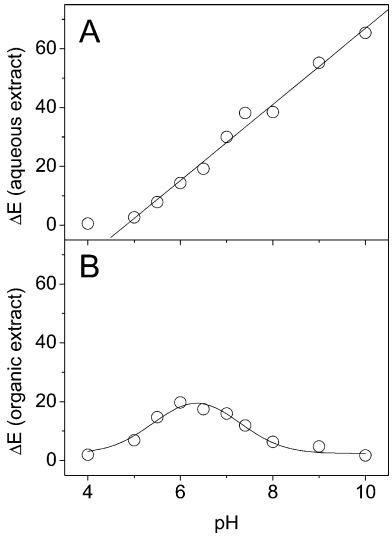



**Figure 4.** Effect of pH on browning development in the reaction between 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) and phenylalanine (**6**). Samples were incubated overnight in 0.3 M sodium citrate (pH 4–6), sodium phosphate (pH 6–8), or sodium borate (pH 8–10) buffer and then extracted with chloroform. Colors in (**A**) aqueous and (**B**) organic extracts were determined spectrophotometically.

pearance of the latter confirmed its production at 37 °C. In addition, 2-ethylpyridine (**15a**) was also identified in this reaction.

Analogous results were obtained when 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) was employed. **Figure 2B** shows the total the total ion chromatogram of GC-MS analysis for the reaction mixture of 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**) and phenylalanine (**6**) after overnight incubation in acetonitrile−water (2:1) at 37 °C and derivatization with cysteamine. This reaction produced the corresponding aldehyde **11bT** (hexanal, which is the aldehyde produced from $n - 6$ fatty acid oxidation) and the Strecker aldehyde phenylacetaldehyde (**14T**), therefore confirming the formation of this last aldehyde at 37 °C from lipid oxidation products. In addition, 2-pentylfuran (**PF**) and 2-pentylpyridine (**15b**) were also identified, analogously to the above-described direct injection of 4,5(*E*)-epoxy-2(*E*)-decenal/phenylalanine reaction mixture.

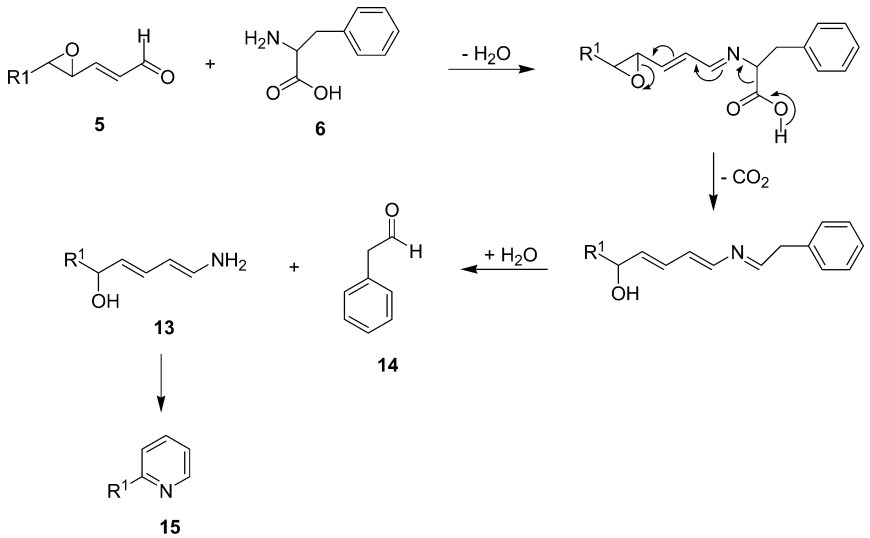
**Effect of pH on Phenylacetaldehyde Formation and Browning Development in Epoxyalkenal/Phenylalanine Reaction Mixtures.** Phenylacetaldehyde (**14**) formation was pH-dependent and was mainly produced at pH 6−7. **Figure 3** shows the formation of phenylacetaldehyde as a function of pH. It followed a Gaussian curve ($r^2 = 0.92$) with the center at pH 6.9. Its formation was not related to color development in these reactions, which is believed to be a consequence of the polymerization of N-substituted 2-(1-hydroxyalkyl)pyrrole (**7**) and favorably produced under basic conditions (*19*, *22*). However, when epoxyalkenal/phenylalanine reaction mixtures were extracted with chloroform, the effect of pH on reaction browning was different for both aqueous and organic extracts (**Figure 4**). Thus, although the main browning was observed in the aqueous extract, which increased linearly ($r = 0.994$, $p < 0.0001$) between pH 5 and 10, the browning in the organic extract followed a Gaussian curve ($r^2 = 0.96$) with the center at pH 6.4. The similarity of this last curve with the curve of phenylacetaldehyde formation (**Figure 3**), and their apparent correlation ($r = 0.92$, $p = 0.000\,13$), suggested a relationship between the formation of phenylacetaldehyde and the browned pigments extractable with chloroform.

## DISCUSSION

Fats and oils are notorious for their role in the development of off-flavors through autoxidation, and their mechanisms of formation have been broadly studied (*23*−*25*). Thus, aldehydes and ketones are the main volatiles from autoxidation, and these compounds can cause painty, fatty, metallic, papery, and candlelike flavors in foods when their concentrations are sufficiently high. On the other hand, the pleasant flavors that contribute to general nutty, meaty, roasted, burnt, floral, plant, or caramel odors in processed foods have been traditionally believed to be produced as a consequence of Maillard reactions (*25*, *26*).

The results obtained in this study suggest that this difference among flavors produced as a consequence of either lipid oxidation or Maillard reaction may be not so clear, and flavors traditionally assigned to Maillard reactions may also be produced as a consequence of lipid oxidation. This is the case of the Strecker-type degradation of amino acids that have been found to be produced by the lipid oxidation products 4,5-epoxy-2-alkenals.

**Scheme 3.** Strecker-type Degradation of Phenylalanine Produced by 4,5-Epoxy-2-alkenals[a]




[a] For **5a**, **13a**, and **15a**, $R^1 = CH_3CH_2$; for **5b**, **13b**, and **15b**, $R^1 = CH_3(CH_2)_4$. **T** is employed for the thiazolidines of the corresponding aldehydes.

The mechanism of the reaction between epoxyalkenals and amino acids leading to the formation of Stecker aldehydes is suggested in **Scheme 3**. Analogously to the Strecker degradation produced by α-dicarbonyl compounds, the imine is produced in a first step. However, and in contrast to the evolution to the pyrrole derivatives indicated in **Scheme 2**, the imine decarboxylation and its later hydrolysis must produce the Strecker aldehyde **14**. This mechanism is supported by the hydroxyl amino derivative produced (**13**). This derivative should produce 2-ethylpyridine (**15a**) or 2-pentylpyridine (**15b**) when starting from 4,5(*E*)-epoxy-2(*E*)-heptenal (**5a**) or 4,5(*E*)-epoxy-2(*E*)-decenal (**5b**), respectively. Both 2-ethylpyridine (**15a**) and 2-pentylpyridine (**15b**) have been detected in these reactions, and this may be one of the origins of the appearance of these compounds in foods (*27*−*29*). In addition, the hydroxyl amino derivative produced (**13**) might also contribute, by polymerization, to the browning color that is extracted by chloroform.

All these data indicate that Strecker-type degradation of amino acids is produced at 37 °C by some lipid oxidation products. Although additional studies are needed to know if this reaction is also produced by other lipid oxidation products and to fully elucidate the real importance of these reactions in final food flavors produced during food processing and storage, the results obtained in this study constitute a new proof of the interrelations between lipid oxidation and Maillard reaction, which are able to produce common products, both volatile—as described above—and nonvolatile (*30*), by analogous mechanisms and starting from different reactants.

## ACKNOWLEDGMENT

We are indebted to Dr. Jorge Ruiz, University of Extremadura, for valuable discussions and to José L. Navarro for technical assistance.

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**Received for review July 7, 2004. Revised manuscript received August 20, 2004. Accepted September 5, 2004. This study was supported in part by the European Union and the Plan Nacional de I + D of the Ministerio de Educación y Ciencia of Spain (Project AGL2003-02280).**

JF048883R