DEHYDRATED FOODS
Chemistry of Browning Reactions in Model Systems

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Many different types of organic reactions lead to the production of brown pigments at moderate temperatures. In spite of many reviews of the subject, there has been no comprehensive organization of the reactions. In this review some relationships are shown to exist among the carbonyl-amino, the nonamino, and the oxidative types of browning. Recent findings have provided the basis for an integration of the several isolated partial theories of browning (Maillard, sugar fission, ascorbic acid, furaldehyde) heretofore proposed. The significance of the occurrence of the Amadori rearrangement in the Maillard reaction is stressed, and a mechanism for browning in sugar-amine systems based upon the rearrangement is outlined. Attention is directed to the little-studied but important role of dehydrogenated reductones in both enzymatic and nonenzymatic browning reactions. Investigations of browning reactions in model systems during the past 3 years are reviewed, with the pertinent older studies, and the results of most of these are shown to fit into the proposed scheme of reactions. A classified directory to the major part of 201 references on browning in nitrogenous model systems (1940 to March 1953) is included.

THE CHEMISTRY OF BROWNING REACTIONS in nitrogenous model systems has been adequately reviewed to the year 1950 by Danehy and Pigman (*33*). However, some important findings in model system studies since 1950 are interrelated.

More recent reviews of browning reactions (*9, 30, 96, 97, 98, 107, 113, 131, 136, 137, 138, 156, 158, 177, 183, 201*) have, in general, emphasized the complexity of the subject and the lack of specific knowledge on the chemical reactions and intermediates involved. The complexity of browning reactions has recently been demonstrated (in a superficial way), for chromatographic investigations have shown the presence of at least 15 components in a glucose–aqueous ammonia system (*78*) and 24 or more definite compounds in a glucose-glycine reaction mixture (*28;* cf. *70, 139*). However, along with such perplexing disclosures have come revealing demonstrations, through the characterization of a few isolated compounds, that certain definite types of reactions are involved. Because these scattered disclosures of reaction types are capable of orderly arrangement, a perspective view of the entire group of chemical reactions known as browning can now be drawn. As many gaps in our present knowledge are indicated, the correlation should be of value in programming future studies.

Model Systems Important For Browning Research

Three broad types of browning reactions are recognized in food technology. The most common type, carbonyl-amino reactions, includes the reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, and proteins. Another type, called caramelization, occurs when polyhydroxycarbonyl compounds (sugars, polyhydroxycarboxylic acids) are heated to relatively high temperatures in the absence of amino compounds. This type of browning characteristically requires more energy to get started than the carbonyl-amino reactions, other conditions being equal. Both acids and bases are known to catalyze caramelization reactions, but little more than this is known. The chemistry of caramelization is astonishingly underdeveloped (see reviews *117, 201*). Neither carbonyl-amino nor caramelization reactions are dependent upon the presence of oxygen to produce browning. A third broad type of browning frequently encountered by the food processor is the group of oxidative reactions which, for example, convert ascorbic acid and polyphenols into di- or polycarbonyl compounds. These oxidations may or may not be enzyme-catalyzed. A comprehensive review of the enzyme-catalyzed oxidative browning reactions has been published by Joslyn and Ponting (*82*).

Browning, of whatever type, is caused by the formation of unsaturated, colored polymers of varying composition (*33, 39, 41, 45, 46, 82, 96, 180, 197*). Compounds that engender browning usually contain a carbonyl or potential carbonyl grouping. [Pyrroles are an exception, but their participation in food browning has not been adequately demonstrated (cf. *79*).] Polyhydroxy compounds (*87, 168, 196*) and sugars in which the carbonyl function is blocked (*116, 167, 170, 196*) do not give rise to browning. Moreover, the extraction of a browning system with a solvent that removes, *inter alia*, carbonyl compounds will retard or eliminate browning (*69, 180*). Browning can also be inhibited by the addition of reagents that will combine with or eliminate carbonyl groups. A discussion of browning inhibitors comes later—the point to be made here is that browning can be regarded as stemming from carbonyl compounds. With this viewpoint, the relationships among the carbonyl-amino, the nonamino, and the oxidative types of browning reactions are more readily apparent.

The naturally occurring compounds from which browning originates usually contain, not only one, but a multiplicity of potential carbonyl groups. Examples of such compounds are catechols and

the vicinal polyphenols, ascorbic acid, and the reducing sugars. Catechols are easily oxidized to quinones. Likewise, ascorbic acid and other reductones [the term "reductone" is used in its general sense (*53*) throughout this paper] even more readily yield vicinal tri-, α-di-, or conjugated dicarbonyl compounds. Since these oxidations may be auto- (*81*) or enzyme-catalyzed (*82*) or reagent-induced, it is apparent that no real difference need exist between enzymatic and nonenzymatic browning after the initial oxidations. Sugars are also examples of naturally occurring compounds that can yield a multiplicity of carbonyl groups. Reducing sugars are transformed (by dehydration and/or fission) to enediols and osones, and to reductones and dehydro reductones, which provide α-dicarbonyl or conjugated dicarbonyl groups and color.

Some comparatively simple organic compounds will undergo polymerization and browning at ordinary temperatures, even when they are prepared in a state of high purity. Especially important in this class are the α,β-unsaturated aldehydes—e.g., furaldehydes—and the α-dicarbonyl compounds—e.g., pyruvaldehyde, diacetyl, and reductones in the dehydro form. Apparently the presence of oxygen or oxidation products is required for the browning of these types of compounds in the pure state (*34, 92, 162*). In the presence of amino compounds, the browning is even more rapid and intense (*36, 144, 153, 168, 170, 199*). The interaction of reducing sugars and amines readily produces such spontaneously reactive compounds as those named above.

It can be concluded that the most important model systems for studies of browning reactions are those in which α-hydroxycarbonyl (or α-aminocarbonyl) compounds are transformed into unsaturated colored polymers.

Mechanism of Browning in Sugar-Amine Systems

Nearly all of the research on browning reactions in model systems published since 1940 has involved reactions of carbonyl with amino compounds. Classified references to these studies (through March 1953) are given in Table I.

The literature on sugar-amine browning reactions in model systems reveals seven different types of reactions which are known to occur during browning. These type reactions (lettered A through G) may be classified for discussion purposes according to the three stages of development:

I. Initial stage (colorless; no absorption in near-ultraviolet)
   - A. Augar-amine condensation
   - B. Amadori rearrangement

II. Intermediate stage (colorless or yellow, with strong absorption in near-ultraviolet)
   - C. Sugar dehydration
   - D. Sugar fragmentation
   - E. Amino acid degradation

III. Final stage (highly colored)
   - F. Aldol condensation
   - G. Aldehyde-amine polymerization; formation of heterocyclic nitrogen compounds

As reaction types B, C, D, E, F, and G can follow spontaneously from A, a reaction scheme that unifies the individual reaction types can be formulated. The interrelationships of these reactions are outlined in Figure 1, which shows how the partial mechanisms for browning—i.e., the furfural, sugar-fission, and ascorbic acid (reductone) theories discussed by Stadtman (*177*)— can be considered as part of the total sugar-amine condensation (Maillard) theory. All the reactions shown in Figure 1 are known to occur in model browning systems, but the extent to which each represents the browning processes of natural products is unknown.

In this review the types of reactions which have been found to occur in model systems are discussed and correlated according to the scheme of reactions presented in Figure 1 (lettered paragraphs of the text correspond to the lettered reactions in Figure 1). An attempt has been made to include all published work since the time of Danehy and Pigman's review (*33*) and through March 1953. Only such older work as is pertinent to the discussion is mentioned.

Initial Stage of Sugar-Amine Reaction

A. Sugar-Amine Condensation

Stadtman (*177*) and Danehy and Pigman (*33*) suggested that the term "Maillard reaction" be reserved for browning reactions involving the interaction of reducing sugars and amino compounds. It has not been clear, however, to what extent sugar-amine condensation is involved in the Maillard reaction—for example, some authors have pointed to the catalytic effects of amino groups on reducing sugar transformations without apparent sugar-amine condensation. This seeming divergence of views can now be explained. The evidence indicates that sugar-amine condensation is the first step in the so-called catalysis of amino compounds. After condensation and rearrangement, the sugar moiety is dehydrated and easily polymerizable and unsaturated compounds are formed in which the amine moiety is labile. Eventually, the amine is split off (in part) from the dehydrated sugar residue to give the effect of a true catalysis.

It has been shown (*16, 36, 174*) that sugars and amines undergo browning in aqueous solution in proportion to the basic strength of the amines employed. For example, the strongly basic D-glucosyl-piperidine (0.2 *M*) in aqueous solution with glycine (2.0 *M*) at 25° C. (pH 8.4) undergoes browning at approximately the same rate as does D-glucose (0.2 *M*) in an aqueous solution of sodium hydroxide at the same pH (*76*). Also, the ultraviolet absorption spectra of heated dilute aqueous solutions of D-glucosyl-*n*-butylamine correspond closely to that of D-glucose in sodium hydroxide (*27*). Nevertheless, one should not conclude, at least in the case of the less strongly basic glycosylamines or of more concentrated solutions, that browning is the result of only an alkaline sugar degradation (*16*, cf. *27*). Generally, *N*-substituted glycosylamines remain undissociated in aqueous solutions to appreciable extents (*27, 122, 148*); hence, the irreversible transformations that the undissociated molecules undergo must be considered. The end results in the two cases (glycosylamine *vs.* glycose decomposition) should differ, but the exact nature of the alleged differences is not known.

Recent researches have established that sugars do condense with amino acids in aqueous solutions. As the solutions are concentrated, as in food dehydration, the reversible sugar-amine condensation (Reaction A) goes toward completion (*70, 101, 119*). In the final concentrated solution (or dehydrated food) there are *N*-glycosyl derivatives of amines, amino acids, and proteins.

In a kinetic study of sugar–amino acid condensation, Haugaard, Tumerman, and Silvestri (*73*) used a novel solubility technique. They measured the rate of solution of amino acids from the solid phase of a saturated aqueous solution containing an aldose (pH 9). The initial reaction was reversible, and the amino acid combined with the aldose in a 1 to 1 ratio. Later, irreversible decomposition of the sugar-amine condensation product was indicated.

In another kinetic study, Katchalsky and Sharon (*85*) added alkali continuously to the aqueous solutions of aldoses and amino acids to keep the pH of the reaction mixture constant as the sugar-amine condensation reactions occurred. Their proposed mechanism involved dual catalysis by both hydrogen and hydroxyl ions. The rate of the primary reaction increased with the basicity of the amino component; the velocity of the reaction decreased with increase of pH; and the extent of the reaction decreased with decreasing pH. The rate constants for the primary reaction of glycine with the various aldoses showed that the ease of opening of the pyranose ring (percentage of aldehyde form present) was a contributing factor. In agreement with the latter conclusion are the findings of Speck (*174*) and Traitteur (*183*) that color formation in aqueous reducing sugar–amino acid mixtures is directly proportional to the percentage of aldehyde form of the reducing sugar. Katchalsky used the same method (keeping the pH constant) in an earlier study with Frankel (*57*) and could find

Table I. Studies Pertaining to Browning in Nitrogenous Model Systems

(Published since 1940)

N-Substituted glycosylamines	(*4, 15, 16, 27, 36, 63–65, 74, 75, 76, 122, 125, 132, 148, 149*)
Amadori rearrangement products	(*4, 15, 63–65, 76, 122, 172*)
Ammonia and ammonium salts	
Reactions with	
Hexoses	(*14, 36, 47, 78, 151, 169, 183, 186*)
Aldehydes	(*141, 142, 153*)
Amines, amine salts, and imines	
Reactions with	
Hexoses	(*16, 27, 36, 48, 122, 151, 174, 183, 186*)
Pentoses	(*174, 180*)
Trioses	(*4, 16, 171, 172*)
Biose	(*16, 26*)
Aldehydes	
Fatty	(*35, 126, 176, 184*)
Unsaturated	(*55, 193*)
Ketones and ketols	(*8, 26, 171*)
Reductones	(*29, 53, 189*)
Dehydro reductones	(*51, 72*)
Amino acids	
Reactions with	
Hexoses	
Aldoses	(*1, 9, 21, 28, 31, 32, 36, 47, 58, 59, 63–67, 70, 71, 73, 76, 77, 84, 85, 89, 91, 94, 96, 98, 107, 109, 110, 114, 115, 118, 119, 132, 133, 135, 140–143, 145, 168–170, 174, 178, 181, 183, 185, 186, 195–197, 199*)
Ketoses	(*1, 32, 77, 89, 133, 145, 168, 185, 186*)
Pentoses	(*9, 21, 36, 77, 79, 96, 145, 174, 179, 183, 195, 197*)
Trioses	(*16, 36, 65, 67, 174*)
Biose	(*16*)
Hexose phosphates	(*36, 167*)
Deoxy sugars	(*77, 79, 80*)
Methylated sugars	(*174, 196*)
Disaccharides	(*36, 67, 83, 89, 127*)
Oligosaccharides (other than di-)	(*36, 194*)
Glyconic and glycaric acids	(*168*)
Uronic acids	(*36, 86, 168, 183*)
Keto acids	(*36, 67, 145, 164*)
Aldehydes	
Fatty	(*24, 40, 67, 126, 143*)
Keto	(*1, 36, 39, 40, 44, 45, 164, 174*)
Unsaturated	(*36, 40, 67, 153, 179*)
Ketones and ketols	(*36, 79, 143, 164, 171*)
Reductones	(*6, 29, 31, 32, 53, 76, 88, 140, 144, 145*)
Dehydro reductones	(*88, 140, 144, 164*)
Peptides and proteins	
Reactions with	
Hexoses	
Aldoses	(*13, 74, 84, 87, 89, 100, 98–105, 111, 124, 134, 140, 165, 166*)
Glucosamine	(*98, 105, 106*)
Ketoses	(*111, 165*)
Pentoses	(*111, 165*)
Deoxy sugars	(*98, 105*)
Methylated sugars	(*13*)
Disaccharides	(*56, 99, 165*)
Uronic acids	(*111*)
Aldehydes	(*87, 126*)
Reductones	(*20, 182*)
Amides	
Formamide, dehydroascorbic acid	(*144*)
Acetamide	(*122, 126*)
Pyrroles	(*79, 143*)
No browning (under conditions used)	
Nonamino compounds	
Hexitols	(*87, 168, 196*)
Glucose-1-phosphate	(*167*)
Methylated glucoses	(*13, 16, 73, 196*)
O-Glycosides	(*170, 196*)
Sucrose	(*9, 21, 36, 76, 87, 89, 99*)
Lactic acid	(*67*)
Maleic acid	(*186*)
Amino compounds	
Triethylamine	(*183*)
Triethanolamine	(*126, 174*)
p-Aminobenzoic acid	(*79*; cf. *67*)
N,N-Dimethylglycine	(*174, 196*)
Urea	(*186*)
Succinimide	(*186*)
Aminoanthraquinones	(*186*)

no evidence for a reaction between fructose and various amino acids in dilute aqueous solutions. However, fructose is known to condense with amines under other conditions.

Haugaard *et al.*, Katchalsky *et al.*, and Täufel and Iwainsky (*179*) were unable to use the Van Slyke or formol titration procedures to determine amino groups in kinetic studies. The use of these methods in the past has led to conflicting conclusions (*33*; cf. *21*). Täufel and Iwainsky also showed the inadequacy of copper reduction methods for the determination of reducing sugars in sugar–amino acid reactions, since strongly reducing substances are generated in the reactions. They isolated the reducing sugar chromatographically before analysis and determined amino acids by the method of Pope and Stevens (*150*), which involves the solubilization of copper from copper phosphate by the amino acid.

Hannan and Lea (*70, 71*) demonstrated 1 to 1 reaction between glucose and the free amino groups of α-*N*-acetyllysine, polylysine, and casein. Concentrated solutions of the reactants at pH 8.5 were freeze-dried and stored at 37° C., pH 6.4, at different humidities. In all three cases the glucose (determined by glucose oxidase) and the free amino groups [determined by the Van Slyke method and confirmed by Sanger's fluorodinitrobenzene reaction (*101, 166*)] disappeared in a 1 to 1 ratio in the initial colorless stage of the reaction. Galactose reacted with casein, as did glucose (*105*). Mohammad, Fraenkel-Conrat, and Olcott (*124*) showed that glucose and the free amino groups of bovine serum albumin and other proteins and peptides combined in a 1 to 1 ratio preliminary to browning.

Crystalline glucose-glycine condensation products isolated from browning reactions were reported by Patron (*137*) and Mackinney (*115*). Only small amounts of these compounds were isolated, and their structures have not been determined; both compounds gave analyses indicating a mole for mole reaction between glucose and glycine.

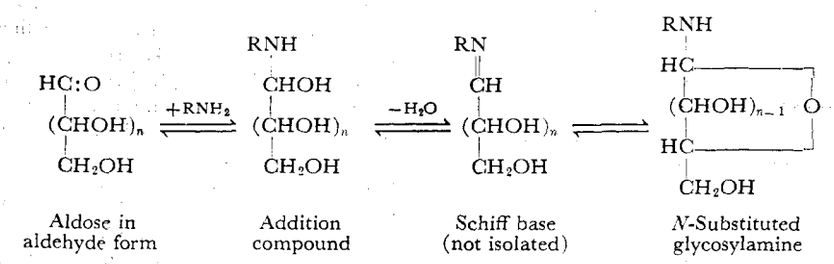
Three acidic glucose–glycine reaction products (neither crystalline nor homogeneous) were isolated in low yields by elution of the reaction mixture (concentrated aqueous solution heated at 45°C., pH 5) from an anion-exchange column by Barnes and Kennedy (*10*). Determinations of carbon, hydrogen, and molecular weight gave: (*a*) $C_8H_{17}O_8N - 1.5H_2O$, (*b*) $[C_8H_{17}O_8N - 2H_2O]_2$, and (*c*) $[C_8H_{17}O_8N - 3H_2O]_{3 \text{ or } 4}$. The monomeric product, (*a*), was nearly colorless, but it produced color on heating in water. Products (*b*) and (*c*) were more highly colored. All three condensation products were more stable on standing in air than normal *N*-substituted glycosylamines.

N-Glucosyl derivatives of the sodium salts of glycine, serine, and lysine (2 moles of glucose reacted with the two amino groups of lysine) were prepared by Micheel and Klemer (*119*) by evaporating aqueous solutions of the components to dryness. These compounds were analytically pure, in contrast to the impure calcium and barium salts reported previously by Kuzin and Polyakova (*94*).

Several chromatographic studies which demonstrate sugar-amine condensation have been reported (*28, 65, 70, 139, 179*). From the browning reactions of reducing sugars and amino acids, spots were obtained on paper chromatograms which reacted with both sugar spray reagents and ninhydrin, and also with *p*-dimethylaminobenzaldehyde in the manner of glycosylamine and amino sugar derivatives.

Chichester, Stadtman, and Mackinney (*28*) used C^{14}-labeled glucose and C^{14}-labeled glycine with unlabeled glycine and glucose, and showed that the major reaction product was derived from both glucose and glycine. Elution of this major intermediate from a cellulose column gave a crystalline compound which analyzed for carbon, hydrogen, nitrogen, and molecular weight in agreement with the calculated values for a monohydrated glucose–glycine 1 to 1 condensation product (*115*). Another 1 to 1 compound of a reducing sugar and an amino acid was isolated similarly by Hannan and Lea (*70*) after paper chromatography of a freeze-dried glucose–α-*N*-acetyllysine reaction mixture.

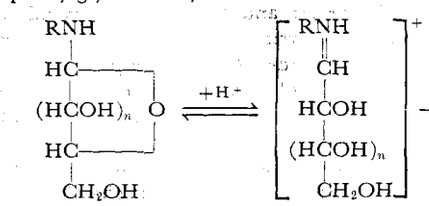
It can be concluded that reducing sugars and amines condense in an equimolar ratio in browning reactions. The initial reaction is reversible, but irreversible reactions soon follow (*70, 73, 103*). The accepted mechanism for sugar-amine condensation involves opening of the ring form of the sugar, addition of the amine to the carbonyl group, and subsequent elimination of a molecule of water to form the *N*-substituted glycosylamine (cf. *85*).



Aldose in aldehyde form | Addition compound | Schiff base (not isolated) | *N*-Substituted glycosylamine

As the *N*-glycosyl derivatives of ammonia and of many primary aliphatic amines crystallize initially with a mole of "water of hydration" (*68, 122, 125, 148*), the final step of water elimination may not actually occur in some cases.

Diglycosylamines may be formed in sugar-amine browning reactions. Smith and Anderson (*171*) have shown that, without an excess of aniline, 2 moles of β-phenylglyceraldehyde will react in the



N-substituted aldosylamine | Cation of Schiff base

cold with one mole of aniline to form the crystalline compound, $C_6H_5N(CHOH.CHOH.C_6H_5)_2$, which readily decomposes to a brown tar. Diglycosylamines are readily formed from reducing sugars and ammonia in aqueous alcohols, as has been shown on paper chromatograms by Bayly, Bourne, and Stacey (*14*) and Raacke-Fels (*151*). Finally, the researches of Mohammad, Fraenkel-Conrat, and Olcott (*124*) and of Lea and Hannan (*70, 101, 102*) revealed that, although reducing sugars and the free amino groups of proteins combine initially in a 1 to 1 ratio, the ratio increases and approaches 1.5 to 1 during the latter stages of the reaction. It is significant here that one mole of water was liberated for each mole of sugar combined with the protein (*101, 104*). As yet, diglycosylamine formation remains to be demonstrated in sugar–amino acid or sugar-protein browning systems.

B. Amadori Rearrangement

Speculations that the Amadori rearrangement of *N*-substituted glycosylamines could be involved in nonenzymatic browning reactions have appeared in the literature, starting in 1945 (*13, 15, 65, 71, 79, 103, 148*), but its occurrence was not actually demonstrated until Gottschalk (*63*) and Hodge and Rist (*75*) reported their results in 1952. Earlier, Barnes and Kennedy (*10*), Gottschalk and Partridge (*65*), and Lea and Hannan (*103*) had indicated how the rearrangement of first-formed glycosylamines would explain some of their experimental findings. The available evidence that the Amadori rearrangement is a key reaction for browning in aldose-amine, and ketose-amine systems is presented below.

Aldoses. The Amadori rearrangement (*93, 190*) is the isomerization of *N*-substituted aldosylamines to 1-amino-1-deoxy-2-ketoses. According to Weygand (*190*), the reaction is acid act-alyzed:

N-substituted aldosylamine | Cation of Schiff base | Enol form | *N*-Substituted 1-amino-1-deoxy-2-ketose, keto form

It has never been demonstrated that the Amadori rearrangement product is in equilibrium with the *N*-substituted glycosylamine in solutions. Whereas this rearrangement had been considered previously to occur only with *N*-glycosyl derivatives of primary aromatic amines, Hodge and Rist (*75, 76*) demonstrated that the rearrangement occurs also with *N*-glycosyl derivatives of both primary and secondary aralkyl and alkylamines. Furthermore, the rearrangement of *N*-substituted glycosylamines occurred spontaneously in the dry or nearly dry state at 25° C. When the C-2 hydroxyl of a crystalline *N*-substituted glycosylamine was unsubstituted, the compound was slowly transformed during storage to a dark brown tar. From the tarry mixture, the Amadori rearrangement product was isolated in 30 to 50% yields. On the other hand, if the C-2 hydroxyl was substituted (as in 2-*O*-methyl-D-glucosylpiperidine), then the derivative remained white and stable after storage for 2 years at 25° C. Under experimental conditions that caused the rapid rearrangement of D-glucosylpiperidine, 2-*O*-methyl-D-glucosylpiperidine was recovered unchanged. Thus, it was shown that blocking the Amadori rearrangement of an *N*-substituted glycosylamine blocked the browning which otherwise would have occurred.

Several other groups of workers (*16, 73, 76, 80, 196*) have used different types of model systems composed of 2-deoxy or 2-substituted aldoses and amino acids. Each group showed that the C-2 hydroxyl of an aldose is essential for the occurrence of a significant degree of browning. Only one conflicting instance has been reported: Lea and Rhodes (*105*) found that 2-deoxy-D-galactose underwent a more rapid browning reaction with casein than D-galactose, although it reacted with the free amino groups more slowly than D-galactose. There was evidence in their study (cf. *106*) that the browning of 2-deoxy-D-galactose with casein, like that of 2-deoxy-2-amino-D-glucose with casein, did not proceed mainly through carbonyl-amino condensation.

It is concluded that autoisomerization is a general reaction of *N*-substituted aldosylamines on standing in moist atmospheres, since most of them (un-

substituted at the sugar hydroxyls) show decomposition with browning to some extent on storage (*15*, *75*, *76*, *125*, *132*). *N*-Glycosyl derivatives of proteins should react similarly (cf. *103*). Although the Amadori rearrangement products are more stable than the original glycosylamines in moist, acidic atmospheres, they are still heat-labile, even in the dry state. On heating, they undergo dehydration and fission and yield colorless reductones as well as brown fluorescent substances (*76*).

The Amadori rearrangement products undergo browning decomposition alone in aqueous solutions at alkaline pH, and the rate of browning is considerably enhanced in the presence of amino acids (*76*). An alkaline pH is not necessary for browning, however; the browning reactions of 1-deoxy-1-piperidino-D-fructose with glycine ethyl ester hydrochloride at pH 6.3 (initial) were more rapid than those of D-glucosylpiperidine or of D-glucose with glycine at pH 8.4.

The recent researches of Gottschalk (*63*) with D-glucose and phenylalanine and of Hodge and Rist (*76*) with D-glucosylglycine ethyl ester showed that the Amadori rearrangement occurs also with *N*-glycosyl derivatives of amino acids. More experiments of this type are needed, however; for Gottschalk's product was a hygroscopic sirup, and Hodge and Rist's hygroscopic crystalline product was not entirely homogeneous. Nevertheless, both products analyzed for an isomer of the 1 to 1 sugar-amine condensation product and gave the characteristic reducing behavior of 1-amino-1-deoxy-2-ketoses.

A comparative tabulation of the properties of *N*-substituted glycosylamines and the corresponding Amadori rearrangement products is given in Table II. From the Amadori rearrangement product, none of the aldose is recoverable after acid hydrolysis, although about 50% of the amine moiety is released. This was the behavior of Gottschalk's rearrangement product obtained from the reaction of glucose with phenylalanine (*63*). Hannan and Lea's 1 to 1 glucose–α-*N*-acetyllysine compound also gave no glucose on acid hydrolysis (*70*). Chichester and Mackinney's crystalline glucose-glycine reaction product (described under A) gave no glucose upon acid hydrolysis and 50% of the glycine was recovered. Furthermore, on heating with dilute acetic acid, 5-hydroxymethylfurfural (HMF) was formed. It is apparent from Gottschalk's study (*63*) that hydroxymethylfurfural is much more readily formed from 1-amino-1-deoxy-D-fructose derivatives than from D-fructose itself under equivalent conditions.

Chichester and Mackinney's crystalline compound was colorless, but it darkened readily on warming. It gave a relatively large levorotation, $[\alpha]_D$ − 40°. [For comparison, the sodium salt of D-glucosylglycine in water gives $[\alpha]_D$ − 8 (10 minutes) → +20° (*119*), and D-glucosylglycine ethyl ester in ethanol gives $[\alpha]_D$ − 8 (5 minutes) → +2° (*76*, *199*).] But, most revealing was the fact that a solution of the compound in 0.1 *N* sodium hydroxide reduced dichlorophenolindophenol solution readily at room temperature (unpublished experiment performed jointly by Chichester and the author, March 12, 1953). The total physical and chemical behavior of this compound, which was isolated from a browning reaction conducted under Maillard's conditions (*28*), indicates that it is probably a monohydrate of the Amadori rearrangement product of D-glucosylglycine—namely, 1-(*N*-carboxymethyl)-amino-1-deoxy-D-fructose.

Patron (*137*) heated an aqueous solution of glucose (1 *M*) and glycine (1 *M*), adjusted to pH 4.5 with acetic acid, at 100° C. for 30 minutes. The reaction mixture exhibited strong blue fluorescence in ultraviolet light, although there was only moderate darkening of the solution at this point. The fluorescent material was separated as a silver salt by a method described by Lewis (*107*, *108*). After liberation of the compound with hydrochloric acid (in which the compound was stable), about 100 mg. of hygroscopic, yellowish white crystals were isolated. The compound in aqueous solution was colorless, acidic, and strongly fluorescent, and it reduced alkaline silver nitrate in the cold to form a silver mirror. It gave only a faintly positive Seliwanoff test; but, after reaction with nitrous acid (fleeting pale blue color), the Seliwanoff test was strongly positive, indicating the presence of hydroxymethylfurfural. Analysis gave 5.3 ± 0.2% nitrogen, the same as for Chichester and Mackinney's compound (calculated for $C_8H_{15}O_7N.H_2O$: 5.5% N). Because no further data were obtained, it is not known whether this compound was the glycosylamine or the rearrangement product; however, its stability to hydrochloric acid, its strong reducing power, and its easy conversion to hydroxymethylfurfural all indicate the rearrangement product. Rigorous proof of structure for all of the above-discussed glucose–amino acid compounds is still needed.

Smith and Anderson (*171*, *172*) studied the rearrangement of trioses. Their experiments showed that both a primary aromatic amine and acetic acid were necessary to effect the rearrangement of β-phenylglyceraldehyde to β-phenylpyruvaldehyde. Dimeric β-phenylglyceraldehyde and *N*-methylaniline (*172*), when refluxed in absolute alcohol containing a little acetic acid, apparently underwent condensation and a typical Amadori rearrangement to yield (probably): $C_6H_5N(CH_3).CH_2$-$CO.CHOH.C_6H_5$. (The carbonyl and hydroxyl groups could have formed on carbons 3 and 2, respectively.) The white crystalline product reduced Fehling solution and alkaline methylene blue at room temperature. The corresponding compound from phenylalanine ethyl ester also reduced Fehling and alkaline methylene blue solutions and decomposed with browning after standing only 1 hour at room temperature.

Ketoses. Although it is now established that the Amadori rearrangement occurs after aldose-amine condensation in model systems which undergo browning, very little is known about the first reactions by which ketoses undergo browning. Maillard (*116*) reported that fructose browns with amino acids at a rate somewhat faster than glucose. This finding has been corroborated under various conditions by several others (*36*, *145*, *168*, *174*, *183*, *186*). However, with casein, fructose browns at a very slow rate, even more slowly than the disaccharides, maltose, and lactose (*110*).

Ketoses condense with aliphatic amines in aqueous alcoholic media more readily than aldoses, according to the recent communication of Erickson (*48*). Conversely, aldoses condense with aromatic amines more readily than do ketoses, according to Barry and Honeyman (*12*).

Table II. Properties of *N*-Substituted Glycosylamines and Corresponding Amadori Rearrangement Products

	N-Substituted Glycosylamines	*1-Amino-1-deoxy-2-ketoses*
Reducing power		
In dilute NaOH at 25° C.		
o-Dinitrobenzene	Purple after 1 hour	Purple after 1 minute
Methylene blue	Very slow decolorization	Rapid decolorization
Dichlorophenolindophenol	Very slow decolorization	Rapid decolorization
Fehling solution		
At 25° C.	Little or no reduction	Reduction
At 100° C.	Reduction	Rapid reduction
Tollens reagent, 25° C.	Little or no reduction	Reduction
Browning with amino acids at 25° C.	Slow	Rapid
Ninhydrin reagent with heating	Color	Color[a]
Products of acid hydrolysis (*63*)	Glucose, amine, little HMF	Glucose, some amine, much HMF

[a] Reported to be produced more rapidly with 1-deoxy-1-piperidinofructose than with D-glucosylpiperidine (*76*).

In Erickson's experiments more than one mole of octadecylamine apparently condensed with fructose or sorbose, as though multiple Amadori-type rearrangements had occurred along the ketose chain. Raacke-Fels (*151*) also had some indications from spot reactions on paper chromatograms that fructose combined with ethylamine and propylamine in aqueous alcoholic solutions.

From the reactions of amines and amino acids with model ketoses (such as acetol, hydroxyacetophenone, α,β-dihydroxypropiophenone, dihydroxyacetone, phenyldihydroxyacetone, acetoin, benzoin, and propionoin), as reported by Hurd (*79*), Smith and Anderson (*171*), and Carson (*26*), it appears that an Amadori-type rearrangement is to be expected for *N*-substituted ketosylamines. The similarities of these reactions with the Amadori rearrangement are pointed out by Anderson (*4*). The necessity for oxidation in the formation of a dicyclohexylimine derivative of diacetyl from acetoin and cyclohexylamine, as reported by Carson (*26*), is an important point for consideration in future studies of the browning reactions of ketoses.

According to Hurd (*79*), the model ketoses, acetoin and acetol, undergo rapid browning with alanine; furthermore, 2,4-dimethylpyrrole (which could be formed from acetol and alanine) undergoes rapid browning with acetol. Acetol and alanine formed 2,5-dimethylpyrazine also, but the pyrazine derivative did not brown rapidly with acetol. Benzoin reacted with alanine to yield both tetraphenylpyrrole and tetraphenylpyrazine. In view of the likelihood of Amadori-type rearrangements with both aldoses and ketoses in browning reactions, pyrrole and pyrazine derivatives should be sought in sugar-amine reactions and their role in the browning established. Both fructosamine (1-amino-1-deoxyfructose) and glucosamine (2-amino-2-deoxyglucose) have long been known to undergo self-condensation to yield substituted pyrazines. Because of the uncertainty that exists, the formation of heterocyclic nitrogen compounds in sugar-amine browning via the Amadori rearrangement has not been included in Figure 1.

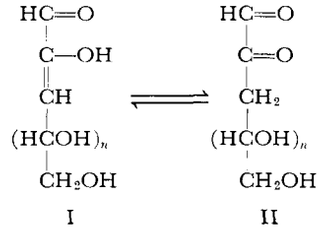
Intermediate Stage

The initial stage of browning (Reactions A and B) cannot be detected by spectrophotometric measurements in the ultraviolet; however, before visible browning has begun, a strong absorption appears to announce the beginning of the intermediate stage. The initial strong absorption occurs at 2770 to 2850 A. (the furfural region) for the reaction of reducing sugars with most amino acids (*28*, *59*, *70*, *170*, *199*). The conjugated unsaturation indicated by the spectra could develop either by sugar dehydration alone or by sugar fission and dehydration of the (recombined) fragments. A third reaction, the Strecker degradation of α-amino acids, is included in the intermediate stage, although it follows fission and/or dehydration or dehydrogenation of the sugar residue.

C. Dehydration Of Sugar Moiety

Two types of sugar dehydration reactions are now known. In acidic systems, furfurals are formed. In the presence of amines in nearly anhydrous systems, 6-carbon and other reductones are formed. This reductone-producing reaction may provide an important heretofore missing link in the browning mechanism.

Furfural Formation. The accelerating effect of glycine, aspartic acid, and arginine (also ammonium chloride, sodium pyruvate, diacetyl, morpholine, and hydrogen peroxide) on the color-producing reactions in aqueous solutions of furfural was demonstrated by Rice, Kertesz, and Stotz (*153*). Wolfrom and coworkers (*179*, *199*) showed that glycine accelerates the production of furfural and color from D-xylose, and of hydroxymethylfurfural and color from D-glucose, in dilute aqueous solutions. They followed the reactions by means of ultraviolet absorption spectra and proposed a mechanism for the dehydration based upon the spectra. The mechanism involved the initial loss of one mole of water with production of the α-enolic-α,β-unsaturated aldehyde (I), which would exist in equilibrium with the 3-deoxyosone (II).



I II

The argument for a mechanism involving 1,2-enolization, rather than dehydration, as the first step was given by Newth (*128*), and a correlation of the enolization and dehydration mechanisms was presented more recently by Petuely (*146*). The role of furfurals in browning has been discussed by Stadtman (*177*) and Danehy and Pigman (*33*).

The formation of hydroxymethylfurfural in glucose-glycine browning reactions under Maillard's conditions was demonstrated chromatographically by Chichester, Stadtman, and Mackinney (*28*). Compound 7 on their illustrated chromatogram has since been found by them to be isomeric with hydroxymethylfurfural (*28*). A crystalline isomer of hydroxymethylfurfural identified as 2-(hydroxyacetyl)furan, has been isolated recently by Miller and Cantor (*121*); but whether or not this compound is formed in browning reactions is still unknown.

Täufel and Iwainsky (*181*) heated a neutral solution of glucose (20%) for 37 hours at 96° C. without detectable change when developed on a paper chromatogram. After 42 hours of heating a spot appeared which corresponded in R_f and color reactions to hydroxymethylfurfural, and the spot increased in size and intensity on further heating. In the presence of amino acids, the spot hydroxymethylfurfural appeared after only 14 hours of heating. Thereby, the accelerating effect of amino acids on sugar dehydration was again demonstrated.

The results of Gottschalk (*63*) explain the "catalysis" of furfural formation by amines. When fructose was heated at 100° C. in 2 *N* acetic acid for 2 hours, no hydroxymethylfurfural was detectable chromatographically. A considerable amount of hydroxymethylfurfural was obtained under these conditions, however, either from the D-glucose–phenylalanine Amadori rearrangement product or from 1-deoxy-1-*p*-toluino-D-fructose. D-Glucosyl-*p*-toluidine was hydrolyzed under these conditions to D-glucose and *p*-toluidine. The explanation for the liberation of amine, but not sugar, by the hydrolysis of Amadori rearrangement products is now evident. The amine is hydrolyzed from the Schiff base of hydroxymethylfurfural after the dehydration of the sugar moeity. The deoxyaminoketose per se is not hydrolyzed. The catalysis of furfural formation and resultant browning as first outlined by Gottschalk and Partridge (*65; cf. 63*) has been incorporated in Figure 1. After condensation of the sugar and the amine (Reaction A) and rearrangement to the easily dehydrated deoxyaminoketose or tautomeric 1,2-enolic structure (Reaction B), the loss of 3 molecules of water (Reaction C) occurs readily (acid catalyzed) to form the Schiff base of the furfural. The amine is subsequently liberated from the Schiff base (but only in part) by hydrolysis. The fraction of the amine not liberated is combined in the melanoidins (humin substances) which accompany the reactions (*63*).

Reductone Formation. Hodge and Rist (*76*) have shown that when D-glucose and piperidine are heated together at 70° to 80° C. in the presence of malonic acid and an excess of piperidine, the Amadori rearrangement occurs first; then, on longer heating, the sugar chain is dehydrated with the loss of 2 molecules of water. The crystalline product, $C_{11}H_{17}O_3N$ (isolated in 20% yield), had the properties of a true reductone (*53*, *147*). The reductone (considered to be $C_6H_8O_4$) retained the piperidyl radical, and the reductone-piperidine compound,

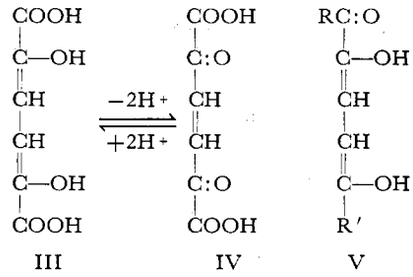
$C_{11}H_{17}O_3N$, was neutral in aqueous solution (internal neutralization of the acidic enediol grouping by the amine radical). Glucosylpiperidine and galactosylpiperidine gave the same optically inactive reductone. Galactosylpiperidine gave this compound (12%) after it had stood at 25° C. for 2 years in the "dry" state. An aqueous solution of the piperidyl-reductone turned brown of itself in air at pH 6. In the presence of amino acids, a more rapid browning reaction occurred.

The same piperidyl-reductone was obtained from the brown residue formed by heating glucosylpiperidine, galactosylpiperidine, or 1-deoxy-1-piperidinofructose in the dry state at 110° C. (cf. *125*). When the heating was done in a vacuum of 0.3 mm., a sirupy distillate (ca. 15%) which possessed reductone properties was also formed. The distillate was water-soluble initially, but underwent very rapid browning on standing in air and finally formed a dark water-insoluble polymer.

The *N*-glucosyl derivative of a primary amine (monoethanolamine) also was spontaneously dehydrated on standing to produce a reductone (*76*). However, in this case, the product was amorphous and melanoidinlike, and may have been slowly polymerized. The *N*-hydrogen atom in the glucosylethanolamine probably allowed further condensations, which could not have occurred in the case of the tertiary glycosylpiperidines.

It is now apparent that the sugar in sugar-amine browning reactions can be dehydrated in two ways. In neutral or acidic aqueous systems, furfurals are formed. In the dry state, or in nonaqueous solvents in the presence of amines, reductones are formed. It is possible that the reductones possess the structure of furfurals without furan ring closure. Whether or not the 6-carbon reductone is formed without fission, or by fission and recombination of the fragments, is not known.

Petuely and Künssberg (*147*) showed by polarographic analysis that α,α'-dihydroxymuconic acid (III) is a true reductone. It is a conjugated dienediol, rather than an enediol-(α); nevertheless, the two enolic hydroxyls are readily dehydrogenated to give the dehydro form (IV). Furfurals, by hydrolytic scission of the ring, could yield the conjugated dienediolic structure (V; R = H; R′ = H, CH_2OH, or CH_3). 2-Hydroxyacetylfuran (*121*) also would yield V (R = CH_2OH, R′ = H) which, by analogy, probably is also a reductone (cf. *147*). As a matter of fact, furfurals do produce reductonelike reducing power upon acid or alkaline hydrolysis (*147*) although the structures of the reductone, formed are not known. A ring opening of furfural, such as shown in V, is known to occur with aromatic amine hydrochlorides in the Stenhouse reaction (*55*, *193*). Open-chain dienediolic intermediates obviously may form upon sugar dehydration and never undergo the ring closure to furan derivatives but polymerize instead to form melanoidins.

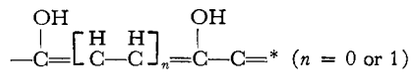


III IV V

The recent communication of Wolfrom, Schlicht, Langer, and Rooney (*197*) cites results indicating that the repeating units in the water-soluble, nondialyzable aldose-glycine melanoidins of their study approach the sugar dehydration stage of a furfural-glycine condensation product. However, the results "do not require that the furan rings are necessarily present in their intact cyclized forms." Under their conditions sugar dehydration (whether to furfurals or to open-chain reductones) apparently is the dominant reaction in the formation of sugar-amine melanoidins. However, sugar fission and recombination of the fragments to 6-carbon dehydrated residues are not ruled out by their study.

Browning Reactions of Reductones. The development of reductonelike reducing power is a well-known characteristic of sugar-amine browning reactions (*22*, *40*, *42*, *103*, *179*, *187*). Furthermore, the brown polymers which form are known to possess strong reducing power and a redox system similar to those of reductones (*40*, *42*, *195*). In spite of these facts, the structures of the reductones formed during sugar degradation are not known with certainty, and in only a few model systems has the browning of reductones with amines been studied (Table I).

Ascorbic acid is the best known reductone, and most of the browning studies have involved this single enediol. It is probable that all reductones will be found to undergo similar browning reactions, as they all have the same type of conjugated unsaturation shown in the following general formula:



The unsaturation shown at the asterisk is frequently, but not always, that of a carbonyl group (cf. *53*); conjugated unsaturation is necessary for stability of the reductone. It is not the reductone per se, but the dehydro form of the reductone, that is the prime source of browning. The browning of reductones therefore belongs to the class of oxidative browning reactions. This is not to say that carbonyl-amino reactions are excluded; on the contrary, the dehydro reductones, like the furfurals, brown more rapidly in the presence of amino compounds than alone.

Seaver and Kertesz (*168*) reported that L-ascorbic acid undergoes browning with glycine (90° C., pH 4.3) at a faster rate than the common sugars; however, no attempt was made to exclude air in their experiments. Patron (*137*) found that it is the dehydro form of ascorbic acid that undergoes the browning reactions. When oxygen was excluded from solutions of L-ascorbic acid and glycine, there was no browning; dehydroascorbic acid and glycine readily produced browning. Another reductone, dihydroxymaleic acid (which is more easily oxidized than ascorbic acid), underwent browning very easily with glycine or amines, and this reaction was accelerated in acetic acid–buffered solutions at pH 4.0.

Pecherer (*144*) showed that dehydroascorbic acid (from the crystalline methanol complex) gave a pink color with glycine or α-alanine (and other amino acids, some not α) when refluxed in water or in alcohol (cf. *20*, *88*, *140*); the pink color deepened to cherry red within 1 minute and became dark brown after 50 minutes. Evaporation of the solvent gave an intractable brown, amorphous material.

Proteins readily form complexes with copper, according to Thompson, Kocher, and Fritzsche (*182*), and the copper-proteins undergo a browning reaction with reductones at 30° C., pH 6.5 to 7. The amorphous, fluorescent red-brown product from the reaction of copper-casein with ascorbic acid contained no reduced reductone groupings (indophenol titration) but did contain the dehydro form of a reductone (dinitrophenylhydrazine reaction). There was no browning if any one of the three components (copper, casein, or ascorbic acid) was omitted. Other reductones (reductic acid, catechol, hydroquinone) reacted as rapidly as ascorbic acid with the copper-casein complex. Furthermore, furfural and furfuryl alcohol, which (in the open-chain form) could form dehydro reductones, also showed a positive but weaker browning reaction.

In a series of model systems representing the constituents of orange juice, Curl (*31*, *32*) measured the browning and carbon dioxide evolution of various synthetic combinations of ascorbic acid, sugars (glucose, fructose, sucrose), and amino acids (alanine, asparagine, arginine). The citrate-buffered solutions were sealed in tin cans and stored at 49° C., pH 3.75. The color density increased only slightly in 60 days for ascorbic acid alone, for the mixture of sugars alone, and for ascorbic acid plus the mixture of amino acids. Appreciable browning was obtained, however,

in the solution containing sugars and amino acids. The carbon dioxide production of the sugar–ascorbic acid systems (*31*) was greatly increased by the addition of copper, iron, tin, or mercury salts. Mixtures containing sugars, ascorbic acid, and amino acids yielded about the same amount of carbon dioxide as concentrated orange juice.

Acceleration of the decomposition of L-ascorbic acid to carbon dioxide in the presence of amino acids was reported by Ashikaga (*6*). In the light of present knowledge, the carbon dioxide could have come from the carboxyl group of the amino acid by way of the Strecker degradation (*144*, *163*) as well as from the carboxyl group of α,β-diketo-L-gulonic acid. The latter compound is produced by opening of the lactone ring of dehydroascorbic acid prior to decarboxylation (*50–52*). Both decarboxylation mechanisms would require preliminary dehydrogenation of the reductone.

Most of the studies on the browning reactions of ascorbic acid have involved a natural product (cf. *177*). According to both Krijt (*90*) and Miller (*120*), reductones other than ascorbic acid are not present in fresh fruits and vegetables, but, after storage, all dehydrated and processed foods contain increasing amounts of the other reductones with increasing storage time and temperature (cf. *40*, *42*). Baraud (*7*) recently reported that an unknown substance, which reacts with *p*-sulfophenylhydrazine in the manner of dehydrogenated reductones, is formed from glucose in various wines. The unknown dicarbonyl compound was not derived from triose-reductone, dihydroxymaleic acid, or ascorbic acid. The same reductone-like substance was formed in borate-buffered glucose solutions which were heated on a boiling water bath. Greatly increasing amounts of the substance were obtained with increasing increments of pH in the range 5.6 to 7.8. Wendland (*187*) showed that reductones were produced from buffered glucose solutions by heating on a boiling water bath (pH 6.9 and up) or on long standing at room temperature. The addition of glycine or protein catalyzed the reaction.

It is apparent that more intensive study of reductone formation from the sugars and the manner in which dehydrogenated reductones form brown pigments would throw much needed light upon both enzymatic and non-enzymatic browning processes.

As for the strong browning reactions given by uronic acids and pectic substances (*36*, *86*, *111*, *168*), it should be remembered that uronic acids are decomposed on heating in alkaline solution to yield indophenol-reducing substances (reductones) (*52*, *86*). In strong acids, furfural and reductic acid are formed (*86*). Recently, Euler and Hasselquist (*52*) have shown that glucurone (a lactone of glucuronic acid), when dissolved in 2 *N* sodium hydroxide at 90° C., is completely converted, and one or more reductones containing the equivalent of one enediol group per molecule of glucurone are formed. With these evidences of reductone formation from uronic acids, the browning may occur through the dehydro form of the reductones as well as furfural formation.

D. Fragmentation Of Sugar Moiety

The fission products of the sugars vary considerably in their potential for browning. Fragments which retain the α-hydroxycarbonyl grouping will undergo browning alone in aqueous solutions; and, in the presence of amino compounds, the browning is greatly accelerated. The most highly reactive compounds are glycolaldehyde (*16*, *26*), glyceraldehyde (*36*, *65*, *174*, *183*), pyruvaldehyde (*36*, *39*, *44*, *174*, *183*), acetol (*79*, *158*, *188*), dihydroxyacetone (*174*, *183*), acetoin (*26*, *79*, *158*), and diacetyl (*26*, *36*, *67*, *174*). Acetaldehyde (*35*, *39*, *126*) is only slightly less reactive, aldol is still a little slower (*67*, *79*, *126*), and propionaldehyde is a very slow reactant (*126*). Even slower reacting are the keto acids, pyruvic (*36*, *67*) and levulinic (*67*, *170*), while the saccharinic acids, lactic and acetic, do not react with amino compounds (*67*). Formic acid has not been shown to be a source of browning, and formaldehyde is not only inactive (*126*), but is actually an inhibitor for furfural (*69*), caramelization (*83*), and carbonyl-amino (cf. *33*, *177*) types of browning.

Triose-reductone, which is known to be produced in the alkaline degradation of a large number of reducing sugars (*52*, *86*, *189*), forms crystalline condensation products with amines and amino acids (*29*, *53*, *189*). A comparative rate of browning has not been reported for triose-reductone. By analogy with other reductones, it would be expected to undergo rapid browning reactions in the dehydro form—i.e., as mesoxalic dialdehyde, CHO.CO.CHO.

The fragmentation of sugars in neutral and slightly acidic solutions was first reported by Nodzu (*129*) and Enders and Marquardt (*44*). Nodzu identified acetol and/or pyruvaldehyde, pyruvic acid, and diacetyl. Enders assumed that their product was pyruvaldehyde, but Sattler and Zerban (*158*, *159*) showed that Enders could have had acetol instead of, or in addition to, pyruvaldehyde, since their bishydrazone derivatives are identical. Pyruvaldehyde and diacetyl were positively identified in the distillates from solutions of sugars and amino acids by Speck (*174*). Other volatile substances which reduce periodate—e.g., acetol, glycolaldehyde—were indicated (by difference) from periodate oxidation analyses of the distillates. Although the amounts of the volatile fragments recovered were small, it is conceivable that much larger amounts could have been present initially in the reaction mixture but were held back by melanoidin formation (*38;* cf. *188*). Fructose gave much more pyruvaldehyde than did glucose with both glycine and β-alanine in Speck's experiments.

The accepted mechanism for sugar fragmentation is dealdolization—i.e., the reverse of aldol condensation. As amines or amine salts are known to catalyze aldol condensation (*24*, *54*, *92*, *184*), they will also catalyze the reverse reaction, if the reaction is truly reversible. Speck and coworkers (*174*) investigated the types of catalysts effective for the dealdolization of diacetone alcohol. They found that the dealdolization is subject to amine catalysis, but not to general base catalysis. The anions, not the zwitterions, of amino acids functioned as catalysts. The magnitude of the catalytic constant for methylamine in this reaction was approximately 20 times that for the β-alaninate ion and 65 times that for the α-alaninate ion.

Solutions of xylose or glucose with ethanolamine, diethanolamine, or triethanolamine were adjusted to pH 6.0 with sulfuric acid and distilled. Monoethanolamine sulfate produced severe browning with the formation of black insoluble melanoidins, and pyruvaldehyde was found in the distillate. Relatively large amounts of furfural were found in the distillate from the xylose solution. Diethanolamine sulfate produced a similar but less marked effect. No browning occurred with triethanolamine sulfate, and only a small amount of pyruvaldehyde was found in the distillate. Speck concluded that amines and amino acids which accelerate the degradation and browning of reducing sugars do so through the participation of the primary or secondary amino groups. Figure 1 shows how the amines participate in the reactions. After sugar-amine condensation (A), the Amadori rearrangement (B) produces the 1,2-enol or 2-ketose configuration in which the C—C bond α,β to the carbonyl group is weakened. The amino compounds present then accelerate the dealdolization to triose and other fission products.

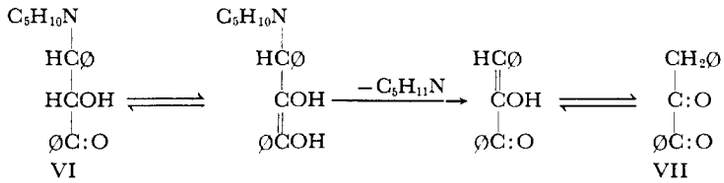
The isolation of 4(5)-methylimidazole and 2-methyl-5- and 2-methyl-6?-(D-*arabo*-tetrahydroxybutyl)pyrazine from the browning reaction of D-glucose in aqueous ammonia at 37° C. (*78*), and the detection of 4(5)-methylimidazole in the browning reaction of glucose and glycine (*43*) provide additional evidence for the fragmentation of sugars during browning.

The Amadori rearrangement product, 1-deoxy-1-piperidino-D-fructose, was shown by Hodge and Rist (*76*) to undergo fission on heating to yield, *inter alia*, piperidine acetate. Evidently, piperidine was split from the compound

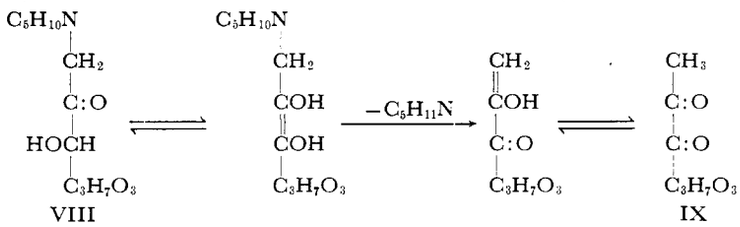
and the sugar chain was rearranged to yield acetic acid. The piperidine acetate formed in this decomposition would catalyze the aldol condensation of sugar fission products with accompanying dehydration of the aldols (cf. *92*, *184*). Hence, this is a mechanism to be considered for the formation of the 6-carbon reductone.

The volatile acid liberated by the treatment of glucose with 0.035 *N* sodium hydroxide at 35° C. in the absence of oxygen is acetic acid, as was determined by Sowden and Schaffer (*173*). It would not be surprising to find other analogies between the decomposition reactions of Amadori rearrangement products, on the one hand, and of the Lobry de Bruyn–Alberda van Ekenstein rearrangement products on the other.

Barker and Cromwell (*8*) reported the spontaneous decomposition in the solid state of α-hydroxy-β-piperidyl-β-phenylpropiophenone (VI) to brown tarry substances which contained phenyl benzyl diketone (VII).



Therefore, it is not unreasonable to assume that 1-deoxy-1-piperidinofructose (VIII) might lose piperidine similarly to yield IX.

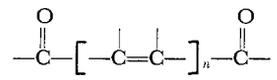


Fragments such as glycolaldehyde and acetylglycolyl could be formed from IX by dealdolization. Glycolaldehyde could yield acetic acid by a saccharinic acid rearrangement, and acetylglycolyl could yield it by oxidative scission. Piperidine acetate may have arisen by such transformations on heating VIII.

Weygand and Bergmann (*191*) oxidized Amadori rearrangement products in an ammoniacal solution with oxygen and a platinum catalyst and obtained arabonic acid (ammonium salt), the amine, and carbon dioxide. This type of oxidative fission may be a possible source of carbon dioxide from the sugar radical (carbon 1) for sugar–amine browning reactions that occur in the presence of oxygen.

E. Strecker Degradation Of Amino Acid Moiety

The Strecker degradation (*163*) of α-amino acids (to aldehydes containing one carbon less than the amino acid, with the liberation of carbon dioxide) has not been properly recognized in previous reviews as an important partial mechanism for browning. Evidence for its existence is scattered throughout the literature back to the time of Maillard (*116*, cf. *2*, *3*). Recently the degradation has been reviewed experimentally by Schönberg, Moubacher, and Mostafa (*164*), who found that only carbonyl compounds containing the structure:



where *n* is zero or an integer, are capable of initiating the degradation. Compounds easily converted to such dicarbonyl structures—e.g., reductones by dehydrogenation, imino analogs by hydrolysis—are also reactive. The total reaction is illustrated by the following equation:

$$R\cdot CO\cdot CO\cdot R + R'\cdot CHNH_2\cdot COOH \rightarrow R'\cdot CHO + CO_2 + R\cdot CHNH_2\cdot CO\cdot R$$

The transamination shown in the above equation could be an important reaction for incorporation of nitrogen into the brown polymer. Akabori (*2*) found that very little ammonia was liberated in the degradation of amino acids by glucose or furfural. The likelihood of transamination should also be remembered in using the Van Slyke determination of α-amino nitrogen in browning reactions. If the α-dicarbonyl compound is an osone—e.g., Compound II or pyruvaldehyde—the α-amino nitrogen is not lost in the reaction, but merely transferred to the α-position of the osone moiety where it could continue to react with nitrous acid.

Maillard (*116*) concluded that the carbon dioxide liberated in his browning reactions must have come from the carboxyl group of the α-amino acid, rather than from the sugar radical. Recent tracer experiments with carboxyl-labeled glycine have upheld Maillard's hypothesis. Stadtman, Chichester, and Mackinney (*178*) showed under Maillard's conditions that over 80% of the carbon dioxide generated from glucose and glycine came from the carboxyl group, and less than 10% came from the reaction of nonselectively labeled glucose with inactive glycine. Wolfrom *et al.* (*197*) have reported even higher percentages (90 to 100%) of carbon dioxide arising from carboxyl-labeled glycine.

Ambler (*3*) reported long ago that aldehyde production paralleled carbon dioxide evolution in sugar–amino acid browning reactions. More recently, carbon dioxide evolution was found to parallel color production (*96*, *109*, *195*) and show about the same dependence upon pH (*195*); however, Patron's experiments (*137*) under similar conditions did not confirm these reports. Stadtman *et al.* (*178*) found that less carbon dioxide was produced per unit of optical density at low temperatures than at high temperatures, and the melanoidins formed at 56.5° C. contained more labeled carbon from carboxyl-labeled glycine than those formed under Maillard's conditions at 100° C.

In a xylose–glycine system under nitrogen at 95° C., pH 4 to 5, 2 moles of xylose reacted to produce one mole of carbon dioxide, and the reaction stopped at that point, according to Langer and Wolfrom (*96*, *195*, *197*). In the presence of air the sugar could decarboxylate an excess of glycine. The osone structure (II) or a sugar fragment, such as pyruvaldehyde, were mentioned as likely α-dicarbonyl intermediates which could have caused the Strecker degradation.

The Amadori rearrangement product, 1-deoxy-1-piperidinofructose, produced the Strecker degradation of α-aminophenylacetic acid in aqueous solution in the absence of air (*76*). The transformation VIII → IX (shown under Reaction D) would give the α-dicarbonyl structure necessary for the Strecker degradation. The fission products of VIII, such as pyruvaldehyde and diacetyl, as well as reductones dehydrogenated by dismutation, are other possible sources of the required dicarbonyl compounds.

Pecherer (*144*) showed that dehydroascorbic acid in methanol degraded α-alanine to carbon dioxide and acetaldehyde with concurrent browning. Quinones, which are formed from polyphenols by oxidases, would also cause the Strecker degradation of α-amino acids (*163*, *164*). The low temperatures which prevail during enzymatic browning may strongly limit the extent of the Strecker degradation, but the temperatures and other conditions necessary for significant reaction have not been satisfactorily fixed (cf. *2*, *163*).

The aldehydes formed by the Strecker degradation are a source of browning. They could condense with themselves, with sugar fragments, with furfurals and other dehydration products, or with aldimines (*134*) and ketimines to form brown pigments. Mohammad, Olcott, and Fraenkel-Conrat (*126*) demonstrated that both proteins and amino

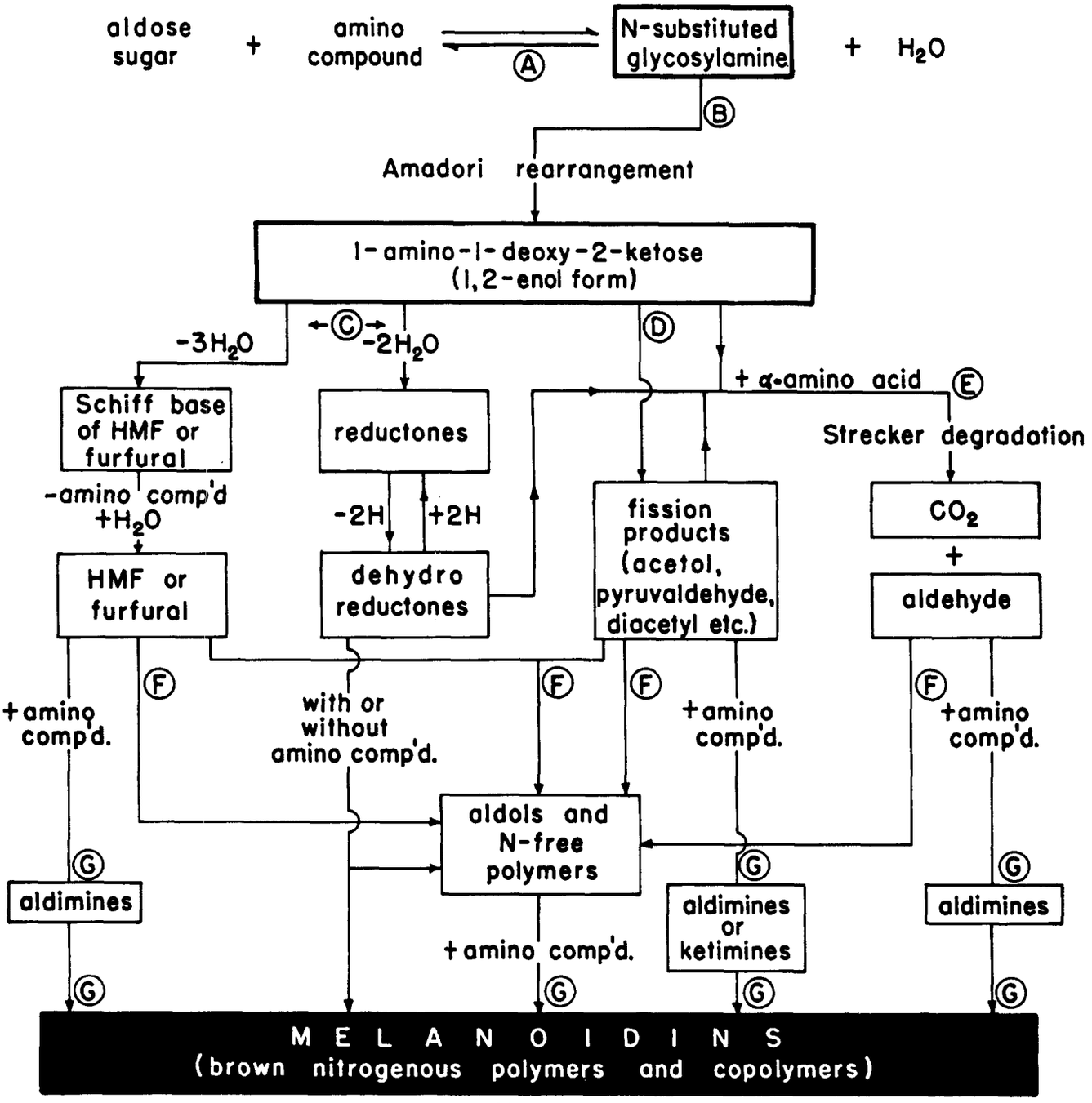


Figure 1. Amadori rearrangement in integration of known reactions leading to browning in sugar-amine systems

acids undergo very rapid browning reactions with acetaldehyde, but no browning was obtained with formaldehyde. Since glycine (which yields formaldehyde by the Strecker degradation) browns in some cases more and in other cases nearly as rapidly as alanine (which yields acetaldehyde), it is apparent that the production of aldehydes from amino acids by the Strecker degradation could not be the major color-producing reaction. Another argument is that some non-α-amino acids, which supposedly are not active in Strecker-type degradations, react more rapidly with reducing sugars to produce melanoidins than the corresponding α-amino acids (*65*, *67*, *71*, *91*, *174*, *183*). Therefore, it is concluded that the Strecker degradation is a nonessential but possibly important concomitant reaction leading to melanoidin formation.

The source and amounts of carbon dioxide formed in browning reactions where the Strecker degradation could not occur—e.g., with ammonia, amines, and amino acids other than α—have not been fully investigated. Lewis, Esselen, and Fellers did show, however, that appreciable amounts of carbon dioxide were liberated during the reaction of glucose with *p*-aminobenzoic acid (*108*), as well as with some nitrogen-free organic acids (*109*). The participation of amines in the decarboxylation of ascorbic acid and pyruvic acid has been discussed by Euler and Hasselquist (*51*).

Final Stage

In the final stage of browning, the intermediates polymerize and unsaturated, fluorescent, colored polymers are formed. The chief reactions involved are thought to be aldol condensation, aldehyde-amine polymerization, and the formation of heterocyclic nitrogen compounds, such as pyrroles, imidazoles, pyridines, and pyrazines. The chemistry of the melanoidins has been reviewed by Enders (*41*, *42*), Enders and Theis (*46*), Lüers (*113*), Traitteur (*183*), Danehy and Pigman (*33*), and Langer (*96*).

The relationships between color production and fluorescence were studied recently in model systems (*59*, *133*;

cf. *132*). The results led to the conclusion that the fluorogens are precursors of the brown pigments but are not identical with them. In the presence of bisulfite, color formation was inhibited, yet the fluorogens accumulated. The nonidentity of the fluorogens and the pigments was shown also by the isolation of colorless fluorescent intermediates from a glucose–glycine system (*137*), from a glucose–α-*N*-acetyllysine system (*70*, *71*), and from potatoes (*111*). Although these compounds were not identified, evidence was given that all three compounds were formed by sugar-amine condensation. The kinetics of fluorescence production in glycose–glycine (*141*, *142*) and in acetaldehyde–ammonia (*141*) systems was reported by Pearce.

Because of the similarity in composition and properties of the melanoidins derived from the reaction of glycine with glucose, mannose, xylose, furfural, or pyruvaldehyde, it has been assumed that all were derived from similar intermediates (*39*, *45*, *179*, *197*). Traitteur (*183*) has now reported significant differences among furfural-glycine melanoidin (high content of ether-bound oxygen), glucose-glycine melanoidin (high content of alcoholic hydroxyl groups), and pyruvaldehyde-glycine melanoidin (high content of enolic hydroxyl groups and low content of ether-bound oxygen). Glucose-glycine melanoidin was considered to occupy an intermediate position between the two extremes represented by furfural and pyruvaldehyde melanoidins. The melanoidins derived from acetaldehyde-glycine or pyruvic acid–glycine gave colors in incident ultraviolet light which are different from that of the first-named group, indicating possible differences in structure (*45*). In unpublished work, Carson and Olcott found that the melanoidins which are produced from acetaldehyde and amino compounds are not acidic, contrary to the case with sugar-amine melanoidins. As sugar-amine melanoidins contain reductone-like reducing power (*40*, *42*, *195*), their acidity may be caused chiefly by enediolic hydroxyl groups.

The naturally formed melanoidin isolated from nonenzymatically browned apricots by Weast and Mackinney (*186*) was shown by Sattler and Zerban (*160*) not to contain 6-carbon sugar residues, as it gave a negative test for hydroxymethyl furfural with the anthrone reagent. On the other hand, the synthetic melanoidin prepared by Weast and Mackinney (*186*) from the reaction of fructose with asparagine at 100° C. did contain a 6-carbon skeleton, as it yielded hydroxymethyl furfural and a positive anthrone test. Sattler and Zerban suggested that the melanoidins which were formed at low temperatures (as in the sun-drying of apricots) may have contained polymerized 3-carbon sugar fragments (which give no anthrone reaction); whereas those formed at higher temperatures contained polymerized 6-carbon residues from nonfragmented sugars. Wolfrom *et al.* (*197*) obtained experimental data which they interpreted as support for the latter case.

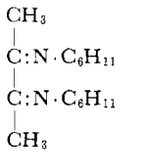
Inhibitors for browning have been shown to be, chiefly, carbonyl reagents, such as cyanide (*9*, *25*), Dimedon (*42*, *44*, *89*, *96*, *155*), hydroxylamine (*9*, *25*), hydrazines (*9*), mercaptans (*67*), and bisulfite (*9*, *47*, *59*, *76*, *110*, *130*, *177*, *188*). However, mercaptans and bisulfites (which are the best of the above-listed inhibitors from a practical standpoint) are also reducing agents; and, as such, they would keep reductones in the inactive reduced form, rather than the active dehydro form. Other inhibiting factors, such as the lowering of pH, temperature, water content, and removal of reactants, have been reviewed by Olcott (*130*, *131*).

When a model system has browned only as far as the yellow-orange stage, the color can be appreciably diminished by adding sulfhydryl or sulfite compounds; but, after further browning to a dark brown color, very little diminution in color is obtained (*67*). Guss (*67*) also found that a few drops of β-mercaptoethanol (and several other mercaptans) would inhibit the browning of glucose (3 grams) in aqueous solutions (5 ml.) containing glycine, lysine hydrochloride, or sodium glutamate (0.5 gram), respectively, at 95° C. As mercaptans did not inhibit the browning of furfural-glycine, it appeared that the inhibition occurred before the dehydration or ring closure to form furfurals.

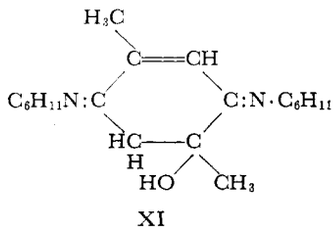
The critical role of water in sugar-amine browning reactions has been investigated by Olcott and Dutton (*132*), Lea and Hannan (*70*, *98*, *100*, *101*, *104*, *105*), Rooney (*155*), Mashkovtsev (*118*), Volgunov and Pokhmo (*185*), and Pigman and Rosen (*149*). These studies confirmed a point well known to the dehydrator—viz., browning of a thoroughly dehydrated food increases and passes through a maximum as the water content is increased. There is a striking resemblance in the shape of the curves obtained by Rooney (*155*) on plotting browning against water content for the model system, xylose-glycine, and the potato dehydration - browning curves obtained by C. E. Hendel (reported by Olcott, *130*).

F. Aldol Condensation Several independent investigations (*24*, *54*, *94*, *184*) have shown that amino compounds (particularly amine salts), including peptones and egg albumin, are effective catalysts for the aldol condensation of acetaldehyde and crotonaldehyde. Since amine catalysts are present, and aldehydes can be generated by sugar dehydration (C), by sugar fission (D), and by the Strecker degradation (E), it is evident that aldol condensation (F) is a highly probable reaction for melanoidin formation. Browning reactions have been demonstrated for pyruvaldehyde alone (*36*), for furfurals alone (*36*, *153*, *161*, *170*, *179*, *198*, *199*), and between pyruvate and furfural (*153*). Sugars alone show less browning under equivalent conditions (*199*). The aldol condensation of pyruvaldehyde has been postulated by Enders and Sigurdsson (*45*) as the initial reaction in melanoidin formation. Since aldol, $CH_3 \cdot CHOH \cdot CH_2 \cdot CHO$, is known to undergo browning with glycine (*67*, *96*), it is likely that nitrogen-free aldols in general will react with amino compounds, aldimines, and ketimines to produce nitrogenous melanoidins, as indicated in Figure 1.

The aldol condensation of diacetyl proceeds by first forming the ordinary aldol between two molecules, then intramolecular condensation takes place to yield 2,5-dimethyl-*p*-quinone. Carson (*26*) recently investigated the reaction of diacetyl with cyclohexylamine at 0° C. He isolated crystalline diacetyl dicyclohexylimine (X) and a highly reactive, colorless crystalline compound, $C_{20}H_{32}N_2O$, for which the structure (XI) was proposed. The colorless intermediate (XI)



X



XI

browns on exposure to moist air at room temperature and decomposes spontaneously with color formation in many organic solvents. In methanol at 25° C., XI is dehydrated and yields 2,5-dimethyl-*p*-quinone dicyclohexylimine, a yellow crystalline compound. Since α-dicarbonyl compounds—e.g., diacetyl, osones, dehydro reductones—are generated in sugar-amine browning reactions, quinones and quinone imines (which are so readily formed at low temperatures) could conceivably be involved in melanoidin formation.

G. Aldehyde-Amine Polymerization and Formation of Heterocyclic Nitrogen Compounds According to Sprung (*176*), simple aldehydes and amines, even acetaldehyde

and ammonia, react readily at low temperatures and form polymeric colored products the structures of which are not known. α,β-Unsaturated aldehydes react with amines at temperatures above $-10°$ C. to produce resins and complex aldollike condensation products.

Patrick (*134*) showed that aliphatic aldehydes will condense with aldimines to form two different types of products: a dimeric, unsaturated, conjugated aldimine—e.g., $RCH_2C:C(R')CH:NR''$—and a substituted dihydropyridine. Both types of condensations could occur also in melanoidin formation, as both aldehydes and aldimines are present. The formation of pyridines in this way might explain the fact that pyridines have been distilled from melanoidins (*41*, *46*). Pyrroles, also, have been distilled from glucose-glycine melanoidins in the presence of zinc dust (*41*, *46*, *60*, *157*).

Positive color reactions have been obtained from model browning mixtures with such nonspecific tests as the pine-splinter (*60*, *157*), Paulys (*44*), and Elson-Morgan (*64*, *65*, *70*, *71*, *77*), which indicated the presence of pyrroles, imidazoles, or other nitrogenous heterocyclic compounds. Additional inconclusive evidence for the presence of pyrroles in model browning systems was given by Pearce and Bryce (*143*). They heated solutions of sugars (glucose, mannose) and sugarlike substances (acetoin, diacetyl) with amino acids (glycine, glutamic acid) and showed that the fluorescence spectra of these solutions were similar to the spectra of pyrroles. (Glycols, which do not undergo browning with amino acids, also gave pyrrolelike fluorescence spectra when heated with amino acids.) On the other hand, 4(5)-methylimidazole (*43*, *78*), tetraphenylpyrrole (*79*), and several substituted pyrazines (*64*, *78*, *79*), have been detected and actually isolated from sugar-amine browning reactions in model systems. Nevertheless, the presence of such heterocyclics in the melanoidins remains to be proved.

Browning in Nonamino Systems

It is known that sugars, polysaccharides, polyhydroxycarboxylic acids, reductones, α-dicarbonyl compounds, and quinones will undergo browning in the absence of amino compounds (cf. *68*). Such decompositions, even in the absence of catalysts, are of some importance to the food chemist; but they occur mainly at high temperatures not often encountered in normal food processing. In most foods these nonamino reactions would occur in the presence of accelerators, such as carboxylic acids and their salts, phosphates, and metallic ions, and thereby they would approach (but not equal) the low energy requirements of sugar-amine reactions.

Lewis, Esselen, and Fellers (*109*) found that the sodium salts of nitrogen-free organic acids cause a more or less typical browning reaction with sugars. As in sugar-amine systems, the rate of color formation is markedly increased by increased pH (addition of sodium hydroxide), carbon dioxide is liberated, the pH drops during the reaction, the color development is inhibited by sulfur dioxide, and an oxygen atmosphere has only a slight enhancing effect on the amount of color produced. The source of the carbon dioxide and the mechanism by which it is produced remain obscure.

It is apparent from Lewis' data (*107*, *109*) that sodium acetate, oxalate, tartrate, lactate, and fumarate do not produce browning as rapidly with glucose at 100° C., pH 7.2, as does glycine; on the other hand, sodium citrate causes even more browning than glycine under these conditions. All of these carboxylic acid salts accelerated browning considerably over what is known to occur with glucose alone at 100° C., initial pH 7.2. However, in the latter unbuffered case, the pH drops more and faster during the browning, thereby providing a self-inhibiting action on color production. The apparent accelerating effect of fatty acid salts could possibly be caused by approximate maintenance of the initial pH. In such case the catalysis is only that of bases in general. The sugar–carboxylic acid systems of Lewis were reinvestigated for browning in acid solution by Patron (*137*). He concluded that temperatures upwards of 100° C. were required, or very little browning would occur in the absence of amino compounds—for example, aqueous solutions of 0.5 *M* glucose which contained either 0.05 *M* citric or malic acid gave virtually no browning after 10 days at 50° C.

The browning of fructose in the presence of malic acid at 60° to 70° C. in acid solutions was investigated by Livingston (*112*). The browning reaction was more rapid than that of fructose with hydrochloric acid at the same pH. Absorption spectra measurements and control experiments indicated that the fructose was dehydrated to hydroxymethyl furfural. [From the recently published results of Petuely (*146*), it can safely be said that the fructose was also 1,2-enolized in the acid solution.] Fructose, sucrose (cf. *145*), hydroxymethyl furfural, and ascorbic acid gave browning in the presence of malic acid; but glucose, furfural, pyruvic, and levulinic acids did not. By heating various dicarboxylic acids with hydroxymethyl furfural, it was shown that an α-hydroxyl group in the dicarboxylic acid is required for the occurrence of browning.

The model system studies of Pederson, Beattie, and Stotz (*145*) indicated that organic acids, phosphates, and amino acids accelerated the discoloration of reducing sugar solutions, but they did so by acting synergistically rather than separately. Malic acid gave more color than citric or tartaric acids in their systems. A similar study by Patron (*137*) showed that the phosphate, iron, and copper salts of a synthetic mixture (representing the minerals in orange juice) also acted synergistically on the browning of glucose–organic acid and glucose–glycine–organic acid systems in the presence of oxygen. The effect was much greater when glycine was present. It is apparent from these two reports that the presence of small amounts of minerals and organic acids in natural systems may have important effects on the browning reactions.

The accelerated decomposition of reducing sugars with color production by phosphates and other buffers (acetates, citrates, phthalates) is frequently mentioned in the literature (*22*, *38*, *47*, *83*, *96*, *109*, *129*); but it is not known with certainty which of these salts have specific action and which have only the general accelerating action of bases. Phosphates have been reported to have a specific accelerating effect on sugar-amine condensation (*1*), sugar fission (*38*, *61*, *129*), production of reductones (*22*, *123*), and browning (*47*, *145;* cf. *83*). Other reports (*64*, *124;* cf. *83*) indicate that there is no specific acceleration of browning by phosphate. Obviously, experiments expressly designed to settle the question are needed. Until the question of specific *vs.* general base effect on the decomposition is settled for the various buffer salts, stability studies on sugars, such as that of McDonald (*114*) (who used phosphate and glycinate buffers), cannot bear a general interpretation.

The main reactions that occur in carbonyl-amino browning also occur in nonamino browning. For example, 1,2-enolization, sugar dehydration to furfurals, and sugar fission are known caramelization reactions. Just as the Amadori rearrangement (1,2-enolization of glycosylamines) is now known to provide a more labile configuration of the 1-amino sugar chain, so the Lobry de Bruyn–Alberda van Ekenstein rearrangement (1,2-enolization of reducing sugars) provides the labile sugar chain for nonamino browning reactions.

Transformations of reducing sugars of the Lobry de Bruyn type that occur in weak alkali are known to cause browning (*62*), and to occur in acid as well as in alkaline media. Wolfrom and Shilling (*200*) obtained 0.1% glucose by column chromatography after refluxing an aqueous solution of fructose (80%) at 113° for 16 hours (pH 6.9 → 2.7). The cooled solution was very dark brown and had a strong burnt sugar odor. Petuely (*146*) and Berner and Sandlie (*19*) also used chromato-

graphic methods to identify fructose in heated, acidic glucose solutions. Petuely detected very small amounts of mannose in the acidic glucose reaction mixture. In addition, glucose was isolated and identified in the reaction mixture obtained by heating an aqueous solution of fructose and tartaric acid in an autoclave at 140° C. for 1.5 hours. Furfural formation was a competing reaction, strongly so below pH 3. Speck and Forist (*175*) reported that both weak organic acids and their salts are capable of catalyzing the Lobry de Bruyn–Alberda van Ekenstein rearrangement of glyceraldehyde and dihydroxyacetone. Simultaneous dehydration occurred to yield pyruvaldehyde. The products of this latter transformation, starting with the 3-C-phenyltrioses and acetic acid, were isolated and identified by Smith and Anderson (*4*, *171*).

Following 1,2-enolization of the sugars, dehydration and fission occur. However, dehydration without fission is not known in alkaline media. Furfural formation is strongest in strongly acid media (below pH 3), but it occurs to a measurable extent at elevated temperatures even in the pH range 6.0 to 6.7 (*17*, *158*, *161*, *170*, *198–200*). Sugar fission is known to occur to a limited extent in weakly acidic media (*37*, *61*, *129*, *158*), and it increases rapidly with increasing pH (*37*, *38*, *188*; cf. *7*). If sulfite is used to inhibit color development in heated glucose solutions (alkaline pH), the yield of acetol obtainable from the distillates is significantly increased (*188*).

According to Berl and Feazel (*18*), the ultraviolet absorption spectra of sugars in alkali at pH 8 to 10 are simple and correspond neither to pyruvaldehyde, acetol, aldehydo sugars, nor exactly to triose-reductone. The spectrum is identical with that of triose-reductone, except the shift of the maximum from 2950 to 2650 A. comes at pH 7, rather than pH 5. [From recent polarographic measurements on triose-reductone (*23*) a shift in $\frac{\Delta E_{1/2}}{\Delta \mathrm{pH}}$ was observed at pH 5.5 and also at about pH 8.5. Moreover, the value was the same above and below these limits of pH.] The intensity and rate of development of the spectrum are much greater for the trioses than for the pentoses and hexoses, suggesting that the chromogen is formed from the latter through the condensation of 3-carbon or smaller intermediates.

Like the sugars, polyhydroxycarboxylic acids will caramelize if heated to sufficiently high temperatures. Lamure (*95*) demonstrated that potassium tartrate undergoes browning and decomposes rapidly on heating the pure hemihydrate at 240° C. Water, carbon dioxide, potassium pyruvate, potassium acetate, and carbon were identified as products of the reaction. This pyrolysis involves the dehydration of a polyhydroxycarbonyl compound to produce an α-dicarbonyl compound and a saccharinic acid (acetic acid), thereby resembling sugar browning reactions.

It is concluded that organic acids and their salts accelerate the caramelization of sugars by promoting enolization of the sugar. Enolization occurs in both acid and alkaline media (*146*, *196*), but far more easily with increasing alkalinity. The enolized sugar is more easily dehydrated and fragmented than the initial ring form. The dehydration and fragmentation products contain (as the most reactive intermediates) unsaturated osones and dehydro reductones (α-dicarbonyl compounds) which so readily form polymeric brown pigments. Thus, the browning of nonamino and carbonyl-amino systems is similar. There is one outstanding difference: The acceleration of browning is appreciably greater through glycosylamine enolization (the Amadori rearrangement) than through enolization of the unsubstituted sugar (Lobry de Bruyn–Alberda van Ekenstein rearrangement).

Nonamino Browning Of Reductones The heating or long standing of pure glucose solutions produces reductones (*7*, *187*). Furthermore, the reductones are unstable and apparently undergo oxidative degradation (*187*). Ascorbic acid alone in aqueous solution will undergo browning on heating at 98° C. or above (*168*, *170*), forming furfural and carbon dioxide (*170*, *153*). The browning is increased with increasing pH, and above pH 7 autoxidation and color production occur readily at 25° C. (*11*, *137*). Neutral solutions of the reductones derived from glycosylpiperidines (Section C) also gave color on standing in air at 25° C. Patron (*135*) found that crude dihydroxymaleic acid turned brown in the solid state and produced an odor like maple sirup. Apparently all reductones, including the polyphenols, undergo strong darkening in alkaline solution in contact with air (*107*, *137*, *201*). As in the case of the ascorbic acid–amino systems previously discussed (Section C), it is the dehydro form of the enediol that is so unstable (*50*, *52*, *137*). According to the chromatographic experiments of Euler and Hasselquist (*52*), dehydroascorbic acid is hydrolyzed by acid and yields three different reductones. The promotion of color formation through the catalytic oxidation of reductones by copper (*11*, *145*, *182*) and the stoichiometric oxidation with complex formation by trivalent iron and titanium compounds (*5*, *49*, *50*, *107*, *138*, *192*) are also known mechanisms for darkening in foods.

Enzymatic Browning Darkening of the surfaces of freshly cut fruits is attributed to one or more oxidases which act on tyrosine, catechol, or other polyphenolic substrates to form quinones. Catechols are enediols in a conjugated system and are classed as reductones (*53*). Although aromatic enediols are easily oxidized, nonaromatic reductones are oxidized even more readily (*5*, *53*). Some of the reactions of the quinones after they are formed may conceivably be enzyme-catalyzed, but nonenzymatic browning reactions are believed to predominate (*82*). Ascorbic acid is also a substrate for enzymatic oxidation. The dehydroascorbic acid formed would undergo browning as described above.

Little is known about the browning mechanism for quinones (*82*). It is known, however, that quinones (also dehydroascorbic acid) will cause the Strecker degradation (Reaction E) of amino acids (*163*, *164*). In addition, quinones (*72*), and probably also formaldehyde (*152*), will oxidize ascorbic to dehydroascorbic acid, which will undergo browning of itself. Since *p*-quinones can be formed in typical sugar-amine browning systems (see Reaction F), the reductones present could be dehydrogenated by the quinones; thereby, browning of the reductones could occur in the absence of oxygen.

Conclusion

The several types of browning, the many different reactants and reactions involved in each type, and the large number of controlling variables make the chemistry of browning reactions an intricate subject. It should be possible to analyze the browning complex by identifying the individual reactions and studying each in model systems; yet, even with all the possible pathways to melanoidin formation outlined, it would still remain to be determined which of these routes are operative and to what extent one affects the other in a given food (or other system) under a given set of conditions. Although we are still in the first stages of solving the problem, chromatographic methods and isotopic tracer techniques give a promising outlook for an acceptable solution in the future. As a step toward eventual solution, this review has discussed the reactions involved in browning, proposed a mechanism for browning in sugar-amine systems which correlates the known reactions, and pointed out important unexplored areas.

The mechanism for sugar-amine browning presented here (Figure 1) contains several distinct routes to melanoidin formation, all of which stem from the Amadori rearrangement of sugar-amine condensation products. In interpreting Figure 1 it should not be concluded that all sugar-amine browning passes through the Amadori rearrangement (*27*, *75*, *199*); and the term Amadori rearrangement should not be construed as meaning the complete

transformation of an aldose to the 2-ketose configuration. For example, 1,2-enolization of a glycosylamine, as postulated by Gottschalk (*63, 64*), comes within the scope of the term, as this enolization yields the tautomeric form of the ketose.

A mechanism for browning providing for 1,2-enolization of the sugar radical of a glycosylamine without passing through the Amadori rearrangement—i.e., by elimination of the amine radical to produce the unsubstituted sugar enol—has been proffered by Patron (*137*). Others (*174, 196*) have suggested 1,2-enolization of the sugar without sugar-amine condensation to provide the labile intermediate for both dehydration (*128, 146*) and fission (*174*) of the sugar. It has been the purpose of this article to show that in concentrated solutions of aldose sugars and amino compounds, and in the nearly dry state, the experimental evidence favors the mechanism outlined in Figure 1. Sugar-amine condensation and the Amadori rearrangement are the key reactions leading to the 1,2-enol and, from it, the accelerated production of brown pigments occurs through several different routes.

One striking feature of the proposed mechanism (Figure 1) is the extent to which simple addition reactions, condensations, enolizations, dehydrogenations, and rearrangements are represented. All of these are considered to be proton-transfer reactions. Such an explanation is required by the known independence of sugar-amine browning from atmospheric oxidation. This is not to say, however, that oxidation by the atmosphere plays no part in sugar-amine browning. In connection with the browning of reductones, some type of oxidation is essential, but need not be provided by the atmosphere. Air-oxidation might also diminish the extent of color formation by converting very reactive compounds—e.g., pyruvaldehyde—into less active compounds—e.g., pyruvic acid. Of the three main routes to melanoidins outlined in the intermediate stage, sugar dehydration and sugar fission are of prime importance; amino acid degradation is secondary. Doubtless, dehydration and fission go hand in hand. Whether one precedes the other is not known. The relative importance of each may vary with the controlling conditions.

The formation of reductones, α-dicarbonyl, and conjugated dicarbonyl compounds (dehydro reductones) has been encountered repeatedly in this correlation. Yet, very little study has been given to the browning reactions of these compounds in model systems. More research on the decomposition and polymerization reactions of pertinent dicarbonyl compounds should help establish the mode of melanoidin formation for many of the browning pathways.

The value of this correlation of browning reactions will be directly proportional to the degree to which it promotes solutions to the problems of food technology. At least two areas for practical applications are now apparent:

For systems in which the Amadori rearrangement of sugar-amine condensation products is the key reaction, methods should be sought for blocking the rearrangement. No research directed specifically toward this end has yet been tried. The multitudinous reactions which follow the rearrangement would then be eliminated.

The reaction of glucose with piperidine yields a new type of stable crystalline reductone. No doubt antioxidants of the ascorbic acid type would find greater use in food preservation, if they could be made cheaply. In the piperidyl-reductone and its analogs there is the possibility of a relatively cheap, neutral antioxidant, which could be tailored for various uses by variation of the amine or amino acid radical.

Dehydrators would think of browning as an undesirable reaction; yet, in many instances a particular food—e.g., coffee, fresh bread crust, maple sirup, roasted nuts—could not be identified were it not for its characteristic desirable "browned" flavor and odor. The control of browning reactions to produce only wanted flavors and odors is an intriguing possibility. Control of browning to do man's will is the ultimate goal of browning research, but progress toward this goal can be made only as the reaction mechanisms are better understood.

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