Prolog

A general meeting held in Chicago, October 28-30, 2002 evaluated the significance of food-borne acrylamide in foods. The information presented in the following paper attempted to bring together acrylamide mechanisms proposed by various authors between April and October of 2002. This period marked a very fertile 6-month period of scientific inquiry concerning the chemical origins and abundance of acrylamide in American and European diets. World-renowned experts in acrylamide research and food chemistry received the paper prior to attending the October meeting. The mechanism paper presented below, along with the preliminary papers for the other 4 working groups were called "straw-man papers". As such, they offered a tangible effigy for scientific assault. Perhaps the piñata would have been a like-fitting metaphor. The superfluous layers were cudgeled off to expose the prized matter inside. The paper below is the piñata before the stick. By the meeting's end, much had been removed. In particular, fat and acrolein pathways had largely been dismissed as significant pathways of acrylamide formation. The workshop also re-evaluated the minimum requirements for acrylamide formation through asparagine. Originally, researchers believed that a dicarbonyl-asparagine reaction was a required intermediate in acrylamide formation. However, the data indicated that monocarbonyls also reacted with asparagine to produce acrylamide. Significantly, this opened the possibility that at least trickles of acrylamide can come from pyrolytic carbonyls of fat, carbohydrate and protein origin. The "post-stick" product of the meeting is contained in the attached summary document from the acrylamide mechanisms working group.

Introduction

Acrylamide is a known neurotoxin and cancers suspect agent in man (Dickson et al, 1999; Coughlin, 2002). Its identification as an n-terminal adduct in human hemoglobin first occurred over 30 years ago. Adduct level is considered to correlate with human exposure (Tareke, et al, 2002). While some elevated blood acrylamide levels correlate with recognizable demographic phenomena such as smoking and occupational exposure, detectable levels appear in all human populations despite considerable diversity in diet and locale. The identification of acrylamide in feral animals suggested that at least some acrylamide might arise from endogenous biochemical pathways.

Recent research conducted by the Department of Environmental Chemistry at Stockholm University, Sweden (Tareke, et al., 2002) has sought to identify unresolved environmental sources of acrylamide. The group examined a cross-section of foods characteristic of the Swedish diet. In general, foods high in fats and carbohydrates prepared at temperatures above 160°C exhibited acrylamide formation roughly in proportion to their degree of browning. Low carbohydrate, high protein foods, such as meats, developed moderate levels of acrylamide. Moderate protein, high carbohydrate foods such as potatoes developed substantially higher levels of acrylamide under heating conditions. The striking health implications of this work led to a pre-publication press announcement of the Swedish findings in April of 2002. The scientific and public concern that followed precipitated a number of enquiry meetings to assess the significance of acrylamide in the human diet. The WHO made food-borne acrylamide

the subject of an international meeting in June 2002. The meeting marked the first international dialogue on the food chemistry of acrylamide in foods. Scientists from many nations met independently to discuss the importance of acrylamide within their borders. Recently, the US FDA sponsored a public meeting assessing the impact of acrylamides in the US diet (Lineback, 2002).

In the short weeks prior to publication of the full peer-reviewed Swedish work in August 2002, bench and theoretical chemists in America and Europe began to speculate on plausible acrylamide mechanisms and to quantify acrylamide levels in their domestic foodstuffs. Confirmation of Swedish assayed levels of acrylamide in American and European foods began to emerge literally within days of the initial press release. The first public discussion of food-acrylamide in America occurred at the annual IFT meeting in Anaheim, California, June 2002. The meeting and its follow up reflections proposed a number of acrylamide mechanisms. These variously considered the relevance of fats, carbohydrates, proteins (especially those containing epsilon amino acids), or free amino acids in acrylamide pathways.

Assessing the relative importance of these components is difficult since various foods limiting in each of these compounds still exhibit some level of acrylamide development. For example, acrylamide from potato chips prepared in fatty acid esters (lacking in exogenous glycerol) parallel and often surpass potato chips prepared in traditional triglyceryl fats (Sadler, 2002). One explanation of this phenomenon might be that fat strictly plays a role as a thermal transfer medium and is not of itself a precursor to acrylamide. This would leave only carbohydrates and amino acids as potential precursors. However, modest levels of acrylamide also arise in seared meats, which are virtually devoid of free sugars. This would imply that amino acids (or proteins) alone give rise to acrylamides. By late September, early October of 2002, breakthroughs at Procter and Gamble (Sanders et al., 2002), The Canadian Health Service (Becalski et al., 2002) and by experimenters in Britain (Mottram et al., 2002), Germany (Stadler et al., 2002) and Australia appear to have corroborated at least one acrylamide mechanism, the creation of acrylamide from the amino acid asparagine and various Maillard-active dicarbonyl and hydroxycarbonyl precursors. This pathway would explain the generally high acrylamide content in fried potato products since potatoes are unusually high in free amino acids, and especially high in free asparagines. Depending on their storage conditions, potatoes may also contain significant quantities of free glucose.

Additional research must explore the possibility of other mechanisms. Even in the fortunate event that the asparagine/carbonyl pathways exclusively explain all acrylamide, the task of regulating the pathway to minimize acrylamide formation remains. This whitepaper examines some of the mechanisms for acrylamide production proposed by the world scientific community during the past few months. Hopefully, interrogating these pathways will lead to a clearer understanding of food-borne acrylamides by:

1. Emphasizing probable pathways

2. Rejecting unlikely pathways

3. Suggesting inhibitory or catalytic agents to discern between various elementary steps in a given mechanism

4. Suggesting pre-emptive treatments/processes for limiting acrylamides in foods

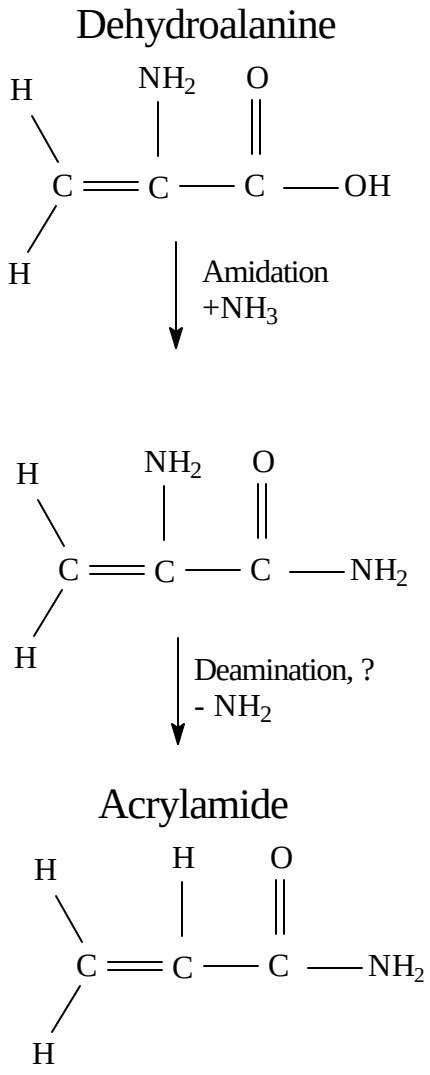
It is a notable credit to the collaborative openness of acrylamide researchers and the rapid electronic coordination and dissemination of data, that key pieces to the acrylamide puzzle are being added almost daily. The dynamic nature of this enterprise adds additional challenges to providing a comprehensive whitepaper. Hopefully, the paper will supply a body of data for dissection. Inevitably, important points will have been missed. Please feel free to provide me with details of obvious omissions and I will endeavor to insert them into the final document.

PATHWAYS

I. AMINO ACIDS ONLY

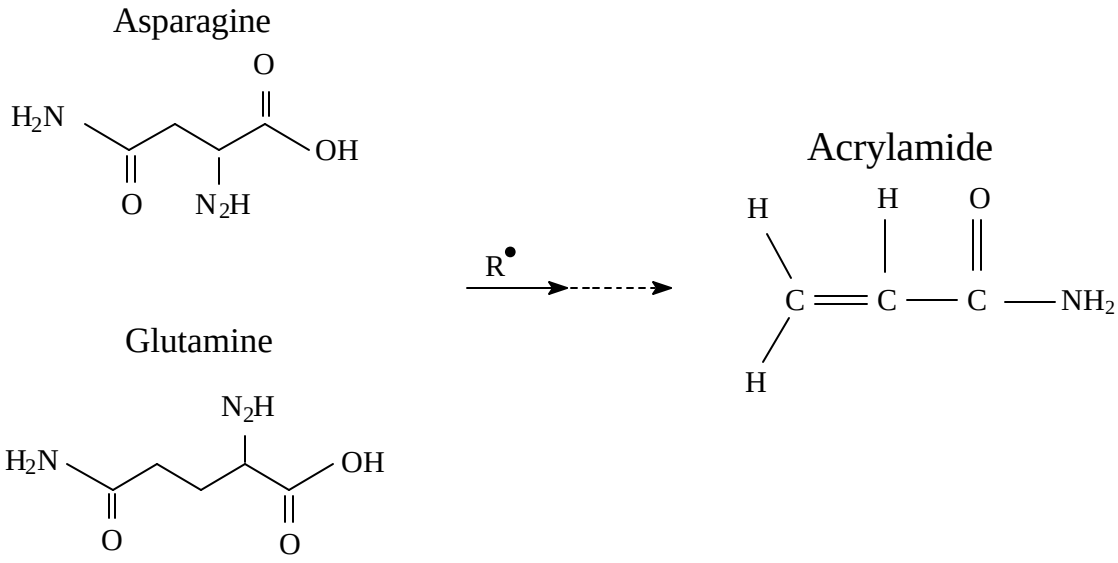
The only certain fact in acrylamide formation is the necessity of a nitrogen-containing precursor. Although free amino acids and proteins are the heads-on favorites for this distinction, researchers should not loose sight of the fact that other biological sources of nitrogen exist in the form of nucleotides and other heterocyclic compounds. Given this caveat for the sake of comprehensiveness, several acrylamide mechanisms based solely on amino acids have been proposed (DeVries, 2002); Lindsay (2002), Friedman (2002) :

A. From alanine (Lindsay, 2002)

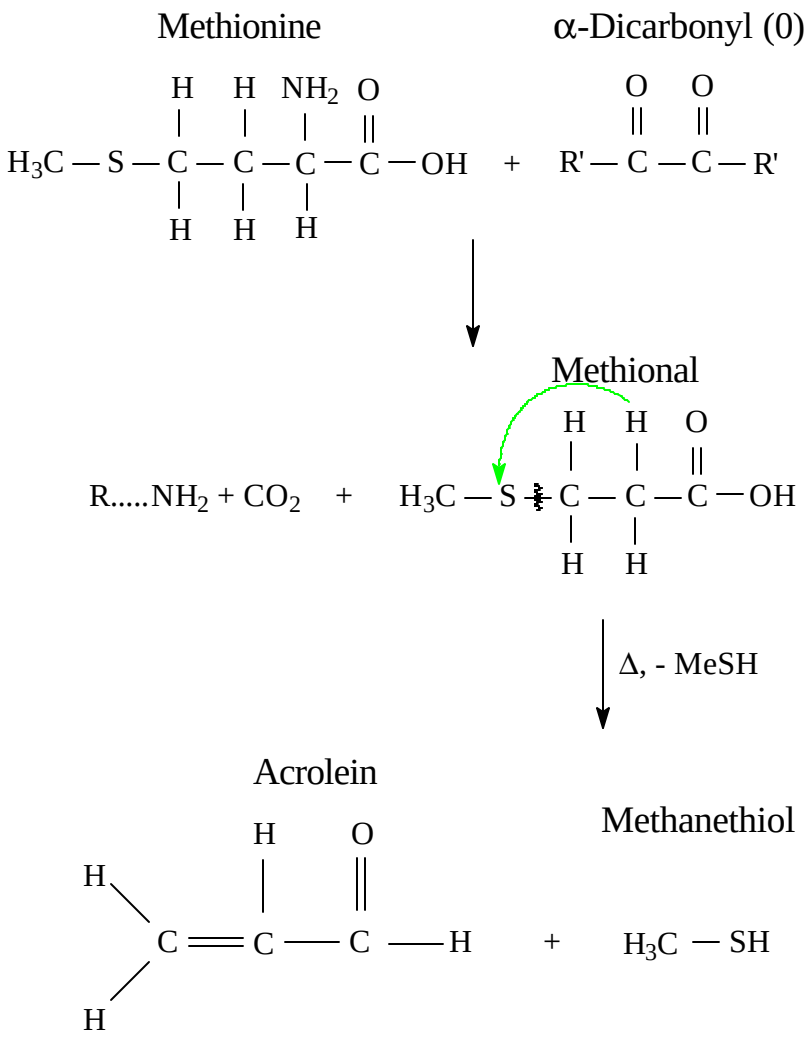


B. Lindsay (2002) and Friedman (2002) proposed mechanisms directly from asparagine and glutamine as early as June,2002. Recent research, shown in a later section, has confirmed asparagine's role in acrylamide formation and strongly implicated glutamine in a low yield acrylamide pathway. Lindsay's proposed the following mechanism:

Radical mediated cleavage of asparagine (or glutamine):



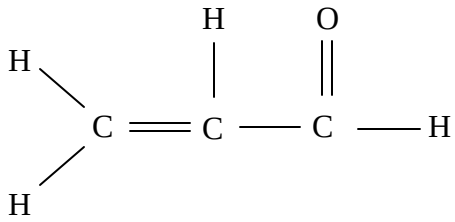
C. A methionine/dicarbonyl reaction was also proposed (Lindsay, 2002)



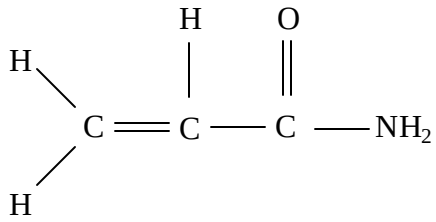
II. PATHWAYS BASED ON AN ACROLEIN INTERMEDIATES

Several pieces of circumstantial evidence made acrolein an early suspect in acrylamide formation. Like acrylamide, it appears in thermally abused foods high in fat. It has certain structural similarities to acrylamide (illustrations below). Acrolein concentrations produced at high temperatures are sufficient to support acrylamide formation in a theoretical mass balance. Acrolein detection is common in samples testing positive for acrylamide (Sadler, 2002). The ubiquity of acrylamide in compositionally diverse foods could be explained by the fact that acrolein can arise from degradative pathways of fats, carbohydrates, and proteins. Potential pathways are shown below.

Acrolein (2-Propenal)

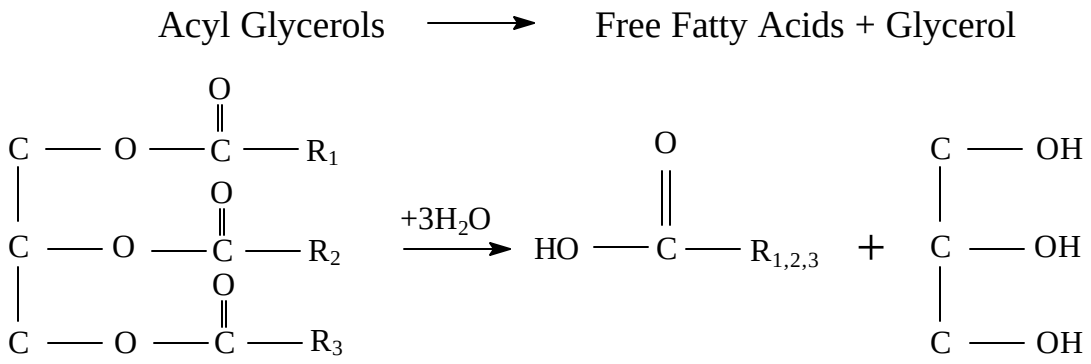


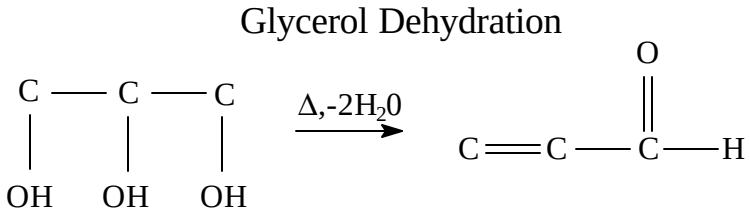
Acrylamide



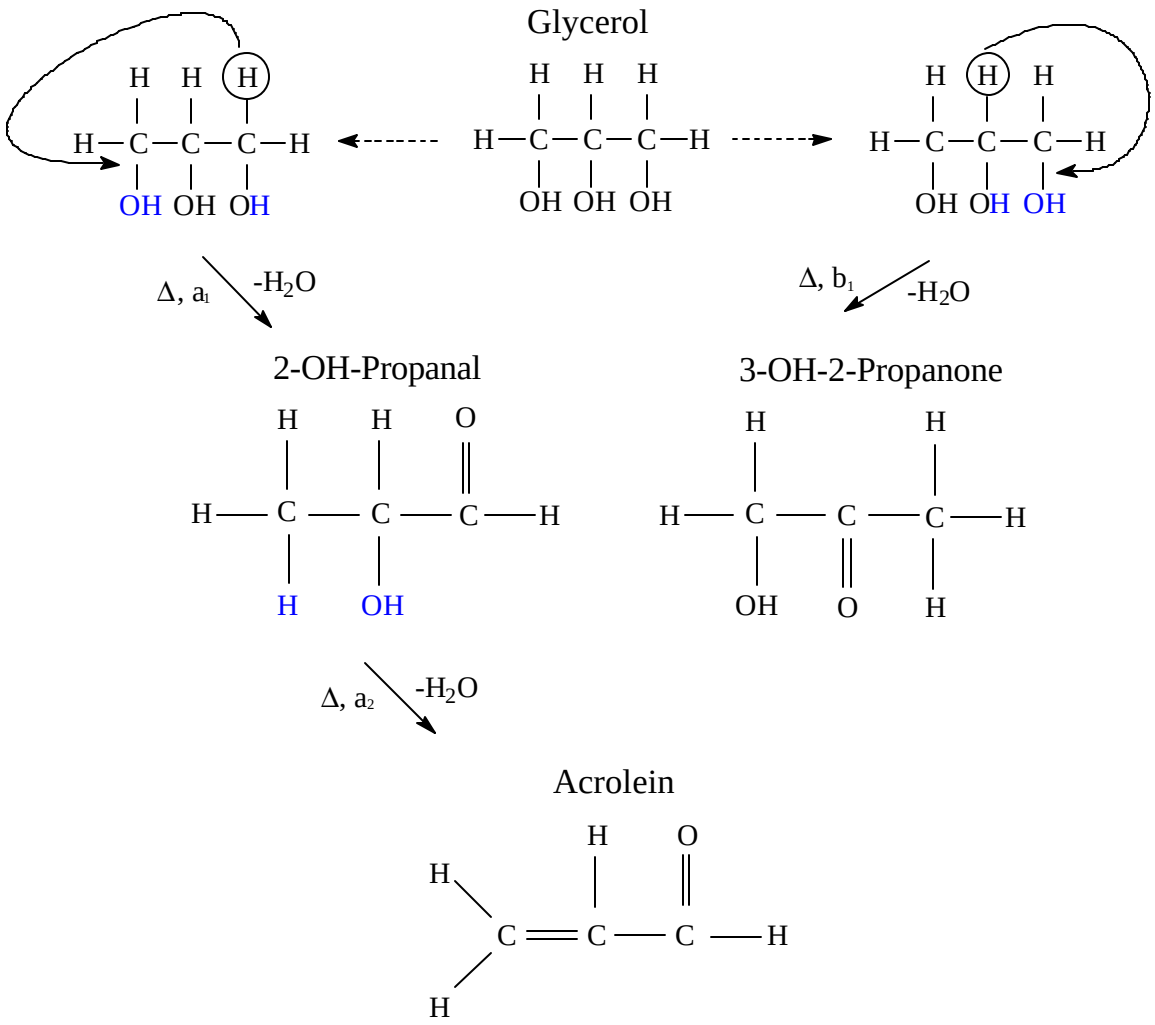
A. Lipid Pathways for Acrolein

1. A possible mechanism from triglycerides has been proposed (Lindsay, 2002)



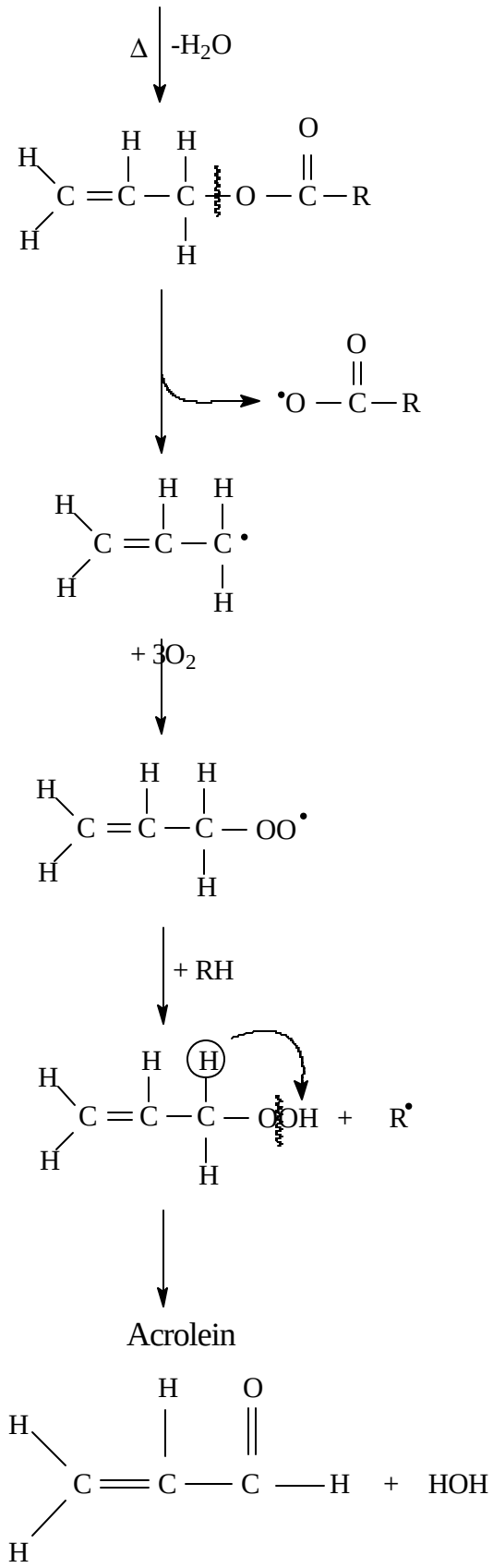


2. And through glycerol dehydration as follows (after Lindsay, 2002 and Friedman, 2002).

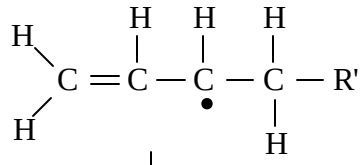


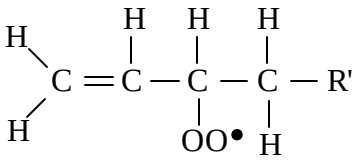
3. Lindsay (2002) also proposed a possible monoglyceride oxidation pathway for acrolein:

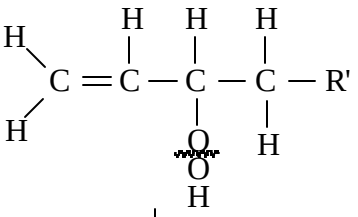
MONOGLYCERIDE

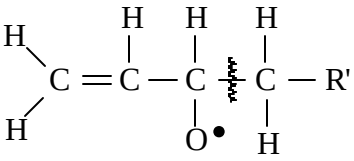


or similarly:

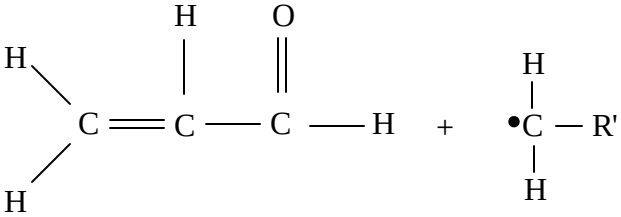




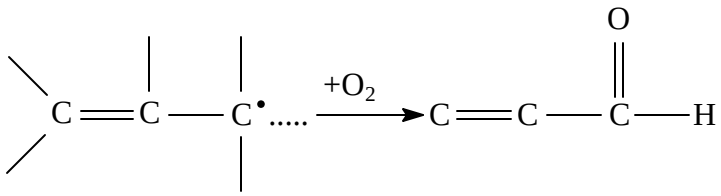




Acrolein



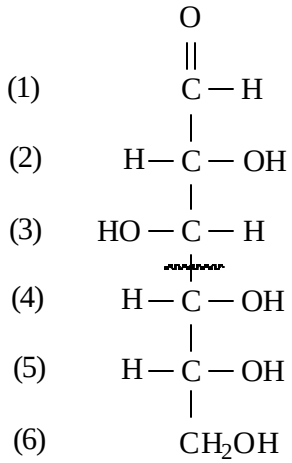
4. Recombination of lipid fragments has also been proposed (Lindsay**,** 2002)



B. Acrolein from Starches and Sugars

A. Acrolein pathways from sugars also exist. While these are not traditional Maillard type reactants, some evidence for this pathway appears to exist from research on tobacco pyrolysis.

Starch ⟶ Glucose ⟶ Fragments



Δ, 1) and 2)....

1) Cleavage

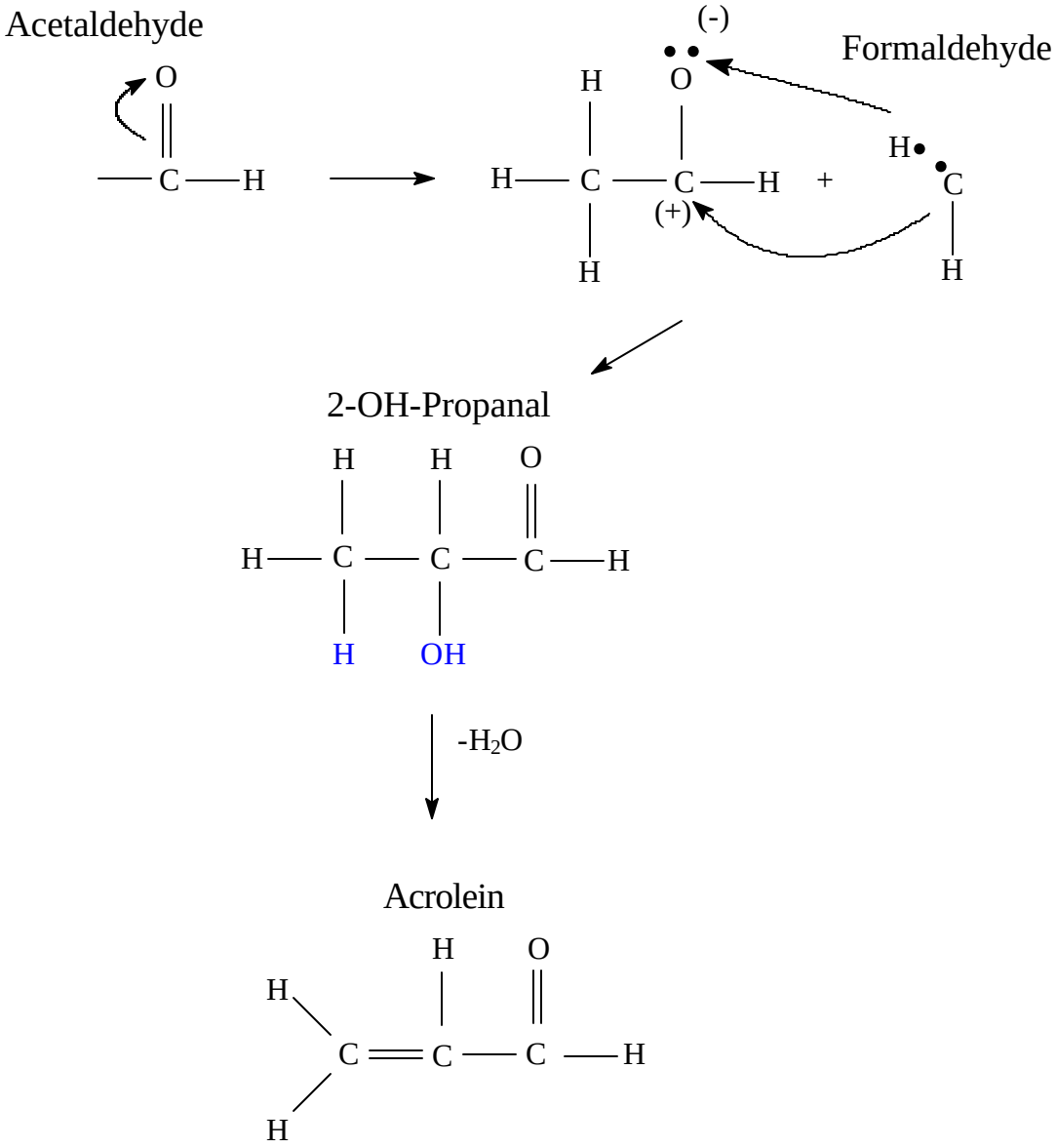
2) - H_2O

Acrolein

(Greater amounts of acrolein from $C_{4,5,6}$ than $C_{1,2,3}$)

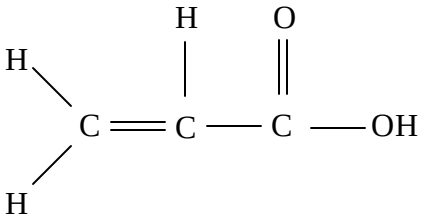
C. Acrolein from Recombination of Simple Aldehydes

A. Acetaldehyde and formaldehyde appear to be ubiquitous byproducts of pyrolysis, oxidation and irradiation of fats, proteins and carbohydrates. Acetaldehyde and formaldehyde have been proposed to produce acrolein in the following way:



III. PATHWAYS BASED ON AN ACRYLIC ACID INTERMEDIATE

Acrylic Acid

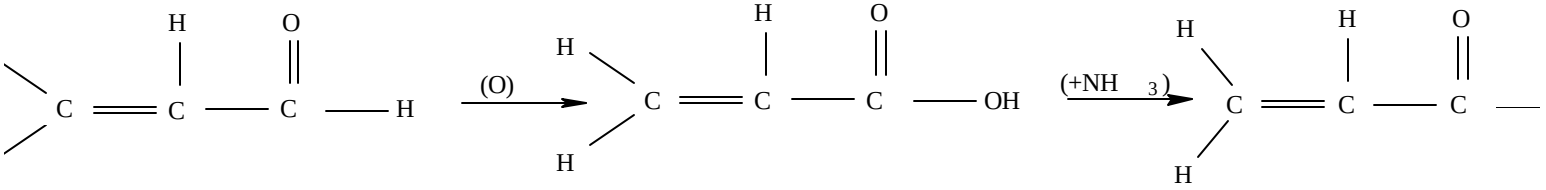


While acrolein exhibits many attractive qualities as an acrylamide precursor, its direct conversion into an amide is difficult to envision. Acrylic acid/ammonia reaction offers a much more compelling and, from the standpoint of organic chemistry, a better documented pathway for amide synthesis (Friedman, 2002). Two mechanisms have been proposed for conversion of acrolein to acrylamide (Lindsay, 2002). These occur either by direct oxidation through an acrylic acid intermediate or through amination which may or may not have an acrylic acid intermediate.

A. Acrolein Conversion:

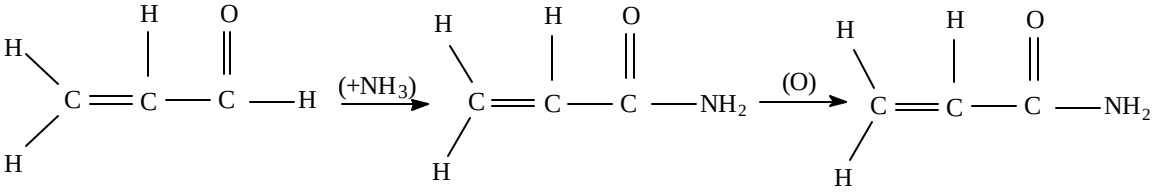
1) Direct Oxidation

Acrolein → Acrylic Acid → Acrylamide



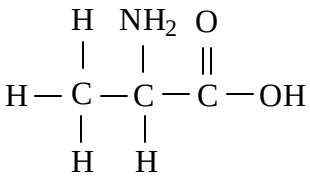
2) From Amination:

Acrolein → Adduct → Acrylamide

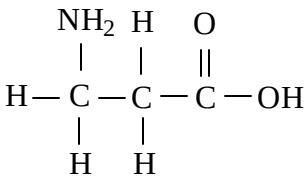


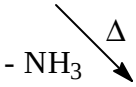
B. Acrylic Acid Formation Has Been Proposed Directly Through Alanine**:**

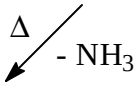
α - Alanine



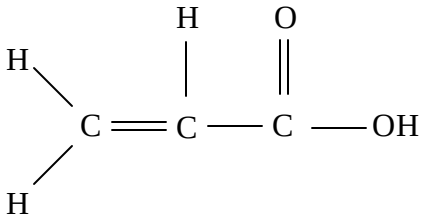
β - Alanine



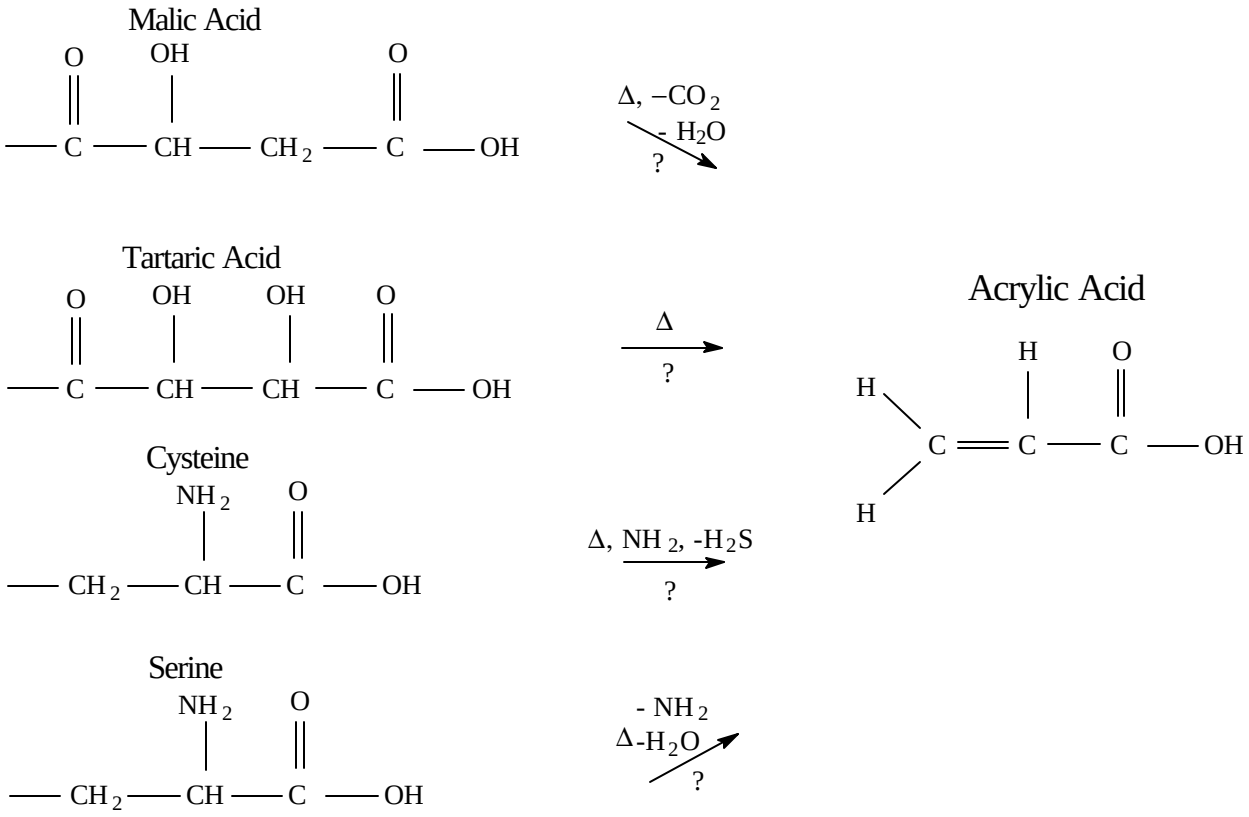




Acrylic Acid



C. And Assorted Di-acids and Amino Acids



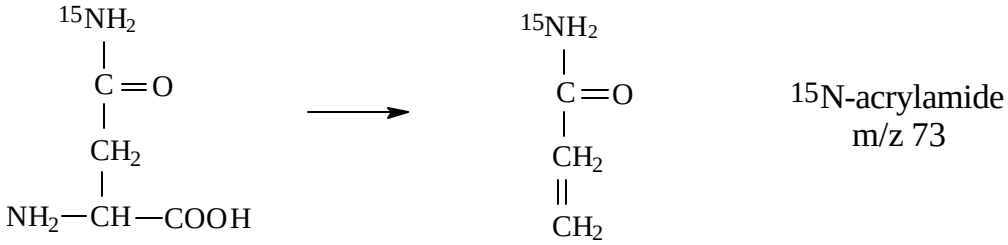
Acrylamide production based on the reaction of ammonia with acrylic acid is straightforward and founded on an amination process well-documented in organic chemistry. Thermal desorption GC/MS confirms the presence of ammonia and acrylic acid in many acrylamide-containing foods (Sadler, 2002). The significance of this mechanism is easy to test. Unless acrylic acid is limiting, increasing available ammonia would force higher acrylamide yields. However, ammonia addition in the form of heat labile ammonium carbonate does not significantly influence acrylamide production (Becalski, 2002). While pH and moisture could greatly influence this reaction in ways as yet not fully researched, it appears at present that the reaction between ammonia and acrylic acid plays at best a minor role in acrylamide formation.

IV. PATHWAYS BASED ON MAILLARD BROWNING PRECURSORS

Early analytical work observed acrylamides primarily in foods browned during preparation. While across the diet browning correlates poorly with acrylamide level, within a batch, a given food tends to increase in acrylamide as browning progresses. This led to the conjecture that Maillard browning precursors might be stoking the acrylamide pathway. Perhaps poor global correlation between browning and acrylamides suggest that differences in reducing sugars, amino acids and proteins play an important role in the ultimate yield of acrylamide.

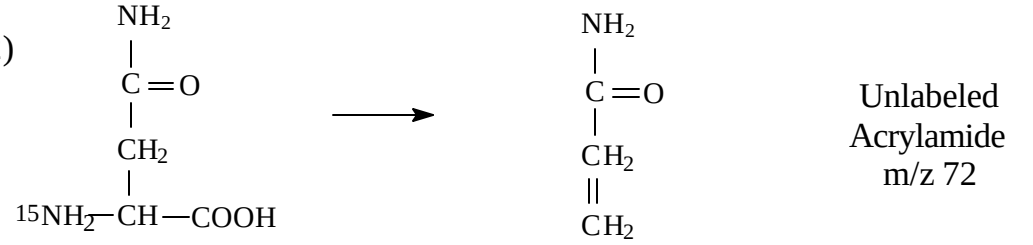
Within a three week period in late September, early October 2002, several research groups documented a clear cut role for asparagine in acrylamide formation. Researchers at Health Canada (Besalski et al., 2002) and Procter and Gamble (Sanders et al., 2002) presented data at the annual AOAC meeting in Los Angeles California which both suggested a key role for asparagine. Both researchers used stable isotopes to map reaction pathways. Three verification experiments reported by Sanders et al. are illustrated below. Step 1 indicates the alpha carbon of asparagine is the focal point of reaction. It also indicates that an asparagine pathway exists exclusive of reducing sugars. Step 2 suggests that the alpha amino group does not participate in acrylamide formation. Step 3 suggests that the amino acid R group in asparagines remain intact instead of being assembled from pyrolytic fragments.

#1)



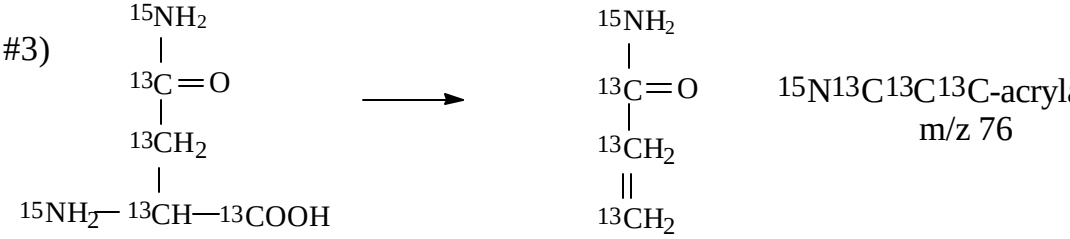
^{15}N-acrylamide
m/z 73

#2)



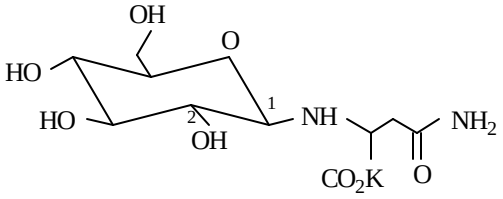
Unlabeled
Acrylamide
m/z 72

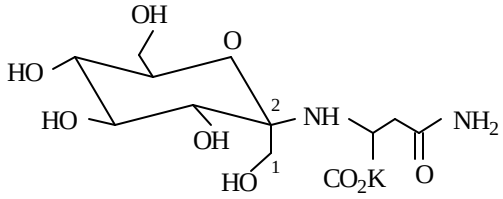
#3)

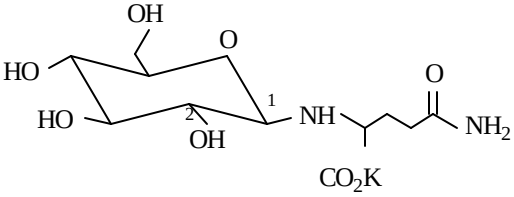


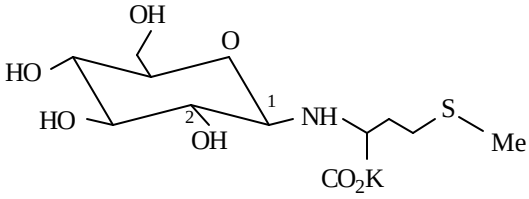
^{15}N^{13}C^{13}C^{13}C-acrylamide
m/z 76

While thermal degradation of pure asparagine produces acrylamide, the presence of reducing sugars and certain carbonyl containing compounds increase the reaction up to 100 fold (Sanders et al., 2002). Stadler et al., (2002) heated certain N-glycosides (early Maillard precursors) of various amino acids to 180°C and analyzed reaction products for acrylamide. Heating asparagine, glutamine, cysteine and methionine with fructose, galactose, lactose or sucrose produced significant quantities of acrylamide. Interestingly, at pyrolytic temperatures, equivalent acrylamide yields were reported for reducing (fructose and galactose) and non-reducing (lactose, sucrose) sugars. Certain of the proposed acrylamide-producing N-glycosides included:

1. 

2. 

3. 

4. 

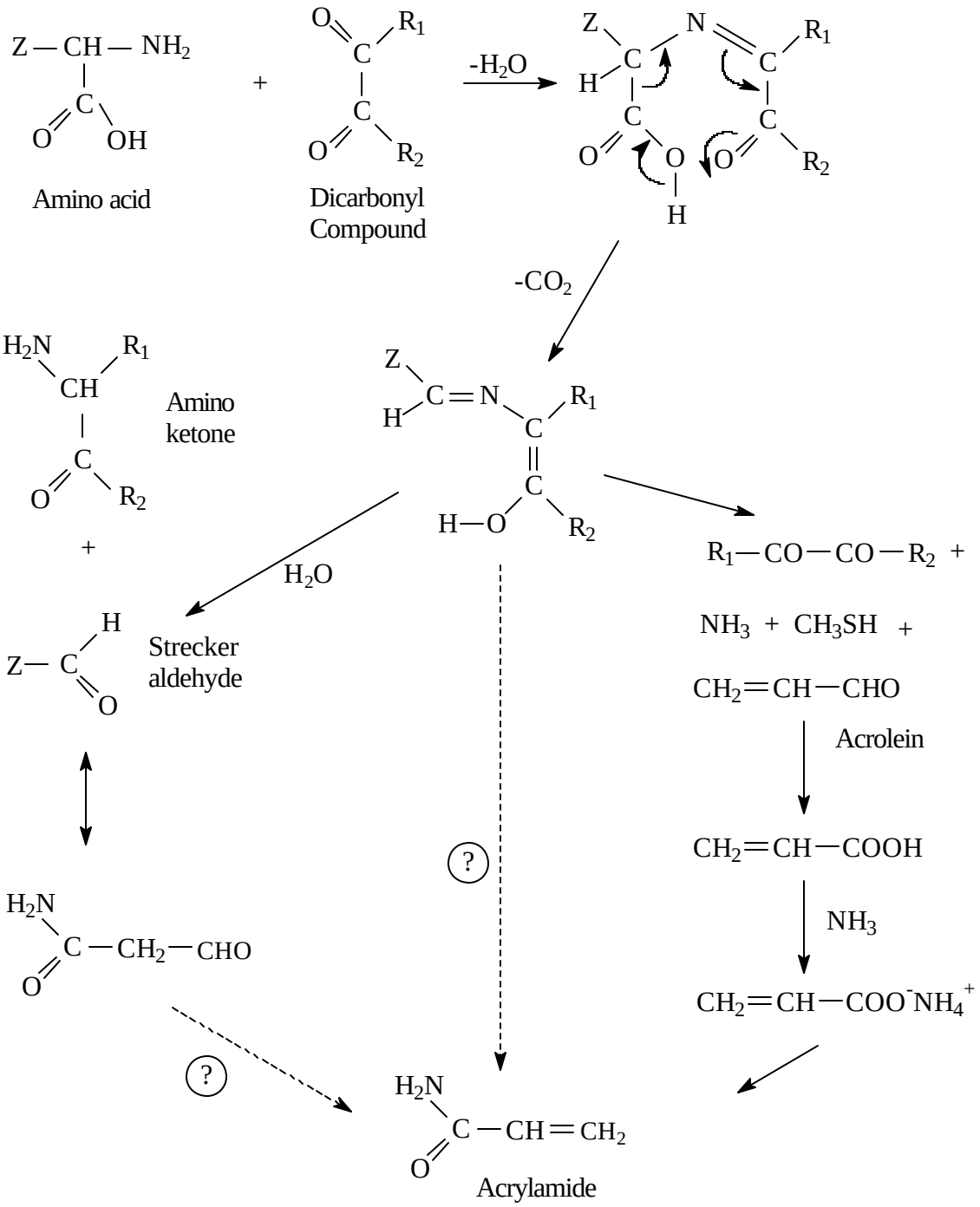
1. N-(D-glucos-1—yl)-L-asparagine

2. N-(D-fructos-2—yl)-L-asparagine

3. N-(D-glucos-2—yl)-L-glutamine

4. N-(D-glucos-2—yl)-L-methionine

Sanders et al. (2002) indicated a ribose/asparagine combination also produced acrylamide. Acrylamides in meats and other foods low in reducing sugars may arise from pyrolytic products of ribose in DNA and RNA. They also observed acrylamide formation through glyoxal, glyceral and 2-deoxy-glucose combinations, which suggests many dicarbonyl and dicarbonyl-forming compounds participate in amide formation. Lindsay (2002), Mottram et al. (2002), Sanders et al. (2002) and Becalski et al. (2002) have all suggested involvement of dicarbonyl compounds or their hydroxy carbonyl precursors in acrylamide formation. The mechanism proposed by Mottram et al. (2002) suggests one spur of the mechanism produces an acrolein intermediate.


Amino acid
Dicarbonyl Compound
-H2O
-CO2
Amino ketone
H2O
Strecker aldehyde
R1—CO—CO—R2 +
NH3 + CH3SH +
CH2=CH—CHO
Acrolein
CH2=CH—COOH
NH3
CH2=CH—COO-NH4+
?
?
Acrylamide

V. CONCLUSIONS

It appears unmistakable that some pyrolysis fragments of asparagine find their way into an acrylamide endpoint and that acrylamide formation is greatly facilitated by collateral pyrolysis of sugars, both reducing and non-reducing. The role of fats, while perhaps lacking the pre-eminence initially postulated, may still play some role. It appears that expedited acrylamide formation may only demand asparagine plus a carbonyl compound having an alpha hydroxyl group. Production of such a compound from glycerol or fatty acid pyrolysis is easy to envision. Indeed, several triglyceride and fatty acid processes posted above anticipate such molecules. The role of amino acids other than asparagine remains to be elucidated. Strong collaborative evidence suggests that glutamine produces some (albeit by asparagine standards rather petty amounts) of acrylamide. Cysteine and methionine appear to produce acrylamide in certain studies but not in others. Future work should clarify these discrepancies. If nothing else, such ambiguity reminds us that research probing the differential behavior of amino acids demands reagents of uncommon purity.

Perhaps the most exciting detail in the chase for an acrylamide pathway has been the heartiness of the race. The first announcement of the Swedish study appeared in early spring. By summer's end, no less than 5 research groups in as many countries independently described acrylamide pathways which agreed in most significant details. Is the quest for the food origins of acrylamide complete? No. Questions remain. The role and relative importance of fats, certain amino acids, and carbonyl precursors should be clarified as a necessary prerequisite to planning intervention strategies. Pathways glimpsed from model foods deserve confirmation in whole foods. An audit of asparagine-traceable acrylamide in whole foods would reveal the presence and importance of non-asparagine pathways. The few conflicting details of identified pathways deserve reconciliation. While significant advances in understanding acrylamide origins have occurred, these are only academic until acrylamide chemistry is examined with an eye toward developing mechanisms of prevention.

VI. REFERENCES

Becalski, A.; Lau, B.; Lewis, D.; and Seaman, S. 2002. Acrylamides in Food: Occurrence and Sources. Poster 116th Annual International AOAC Meeting, September 26th, Los Angeles, CA., USA.

Coughlin, L. 2002. Overview of Acrylamides in Foods. Presentation 116th Annual International AOAC Meeting, Los Angeles, CA., USA.

DeVries, J. 2002. Personal Communication.

Dickson, S.; Heenam, M.; Caiens, S.; Sage, H.; Bergmak, E. 1999. Acrylamide. 9th Annual Report on Carcinogens. TIAFT Abstracts 035. Hair Analysis.

Friedman, M. 2002. Mechanism of Formation of Acrylamide in Food Products. Notes from Conference Call, May 23, 2002.

Lindsay, R. Generalized Potential Origins of Acrylamide in Foods. Workshop on FRI Acrylamide Project Chicago, O'Hare Hilton June 4, 2002

Lineback, D. 2002. US Food and Drug Public Meeting [Docket No. 02N-0393] September 30, College Park, MD USA.

Mottram, D.; Wedzicha, B.; and Dodson, A. 2002. Nature 419:448.

Sadler, G. 2002. Research Notes, Thermal Desorption of Browned Baked and Fried Foods

Sanders, R.; Zyzag, D.; Stojanovic, D.; Tallmadge, B.; Ebehart, D.; and Ewald, D.; An LC/MS Acrylamide Method and its Use in Investigating the Role of Asparagine. Presentation 116th Annual International AOAC Meeting, September 26th, Los Angeles, CA., USA.

Stadler, R.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P.; Robert, M.; Riediker, S. 2002. Nature 419:449.

Tareke, E.; Ryberg, P.; Karlsson, P.; Ericsson, S. and Tornqvest, M. 2002. J. Ag. and Food Chem