JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Strecker-type Degradation of Phenylalanine by Methyl 9,10-Epoxy-13-oxo-11-octadecenoate and Methyl 12,13-Epoxy-9-oxo-11-octadecenoate

Rosario Zamora, Emerenciana Gallardo, José L. Navarro, and Francisco J. Hidalgo*

Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Avenida Padre García Tejero 4, 41012 Seville, Spain

The reaction of methyl 9,10-epoxy-13-oxo-11(*E*)-octadecenoate, methyl 12,13-epoxy-9-oxo-11(*E*)-octadecenoate, 4,5(*E*)-epoxy-2(*E*)-heptenal, and 4,5(*E*)-epoxy-2(*E*)-decenal with phenylalanine in acetonitrile−water (2:1, 1:1, and 1:2) at 80 °C and at different pHs and carbonyl compound/amino acid ratios was investigated both to determine if epoxyoxoene fatty esters were able to produce the Strecker-type degradation of the amino acid and to study the relative ability of oxidized long-chain fatty esters and short chain aldehydes with identical functional systems to degrade amino acids. The studied epoxyoxoene fatty esters degraded phenylalanine to phenylacetaldehyde. The mechanism of the reaction was analogous to that described for epoxyalkenals and is suggested to be produced through the corresponding imine, which is then decarboxylated and hydrolyzed. This reaction also produced a conjugated hydroxylamine, which was the origin of the long-chain pyridine-containing fatty ester isolated in the reaction and characterized as methyl 8-(6-pentylpyridin-2-yl)octanoate. Epoxyoxoene fatty esters and epoxyalkenals exhibited a similar reactivity for producing phenyl-acetaldehyde, therefore suggesting that nonvolatile lipid oxidation products, which are produced to a greater extent than volatile products, should be considered for determining the overall contribution of lipids to Strecker degradation of amino acids produced during nonenzymatic browning. In addition, the obtained data confirm that, analogously to carbohydrates, lipid oxidation products are also able to produce the Strecker degradation of amino acids.

INTRODUCTION

Strecker degradation of amino acids 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 with an amino acid. After rearrangement, decarboxylation and hydrolysis, an α-amino carbonyl compound and the corresponding Strecker aldehyde are generated (see, for example, ref *5* for a general scheme of Strecker degradation produced by α-dicarbonyl compounds). α-Amino carbonyl compounds are precursors of pyrazines (*6−8*) and many Strecker aldehydes are significant flavor compounds (*9*).

Strecker-type degradation of amino acids can also be produced by the lipid oxidation products 4,5-epoxy-2-alkenals (*5*). The reaction takes place in a similar way to that described for α-dicarbonyl compounds. Consequently, the imine is produced

in a first step. After rearrangement, decarboxylation, and hydrolysis, a conjugated hydroxylamine and the corresponding Strecker aldehyde are generated. The conjugated hydroxylamines are the precursors of 2-alkylpyridines.

In addition to 4,5-epoxy-2-alkenals, other lipid oxidation products with analogous structures should also be able to produce an analogous degradation of amino acids. Particularly, epoxyoxoene fatty acids with analogous 4,5-epoxy-1-oxo-2-pentene system are produced when fatty acid hydroperoxides are treated with different catalysts containing iron, such as Fe(II) (*10*), Fe(III)−cysteine (*11*), and a soy extract (*12*). These compounds react with amines and amino acids, producing long-chain pyrrole derivatives (*13*), and they should also be able to produce Strecker aldehydes analogously to 4,5-epoxy-2-alkenals.

The first objective of this study was to determine if epoxy-oxoene fatty esters are able to degrade amino acids analogously to 4,5-epoxy-2-alkenals and to find out the mechanism of the reaction. The second objective was to compare the reactivities of aldehydes and ketones for the Strecker-type degradation of

* To whom correspondence should be addressed. Tel: +(34) 954 611 550. Fax: +(34) 954 616 790. E-mail fhidalgo@ig.csic.es.

amino acids by quantifying the formation of the corresponding Strecker aldehyde in diverse systems containing either 4,5-epoxy-2-alkenals or epoxyoxoene fatty esters. All of these studies were carried out with phenylalanine because its aldehyde derivative phenylacetaldehyde has a high boiling point (195 °C), can be easily determined by gas chromatography (GC), and is a very powerful odorant (*9*).

EXPERIMENTAL PROCEDURES

Materials. A mixture of methyl 9,10-epoxy-13-oxo-11(*E*)-octadecenoate (**1a**) and methyl 12,13-epoxy-9-oxo-11(*E*)-octadecenoate (**1b**) was prepared as described previously (*13*, *14*). Briefly, linoleic acid was oxidized by soybean lipoxygenase to produce mainly the corresponding 13-hydroperoxy derivative. Reduction of the hydroperoxides with sodium borohydride, followed by oxidation, esterification, and epoxidation, yielded the corresponding epoxyoxoene fatty esters (**1**). 4,5(*E*)-Epoxy-2(*E*)-heptenal and 4,5(*E*)-epoxy-2(*E*)-decenal were prepared from 2,4-heptadienal and 2,4-decadienal, respectively, as described previously (*15*). 2-Pentyl-1-phenethyl-1*H*-pyrrole (**7a**) was prepared by reaction of 4-hydroxy-2-nonenal with phenethylamine in tetrahydrofuran (*16*). 4-Hydroxy-2-nonenal was prepared according to Gardner et al. (*17*). Linoleic acid and soybean lipoxygenase were purchased from Fluka Chemie AG (Buchs, Switzerland). 2,4-Heptadienal, 2,4-decadienal, and L-phenylalanine (**2**) were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from reliable commercial sources.

Epoxyoxoene Fatty Ester/Phenylalanine and Epoxyalkenal/Phenylalanine Reaction Mixtures. A solution of 0.025 or 0.05 mmol of oxidized lipid [epoxyoxoene fatty ester (**1**) or epoxyalkenal] in 1 mL of a mixture of acetonitrile-water (2:1, 1:1, or 1:2), containing or not 10 μL of 2 N KOH in methanol, was treated with 0.05 mmol of phenylalanine (**2**) and incubated at 80 °C. The pH of samples with no KOH added was ~5. The pH of samples containing KOH was ~9. After overnight incubation, samples were cooled and treated with 300 μL of ethanol. The pH of incubated samples did not change significantly upon incubation. These incubated samples (10 μL) were then diluted with 190 μL of the corresponding acetonitrile-water mixture (2:1, 1:1, or 1:2), 25 μL of internal standard solution [8.43 mg of 3(*Z*)-nonenol in 25 mL of methanol] was added, and samples were either studied by GC-MS or their phenylacetaldehyde content was determined by GC.

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 °C (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 (11) Content by GC. GC 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 flame ionization detector. Quantification of phenylacetaldehyde (**11**) was carried out by preparing standard curves over a concentration range of 5-90 nmol phenylacetaldehyde (**11**) in the 225 μL of solution prepared for GC injection (see above). For each curve, five different concentration levels of the aldehyde were used. Phenylacetaldehyde (**11**) content was directly proportional to the phenylacetaldehyde/internal standard area ratio ($r > 0.99$, $p < 0.0001$). The coefficients of variation within this range were lower than 5%.

Synthesis of Methyl 8-(6-Pentylpyridin-2-yl)octanoate (12). A solution of 2.5 mmol of epoxyoxo fatty ester (**1**) and 7.5 mmol of phenylalanine (**2**) in 50 mL of dimethyl sulfoxide was heated under reflux for 24 h. After that time, the reaction mixture was diluted with water (50 mL) and extracted four times with 50 mL of diethyl ether. The organic layers were washed twice with 50 mL of water and dried over sodium sulfate. This extract was then fractionated by column chromatography on silica gel 60 using hexane-ether (7:3) as solvent [compound **12** had R_f = 0.21 on silica gel TLC plates using hexane-
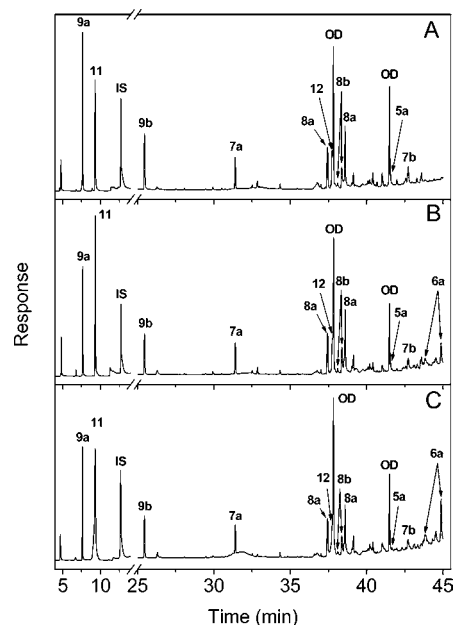

Figure 1. Total ion chromatograms of GC-MS analysis for the reactions of epoxyoxoene fatty esters (**1**) and phenylalanine (**2**) in (**A**) acetonitrile-water (2:1), (**B**) acetonitrile-water (1:1), and (**C**) acetonitrile-water (1:2) after overnight incubation at 80 °C. The structure for the identified compounds are given either in Schemes 1-3 or in the text. The internal standard [3(*Z*)-nonenol] is marked IS.

ether (7:3) as solvent]. This fractionation allowed purifying compound **12** to a high extent. Final purification of compound **12** was carried out by preparative TLC on aluminum oxide N-coated plates, and the solvent employed was hexane-ether (7:3) [R_f = 0.53]: ^{1}H NMR (CDCl$_3$) δ 0.89 (t, 3H, H-18), 1.2-1.4 and 1.55-1.75 (2m, 16H), 2.30 (t, J = 5.4 Hz, 2H, H-2), 2.74 (t, J = 8.1 Hz, 4H, H-8 and H-14), 3.66 (s, 3H, OCH$_3$), 6.94 (dd, J = 1.9 and 7.7 Hz, 2H, H-10 and H-12), and 7.49 (t, J = 7.7 Hz, H-11); ^{13}C NMR (CDCl$_3$) δ 14.06 (C-18), 22.58 (C-17), 24.92 (C-3), 29.06, 29.14, 29.23, 29.72, 29.96, 30.16 (C-14), 31.66 (C-16), 34.08 (C-2), 38.57 (C-8), 51.48 (OCH$_3$), 119.59 and 119.70 (ring C'-3/C'-5), 136.89 (ring C'-4), 161.75 and 161.92 (ring C'-2/C'-6), and 174.36 (C-1); GC-MS m/z (relative intensity, ion structure) 305 (2, M$^+$), 304 (2, M$^+$ − 1), 276 (11, M$^+$ − ethyl), 262 (11, M$^+$ − propyl), 249 (19, methyl 8-(6-methylpyridin-2-yl)octanoate), 190 (15, M$^+$ − (CH$_2$)$_4$CO$_2$CH$_3$), 176 (33, M$^+$ − (CH$_2$)$_5$CO$_2$CH$_3$), 163 (100, 2-methyl-6-pentylpyridine), 120 (63, 2-ethyl-6-methylpyridine − 1), and 107 (53, 2,6-dimethylpyridine).

^{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$_4$Si as internal standard. Two-dimensional NMR was used to assign the ^{13}C NMR spectra.

Statistical Analysis. Phenylacetaldehyde (**11**) determinations are expressed as mean values ± SD of, at least, three experiments. Statistical comparisons among different groups were made using ANOVA. When significant F values were obtained, group differences were evaluated by the Student-Newman-Keuls test (*18*). All statistical procedures were carried out using *Primer of Biostatistics: The Program* (McGraw-Hill, Inc., New York). Significance level is $p < 0.05$ unless otherwise indicated.

RESULTS

Strecker-type Degradation of Phenylalanine (2) Produced by Epoxyoxoene Fatty Esters (1). When a mixture of epoxyoxoene fatty esters (**1**) and phenylalanine (**2**) was heated overnight at 80 °C, the formation of the Strecker aldehyde phenylacetaldehyde (**11**) could be easily observed by GC-MS (Figure 1). In fact, this compound appeared as a major reaction product when a flame ionization detector was employed (data

Strecker-type Degradation by Oxidized Fatty Esters

J. Agric. Food Chem., Vol. 53, No. 11, 2005   **4585**

Scheme 1. Formation of Pyrrole Derivatives in the Reaction of Epoxyoxoene Fatty Esters (**1**) with Phenylalanine (**2**)[a]
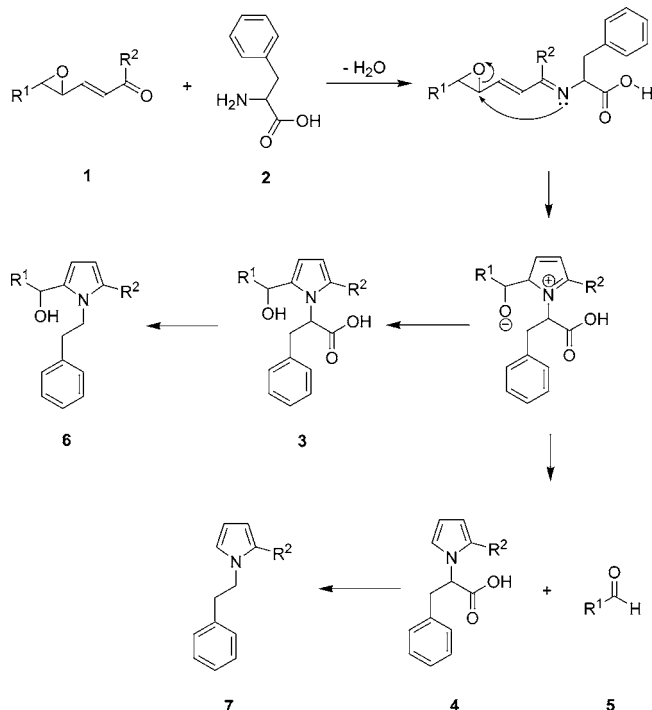
[a] For **1a**, **3a**, **4a**, **5a**, **6a**, and **7a**: $R^1 = (CH_2)_7CO_2CH_3$, $R^2 = CH_3(CH_2)_4$. For **1b**, **3b**, **4b**, **5b**, **6b**, and **7b**: $R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_7CO_2CH_3$.

Scheme 2. Formation of Furan Derivatives from Epoxyoxoene Fatty Esters (**1**) in the Presence of Phenylalanine (**2**)[a]

[a] For **1a**, **5a**, **8a** and **9a**: $R^1 = (CH_2)_7CO_2CH_3$, $R^2 = CH_3(CH_2)_4$. For **1b**, **5b**, **8b**, and **9b**: $R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_7CO_2CH_3$.

not shown). In addition, several other compounds were also produced. These compounds were mostly pyrrole and furan derivatives produced in the reaction between the epoxyoxoene fatty esters and the amino acid by nowadays well-known mechanisms (*13*, *19*).

Pyrrole formation (Scheme 1) 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 α,β-unsaturated carbonyl compounds (*20*). This cis isomer of imine would then convert to the indicated cyclic intermediate, the transformation of which into products **3** and **4** is likely to occur by electronic rearrangement. This electronic rearrangement implies the exit of either an aldehyde (**5**), when compound **4** is produced, or the proton at position 5 of the ring of the intermediate, producing compound **3**. Finally, the thermal treatment of compounds **3** and **4** may produce their decarboxylation to compounds **6** and **7**, respectively. This heating may occur, for example, in the injection port of the chromatograph. On the other hand, this heating did not influence the different pyrrole derivatives produced in the reaction because analogous heterocyclic derivatives were found in similar systems incubated at 37 °C and studied by high performance liquid chromatography (*13*, *19*).

All these compounds could be easily identified in the chromatograms. Thus, the long chain pyrrole fatty ester methyl 9-hydroxy-9-(5-pentyl-1-phenethyl-1*H*-pyrrol-2-yl)nonanoate (**6a**) appeared in the dehydrated form as two isomers at $t_R = 43.9$ and 44.9 min, respectively. It was identified by MS. Its mass spectrum was analogous to the mass spectra of similar long chain pyrrole fatty esters (*13*, *19*). [GC−MS *m/z* (relative intensity, ion structure) of **6a**: 409 (52, M⁺), 352 (55, M⁺ − butyl), 318 (21, M⁺ − phenylethyl), 280 (69, M⁺ − (CH₂)₅-CO₂CH₃), 252 (32), 207 (16), 132 (28), 118 (15), 105 (100, phenylethyl), 91 (37, benzyl), and 79 (20).] Its isomer methyl
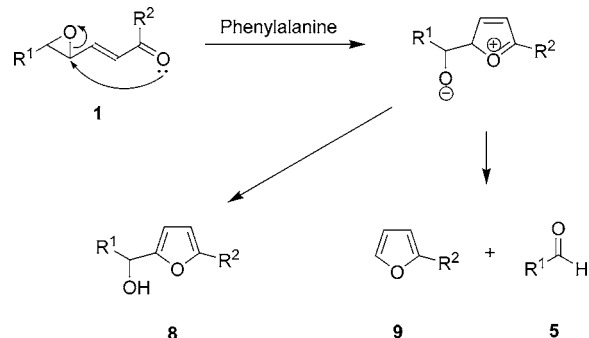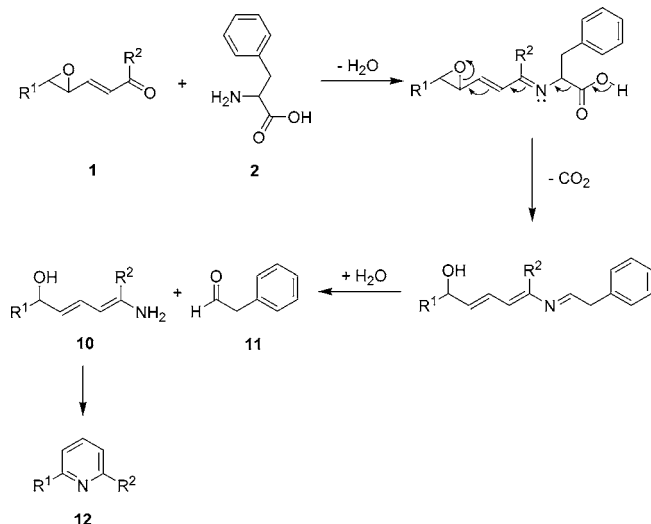
8-(5-(1-hydroxyhexyl)-1-phenethyl-1*H*-pyrrol-2-yl)octanoate (**6b**) could not be unambiguously identified, although two small peaks with the molecular ion at *m/z* 409 and having the fragment ion for this isomer at *m/z* 266 could be detected at retention times close to those of **6a**. Compound **6a** was the only reaction product that seemed to be dependent on the polarity of the incubation media. Thus, it was produced in significant amounts in acetonitrile−water (1:2) (Figure 1C), was produced to a lesser extent in acetonitrile−water (1:1) (Figure 1B), and was absent in acetonitrile−water (2:1) (Figure 1A).

The 1,2-disubstituted pyrroles appeared at $t_R = 31.40$ min for 2-pentyl-1-phenethyl-1*H*-pyrrole (**7a**) and $t_R = 42.7$ min for methyl 8-(1-phenethyl-1*H*-pyrrol-2-yl)octanoate (**7b**). The identity of compound **7a** was confirmed by comparison with authentic compound **7a** synthesized from 4-hydroxy-2-nonenal and phenethylamine (*16*). [GC−MS *m/z* (relative intensity, ion structure) of **7a**: 241 (30, M⁺), 184 (100, M⁺ − butyl), 150 (57, M⁺ − phenylethyl), 105 (63, phenylethyl), 94 (61), 91 (32, benzyl), and 77 (19).] Compound **7b** was identified by analogy of its mass spectrum with those mass spectra of analogous pyrrole derivatives (*13*, *19*). Thus, it exhibited the fragment ions at *m/z* 236 and 184. [GC-MS *m/z* (relative intensity, ion structure) of **7b**: 327 (17, M⁺), 296 (9, M⁺ − CH₃O), 236 (41, M⁺ − benzyl), 184 (100, M⁺ − (CH₂)₆CO₂CH₃), 105 (79, phenylethyl), 91 (77, benzyl), and 55 (47).] Formation of compounds **7** should be accompanied by formation of compounds **5**. However, these carbonyl compounds rapidly react with the amino compound present in the reaction and its identification is not easy (*13*). Thus, in the analogous reaction of epoxyoxoene fatty esters with butylamine, the methyl 9-oxononanoate (**5a**) produced was detected as the imine methyl 9-iminononanoate (*13*). Analogously, a minor compound having a mass spectrum compatible with the imine produced between the methyl 9-oxononanoate and the phenylalanine was detected in the epoxyoxoene fatty esters/phenylalanine reaction mixtures and has been tentatively marked as **5a** in the figure. Its mass spectrum showed the molecular ion at *m/z* 333 and the ion at *m/z* 176, which is considered a fragment ion for the proposed structure. No equivalent product was detected for hexanal (**5b**), which should be produced in smaller amounts.

The formation of pyrrole derivatives was produced in parallel to the formation of furan derivatives. These last compounds are produced without direct participation of the amino acid, but the presence of the amino acid is necessary because furan derivatives were not produced in its absence. The formation of furan derivatives follows the mechanism indicated in the Scheme 2, which is analogous to the above indicated for pyrrole production (Scheme 1). Thus, a cyclic intermediate is produced, the

4586 *J. Agric. Food Chem.,* Vol. 53, No. 11, 2005

Zamora et al.

Scheme 3. Strecker-type Degradation of Phenylalanine (**2**) Produced by Epoxyoxoene Fatty Esters (**1**)[a]

[a] For **10a** and **12**: $R^1 = (CH_2)_7CO_2CH_3$, $R^2 = CH_3(CH_2)_4$. For **10b**: $R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_7CO_2CH_3$.

transformation of which into products **8** and **9** is likely to occur by electronic rearrangement. This electronic rearrangement implies either the formation of an aldehyde (**5**), when compound **9** is produced, or the exit of the proton at position 5 of the ring of the intermediate, producing compound **8**. Furans **8** and **9** are major products of the reaction between epoxyoxoene fatty esters and phenylalanine.

Long-chain furan fatty ester methyl 9-hydroxy-9-(5-pentyl-furan-2-yl)nonanoate (**8a**) appeared in the dehydrated form as two isomers at $t_R = 37.4$ and 38.6 min, respectively. The mass spectrum of this compound was described previously (*19*) and exhibited the expected molecular ion at m/z 306 and the fragment ion at m/z 177, among others. Analogously, the minor long-chain furan fatty ester methyl 8-(5-(1-hydroxyhexyl)furan-2-yl)octanoate (**8b**) appeared in the dehydrated form as two isomers at $t_R = 38.1$ and 38.4 min, respectively. The mass spectrum of this compound was also described previously (*19*) and exhibited the expected molecular ion at m/z 306 and the fragment ion at m/z 163, among others.

The 1,2-disubstituted furans **9** were also produced in parallel to long-chain furan fatty esters **8**. These compounds have also been described previously (*19*), and their mass spectra were computer-matched with the reference mass spectra of the NBS data base. 2-Pentylfuran (**9a**) appeared at $t_R = 7.7$ min and exhibited the expected mass spectrum with the molecular ion at m/z 138 and the base peak at m/z 81. The methyl 8-(2-furyl)-octanoate (**9b**) appeared at $t_R = 25.5$ min and exhibited the expected mass spectrum with the molecular ion at m/z 224 and the base peak at m/z 95.

In addition to pyrrole and furan derivatives, the formation of the Strecker aldehyde phenylacetaldehyde (**11**) was also observed as a major reaction product by GC when a flame ionization detector was employed (data not shown). According to the previously described Strecker-type degradation of amino acids produced by epoxyalkenals (*5*), the reaction should follow the mechanism described in Scheme 3. This mechanism involves the formation of the imine, which after decarboxylation, rearrangement, and hydrolysis produces the phenylacetaldehyde (**11**) and the conjugated hydroxylamine (**10**). This last compound is presumably unstable and should produce the pyridine (**12**). This pyridine is identical when starting from **1a** and **1b**, and
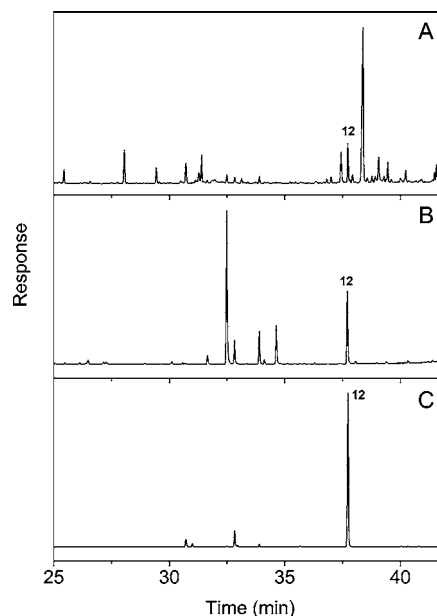
Figure 2. Total ion chromatograms of GC–MS analysis of methyl 8-(6-pentylpyridin-2-yl)octanoate (**12**) in the different steps of its isolation: (**A**) the complete reaction of epoxyoxoene fatty esters (**1**) and phenylalanine (**2**) in dimethyl sulfoxide after 24 h heating under reflux, (**B**) after the first fractionation by column chromatography on silica gel 60 using hexane–ether (7:3) as solvent, and (**C**) after the second fractionation by preparative thin-layer chromatography on aluminum oxide N-coated plates using hexane–ether (7:3) as solvent.

therefore, only one pyridine should be expected. Although partially overlapped by one of the oxodienes (OD) produced in the reaction, the reaction mixture analyzed by GC–MS exhibited one peak at $t_R = 37.7$ min that had a mass spectrum compatible with the pyridine structure. The mass spectrum of compound **12** is collected in the Experimental Procedures section. In an attempt of unambiguously confirming the structure of this compound, it was isolated and characterized by ^{1}H and ^{13}C NMR.

Isolation and Characterization of Methyl 8-(6-Pentylpyr-idin-2-yl)octanoate (12) Produced in the Reaction between Epoxyoxoene Fatty Esters (1) and Phenylalanine (2). The reaction between epoxyoxoene fatty esters and phenylalanine is very complex, and the different products are formed in diverse proportions depending on the solvent employed. This is likely a consequence of the different solubilities of oxidized lipids and amino acids and the different reactions involved. To isolate the pyridine (**12**), different solvents were assayed to find the best conditions for obtaining the highest proportion of this compound. In all assayed solvents, phenylacetaldehyde (**11**) was produced. However, the cyclization reaction of the conjugated hydroxylamine (**10**) to the pyridine (**12**) was produced preferentially in some solvents versus others. Thus, compound **12** was not produced when the reaction was carried out in ethyl acetate and was formed in very low amounts when the reaction was carried out in 1,4-dioxane, ethanol, or 2,6-dimethyl-4-heptanone. Higher amounts of compound **12** were observed in acetonitrile or *N,N*-dimethylformamide, in mixtures of water with acetonitrile, and in different aqueous buffers, including sodium phosphate, pH 6.5, and sodium borate, pH 9.0. Nevertheless, compound **12** was mainly produced when the reaction was carried out in dimethyl sulfoxide. Figure 2A shows the total ion chromatogram of GC–MS analysis for the reaction between the epoxyoxoene fatty esters (**1**) and the phenylalanine (**2**) in

Strecker-type Degradation by Oxidized Fatty Esters

J. Agric. Food Chem., Vol. 53, No. 11, 2005   **4587**

Table 1. Formation of Phenylacetaldehyde (**11**) in Mixtures of Epoxyoxoene Fatty Esters (**1**) or Epoxyalkenals with Phenylalanine (**2**) at Different Lipid/Amino Acid Ratios[a]

lipid	A/W ratio[b]	1:1 (pH 5)	1:1 (pH 9)	1:2 (pH 5)
epoxyoxoene fatty ester (**1**)	2:1	144 ± 12c	61 ± 6c	126 ± 3c
	1:1	121 ± 15cd	38 ± 7cd	93 ± 6de
	1:2	119 ± 7cd	60 ± 14c	102 ± 6e
4,5-epoxy-2-heptenal	2:1	86 ± 10d	28 ± 13d	78 ± 9d
	1:1	96 ± 17d	23 ± 7d	81 ± 6d
	1:2	94 ± 16d	44 ± 4cd	86 ± 7d
4,5-epoxy-2-decenal	2:1	150 ± 17c	43 ± 5cd	120 ± 7c
	1:1	146 ± 18c	47 ± 6cd	102 ± 10e
	1:2	154 ± 22c	41 ± 14cd	124 ± 6c

[a] Values are given in micromole of phenylacetaldehyde per millimole of phenylalanine. The reactions (1 mL) were heated overnight at 80 °C. Means in the same column with different letters are significantly different ($p < 0.05$). Fifty micromoles of each reactant was employed in 1:1 lipid/amino acid ratio reactions and 25 μmol of lipid and 50 μmol of amino acid in 1:2 lipid/amino acid ratio reactions. [b] A/W = acetonitrile/water.

dimethyl sulfoxide after 24 h of heating under reflux. The first part of the chromatogram is not shown because it is hidden by the solvent.

The isolation of compound **12** was carried out in two chromatographic steps to obtain an almost chromatographically pure compound (Figure 2C). The incubated reaction of epoxyoxoene fatty esters (**1**) and phenylalanine (**2**) in dimethyl sulfoxide was first fractionated by column chromatography on silica gel 60 using hexane−ether (7:3) as solvent. This procedure allowed considerable purification of compound **12** (Figure 2B). However, the presence of some impurities was observed. Curiously, these compounds were present in very low amounts in the initial mixture and seemed to be small pyridine derivatives. Final purification of compound **12** was carried out by preparative thin-layer chromatography on aluminum oxide N-coated plates. This procedure allowed compound **12** to be obtained with a high purity (Figure 2C).

Compound **12** was identified as methyl 8-(6-pentylpyridin-2-yl)octanoate on the basis of ^{1}H and ^{13}C NMR spectra. Both spectra were almost identical to those previously published for methyl 8-(6-pentylpyridin-2-yl)octanoate (*21*). The only significant difference was the signal of protons H-10 and H-12, which was described by Lie Ken Jie and Pasha as a doublet with $J = 1.46$ Hz, and the signal is, as should be expected, a double doublet with $J = 1.9$ and 7.7 Hz.

Comparative Formation of Phenylacetaldehyde (11) in the Reactions of Epoxyalkenals and Epoxyoxoene Fatty Esters (1) with Phenylalanine (2). In an attempt of comparing the relative reactivities of aldehydes and ketones for producing Strecker-type degradation of amino acids, formation of phenylacetaldehyde (**11**) was studied in the reaction of epoxyoxoene fatty esters (**1**), 4,5-epoxy-2-heptenal, and 4,5-epoxy-2-decenal with phenylalanine (**2**). Table 1 collects the results obtained in diverse mixtures of epoxyalkenals and epoxyoxoene fatty esters with phenylalanine heated overnight at 80 °C in acetonitrile−water (2:1, 1:1, or 1:2) at different pHs and carbonyl compound/amino acid ratios.

The obtained results showed that epoxyoxoene fatty esters (**1**) did not produce less phenylacetaldehyde (**11**) than analogous epoxyalkenals with independence of the acetonitrile−water ratio, the pH, and the carbonyl compound/amino acid ratio assayed. In fact, ketones seemed to be more reactive than aldehydes for

producing the Strecker-type degradation of the phenylalanine, in particular when the assayed aldehyde was 4,5-epoxy-2-heptenal.

Phenylacetaldehyde (**11**) formation was analogous for the three acetonitrile−water ratios assayed at the different pHs and carbonyl compound/amino acid ratios. Nevertheless, two reactions produced phenylacetaldehyde to a slightly lower extent when the solvent was acetonitrile−water (1:1).

Phenylacetaldehyde (**11**) formation depended on the amount of carbonyl compounds present in the reaction. Thus, phenylacetaldehyde content decreased in the presence of a lower concentration of carbonyl compounds and was not produced when the carbonyl compounds were not present (data not shown). In addition, phenylacetaldehyde was produced in higher amounts at pH 5 than at pH 9, in accordance with the optimal pH found for phenylacetaldehyde formation in the reaction of epoxyalkenals and amino acids (*5*).

DISCUSSION

Although Strecker degradation of amino acids has been traditionally believed to be produced by carbohydrates, a recent study showed that amino acids are also degraded by some lipid oxidation products, in particular short-chain aldehydes having a 4,5-epoxy-1-oxo-2-pentene system (*5*). The results obtained in the present study extend this ability also to long-chain ketones with an analogous functional system.

During lipid peroxidation, lipids are attacked by oxygen species, the result of which is the formation of lipid hydroperoxides. These hydroperoxides can then be converted into a variety of secondary products including both nonvolatile fatty acid derivatives and volatile products. Although volatile products are only a small part of decomposition products of hydroperoxides, for example Fe(II)/Fe(III)-catalyzed linoleic acid hydroperoxide decomposition produced volatiles comprising less than 5 mol % of total products (*22*), these volatile products have received much more attention than nonvolatile products. This is probably due to an easier availability of these compounds. Nevertheless, the above results suggest that, at least for the Strecker-type degradation of amino acids, nonvolatile products may be as reactive as volatile products, and therefore, nonvolatile lipid oxidation products should be considered for determining the contribution of lipids to the overall Strecker degradation of amino acids produced during nonenzymatic browning.

Furthermore, epoxyoxoene fatty esters (**1**), which exhibited a reactivity similar to that of 4,5-epoxy-2-decenal, seemed to be more reactive for the Strecker degradation of amino acids than the shorter epoxyheptenal based on formation of phenylacetaldehyde (**11**). This may be a consequence of the competence among the different reactions that are taking place simultaneously in these systems. Thus, the imine produced between the carbonyl compound and the amino acid may evolve either the cyclic intermediate indicated in Scheme 1 or the decarboxylation indicated in Scheme 3 (these schemes are also valid for epoxyalkenals with $R^2 = H$). For some reason, the presence of a longer chain in R^1 (R^1 is pentyl in epoxydecenal and **1b**, $(CH_2)_7CO_2CH_3$ in **1a**, and only ethyl in epoxyheptenal) either facilitates the decarboxylation of the imine or inhibits the formation of the cyclic intermediate. The similarity of reactivity between epoxyoxoene fatty esters (**1**) and 4,5-epoxy-2-decenal suggests that the group R^2 plays a lesser important role for all these reactions.

Pyrrole formation has been suggested as the last step of the lipid peroxidation process when it takes place in the presence of amino acids, amino phospholipids, and proteins (*20*, *23*−

4588 *J. Agric. Food Chem.,* Vol. 53, No. 11, 2005

Zamora et al.

25). The results obtained in this, and in the previous study (*5*), are in agreement with this hypothesis but suggest that amino group pyrrolization, which may be easily followed spectrophotometrically (*25*−*27*), is only one of the final steps of the lipid peroxidation process. Another final step is the Strecker-type degradation of amino acids, which is produced by aldehydes and ketones with a 4,5-epoxy-1-oxo-2-pentene system and also by other lipid oxidation products such as 4-hydroxy-2-alkenals (Zamora et al., unpublished results). This Strecker-type degradation of amino acids may also be contributing to the appearance of 2-ethylpyridine and 2-pentylpyridine in processed foods (*28*−*30*). However, the presence of the pyridine-containing long-chain fatty ester (**12**) has not been yet described.

All these data confirm that, analogously to carbohydrates, lipid oxidation products are also able to produce the Strecker degradation of amino acids, therefore contributing to the production of Strecker aldehydes with significant flavor properties. Additional studies are needed both to study competitively the ability of lipids and carbohydrates to produce this reaction and to determine the interplays of both macronutrients in the Strecker degradation of amino acids (*31*).

ACKNOWLEDGMENT

LITERATURE CITED

(1) Ho, C.-T. Thermal generation of Maillard Aromas. In *The Maillard Reaction: Consequences for the Chemical and Life Sciences*; Ikan, R., Ed.; John Wiley & Sons: Chichester, U. K., 1996; pp 27−53.

(2) Yaylayan, V. A. Recent advances in the chemistry of Strecker degradation and Amadori rearrangement: Implications to aroma and color formation. *Food Sci. Technol. Res.* **2003**, *9*, 1−6.

(3) Estévez, M.; Morcuende, D.; Ventanas, S.; Cava, R. Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME−GC−MS. *J. Agric. Food Chem.* **2003**, *51*, 3429−3435.

(4) Di, R.; Kim, J.; Martin, M. N.; Leustek, T.; Jhoo, J.; Ho, C.-T.; Tumer, N. E. Enhancement of the primary flavor compound methional in potato by increasing the level of soluble methionine. *J. Agric. Food Chem.* **2003**, *51*, 5695−5702.

(5) Hidalgo, F. J.; Zamora, R. Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. *J. Agric. Food Chem.* **2004**, *52*, 7126−7131.

(6) Maga, J. A. Pyrazines update. *Food Rev. Internat.* **1992**, *8*, 479−558.

(7) Serra-Bonvehi, J.; Ventura-Coll, F. Factors affecting the formation of alkylpyrazines during roasting treatment in natural and alkalinized cocoa powder. *J. Agric. Food Chem.* **2002**, *50*, 3743−3750.

(8) Alasalvar, C.; Shahidi, F.; Cadwallader, K. R. Comparison of natural and roasted Turkish tombul hazelnut (*Corylus avellana* L.) volatiles and flavor by DHA/GC/MS and descriptive sensory analysis. *J. Agric. Food Chem.* **2003**, *51*, 5067−5072.

(9) Fors, S. Sensory properties of volatile Maillard reaction products and related compounds. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 185−286.

(10) Gardner, H. W.; Kleiman, R.; Weisleder, D. Homolytic decomposition of linoleic acid hydroperoxide. Identification of fatty acid products. *Lipids* **1974**, *9*, 696−706.

(11) Gardner, H. W.; Kleiman, R. Degradation of linoleic acid hydroperoxides by a cysteine-FeCl₃ catalyst as a model for similar biochemical reactions. II. Specificity in formation of fatty acid epoxides. *Biochim. Biophys. Acta* **1981**, *665*, 113−125.

(12) Gardner, H. W.; Kleiman, R. A soy extract catalyzes formation of 9-oxo-*trans*-12,13-epoxy-*trans*-10-octadecenoic acid from 13-hydroperoxy-*cis*-9,*trans*-11-octadecenoic acid. *Lipids* **1977**, *12*, 941−944.

(13) Hidalgo, F. J.; Zamora, R. In vitro production of long chain pyrrole fatty esters from carbonyl-amine reactions. *J. Lipid Res.* **1995**, *36*, 725−735.

(14) Hidalgo, F. J.; Zamora, R.; Vioque, E. Syntheses and reactions of methyl (*Z*)-9,10-epoxy-13-oxo-(*E*)-11-octadecenoate and methyl (*E*)-9,10-epoxy-13-oxo-(*E*)-11-octadecenoate. *Chem. Phys. Lipids* **1992**, *60*, 225−233.

(15) Zamora, R.; Hidalgo, F. J. Linoleic acid oxidation in the presence of amino compounds produces pyrroles by carbonylamine reactions. *Biochim. Biophys. Acta* **1995**, *1258*, 319−327.

(16) Zamora, R.; Hidalgo, F. J. 2-Alkylpyrrole formation from 4,5-epoxy-2-alkenals. *Chem. Res. Toxicol.* **2005**, *18*, 342−348.

(17) Gardner, H. W.; Bartelt, R. J.; Weisleder, D. A facile synthesis of 4-hydroxy-2(*E*)-nonenal. *Lipids* **1992**, *27*, 686−689.

(18) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 7th ed.; Iowa State University Press: Ames, IA, 1980.

(19) Hidalgo, F. J.; Zamora, R. Epoxyoxoene fatty esters: Key intermediates for the synthesis of long-chain pyrrole and furan fatty esters. *Chem. Phys. Lipids* **1995**, *77*, 1−11.

(20) Zamora, R.; Hidalgo, F. J. Phosphatidylethanolamine modification by oxidative stress product 4,5(*E*)-epoxy-2(*E*)-heptenal. *Chem. Res. Toxicol.* **2003**, *16*, 1632−1641.

(21) Lie Ken Jie, M. S. F.; Pasha, M. K. Synthesis of novel piperidine- and pyridine-containing long-chain fatty ester derivatives from methyl *iso*-ricinoleate. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1331−1332.

(22) Grosch, W. Degradation of linoleic acid hydroperoxides to volatile carbonyl compounds. *Z. Lebensm. Unters. Forsch.* **1976**, *160*, 371−375.

(23) Hidalgo, F. J.; Zamora, R. Fluorescent pyrrole products from carbonyl-amine reactions. *J. Biol. Chem.* **1993**, *268*, 16190−16197.

(24) Zamora, R.; Hidalgo, F. J. Comparative methyl linoleate and methyl linolenate oxidation in the presence of bovine serum albumin at several lipid/protein ratios. *J. Agric. Food Chem.* **2003**, *51*, 4661−4667.

(25) Zamora, R.; Olmo, C.; Navarro, J. L.; Hidalgo, F. J. Contribution of phospholipid pyrrolization to the color reversion produced during deodorization of poorly degummed vegetable oils. *J. Agric. Food Chem.* **2004**, *52*, 4166−4171.

(26) Hidalgo, F. J.; Alaiz, M.; Zamora, R. A spectrophotometric method for the determination of proteins damaged by oxidized lipids. *Anal. Biochem.* **1998**, *262*, 129−133.

(27) Hidalgo, F. J.; Nogales, F.; Zamora, R. Determination of pyrrolized phospholipids in oxidized phospholipid vesicles and lipoproteins. *Anal. Biochem.* **2004**, *334*, 155−163.

(28) Schieberle, P. Odour-active compounds in moderately roasted sesame. *Food Chem.* **1996**, *55*, 145−152.

(29) Kim, Y.-S.; Ho, C.-T. Formation of pentylpyridines in an oil medium. *J. Agric. Food Chem.* **1998**, *46*, 644−647.

(30) Fukami, K.; Ishiyama, S.; Yaguramaki, H.; Masuzawa, T.; Nabeta, Y.; Endo, K.; Shimoda, M. Identification of distinctive volatile compounds in fish sauce. *J. Agric. Food Chem.* **2002**, *50*, 5412−5416.

(31) Zamora, R.; Hidalgo, F. J. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 49−59.