^{13}C NMR Study of Some Maillard Reaction Products Arising from D-Glucose–DL-Alanine Interactions[1]
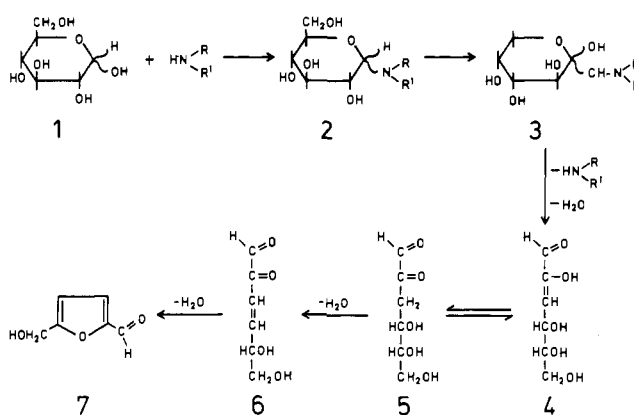
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Water-soluble, nondialyzable Maillard polymers were obtained by reacting D-glucose (and D-fructose) with DL-alanine at 100 °C and pH 3.5 at equimolar concentrations. Compositionally, the polymers appear to be produced via dehydration reactions (elemental analyses). NMR studies of polymers prepared by D-glucose-1-^{13}C, DL-alanine-1-^{13}C, and DL-alanine-2-^{13}C (90 atom %) show that both C-1 and C-2 of alanine are, in part, incorporated into the polymer, probably without change in oxidation state. Carbon 1 of D-glucose, on the other hand, appears to be incorporated into the polymer in a variety of oxidation states, as evidenced by signals in nearly all regions of the spectrum (from 15 to 175 ppm). Dialyzable reaction products were found (by NMR) to be D-glucose and DL-alanine, and Amadori compounds (1-N-alanyl-1-deoxy-D-fructose derivatives) derived from them. These data are generally similar to previous findings using glycine and sugars.

Since Maillard's origional report on the production of polymeric materials, produced as a result of sugar–amino acid interactions, numerous studies have been reported. In the recent literature, Barbetti and Chiappini (1976a,b) have reported results of studies of some model systems, as have Ledl and co-workers (Ledl, 1982a,b; Ledl and Severin, 1982) as well as Velisek and Davidek (1976a,b). More recently, Imasato and co-workers (1981e and Bobbio and co-workers (1981) have described the preparation and fractionation of melanoidins, produced by reaction of various sugars with glycine, and have reported analytical data, including elemental analyses, infrared data, and acetylation data. It is also noteworthy that Danehy (1986) has recently given an excellent review of the Maillard reaction from the point of view of compounds produced that contribute to flavor development.

On the basis of data now available, it is generally conceded that Maillard polymers are probably not formed as

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Scheme I

a result of a single chemical reaction or pathway but result from a multiplicity of chemical reactions that are, in turn, dependent on reaction parameters such as temperature, pH, reactant concentration, and type of reactant (Eriksson, 1982). A prominent (but probably not exclusivee initial reaction (Scheme I) involves the reaction of a sugar (1) with an amino group (2) to produce an Amadori compound (a 1-amino-1-deoxy-2-ketose, 4) via the intermediate glycosylamine (3). Amadori compounds dehydrate (with loss of the amine substituent) to 5-(hydroxymethyl)-2-fur-

aldehyde (Feather and Russell, 1969) (7), via intermediates 4–6. Compound 5, a highly reactive molecule, has been shown to be produced from Amadori compounds (Anet, 1968) as well as at conditions suitable for the Maillard reaction (Kato, 1960).

Indirect evidence also suggests that a Strecker degradation may well be involved in the Maillard reaction. This reaction involves the decarboxylation of an amino acid in the presence of an α,β-dicarbonyl compound to give carbon dioxide (derived from the carboxyl group of the amino acid) and aldehydes (derived from the remainder of the amino acid) (Schonberg et al., 1948; Schonberg and Moubacher, 1952). Both carbon dioxide and aldehydes are produced during a typical Maillard reaction. Recently, Olsson and co-workers (1982) have suggested that dicarbonyl compounds (such as 5) produced from sugars during the reaction may serve as catalysts for such a degradation reaction.

In an earlier paper (Feather and Nelson, 1984), we were able to show that ^{13}C NMR is useful in examining the carbon atoms in a Maillard polymer from a qualitative point of view and allows some information to be gained relative to reaction pathways that might contribute to polymer formation. The present paper reports the preparation of a Maillard polymer from D-glucose (and D-fructose) and DL-alanine, as well as D-glucose-1-^{13}C and DL-alanine-1-^{13}C and -2-^{13}C, and some chemical and spectroscopic data on these polymers. The results reported herein are generally consistent with earlier data reported by this laboratory (Feather and Nelson, 1984), which was concerned with glycine–sugar interactions.

EXPERIMENTAL SECTION

Materials and Methods. D-Glucose-1-^{13}C and DL-alanine-1-^{13}C and -2-^{13}C (all 90 atom %) were obtained from MSD Isotopes, St. Louis, MO. NMR spectra were obtained on a Nicolet NT-300 instrument with samples dissolved in deuterium oxide with dioxane as internal standard (δ 67.2). The NMR data reported are, however, relative to tetramethylsilane (δ 0.00). Optical spectra were obtained on a Varian Cary Model 210 spectrophotometer. Elemental analyses were performed by Microtech Laboratories, Skokie, IL.

Polymer Yield Determinations. In a typical experiment, 18 go of D-glucose (0.1 mol) and 7.5 g of DL-alanine (0.1 mol) were dissolved in 100 mL of phthalate buffer (pH 3.5), and the resultant mixture was heated to reflux in a 200-mL round-bottom flask equipped with a water-cooled condenser. The time required to reach reflux was 0.5 h, and zero time was arbitrarily designated at this point. At hourly intervals, a 10.0-mL aliquot was removed, placed in a 10-mm-diameter dialysis bag (8000–12 000 molecular weight cutoff), and dialyzed for 24 h against running tap water. The contents of the dialysis bag were then quantitatively transferred to preweighed 70-mm-diameter evaporating dishes, and the solution was evaporated to dryness, first at atmospheric pressure and then in vacuo to constant weight. Polymer yields were determined gravimetrically. Polymer preparations so obtained were readily soluble in water for reaction times of up to 10 h.

Polymer Preparation. In a typical experiment, 270 g of D-glucose (or D-fructose) and 112.5 g of DL-alanine were dissolved in 1500 mL of phthalate buffer (pH 3.5), and the solution was heated at reflux for 8 h. After cooling, the solution was placed in 27-mm-diameter tubing (12 000–14 000 molecular weight cutoff) and dialyzed against running tap water for 72 h and then against 10–20-L changes of distilled water over a 10-day period. The material so obtained largely dissolved in distilled water to give a hazy
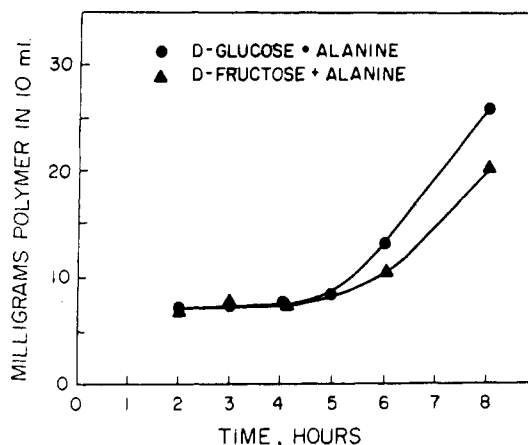

Figure 1. Yields of nondialyzable Maillard polymer as a function of time.

solution that could be clarified by centrifugation at 10 000 rpm for 1.0 h.

^{13}C**-Enriched Preparations.** To 2.5 mL of phthalate buffer (pH 3.5) were added 450 mg of D-glucose-1-^{13}C (90 atom %, 2.5 mmol) and 188 mg of ordinary DL-alanine (2.5 mmol). The resulting solution was sealed in an 8 mm (i.d.) by 15 cm glass tube and heated in an oil bath at 100 °C for 10 h. After the reaction, the solution was transferred to an 11-mm-diameter dialysis bag (3500 molecular weight cutoff) and dialyzed against 1 L of distilled water for 24 h. The dialyzate (external solution) was evaporated to dryness for use in NMR experiments. The dialysis bag was then further dialyzed against 2-L portions of distilled water over a 10-day period with two changes per 24-h period. The polymer preparation, now free of any low molecular weight material, was freeze-dried and used for NMR experiments. Polymer preparations using DL-alanine-1-^{13}C and -2-^{13}C enriched material were processed in exactly the same manner. In all cases, the samples were dissolved in 0.6 mL of deuterium oxide containing a small amount of dioxane (internal standard) and were used for NMR data collection.

RESULTS AND DISCUSSION

Yields of nondialyzable polymer with respect to time (Figure 1) indicate that D-glucose is slightly more reactive than D-fructose. The following elemental analyses were collected. D-Glucose-derived polymer: C, 51.26; H, 5.54; N, 4.39; ash, 4.98. D-Fructose-derived polymer: C, 53.00; H, 5.65; N, 4.84; ash, 2.63. In both respects, these data are similar to earlier data collected for reactions of D-glucose and D-fructose with both glycine and methionine (Feather and Nelson, 1984). In an earlier paper (Ghiron et al., 1987), we described a study of the reaction of 3-deoxy-D-erythroglucosulose with alanine at conditions of the Maillard reaction. In that study, it was found that the compound catalyzed color formation at a much faster rate than did D-glucose and, more importantly, was shown to readily catalyze a Strecker degradation on reaction with phenylalanine, as evidenced by the isolation and unequivocal identification of phenylacetaldehyde as a reaction product. Maillard polymer formation involving a Strecker degradation as a major reaction pathway could also account for the elemental analyses found.

In order to examine the reaction further, ^{13}C NMR data were collected for the system. A natural-abundance ^{13}C NMR spectrum for the Maillard polymer (Figure 2), clearly shows that the carbon atoms in the polymer are distributed over a wide variety of chemical environments as evidenced by the signal positions. The spectrum, while not interpretable, is similar to that reported for a D-glucose–glycine
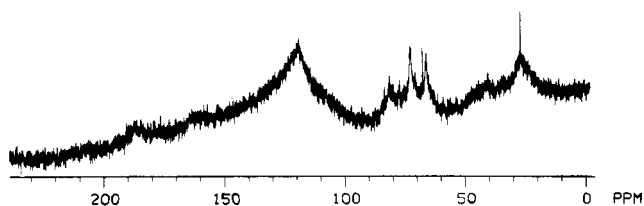
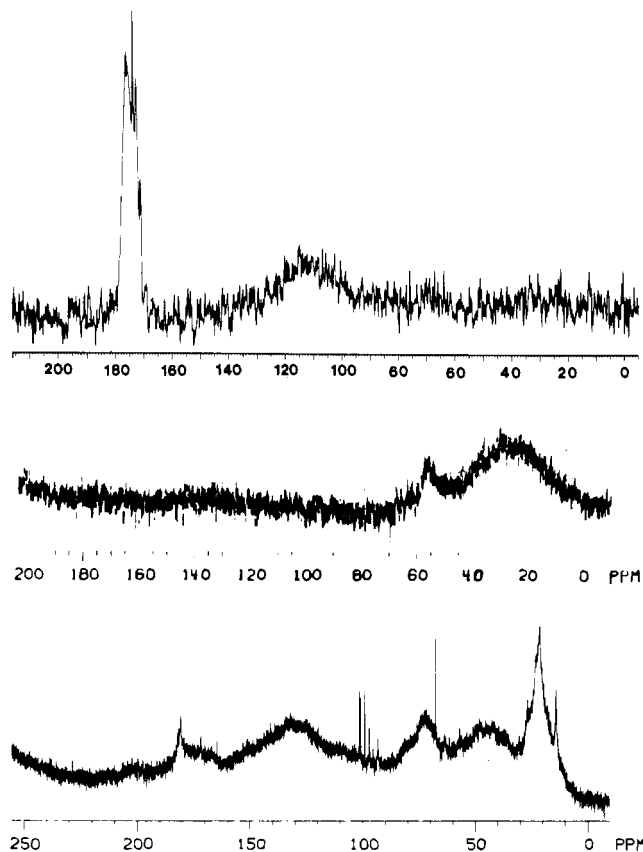**Figure 2.** Natural-abundance ^{13}NMR spectrum of a Maillard polymer prepared from D-glucose and DL-alanine.

Figure 3. ^{13}C NMR spectra of a Maillard polymer prepared from D-glucose and DL-alanine-1-^{13}C (top), D-glucose and DL-alanine-2-^{13}C (middle), and D-glucose-1-^{13}C and DL-alanine (bottom).

Figure 4. ^{13}C NMR spectra of dialyzable materials produced during the reaction of D-glucose and DL-alanine-1-^{13}C (top), D-glucose and DL-alanine-2-^{13}C (middle), and D-glucose-1-^{13}C and DL-alanine (bottom). The signal at δ 67.2 represents dioxane, used as an internal standard.

system reported by Olsson et al. (1982), as well as one by this laboratory (Feather and Nelson, 1984).

More definitive data were collected by using polymers prepared from ^{13}C-enriched precursors. Figure 3 shows spectra for polymers prepared from DL-alanine-1-^{13}C and -2-^{13}C and D-glucose-1-^{13}C, respectively. The spectrum for the polymer derived from DL-alanine-1-^{13}C (Figure 3, top) shows a series of signals centered at δ 170–185, while that for the 2-^{13}C-derived polymer (Figure 3, middle) shows a series centered at δ 15–35 and δ 50–60. The former signals are in the region expected for carboxyl groups and the latter for substituted methyl or aliphatic groups (Stothers, 1972). This suggests that these carbons, when they are incorporated into the polymer, are largely unchanged in their oxidation state. The issue of what percentage of C-1 of the amino acid is incorporated into Maillard polymers vis-à-vis how much is lost as carbon dioxide (presumably via Strecker degradation reactions) is interesting and, as yet, unresolved. Wolfrom et al. (1953) have suggested (based on ^{14}C tracer experiments) that nearly all of the C-1 of the amino acid is converted to carbon dioxide (and, hence, lost from the reaction). More recent experiments (Feather and Huang, 1985), also based on ^{14}C incorporation experiments, clearly show, however, that although much of C-1 is lost (relative to, for example, C-6 of D-glucose),
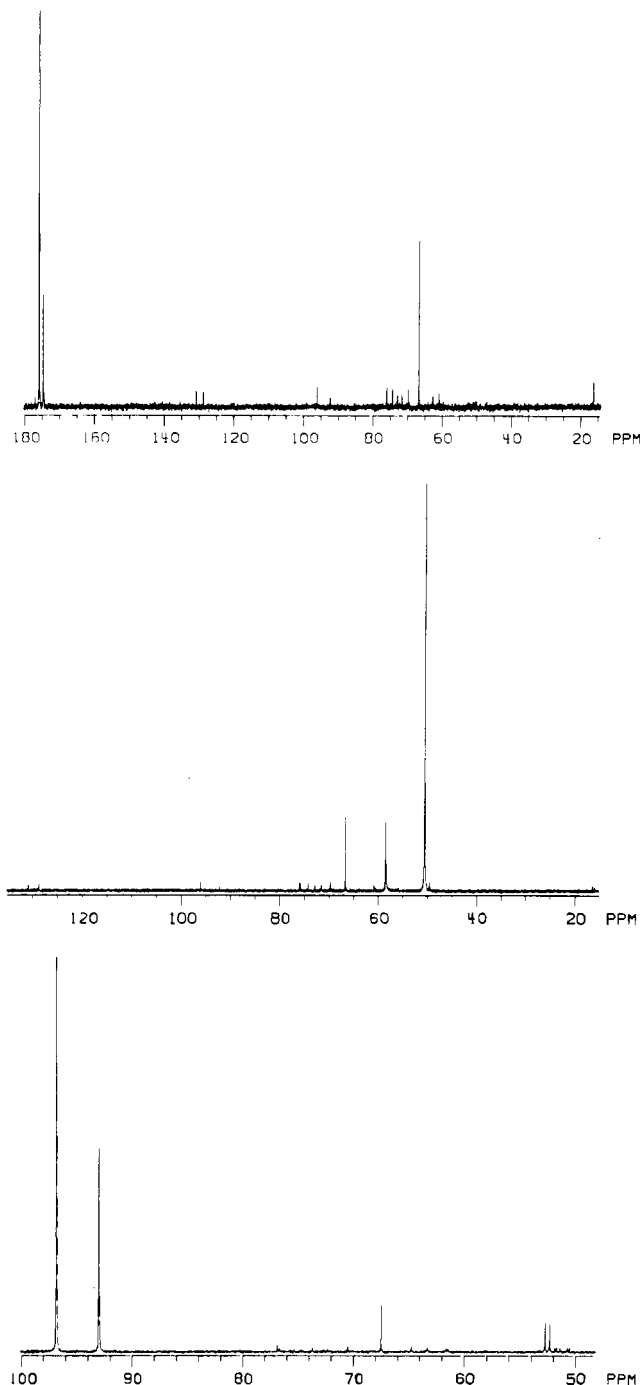
measurable amounts of both C-1 and C-2 of the amino acids are incorporated into the polymer. The NMR data herein, support this finding.

The results regarding the polymer prepared from D-glucose-1-^{13}C (Figure 3, bottom) are more complex. The spectrum shows a variety of signals over almost the entire measurable spectrum, suggesting that C-1 of D-glucose is "scrambled" during the reaction and (in the polymer) exists in a wide variety of environments (and oxidation states). This may not be too surprising, since the signals of C-1 of unreacted D-glucose (a possible reactant) appear at δ 96.7 and 92.9, while C-1 signals from Amadori compounds derived from D-glucose are usually found much further upfield, generally in the range δ 50–55. Thus, early in the reaction, possibly even before extensive polymerization has

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occurred, C-1 of the original D-glucose exists as a carbonyl carbon as well as a substituted methyl group, when it has been converted to an Amadori compound. It is noteworthy that Benzing-Purdie and Ripmeester have reported that the NMR data relative to melanoidins prepared from D-glucose, D-xylose, and glycine are similar, in some respects, to soil humic acid material and that these preparations are highly aliphatic and, in addition, contain aromatic, carbonyl and carboxyl carbons. The findings herein are consistent with their conclusions.

The dialyzate (and largely monomeric) fraction from the reactions (Figure 4) shows that the major products are unreacted starting materials and Amadori compounds (1-*N*-alanyl-deoxy-D-fructose derivatives). Roper et al. (1983) have prepared a number of Amadori compounds, including alanine derivatives, and have assigned the ^{13}C resonances for all the carbon atoms in these derivatives. The data collected herein are consistent with their assignments. For example, for the case of the alanine-*1*-^{13}C preparation (Figure 4, top) the signal at δ 175.6 can be assigned to C-1 of unreacted alanine (Stothers, 1972) and that at δ 174.4 to an Amadori compound derived from it (Roper et al., 1983). In a like manner, for the alanine-2-^{13}C preparation (Figure 4, middle), the signal at δ 50.4 represents C-2 of unreacted alanine, while that at δ 58.3, the Amadori compound. For the D-glucose-*1*-^{13}C preparation (Figure 4, bottom), the anomeric carbons for the and anomers are found at δ 96.7 and 92.9, respectively, while, for the Amadori compound, they are at δ 52.7, 52.2, 51.7, and 51.3.

The assigned signals make up the majority of the visible signals on the spectrum. Although the solutions used were extremely dark, it appears that these compounds are present at very low concentration or are present in such a diversity of compounds that they give rise to no observable signals.

Registry No. DL-Alanine, 302-72-7; glucose, 50-99-7; fructose, 57-48-7.

LITERATURE CITED

Anet, E. F. L. J. "Mechanism of Formation of 3-Deoxy-glycosuloses". *Tetrahedron Lett.* **1968**, *31*, 3525-3528.

Barbetti, P.; Chiappini, I. "Fractionation and Spectroscopic Characterization of Melanoidic Pigments from A Glucose-Glycine Non-Enzymatic Browning System". *Ann. Chim. (Rome)* **1976a**, *66*, 293-304.

Barbetti, P.; Chiappini, I. "Identification of the Furan Group In The Melanoidic Pigments from a Glucose-Glycine Maillard Reaction". *Ann. Chim. (Rome)* **1976b**, *66*, 485-488.

Benzing-Purdie, L.;f Ripmeester, J. A. "Melanoidins And Soil Organic Matter. Evidence of Strong Similarities by Carbon-13 CP-MAS NMR". *Soil Sci. Soc. Am. J.* **1983**, *47*, 56-61.

Bobbio, P. A.; Imasato, H.; Leite, S. R. "Maillard Reactions: Preparation and Characterization of Melanoidins From Glucose and Fructose With Glycine". *An. Acad. Bras. Cienc.* **1981**, *53*, 83-86; *Chem. Abstr.* **1981**, *95*, 62525v.

Danehy, J. P. "Maillard Reactions: Non Enzymatic Browning In Food Systems With Special Reference To The Development Of Flavor". *Adv. Food Res.* **1986**, *30*, 77-138.

Eriksson, C. *Progress in Food and Nutritional Science—Maillard Reactions in Food*; Pergamon: New York, 1982; and references therein.

Feather, M. S.; Russell, K. "Deuterium Incorporation during the Conversion of 1-Amino-1-deoxy-D-fructose Derivatives to 5-(Hydroxymethyl)-2-furaldehyde". *J. Org. Chem.* **1969**, *34*, 2650-2652.

Feather, M. S.; Nelson, D. "Maillard Polymers Derived from D-Glucose, D-Fructose, 5-(Hydroxymethyl)-2-furaldehyde, and Glycine and Methionine". *J. Agric. Food Chem.* **1984**, *32*, 1428-1432.

Feather, M. S.; Huang, R. D. "Maillard Polymers Derived From D-Glucose-1- and -6-^{14}C, Glycine-1- and -2-^{14}C, and, Methyl-^{14}C Methionine". *J. Carbohydr. Chem.* **1985**, *4*, 363-368.

Ghiron, A. F.; Quack, B.; Mawhinney, T. P.; Feather, M. S. "Studies on the Role of 3-Deoxy-D-*erythro*-glucosulose (3-Deoxyglucosone) in Nonenzymatic Browning. Evidence For Involvement In A Strecker Degradation". *J. Agric. Food Chem.* **1988**, following paper in this issue.

Imasato, H.; Leite, S. R.; Bobbio, P. A. "Maillard Reaction. VI. Structural Determinations In emalanoidins From Fructose and Glycine". *An. Acad. Bras. Cienc.* **1981**, *53*, 87-89; *Chem. Abstr.* **1981**, *95*, 62526w.

Kato, H. "Browning Reactions Between Sugars and Amino Compounds. V. Isolation and Characterization of New Carbonyl Compounds, 3-Deoxyosone Formed From N-Glycosides and Their Significance in the Browning Reaction." *Bull. Agric. Chem. Soc. Jpn.* **1960**, *24*, 1-12.

Ledl, F. "Formation of Colored Products In Browning Reactions: Reaction of Dihydroxy Acetone With Furfural". *Z. Lebensm.-Unters.-Forsch.* **1982a**, *175*, 203-207.

Ledl, F. "Formation of Colored Products In Browning Reactions: Reaction of Dihydroxy Acetone With Furfural". *Z. Lebensm.-Unters.-Forsch.* **1982b**, *175*, 349-352.

Ledl, F.; Severin, T. "Formation of Colored Compounds With Hexoses: Studies Relating to the Maillard Reaction". *Z. Lebensm.-Unters.-Forsch.* **1982**, *175*, 262-265.

Olsson, K.; Pernamalm, P. A.; Theander, O. *Progress in Food and Nutritional Science—Maillard Reactions In Food*; Eriksson, C., Ed.; Pergamon: New York, 1982; Vol. 5.

Roper, H.; Roper, S.; Heyns, K.; Meyer, B. "Preparation of Fructoseamino Acids (Amadori Compounds)". *Carbohydr. Res.* **1983**, *116*, 183-195.

Schonberg, A.; Moubacher, R. "The Strecker Degradation of α-Amino Acids". *Chem. Rev.* **1952**, *50*, 176-182.

Schonberg, A.; Moubacher, R.; Mostafa, A. "Degradation of α-Amino Acids to Aldehydes and Ketones by Interaction with Carbonyl Compounds". *J. Chem. Soc.* **1948**, 176-183.

Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic: New York, 1972.

Velisek, J.; Davidek, J. Scientific Papers of the Prague Institute of Chemical Technology, Prague, Czechoslovakia, 1976a, E46; *Food Chem. Abstrc.* **1977**, *87*, 51889a.

Velisek, J.; Davidek, J. Scientific Papers of the Prague Institute of Chemical Technology, Prague, Czecoslovakia, 1976b, E46; *Food. Chem. Abstr.* **1977**, *87*, 51888z.

Wolfrom, M. L.; Schlicht, R. C.; Langer, A. W.; Rooney, C. S. "Chemical Interactions of Amino Compounds and Sugars. 4. The Repeating Unit in Browning Polymers". *J. Am. Chem. Soc.* **1953**, *75*, 1031.