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ARTICLE

# Asparagine Decarboxylation by Lipid Oxidation Products in Model Systems

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The decarboxylation of asparagine in the presence of alkanals, alkenals, and alkadienals, among other lipid derivatives, was studied in an attempt to understand the reaction pathways by which some lipid oxidation products are able to convert asparagine into acrylamide. Asparagine was converted into 3-aminopropionamide in the presence of lipid derivatives as a function of reaction conditions (pH, water content, time, and temperature), as well as the type and amount of lipid compound involved. Alkadienals (and analogous ketodienes) were the most reactive lipids followed by hydroperoxides and alkenals. Saturated carbonyls and polyunsaturated fatty acids, or other polyunsaturated derivatives, also exhibited some reactivity. On the other hand, saturated lipids or monounsaturated alcohols did not degrade asparagine. A mechanism for the decarboxylation of asparagine in the presence of alkadienals based on the deuteration results obtained when asparagine/2,4-decadienal model systems were heated in the presence of deuterated water was proposed. The activation energy ($E_a$) of asparagine decarboxylation by 2,4-decadienal was 81.0 kJ/mol, which is higher than that found for the conversion of 3-aminopropionamide into acrylamide in the presence of 2,4-decadienal. This result points to the decarboxylation step as the key step in the conversion of asparagine into acrylamide in the presence of alkadienals. Therefore, any inhibiting strategy for suppressing the formation of acrylamide by alkadienals should be mainly directed to the inhibition of this step.

**KEYWORDS: Acrylamide; amino acid decarboxylation; 3-aminopropionamide; lipid oxidation; Maillard reaction**

## INTRODUCTION

Food is processed for a variety of reasons including toxin removal, preservation, better marketing and distribution, increased food consistency, etc. Modern food processing also improves the quality of life for people with allergies or some illnesses. On the other hand, the employed procedures can occasionally lead to the formation of toxic compounds. Among them, acrylamide has received increased worldwide attention (*1*) since the 2002 report regarding the presence of elevated levels of acrylamide in certain types of foods processed at high temperatures (*2*).

This toxicant is produced by thermal degradation of asparagine in the presence of reducing sugars as a consequence of the Maillard reaction (*3*−*8*). This reaction seems to be produced by elimination of an intermediate decarboxylated Schiff base [*N*-(D-glucos-1-yl)-3′-aminopropionamide] or the corresponding Amadori product [*N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide], as well as 3-aminopropionamide.

In addition to these routes, recent studies have also pointed to some oxidized lipids as potential inducers in the conversion of asparagine into its vinylogous derivative (*9*). Among them, alkadienals exhibited the highest reactivity for this reaction. The mechanism for this reaction seems to take place in two main steps, namely, the decarboxylation of the amino acid and the later deamination of the produced 3-aminopropionamide. The second step was studied recently, and the mechanism of the reaction was clarified (*10*). However, the role of alkadienals in the decarboxylation reaction of asparagine has not been yet analyzed.

In an attempt to understand the reaction pathways by which carbonyl compounds are able to convert asparagine into acrylamide, this study analyzes the decarboxylation reaction of asparagine in the presence of alkanals, alkenals, and alkadienals, among other lipid derivatives.

## MATERIALS AND METHODS

**Materials.** 3-Aminopropionamide was obtained from TCI Europe (Zwijndrecht, Belgium). All other chemicals were purchased from Aldrich (Milwakee, WI), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany) and were of analytical grade. 2,4-Decadienal (93%) was obtained from Aldrich. It was further purified by column chromatography on silica gel 60 using hexane/acetone (9:0.25) as solvent. The aldehyde recovered from the column was chromatographically pure as determined by GC. The preparation of the following oxidized lipids was described previously (*11*): methyl 13-hydroperoxyoctadeca-9,11-dienoate, methyl 13-hydroxyoctadeca-9,11-dienoate, and methyl 13-oxooctadeca-9,11-dienoate.

**Lipid−Asparagine Reactions.** Model reactions were carried out analogously to the procedure of Granvogl and Schieberle (*7*), with the

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modifications described by Zamora and Hidalgo (*9*). Briefly, mixtures of asparagine (37.5 μmol) and the lipid (0−37.5 μmol) were singly homogenized with 0.063−0.200 mm silica gel (300 mg) (Macherey-Nagel, Düren, Germany), 30 μL of 0.3 M buffer (sodium citrate for pH 2.15−6 and sodium phosphate for pH 6−8), and 0−210 μL of water and then heated under nitrogen at 140 °C in closed test tubes for 30 min, unless otherwise indicated. Most reactions were carried out with 2,4-decadienal. In addition, many other lipid derivatives were also tested, including unoxidized lipids (methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, and methyl 2,4-decadienoate), as well as lipid oxidation products having both long and short chains: hydroperoxides (methyl 13-hydroperoxyoctadeca-9,11-dienoate), alcohols (methyl 13-hydroxyoctadeca-9,11-dienoate, methyl 12-hydroxyoctadecanoate, 2-hexenol, 3-hexenol, and 3-nonenol), ketodienes (methyl 13-oxooctadeca-9,11-dienoate), alkadienals (2,4-decadienal, 2,4-heptadienal, and 2,4-hexadienal), alkenals (2-octenal and 2-pentenal), and alkanals (hexanal). The water activity ($a_w$) of the samples was determined with a Pawkit Decagon analyzer (Pullman, WA). The $a_w$ values determined when 0, 75, and 150 μL of water were added to the reaction mixtures were 0.60, 0.73, and 0.95, respectively. Additions of more than 150 μL of water always resulted in $a_w = 1$.

After cooling (5 min at room temperature and 15 min at −20 °C), 2 mL of 0.25 M sodium bicarbonate, pH 10, was added. Suspensions were stirred for 1.5 min and centrifuged for 10 min at 2250*g*, and the content of 3-aminopropionamide formed was determined in the supernatant.

**Determination of 3-Aminopropionamide.** 3-Aminopropionamide was determined by LC-MS/MS after derivatization with dansyl chloride [5-(dimethylamino)naphthalene-1-sulfonyl chloride]. The derivatization was done according the procedure described by Moret et al. (*12*), which was modified. Briefly, 1 mL of the extract was treated with 50 μL of internal standard solution (23.29 mg of cyclohexylamine in 25 mL of methanol) and 1 mL of dansyl chloride solution (5 mg/mL in acetone). The mixture was stirred for 30 s and left in the dark overnight. To eliminate excessive dansyl chloride, 20 μL of a glycine solution (100 mg/mL) was added, mixed thoroughly, and left for another 15 min at room temperature. The sample was extracted twice with 1 mL of diethyl ether. The combined extracts were dried under a stream of nitrogen, and the residue was redissolved in 1 mL of a 1:1 mixture of acetonitrile/formic acid (998:2) and ammonium acetate (312 mg/L in water) and analyzed by LC-MS/MS.

**LC and MS/MS Conditions.** The HPLC system (Beckman) consisted of a Programmable Solvent Module System Gold 126 and a Rheodyne 7725 manual injector. Samples were fractionated on a 300 × 3.9 mm i.d. reversed-phase column (Nova-Pack C18, 4 μm; Waters) using a 5.5:4.5 mixture of acetonitrile/formic acid (998:2) and ammonium acetate (312 mg/L in water) as solvent. The flow rate was 0.8 mL/min.

A triple-quadrupole API 2000 mass spectrometer (Applied Biosystems, Foster City, CA) was coupled to the HPLC using an electrospray ionization interface in positive ionization mode ($ESI^+$). Mass spectrometric acquisition was performed using multiple reaction monitoring (MRM). The nebulizer gas (synthetic air), the curtain gas (nitrogen), and the heater gas (synthetic air) were set at 40, 45, and 40 (arbitrary units), respectively. The collision gas (nitrogen) was set at 5 (arbitrary units). The heater gas temperature was set at 450 °C and the electrospray capillary voltage to 5.5 kV. The focusing potential was 370 V, and the declustering potential was 26 V. The fragment ions in MRM mode were produced by collision-activated dissociation of selected precursor ions in the collision cell of the triple quadrupole and analyzed the selected products with the second analyzer of the instrument. Three transitions were acquired for the identification of each dansyl derivative.

To establish the appropriate MRM conditions for the individual compounds, the mass spectrometric conditions were optimized using infusion with a syringe pump to select the most suitable ion transitions for the target analytes. Precursor ions and their products ions, used for quantification and confirmation purposes, and the operating parameters are summarized in **Table 1**.

**Quantification of 3-Aminopropionamide.** Quantification of 3-aminopropionamide was carried out by preparing standard curves of this compound in 300 mg of silica gel and following the whole procedure described above. For each curve, six different concentration levels of 3-aminopropionamide (0−10 μmol) were used. 3-Aminopropionamide content was directly proportional to the 3-aminopropionamide/internal standard area ratio ($r > 0.995$, $p < 0.001$). The coefficients of variation at the different concentrations were < 10%.

**Table 1.** Optimized MS/MS Conditions and Transitions Selected in MRM Mode for Identification and Quantification of the Dansyl Derivatives of 3-Aminopropionamide and Cyclohexylamine[a]

| compound | MW | precursor ion (*m/z*) | product ion (*m/z*) | EP (V) | CE (eV) | CXP (V) |
|---|---|---|---|---|---|---|
| 3-aminopropionamide | 321 | 322.2 | 170.1 | 10.5 | 29 | 6 |
| | | | 157.2 | 10.5 | 43 | 6 |
| | | | 115.2 | 10.5 | 57 | 6 |
| cyclohexylamine | 332 | 333.3 | 157.2 | 10.5 | 41 | 6 |
| | | | 128.2 | 10.5 | 59 | 6 |
| | | | 115.2 | 10.5 | 85 | 4 |

[a] The first transition of cyclohexylamine (333.3 → 157.2) was employed for quantification purposes. Abbreviations: EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

**Deuteration Experiments.** Deuteration experiments were also carried out to obtain further insight into the reaction mechanism. In these experiments deuterated water ($D_2O$) was employed, and the resulting acrylamide was analyzed by GC-MS. Experiments were carried out using mixtures of asparagine (37.5 μmol) and 2,4-decadienal (37.5 μmol) that were singly homogenized with 0.063−0.200 mm silica gel (300 mg) and 0−240 μL of $D_2O$ and heated under nitrogen at 180 °C in closed test tubes for 10 min. These conditions were selected because they were previously employed in the study of acrylamide formation in these reactions (*9*). In addition, by using these conditions the formed 3-aminopropionamide suffers an elimination reaction to produce acrylamide.

After cooling (5 min at room temperature and 15 min at −20 °C), 2 mL of 0.3 M sodium citrate buffer, pH 2.2, was added. Suspensions were stirred for 1 min, the supernatant was then filtered, and its acrylamide content was determined by GC-MS after derivatization.

**Analysis of Acrylamide.** Acrylamide was analyzed as the stable 2-bromopropenamide using the method of Castle et al. (*13*), with the modifications described previously (*14*). Briefly, 1 mL of the supernatant was treated with 0.3 g of potassium bromide and 400 μL of saturated bromine solution in water. After 1 h in the dark at 0 °C, the excess of bromine was removed by the addition of 1 M sodium thiosulfate until the solution became colorless, and the solution was extracted with 1 mL of ethyl acetate/hexane (4:1). The organic layer was finally dried with sodium sulfate, evaporated until a volume of ∼50 μL, treated with 50 μL of triethylamine, and analyzed by GC-MS.

The separation of acrylamide analyte after derivatization was performed on GC capillary columns of middle to high polarity. GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (mass selective detector quadrupole type). In most experiments, a 30 m × 0.25 mm i.d. × 0.25 μm HP5-MS capillary column was used. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector temperature, 250 °C; oven temperature, raised from 50 °C (1 min) to 240 °C at 5 °C/min and then to 325 °C at 10 °C/min; temperature of transfer line to MSD, 280 °C; and ionization EI, 70 eV.

The ions monitored for the identification of the analyte, 2-bromopropenamide, were described previously (*14*). However, they changed depending on the deuteration degree. Thus, the nondeuterated 2-bromopropenamide exhibited the ions $[C_3H_4{}^{79}BrNO]^+$ and $[C_3H_4{}^{81}BrNO]^+$ at *m/z* 149 and 151, respectively. The monodeuterated 2-bromopropenamide exhibited the ions $[C_3H_3D^{79}BrNO]^+$ and $[C_3H_3D^{81}BrNO]^+$ at *m/z* 150 and 152, respectively. Finally, the dideuterated 2-bromopropenamide exhibited the ions $[C_3H_2D_2{}^{79}BrNO]^+$ and $[C_3H_2D_2{}^{81}BrNO]^+$ at *m/z* 151 and 153, respectively. All of these ions were employed to determine the relative proportions at which the different deuterated derivatives were produced.

The determination of the relative proportions at which the different deuterated derivatives were produced was calculated from the integration of the acrylamide peak in the trace chromatograms obtained at *m/z* 149, 150, 151, 152, and 153. The areas corresponding to the different derivatives

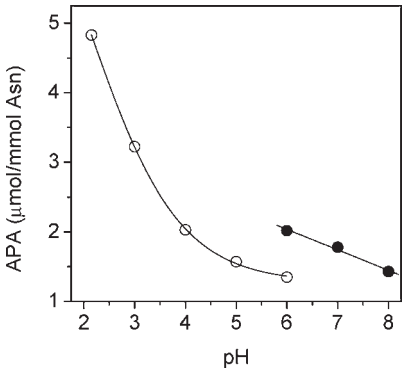


**Figure 1.** Effect of reaction pH on the formation of 3-aminopropionamide (APA) in acrylamide/2,4-decadienal (37.5 $\mu$mol of each) reaction mixtures containing 150 $\mu$L of water and heated at 140 °C for 30 min. The employed buffers were sodium citrate for pH 2.15−6 (○) and sodium phosphate for pH 6−8 (●).

were calculated according to the following equations:

$$\text{nondeuterated acrylamide} = [(\text{area at}m/z\ 151 - \text{area at}m/z\ 153) + \text{area at}m/z\ 149]/2$$

$$\text{monodeuterated acrylamide} = [\text{area at}m/z\ 150 + \text{area at}m/z\ 152]/2$$

$$\text{dideuterated acrylamide} = [(\text{area at}m/z\ 151 - \text{area at}m/z\ 149) + \text{area at}m/z\ 153]/2$$

The percentage of the different derivatives was calculated in each sample according to the equation

$$\text{derivative (\%)} = [(\text{area of the derivative}/\sum \text{areas of the different derivatives})] \times 100$$

## RESULTS

**Effect of Reaction Conditions on the Formation of 3-Aminopropionamide during the Heating of Asparagine with 2,4-Decadienal.** The heating of asparagine with 2,4-decadienal always produced 3-aminopropionamide to an extent that depended on the reaction conditions, including the reaction pH and the $a_w$. **Figure 1** shows the effect of reaction pH on the amount of 3-aminopropionamide determined. As observed in the figure, the amount of 3-aminopropionamide decreased exponentially as a function of the pH when sodium citrate buffer was employed. When the sodium citrate buffer was changed to sodium phosphate buffer, the amount of 3-aminopropionamide determined also continued decreasing as a function of reaction pH. Because the highest amount of 3-aminopropionamide was observed at pH 2.15, this pH was employed in the rest of this study.

The $a_w$ also played a major role in the amount of 3-aminopropionamide produced. As observed in **Figure 2**, there was not a linear relationship between the amount of 3-aminopropionamide formed and the water added. In fact, 3-aminopropionamide content increased until a first maximum, which was observed when 60 $\mu$L of water was added, then decreased slightly, and finally increased again until a second maximum was observed when 150−160 $\mu$L of water was added. This behavior was very similar to that observed in the formation of acrylamide from asparagine/decadienal reaction mixtures (*15*). Because the maximum amount of 3-aminopropionamide was obtained when 150 $\mu$L of water was added, this amount of water was employed in the rest of this study.

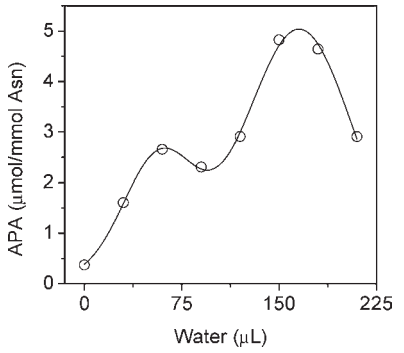


**Figure 2.** Effect of water content in the formation of 3-aminopropionamide (APA) in acrylamide/2,4-decadienal (37.5 $\mu$mol of each) reaction mixtures at pH 2.15 and heated at 140 °C for 30 min.

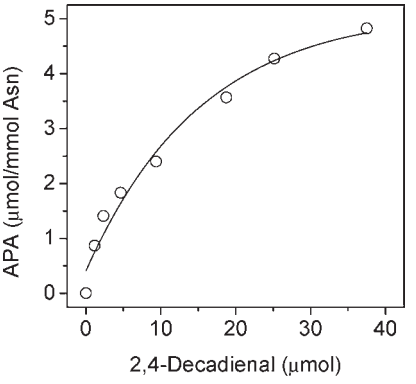


**Figure 3.** Effect of alkadienal concentration in the formation of 3-aminopropionamide (APA) in acrylamide/2,4-decadienal reaction mixtures at pH 2.15 and containing 150 $\mu$L of water.

**Effect of the Concentration of the Aldehyde on the Formation of 3-Aminopropionamide during the Heating of Asparagine with 2,4-Decadienal.** The decarboxylation of asparagine to produce 3-aminopropionamide was a consequence of the presence of the carbonyl compound. Thus, the amount of 3-aminopropionamide increased as a function of the amount of 2,4-decadienal added, although this increase was not linear (**Figure 3**).

**Effect of Time and Temperature on the Formation of 3-Aminopropionamide during the Heating of Asparagine with 2,4-Decadienal.** The decarboxylation of the amino acid also depended on the heating time and temperature. Thus, the amount of 3-aminopropionamide increased linearly ($r > 0.98$, $p < 0.0001$) as a function of time between 100 and 140 °C (**Figure 4**), and the reaction rate increased with the temperature.

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

$$[\text{3-aminopropionamide}] = [\text{3-aminopropionamide}]_0 + kt$$

where $[\text{3-aminopropionamide}]_0$ represents the intercept, $k$ is the rate constant, and $t$ is the time. These rate constants were used in an Arrhenius plot for the calculation of the activation energy ($E_a$) of 3-aminopropionamide formation from asparagine (**Figure 5**). The determined $E_a$ was 81.0 kJ/mol. This $E_a$ was lower than that found, for example, for the decarboxylation of alanine in the presence of glyoxal, which was found to be 105.9 kJ/mol (*16*).

**Effect of the Type of Lipid Compound on the Formation of 3-Aminopropionamide during the Heating of Asparagine in the Presence of Lipids.** In addition to 2,4-decadienal, other lipids were also able to convert asparagine into 3-aminopropionamide to different extents depending on the involved lipid (**Table 2**). The most reactive

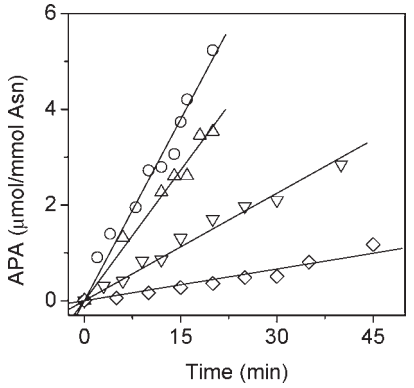


**Figure 4.** Effect of time and temperature on the formation of 3-aminopropionamide (APA) in acrylamide/2,4-decadienal (37.5 $\mu$mol of each) reaction mixtures at pH 2.15 containing 150 $\mu$L of water and heated at 140 (○), 130 (△), 120 (▽), and 100 °C (◇).

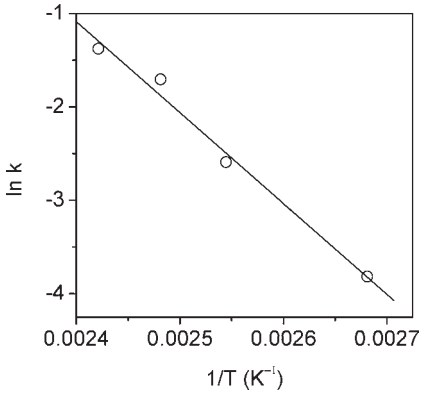


**Figure 5.** Arrhenius plot for 3-aminopropionamide (APA) formation in acrylamide/2,4-decadienal reaction mixtures at pH 2.15.

**Table 2.** Formation of 3-Aminopropionamide in Lipid/Asparagine Reaction Mixtures[a]

| lipid | 3-aminopropionamide ($\mu$mol/mmol of Asn) |
|---|---|
| methyl stearate | 0.02 ± 0.01 |
| methyl oleate | 0.16 ± 0.04 |
| methyl linoleate | 0.46 ± 0.12 |
| methyl linolenate | 0.56 ± 0.18 |
| methyl 2,4-decadienoate | 0.02 ± 0.01 |
| methyl 13-hydroperoxyoctadeca-9,11-dienoate | 2.20 ± 0.35 |
| methyl 13-hydroxyoctadeca-9,11-dienoate | 1.00 ± 0.25 |
| methyl 12-hydroxyoctadecanoate | 0.01 ± 0.01 |
| 2-hexenol | 0.03 ± 0.01 |
| 3-hexenol | 0.02 ± 0.01 |
| 3-nonenol | 0.12 ± 0.03 |
| methyl 13-oxooctadeca-9,11-dienoate | 2.61 ± 0.38 |
| 2,4-decadienal | 4.83 ± 0.91 |
| 2,4-heptadienal | 5.98 ± 0.63 |
| 2,4-hexadienal | 7.76 ± 0.30 |
| 2-octenal | 1.86 ± 0.28 |
| 2-pentenal | 2.65 ± 0.40 |
| hexanal | 0.45 ± 0.09 |

[a] Model systems containing the lipid (37.5 $\mu$mol) and asparagine (37.5 $\mu$mol) were heated for 30 min at 140 °C. Values are mean ± SD for, at least, two independent experiments.

compounds were carbonyl compounds and the lipid hydroperoxide assayed. Among the carbonyl compounds, alkadienals were more reactive than alkenals, and alkanals were the least reactive carbonyl compounds for this reaction. In addition, the chain length also determined the reactivity of the carbonyl compound. In fact, the longer chain, the lesser reactivity. This might also be one of the reasons for the lower reactivity of methyl 13-oxooctadeca-9,11-dienoate as compared to that of 2,4-alkadienals.

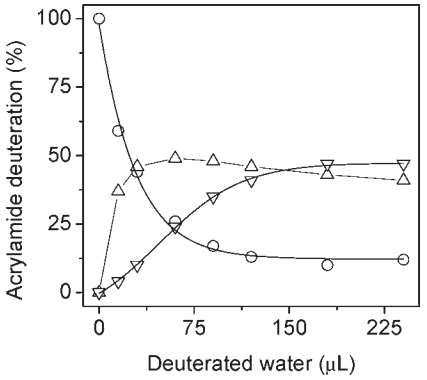


**Figure 6.** Acrylamide deuteration produced as a consequence of employing increasing amounts of deuterated water in asparagine/2,4-decadienal (37.5 $\mu$mol of each) reaction mixtures at pH 2.15. The ratio between three different acrylamides was determined: nondeuterated acrylamide (○), monodeuterated acrylamide (△), and dideuterated acrylamide (▽).

The above-described increase of the reactivity as a function of the unsaturation degree was also observed in the assayed fatty esters. Thus, the reactivity of the unoxidized fatty esters increased in the order methyl stearate < methyl oleate < methyl linoleate < methyl linolenate. This behavior might be related to the oxidizability of the assayed lipid. For the same reason methyl 12-hydroxyoctadenoate was less reactive than methyl 13-hydroxyoctadeca-9,11-dienoate. However, when the two double bonds were conjugated with an ester group, such as in methyl 2,4-decadienoate, the reactivity of the compound was much reduced. In addition, alcohols also showed very much reduced reactivity.

**Effect of the Use of Deuterated Water in the Deuteration Degree of Acrylamide during the Heating of Asparagine with 2,4-Decadienal.** When water was replaced by deuterated water in asparagine/2,4-decadienal reaction mixtures, the formation of three acrylamides with different deuteration degrees was observed: nondeuterated acrylamide, monodeuterated acrylamide, and dideuterated acrylamide. In addition, the ratio among these three compounds depended on the amount of deuterated water added (**Figure 6**). Thus, the proportion of nondeuterated acrylamide decreased exponentially when deuterated water was added. This nondeuterated acrylamide was replaced, in a first step, by monodeuterated acrylamide. However, although this acrylamide increased very rapidly when small amounts of deuterated water were added, its proportion was maintained when > 30 $\mu$L of deuterated water was added. Further decreases of nondeuterated acrylamide did not change the proportion of the monodeuterated acrylamide. However, the proportion of the dideuterated acrylamide increased. The relative proportions among the three acrylamides were maintained when > 100 $\mu$L of deuterated water was added.

## DISCUSSION

Acrylamide formation from asparagine in the presence of lipid-derived carbonyl compounds is a two-step process: first, the decarboxylation of the amino acid and, then, the elimination reaction of the formed 3-aminopropionamide to produce acrylamide. In both steps, lipid-derived carbonyl compounds have been shown to play a major role. Previous studies (*10*) showed that, in the second step, alkadienals were able to decrease the $E_a$ of the reaction from 100−110 kJ/mol, observed in the absence of 2,4-decadienal, to 40−60 kJ/mol. The above results show that carbonyl compounds are also

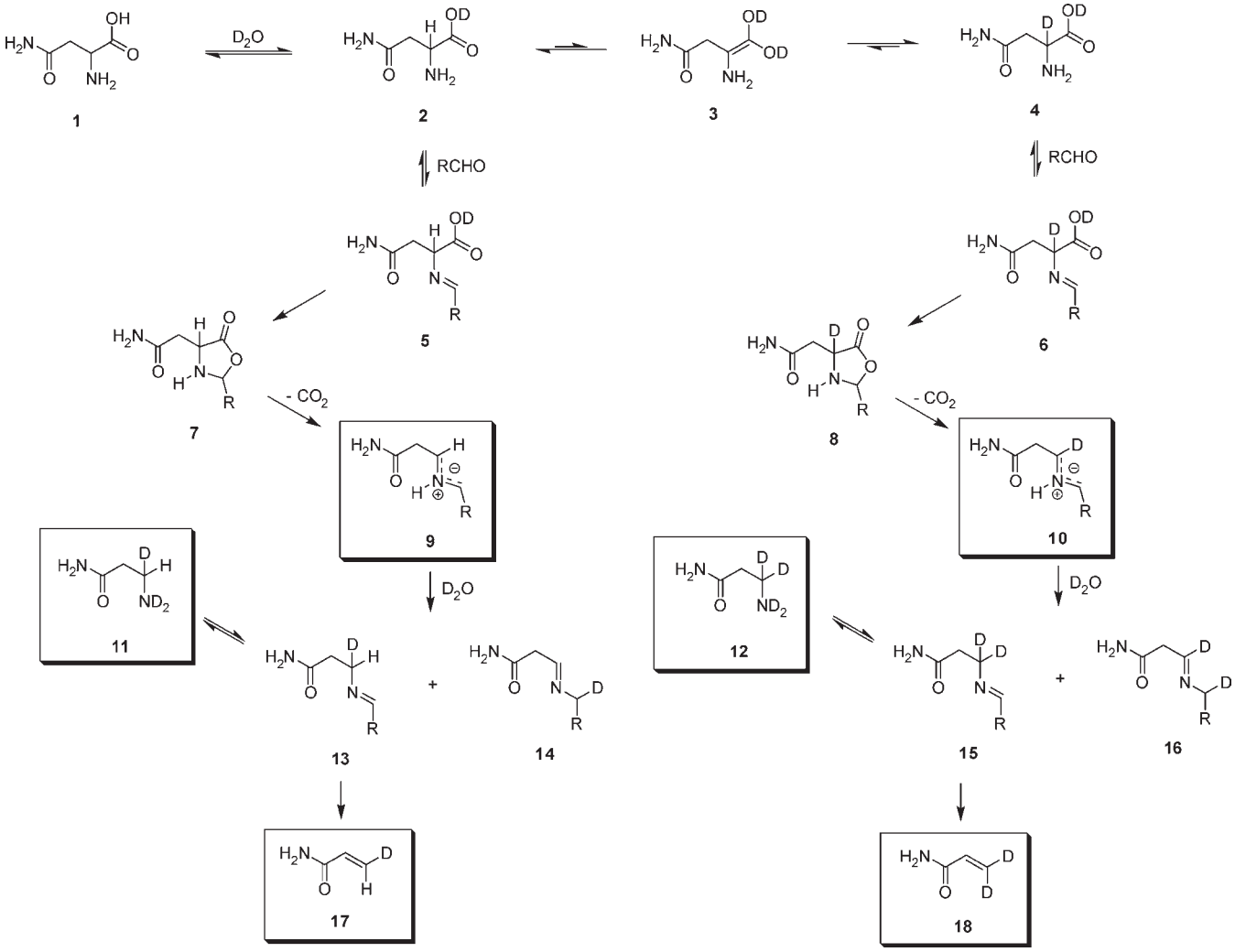


**Figure 7.** Proposed pathways for the decarboxylation of asparagine in the presence of carbonyl compounds. The reaction pathways for the conversion of 3-aminopropionamide into acrylamide were described previously (*10*).

essential for the amino acid decarboxylation. In fact, when carbonyl compounds are either absent or difficultly formed, no decarboxylation was observed under the employed conditions. This is likely the reason for the null results obtained with saturated or monounsaturated alcohols and other saturated lipids (**Table 2**). The presence of unsaturations in the lipid molecule, or the use of reactive derivatives that can produce carbonyl compounds by decomposition, such as lipid hydroperoxides, resulted in significant amounts of 3-aminopropionamide.

The different extents to which the decarboxylation reaction is produced as a function of the unsaturation degree of the alkadienal should be a consequence of the reaction mechanism. This mechanism should also explain the acrylamide deuteration observed when the reaction was carried out in the presence of deuterated water. Because water does not play any role in the elimination reaction of 3-aminopropionamide, in fact, the presence of water inhibits the elimination reaction (*10*), the deuteration should be produced during the decarboxylation.

**Figure 7** proposes a reaction pathway for the formation of 3-aminopropionamide from asparagine and 2,4-decadienal and its later elimination to produce acrylamide. In the presence of deuterated water, the amino acid (**1**) will immediately exchange deuterium at the carboxylic acid site (**2**) and then at the α-position (**4**) due to enolization (**3**). The extent of this last exchange is expected to be low, in agreement with **Figure 6**, in which only high amounts of deuterated water produced similar amounts of mono- and dideuterated acrylamide. The reaction of the carbonyl compound with the deuterated asparagines should produce the imines **5** and **6**, respectively. Both compounds should be stabilized with the presence of conjugated carbon−carbon double bonds in the molecule of the aldehyde. The decarboxylation of these imines is facilitated due to the formation of a relatively stable azomethine ylide (**9** and **10**, respectively) after the loss of carbon dioxide from 5-oxazolidinone intermediates (**7** and **8**, repectively) (*17*, *18*). Azomethine ylide would, finally, evolve into the conjugated imines **13** and **15**, respectively, which are responsible for both the detection of 3-aminopropionamide after hydrolysis and the formation of mono- and dideuterated acrylamides (**17** and **18**, respectively) after the elimination reaction.

Decarboxylation and elimination steps require different reaction conditions and have different $E_a$ values. Therefore, the reaction conditions observed for the conversion of asparagine into acrylamide (described previously in ref *15*) are, in some cases, somewhat intermediate between the conditions of the decarboxylation reaction described above and the conditions of the elimination reaction described in ref *10*. Thus, decarboxylation is mainly produced at acid pH values, and 3-aminopropionamide suffers the elimination reaction mainly at basic pH values. For this reason, the conversion of asparagine into acrylamide mainly occurs at pH 4−6 (a lower pH inhibits the elimination reaction and a higher pH does not favor the decarboxylation). On the other hand, the limiting step of the whole process is the decarboxylation

reaction (it has a higher $E_a$ than the elimination reaction of 3-aminopropionamide in the presence of 2,4-decadienal), and the water plays a major role in the mechanism of this step (**Figure 7**). Therefore, the effects of water content in the decarboxylation reaction and in the whole process were very similar. On the contrary, an increase in the water content rapidly inhibited the elimination reaction.

All of these results point to the decarboxylation step as the key step in the conversion of asparagine into acrylamide in the presence of alkadienals. Therefore, any inhibiting strategy for suppressing the role of alkadienals in the formation of acrylamide should be mainly directed to the inhibition of this step.

## LITERATURE CITED


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