Amadori Compounds as Nonvolatile Flavor Precursors in Processed Foods

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Amadori compounds, derived from the condensation of amino acids with reducing sugars in the Maillard reaction, have been isolated from browned, dehydrated fruits and vegetables, bakery products, cane and beet molasses, and cured tobacco. The nonvolatile compounds in their enolic forms are precursors of flavor compounds, particularly those with burnt and caramel-like aromas. A model basic Amadori compound, 1-deoxy-1-piperidino-D-fructose, was pyrolyzed and the volatile products were isolated. These products, high boiling liquids and sublimable solids, consisted mainly of 4-carbon methyl reductones, α-piperidino-γ-butyrolactone, and piperidine amides of carbonic, formic, acetic, butyric, glycolic, and lactic acids. Only a trace of N-lactylpiperidine was found, indicating a predominance of 4:2 over 3:3 splitting of the hexose moiety. A reaction course through the 4-carbon reductones to the 2- and 1-carbon acid amides is suggested.

Attempts to establish the identity of flavor and aroma compounds often meet with difficult compositional analyses—for example, Bondarovich et al. (1967) indicate that the aroma complex of coffee is made up of several hundred constituents. Other investigators have demonstrated a similar complexity in chocolate, peanut, and bread flavors. The processing of these foodstuffs also involves a common roasting treatment necessary to develop a characteristic browned flavor.

The heating process initiates the condensation of amino acids and reducing sugars, the Maillard reaction, which produces important organoleptic products. Table I lists the free amino acids believed to be involved in the formation of identifying aromas and flavors in four foodstuffs. Pinto and Chichester (1966) indicate that amino acids are important to the production of cocoa flavor. The amino acids listed as peanut constituents are described as agents for producing a desirable peanut aroma when reacting with reducing sugars (Newell and Mason, 1967); and the reduction in amino acid levels resulting from baking bread (Bertram, 1953; Linko and Johnson, 1963; Naumova, 1965) and roasting coffee beans (Wolfrom et al., 1960) indicates these as contributors to the flavor complex.

Caramel-like flavor compounds of the Maillard reaction arise from the reducing sugar moiety. The existence of free reducing sugars and the production of reducing sugars by fermentation and roasting are well documented in research reports (Bertram, 1953; Johnson and Miller, 1961; Linko and Johnson, 1963; Naumova, 1965; Newell and Mason, 1967; Wolfrom et al., 1960). In each foodstuff, characteristic aroma and flavor constituents arose on heating and were assumed to be formed, at least in part, via the Maillard reaction—for example, Figure 1 shows the destruction of reducing sugars and amino acids in cocoa beans (Rohan and Stewart, 1966). The reducing sugars nearly disappeared after a roast of 30 minutes. Although not totally destroyed, the amino acids were substantially degraded. Figure 2 shows the results obtained by Talley and Porter (1968) on amino acid–glucose model systems. Heating threonine and glucose at 103° C. for 8 hours rapidly and extensively destroyed the amino acid. Valine, isoleucine, and lysine gave similar patterns; their curves had the same general shape but varied somewhat in decomposition rate.

According to results summarized in Figure 3, the Maillard reaction contributes to the flavor complex of bread (Johnson and Miller, 1961). Similar ultraviolet-absorbing intermediates from model Maillard reactions—involving the reaction of several amino acids with dextrose—and from a bread crust extract were indicated by ultraviolet absorption curves. In studying the browning of cake that contained a high concentration of reducing sugars, Nordin and Johnson (1957) showed, by paper chromatography, that intermediates produced in the crumb also formed in glycine-glucose and phenylalanine-glucose model systems and during decomposition of the Amadori compound, 1-deoxy-1-piperidino-D-fructose (DPF).

With this evidence in mind, the contribution of the Maillard reaction to flavor and aroma complexes can be fully appre-
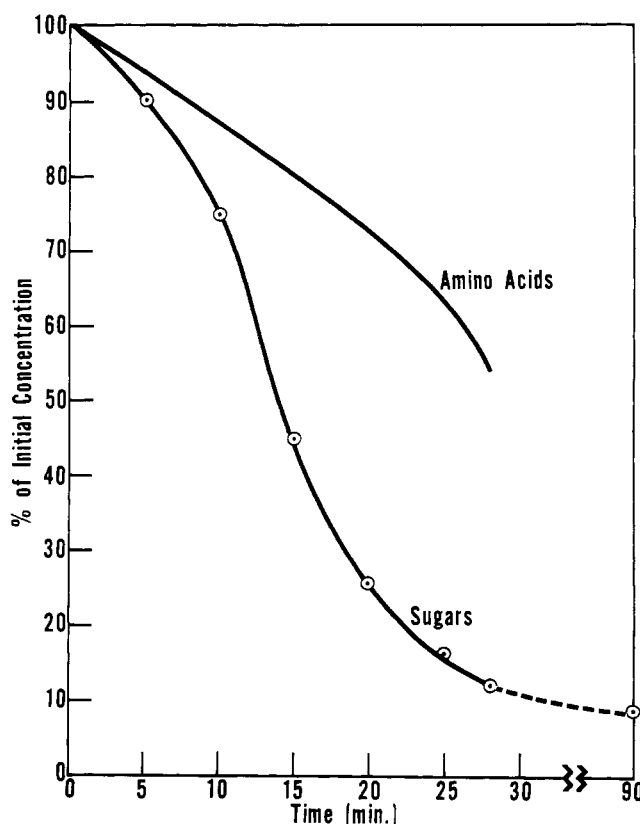

Figure 1. Destruction of amino acids and reducing sugars during roasting of cocoa beans

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Table I. Amino Acid Constituents

Bread[a]	Coffee[b]	Cocoa Beans[c]	Peanuts[d]
Alanine	Alanine	Alanine	Asparagine
Arginine	Asparagine	Glutamic acid	Aspartic acid
Glutamic acid	Aspartic acid	Leucine	Glutamic acid
Histidine	Glutamic acid	Phenylalanine	Glutamine
Isoleucine	Glycine	Threonine	Histidine
Leucine	Leucine	Valine	Phenylalanine
Lysine	Proline		
Phenylalanine	Serine		
Serine	Tyrosine		
Threonine			
Tyrosine			
Valine			

[a] Naumova (1965).
[b] Wolfrom et al. (1960).
[c] Pinto and Chichester (1966).
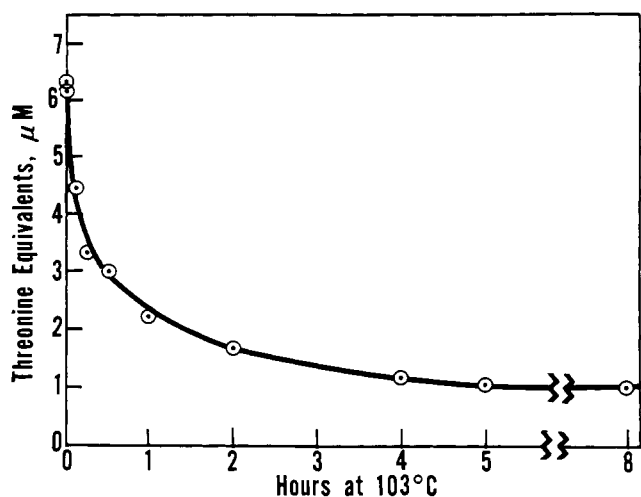[d] Newell and Mason (1967).

Figure 2. Threonine and glucose

Change in concentration of threonine with heating
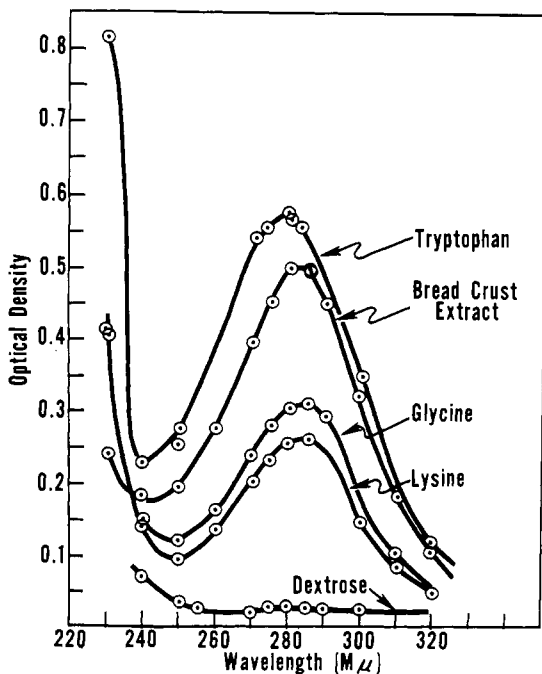

Figure 3. Ultraviolet similarities of model Maillard systems and bread crust

ciated. Although much work has been done on the reaction of reducing sugars with amines, much of the chemistry remains unknown. Knowledge of the heat decomposition products and the sequential reactions that produce them may allow the control of food flavors, odors, stability, and product acceptance.

For a short review of the chemistry involved in the Maillard reaction, the early steps are presented in Figure 4 (Hodge, 1953, 1967). The reaction proceeds through the N-glycosylamine, which undergoes enolization and ketonization to isomeric 1-amino-1-deoxyglycosulose, commonly called an Amadori compound. The authors contend that the Amadori compounds formed in this complex reaction are critical, nonvolatile intermediates affecting the organoleptic properties of processed foods. These intermediates, themselves, should serve as a simple model system to produce the characteristic browned flavors and aromas.

Table II presents the rather general occurrence of Amadori compounds in browned plant and animal tissues. The browning of these Amadori products produces α-dicarbonyl compounds, α,β-unsaturated carbonyl compounds, reductones, fluorescent compounds, and brown pigments (Hodge, 1953; Hodge and Rist, 1953; Hodge et al., 1963). This reaction led to our investigation of the products formed from the heat treatment of DPF (Nordin and Johnson, 1957; Nordin and Kim, 1958). Hodge and Rist (1953) reported that when DPF decomposes, enolic and "reductonelike" products are formed that possess caramel-like odors and antioxidant properties, probably important for the acceptance of processed foodstuffs.
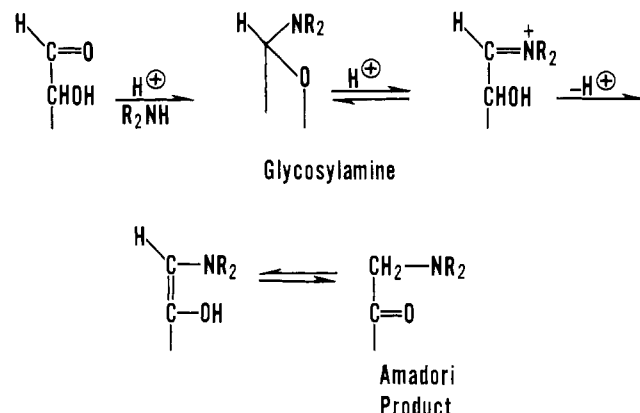

Figure 4. Amadori rearrangement

Table II. Amadori Compounds

Occurrence	Amino Group	Investigator(s)
Browned, dried apricots and peaches	Aspartic acid, threonino, serino, prolino, valino, alanino, glycino, leucino, asparagino	Anet and Reynolds (1956, 1957)
Flue-cured tobacco leaves	L-Prolino	Tomita *et al.* (1965)
Dehydrated carrots	?	Wager (1955)
Beet molasses	Asparagino, serino, glycino, alanino, valino, leucino, tyrosino, glutamic acid, γ-aminobutyric acid	Carruthers *et al.* (1963)
Hog liver	Glutamic acid	Borsook *et al.* (1955)
Liver extract	Alanino, glycino, valino	Heyns and Paulsen (1959)

Table III. Pyrolysis Products of 1-Deoxy-1-Piperidino-D-fructose

Component	Identity	Assignment[a] IR	MS	NMR	GLC	Syn
1+2	Piperidine acetate	+				+
3	*N*-Formylpiperidine	+	+	+	+	+
4	*N*-Acetylpiperidine	+	+	+	+	+
5	*N*-Butyrylpiperidine		+		+	+
6	2-Acetyl-3-piperidinofuran		+			
7	2-Acetyl-3-piperidino-4,5-dihydrofuran		+			
8	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	+	+		+	+
9	2,5-Dimethyl-3-piperidinofuran		+			
12	*N*-Lactylpiperidine	+	+		+	+
13	*N*-Glycoylpiperidine	+	+		+	+
14	Di-*N*-piperidyl carbonyl	+	+	+	+	+
15	α-Piperidino-γ-butyrolactone	+	+	+	+	+
16	Piperidino *C*-methyl reductone	+	+	+	+	+
17	Isopiperidino *C*-methyl reductone	+	+	+	+	+
18	Piperidino-hexose-reductone	+				+

[a] IR, infrared; MS, mass spectrometry; NMR, nuclear magnetic resonance; GLC, gas-liquid chromatography; Syn, synthesis.

Experimentally, DPF was pyrolyzed at 106° C., 1 mm., for 5 hours in a sublimation apparatus. The less volatile compounds in the sublimate-distillate were trapped on a cold finger cooled at 5° to 10° C., while the more volatile compounds escaped into a dry ice trap. The sublimed fraction was then subjected to analytical gas-liquid chromatography (GLC). Figure 5, *A* is the analytical profile of the total pyrolyzate of DPF. At first glance, the chromatogram appears simple; however, during chromatography, the base line region immediately following peak 4 (area *A*) possessed a strong, burnt sugar aroma. Figure 5, *B* resulted from rechromatography of a batch-collected sample of material eluted from area *A*. Peak 8 had a powerful pineapplelike and burnt sugar aroma; peaks 12 and 13 also had some caramel-like odor characteristics. To obtain samples for structural identification, the total cold finger distillate was subjected to preparative GLC and enough of each sample was collected for infrared and mass spectral analyses. For a few samples, nuclear magnetic resonance studies were feasible; in others, GLC and mass spectrometry were combined because of either product instability or paucity of sample.

Table III lists compounds unambiguously identified as pyrolysis products of DPF, together with three piperidino-substituted furans that are only tentatively identified. The amides in this table were synthesized by the reaction of the ethyl esters or acid chlorides of the appropriate acids with piperidine. The component of peak 8 [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone] was produced by the reaction of
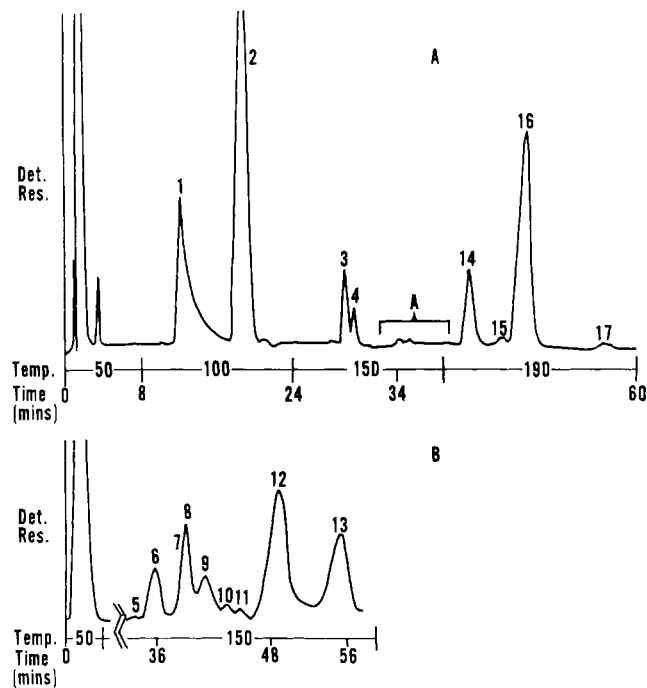
15% Carbowax 20M on 80-100 mesh Gas Chrom Q: 50 ml/min.

Figure 5. Gas-liquid chromatographs

A. 1-Deoxy-1-piperidino-D-fructose
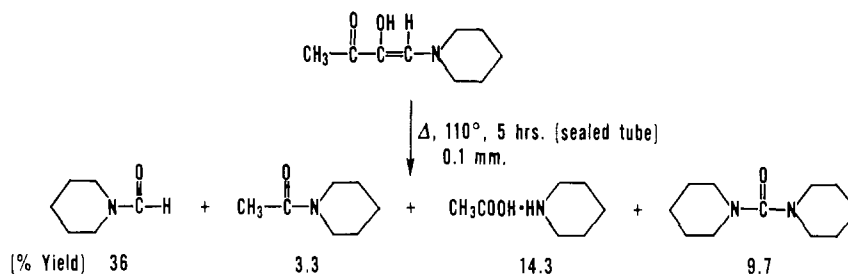*B.* Area *A* constituents

Figure 6. Pyrolysis of piperidino C-methyl reductone

$$\Delta, 110°, 5 \text{ hrs. [sealed tube]}$$
$$0.1 \text{ mm.}$$

[% Yield]   36   3.3   14.3   9.7

Table IV. Structures of Identified DPF[a] Pyrolysis Products

Amides | Reductones | Furans | Lactone

HCOR

CH_3COR $CH_3-\overset{O}{\overset{\|}{C}}-\overset{OH}{\overset{\|}{C}}=\overset{H}{\overset{\|}{C}}-R$

$CH_3(CH_2)_2COR$

$CH_2OHCO\cdot R$ $CH_3-\overset{O}{\overset{\|}{C}}-\overset{R}{\overset{\|}{C}}=\overset{OH}{\overset{\|}{C}}-H$

Acid

$CH_3COOH\cdot RH$

$CH_3CHOHCO\cdot R$

$R\cdot CO\cdot R$

Where R = (structure)N-

[a] 1-Deoxy-1-piperidino-D-fructose.

Table V. Fragment Size and Odor Characteristics

Com- pound	No. of Sugar C Atoms	% of Distillate		Odor
1 + 2	2	43.0		. . .
3	1	1.6		Flowery, musty
4	2	5.1		Flowery, musty
14	1	6.5		Tangy, spearmint
15	4	1.4		. . .
16	4	25.0		Burnt sugar
17	4	3.0		Burnt sugar
5	4		Trace	. . .
6,7	6		Trace	. . .
8	6		Major	Pineapple–burnt sugar
		~ 0.1		
9	6		Trace	. . .
12	3		Major	Weakly caramel
13	2		Medium	Weakly caramel
18	6	10 (over-all)		. . .

$R_2 = C_5H_{10}$

Figure 7. Decomposition of Amadori compounds

$R_2 = C_5H_{10}$

Figure 8. Formation of six-carbon members

L-rhamnose with piperidine (Hodge *et al.*, 1963). The proposed structures of the three furan derivatives, present in trace quantities, are based on mass spectral analyses of portions of peaks from a combination GLC-mass spectrometer. Di-*N*-piperidyl carbonyl was prepared from phosgene and piperidine; α-piperidino-γ-butyrolactone, from α-bromo-γ-butyrolactone and piperidine. The four-carbon reductones were synthesized by the reactions of bromodiacetyl and piperidine (Simon *et al.*, 1965) and by hydrolyzing the product from the reaction of 2-bromo-3-ketobutyraldehyde dimethyl acetal with piperidine. Piperidino-hexose-reductone, isolated from the pyrolysis residue, was identical with an authentic sample prepared from the reaction of glucose with piperidine acetate (Hodge and Rist, 1953). The structures of all the identified
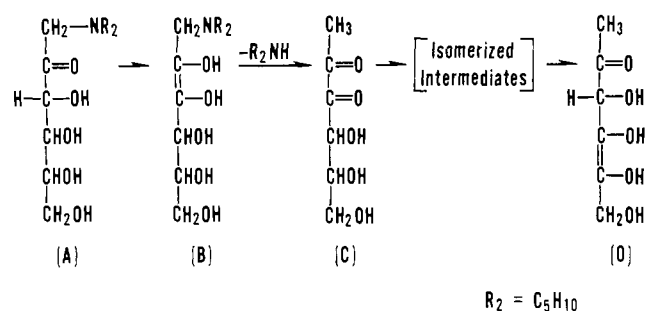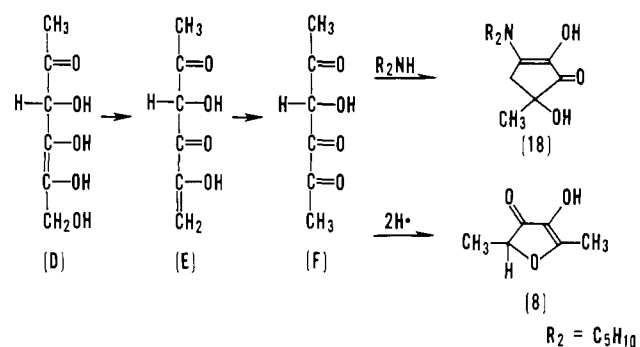
components are listed in Table IV. Yields and odors of these volatile pyrolysis products, and, more importantly, the fragment sizes, which indicate the extent of hexose scission, are given in Table V. The most abundant sugar fragments are composed of 2- and 4-carbon atoms.

When piperidino C-methyl reductone is pyrolyzed in an evacuated sealed tube, under the pyrolysis conditions used to decompose DPF, the following compounds are formed: *N*-formylpiperidine (36%), *N*-acetylpiperidine (3.3%), piperidine acetate (14.3%), and di-*N*-piperidyl carbonyl (9.7%). The route of formation for the major 1- and 2-carbon fragments is summarized in Figure 6. Hence, this aminated 4-carbon reductone (piperidino C-methyl reductone) could serve as a major source of 1-carbon fragments. The rest of the 2-carbon fragments arise from the cleavage of several possible hexose intermediates. This conclusion is substantiated by the compounds obtained from attempted aminations of pyruvaldehyde, diacetyl, and diacetylformoin. These reactive α-dicarbonyl compounds, which have been identified in other carbohydrate decomposition studies, failed to produce the amides of formic or acetic acid, as determined by GLC. In each experiment, amide formation was attempted by reaction of piperidine or piperidine acetate with the appropriate substrate in methanol and in water at various temperatures (0°, 25°, 50°, and 100° C.) and in oxygen or nitrogen atmospheres.

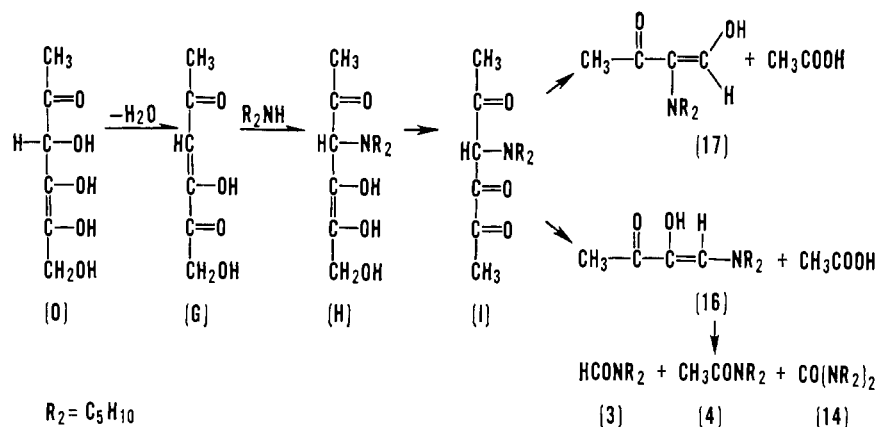From the products isolated in the DPF and the piperidino C-methyl reductone pyrolyses and from the lack of amide for-

$$
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C}=\text{O} \\
| \\
\text{H}-\text{C}-\text{OH} \\
| \\
\text{C}-\text{OH} \\
\| \\
\text{C}-\text{OH} \\
| \\
\text{CH}_2\text{OH} \\
(0)
\end{array}
\xrightarrow{-\text{H}_2\text{O}}
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C}=\text{O} \\
| \\
\text{HC} \\
\| \\
\text{C}-\text{OH} \\
| \\
\text{C}=\text{O} \\
| \\
\text{CH}_2\text{OH} \\
(G)
\end{array}
\xrightarrow{\text{R}_2\text{NH}}
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C}=\text{O} \\
| \\
\text{HC}-\text{NR}_2 \\
| \\
\text{C}-\text{OH} \\
\| \\
\text{C}-\text{OH} \\
| \\
\text{CH}_2\text{OH} \\
(H)
\end{array}
\rightarrow
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C}=\text{O} \\
| \\
\text{HC}-\text{NR}_2 \\
| \\
\text{C}=\text{O} \\
| \\
\text{C}=\text{O} \\
| \\
\text{CH}_3 \\
(I)
\end{array}
$$

From (I):

$$\text{CH}_3-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{C}=\text{C}\overset{/\text{OH}}{\underset{\backslash \text{H}}{}} + \text{CH}_3\text{COOH} \qquad (17)$$

with NR_2

$$\text{CH}_3-\overset{\text{O}}{\overset{\|}{\text{C}}}-\overset{\text{OH}}{\overset{|}{\text{C}}}=\overset{\text{H}}{\overset{|}{\text{C}}}-\text{NR}_2 + \text{CH}_3\text{COOH} \qquad (16)$$

$$\downarrow$$

$$\text{HCONR}_2 + \text{CH}_3\text{CONR}_2 + \text{CO(NR}_2)_2$$
$$(3) \qquad\qquad (4) \qquad\qquad (14)$$

$\text{R}_2 = \text{C}_5\text{H}_{10}$

Figure 9. Formation of scission products

mation with the reactive α-dicarbonyl compounds, a scheme involving aminated hexose moieties must be postulated to account for the major products that were isolated. The rearranged ketonic hexose moiety reacts with the liberated piperidine, undergoes scission, and yields four- and two-carbon fragments.

Figure 7 illustrates the early steps in decomposition of the Amadori compound A (DPF) to produce B; compound B then undergoes allylic elimination of piperidine to produce C (Hodge, 1967). The dicarbonyl compound C can undergo various enolizations and ketonizations to yield numerous isomerized intermediates. One of these intermediates, compound D, could then account for several isolated pyrolysis products (Figure 8). In this scheme, D undergoes allylic hydroxyl elimination from carbon atom 6 to yield E; compound E ketonizes to produce F, diacetylformoin. Compound F then reacts with piperidine to form compound 18 (Table III), piperidino-hexose-reductone, which was isolated from the pyrolysis residue. Simon and Heubach (1965) showed that the reaction of diacetylformoin and piperidine acetate produces this aminated hexose-reductone in 10% yield. This suggested path must be significant, since compound 18 is formed in a high yield, relative to the other identified products, during the pyrolysis.

The production of 4-hydroxy-2,5-dimethyl-3(2H)-furanone, compound 8 (Table III), probably occurs from the reduction of compound F. Hofmann and Eugster (1966) demonstrated that the reduction of F with hydrogen and 30% palladium on charcoal produced compound 8 in 10% yield. Hence, route F to 8 is indicated and may account for the appearance of 8 in several other sugar-amine reactions even though a six-deoxy sugar—e.g., rhamnose or fucose—is not present. Figure 9 indicates a pathway for the formation of the major scission products. Compound D undergoes allylic loss of a hydroxyl group at carbon atom 3 to form the α,β-unsaturated intermediate G. Piperidine adds 1,4 to this system to produce H. Compound H then loses a unit of water to form I, an aminated diacetylformoin. Compound I decomposes by two possible paths; the first yields 17 and the second, 16 or piperidino C-methyl reductone which decomposes to 3, 4, and 14.

The existence of intermediate I is suggested from the lack of success encountered in attempted preparations of 16 by condensation of C-methyl reductone with piperidine or piperidine acetate. Other experiments showed that the reaction of F, diacetylformoin, with piperidine or piperidine acetate failed to produce the isolated 4-carbon reductones 16 and 17. Hence, decomposition of an amino intermediate, such as I, is preferred rather than a fission by dealdolization followed by amination.

CONCLUSIONS

Low-temperature pyrolysis of the strongly basic Amadori compound, DPF, produces compounds that possess important caramel odors. Scission of the fructose moiety produces 4- and 2-carbon fragments. This scission occurs after amination between C-2 and C-3 and to a far lesser extent between C-4 and C-5. Finally, several pathways are suggested for the formation of the products isolated in this pyrolysis study.

Future studies on the pyrolyses of Amadori compounds which contain an amino acid moiety should lead to a better understanding of the chemistry involved in the production of flavors and aromas in processed foods.

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