Formation of 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from Fructose and β-Alanine under Conditions Used for Baking

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The reactions of reducing sugars and amino acids were investigated by the heating of fructose or glucose with amino acids at 150 °C for 10 min as a model system for baking cookies. The products from the reaction were extracted with water and analyzed by HPLC. Fructose heated with β-alanine gave five nitrogen-containing compounds. One was identified as an Amadori compound (1-β-alanino-1-deoxyketose) by NMR and FAB-MS. The other three N compounds indicated the same molecular weight as that of the Amadori compound, *i.e.*, *m/z* 251. The three N compounds are assumed to be 1-formyl-2-β-alaninoglucose, 1-formyl-2-β-alaninomannose, and 1-(hydroxymethyl)-2-β-alanino-2-deoxyketose. One of the unidentified N compounds was converted into the Amadori compound through an intermediate by repeated HPLC analysis. It seems likely that a pathway existed in a part of the reaction from fructose and β-alanine to the Amadori compound; it is concluded that it is the intermediate 1-(hydroxymethyl)-2-β-alanino-2-deoxyhexose or 1-dihydro-1,2-di-β-alanino-1,2-deoxyhexose.

INTRODUCTION

Various chemical reactions occur during food processing. Particularly, the nonenzymatic reaction between amino acids and reducing sugars is most important during browning and flavor development (Hodge and Moser, 1961; Berry and Tatum 1965; Patton, 1950). Recently, this reaction has been postulated to explain age-related changes such as cross-linking and yellowing in long-lived proteins such as collagen and lens crystalline. For the past 80 years or so, glucose has been commonly used as a reducing sugar in these reactions, and numerous investigations have made clear the reaction mechanisms (Hodge, 1953; Njoroge and Monnier, 1989). It is generally understood that when glucose reacts with amino acids, the reaction passes through an Amadori compound such as a ketoamine-linked 1-deoxyhexose (Hodge and Rist, 1953). However, the reaction of fructose with amino acids has not yet been discussed (Suarez, 1989). The rearrangement from the reaction between product fructose and amino acids is known as the Heyns rearrangement, and it is thought that this participation leads to amino-linked adducts of structures analogous to glucose as "glucose–amino acid" (Heyns and Meinecke, 1953). However, it seems that pathways other than the Heyns rearrangement also exist in the reaction of fructose and amino acids (McPherson et al., 1988; Walton et al., 1989).

Fructose is often used with or without sucrose and glucose as a strong sweetener and as a source of favorable color in food processing or cooking. Fructose is also present as the hydrolysis product of sucrose in the processing. Given this situation, it is considered that the reaction pathway of fructose with amino acids or proteins in food is important.

In earlier studies on the reaction of sugars with protein or amino acids heated under the conditions of cookie baking (Nishibori and Kawakishi, 1988, 1990), we noted that 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP) was produced from both fructose and glucose with protein or amino acids. In this paper, the mechanism of formation of DDMP and related compounds from fructose heated with amino acids is investigated in the dry system as a model of the cookies, and the formation mechanism of DDMP is also compared with that from various Amadori compounds.

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EXPERIMENTAL PROCEDURES

Materials. D-Fructose, D-glucose, β-alanine, and *p*-toluidine were purchased from Wako Pure Chemicals (Osaka, Japan). N^{α}-(*tert*-Butoxycarbonyl)lysine (N^{α}-*t*-Boc-lysine) was obtained from Sigma Chemical Co. (St. Louis, MO). 1-Deoxy-1-β-alanino-D-fructose (fructose–β-alanine), 1-deoxy-1-*p*-toluidino-D-fructose (fructose–*p*-toluidine), and 1-deoxy-1-N^{α}-(*tert*-butoxycarbonyl)-lysino-D-fructose (fructose–N^{α}-*t*-Boc-lysine) were prepared according to the procedures of Anet (1957), Lowy and Borsook (1956), and Njoroge et al. (1988), respectively. Other reagents of the best grades were commercially available.

Preparations of Samples. One millimole each of fructose or glucose and β-alanine placed in test tubes was heated in a block bath at 150 °C for 4 min and then extracted with 1 mL of water. The extracts were clarified by filtration and submitted to HPLC.

One hundred milligrams of Amadori compounds, fructose–β-alanine, fructose–*p*-toluidine, and fructose–N^{α}-*t*-Boc-lysine, was placed in test tubes and heated in the same way.

High-Performance Liquid Chromatography (HPLC). Chromatographic analyses of products from the reaction of sugar and β-alanine were done by a Toyo Soda Model HLC-803D on a Develosil NH_2-5 column (250 × 4.6 mm i.d.). Samples were eluted at a rate of 1.5 mL/min with acetonitrile/water (7:3). The detection of elution was monitored continuously by refractive index (RI) and ultraviolet (UV) 210 nm. The analyses of DDMP from Amadori compounds through heating at 150 °C were performed on a Develosil ODS-5 column (250 × 4.6 mm i.d.). These samples were eluted at 0.8 mL/min with water/methanol (6:1). The elution was detected by UV 283 nm.

Isolation. FAC-1 and GAC in Figure 1 were isolated and purified repeatedly from the preparative HPLC by RI. Peaks 1 (P1), 2 (P2), 3 (P3), and 4 (P4) were detected by UV 210 nm.

Spectrometric Analyses. NMR spectra were measured by a JEOL GX-200 (^1H, 200 MHz; ^{13}C, 50 MHz) in D_2O as solvent. All samples were sealed in 4 mm i.d. NMR tubes. Fast atom

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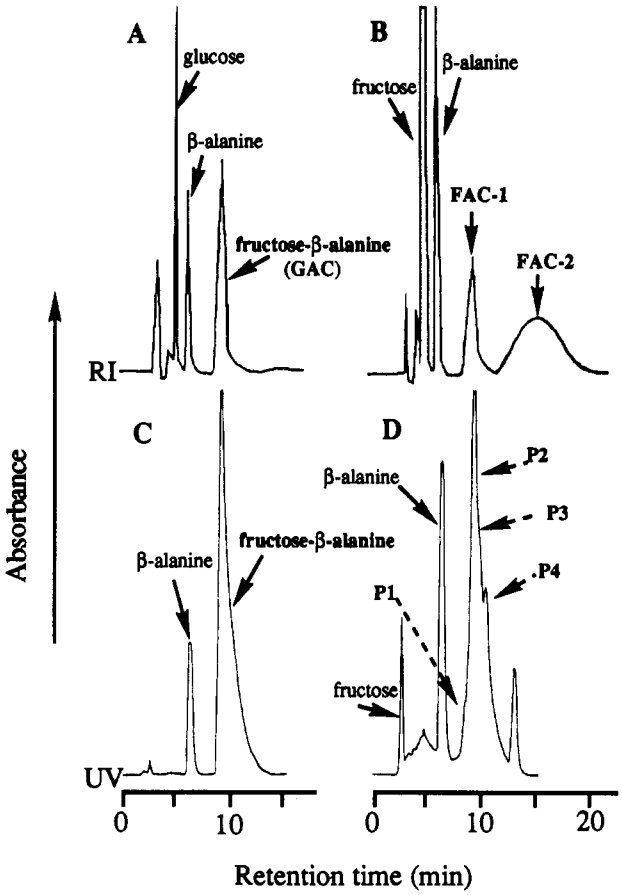

Figure 1. Chromatogram of the products from 1 mmol of either glucose (A and C) or fructose (B and D) with 1 mmol of β-alanine heated at 150 °C for 4 min. RI: column NH_2-5, solvent system $CNCH_3/H_2O$ (7:3 v/v), detector RI. UV: column ODS-5, solvent system $MeOH/H_2O$ (1:6 v/v), detector UV 210 nm.

bombardment mass (FAB-MS) spectral analysis was carried out on a JEOL DX-303 with glycerol as matrix.

RESULTS

Isolation of the Reaction Products of Sugars and Amino Acid and Their Identification. Figure 1 shows HPLC profiles of the water extracts from the reaction mixtures of fructose or glucose with β-alanine. In the reaction of fructose and β-alanine, two peaks of FAC-1 and FAC-2 were determined by RI. In the case of glucose, GAC was the only product.

On the detection by UV 210 nm, FAC-1 was further separated into four peaks, P1, P2, P3, and P4. From FAB-MS data of these peaks, the molecular weights of P1, P2, and P4 were identical with their molecular weights by FAB-MS data [m/z 252 ($M^+ + H$)]. FAC-2 was 251. These three peaks and FAC-2 suggested the existence of Heyns products from fructose and amino acids, that is, the Heyns rearrangement (McPherson et al., 1988; Suarez, 1989). HPLC analyses of P2 showed agreement with GAC for retention time. Also, P2 and GAC resembled each other in the chemical shift of their ^1H NMR data in D_2O. These coupling constants were further checked against those of D-fructose, D-glucose, and fructose–β-alanine. The ^1H NMR spectrum of P2 was found to accord with that of GAC; *i.e.*, the proton signals of the major pyranose component at 3.57–3.83 ppm, doublet of methylene proton adjacent to the nitrogen atom at 3.08 ppm, and triplet of two methylene protons derived from β-alanine at 2.37 and 3.07 ppm were assigned. Both P2 and GAC contained nine carbon atoms from ^{13}C NMR data in D_2O. The presence of four methylene groups (44.2, 59.3, 64.5, 73.3 ppm), three methine groups (79.6, 80.2, 80.6 ppm), one hemibetal carbon (108.3 ppm), and one carboxyl group (189.4 ppm) was assumed.

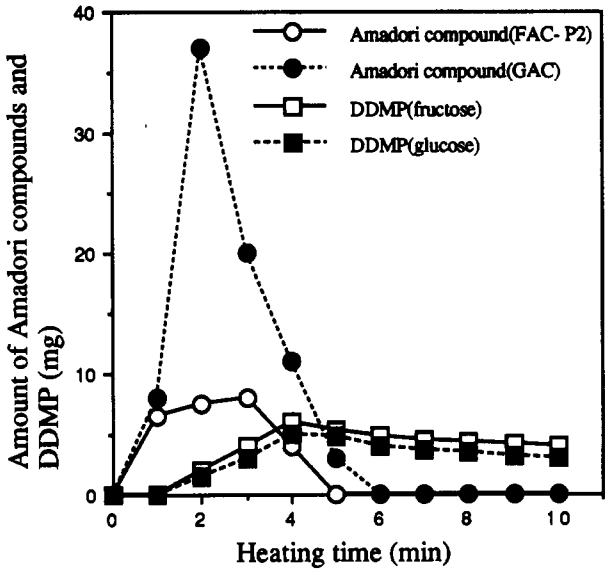

Figure 2. Formation of Amadori compounds and DDMP from 1 mmol of either glucose or fructose with 1 mmol of β-alanine heated at 150 °C for 10 min.

From these results, P2 and GAC were identified as fructose–β-alanine. Gallop et al. (1981) and Reynolds (1963) report that Amadori compounds in solution exist predominantly in the cyclic hemiketone acetal forms. The signal of one carbonyl group in this study was assigned a higher magnetic field (108.3 ppm) than that of the general carbonyl group (180–220 ppm). Fructose–β-alanine, that is, P2 and GAC, may form the cyclic hemiketone acetal configuration.

Formation of DDMP and Amadori Compounds from Sugars and β-Alanine. Figure 2 shows the relationship between the formation of Amadori compound (P2 and GAC) and DDMP in the reaction with reducing sugars and β-alanine heated at 150 °C. The formation of P2 and GAC reached its maximum for 2–3 min, and the formation of GAC was especially remarkable. Subsequently, their formation amounts rapidly decreased. DDMP increased during the first 4–5 min in the reaction of fructose or glucose heated with β-alanine. Moreover, the amount of DDMP from the fructose was slightly higher than that from glucose. When the heating time was longer than 5 min, DDMP formation from both sugars gradually decreased.

DDMP Formation from Other Amadori Compounds. Three kinds of Amadori compounds, fructose–β-alanine, fructose–*p*-toluidine, and fructose–N^{α}-*tert*-Boc-lysine, were prepared by the procedures set out in the literature (Anet, 1957; Lowy and Borsook, 1956; Njoroge et al., 1988). They were heated at 150 °C for 10 min, and the degradation products were analyzed by HPLC. The chromatograms are shown in Figure 3. In the case of all Amadori compounds, DDMP was produced in great quantities. Particularly, fructose–β-alanine and fructose–N^{α}-*tert*-Boc-lysine formed DDMP preferentially.

The formation amounts of DDMP and HMF generated from 1 mmol of fructose–β-alanine by heating are shown in Figure 4. Both DDMP and HMF were increased during heating, and their yields were 16 (1.6%) and 2 μmol (0.2%) at 10 min, respectively.

Effects of the Reaction Temperature on DDMP and HMF Generation from the Mixture of Fructose and β-Alanine. The mixture of fructose and β-alanine was heated at 100, 150, 200, and 250 °C for 10 min. DDMP

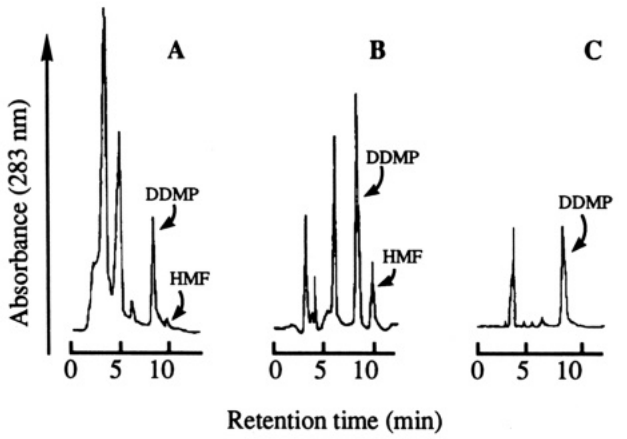

Figure 3. Formation of DDMP and HMF from Amadori compounds heated at 150 °C for 10 min using column ODS-5, solvent system MeOH/H_2O (1:6 v/v), and wavelength 283 nm. (A) Fructose–β-alanine; (B) fructose–*p*-toluidine; (C) fructose–N^{α}-*t*-Boc-lysine.

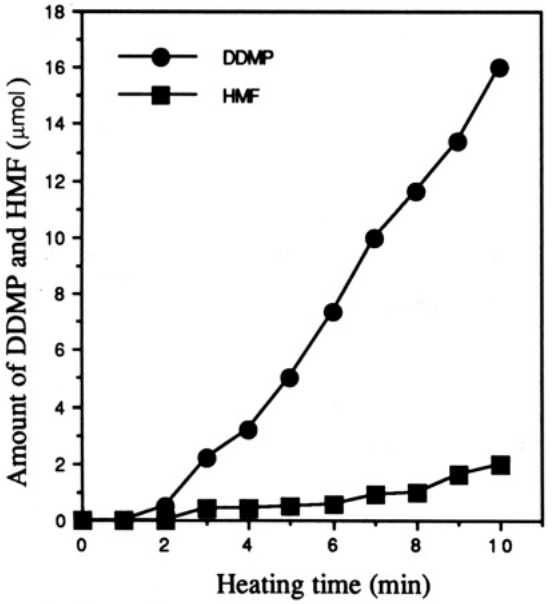

Figure 4. Formation of DDMP and HMF from 1 mmol of fructose–β-alanine heated at 150 °C for 10 min.

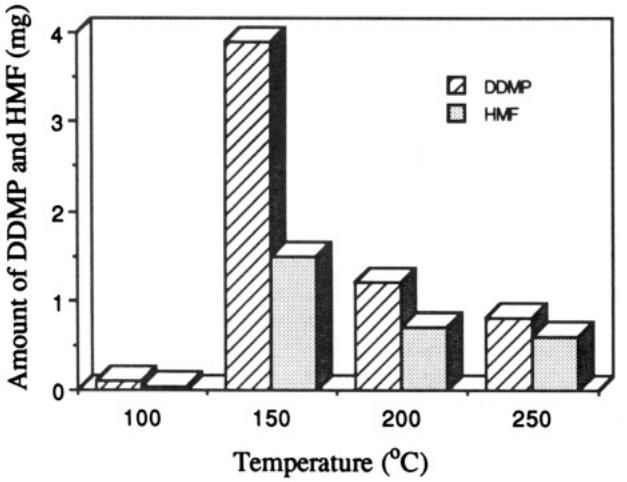

Figure 5. Formation of DDMP and HMF from the reaction of 1 mmol each of fructose and β-alanine heated at 100, 150, 200, and 250 °C for 10 min.

and HMF from the respective reaction mixtures were measured by HPLC as shown in Figure 5. Their formation amounts reached their highest levels at 150 °C compared with the other conditions. DDMP and HMF were hardly detected in the mixture heated at 100 °C.

DISCUSSION

On the nonenzymatic reaction between fructose and amino acids, the Heyns rearrangement (Heyns and Meinecke, 1953) is known and carbon 1 (C_1) of fructose participates mainly in this rearrangement. McPherson et al. (1988), however, suggest a pathway in which amino acid attaches directly to the C_1 of fructose under physiological conditions (Figure 6).

In this paper, we report that FAC-1 was detected from the reaction of fructose and β-alanine and that GAC from glucose and β-alanine is fructose–β-alanine, that is, an Amadori compound. As shown in Figure 1, FAC-1 was further separated into four peaks (P1, P2, P3, and P4) by UV detection, and the peaks P1, P2, and P4 revealed the same molecular weights [m/z 252 (M^{+1} + H)] by MS analyses. FAC-2 has only one peak. These results suggest there are four kinds of theoretical N compounds (**5–8**), including a Heyns product (**7**), as shown in Figure 6. We observed that FAC-2 (molecular weight 251) gradually transformed to the other product with a different retention time by repeated HPLC. The retention time of FAC-2 finally agreed with that of GAC (Amadori compound). These facts suggest a possible rearrangement from Heyns products to Amadori compound. Kitaoka and Onodera (1962) report the existence of a 1,2-diaminosugar in a solution of glucose reacted with amino acid, but we could not identify a 1,2-diaminosugar such as compound **4**. If an Amadori compound was generated through the pathway of Heyns products, a 1,2-diaminosugar should be detected as an intermediate of the reaction. From these results and our data, it is possible that fructose was transformed to compound **2** in the initial term and the aldehyde group of compound **2** reacted with the amino group of β-alanine. As a result of this reaction, fructose–β-alanine would be generated. In this system, P2 formation from fructose with β-alanine was a small amount, and it was 20% of GAC formation from glucose with β-alanine.

On the other hand, Ledl et al. (1985) propose that fructose rearranges into a glucose form, the so-called glucosylation. In the initial stage of the present study, it seemed that fructose easily isomerized to glucose via a 1,2-enediol form like compound **1** in Figure 6. An amino acid would react to C_1. However, Heyns and Breuer (1958) report that glucose was not detected among the reaction products between fructose and amino acids. It is possible that compound **2** exists in the pathway to Amadori compound formation from fructose as mentioned above.

There have been many studies over the past 40 years on the ring and the open-chain forms of Amadori compounds. In the present study, we found evidence from analytical data of ^{13}C NMR on fructose–β-alanine that the Amadori compound (Figure 6) may exist in the ring form. This is so because the carbonyl group of C_2 in fructose–β-alanine was detected at 108.3 ppm. If the Amadori compound exists in the open-chain form, signs of a carbonyl group must be observed at low magnetic field. The ring form structure may also facilitate getting DDMP from fructose and β-alanine compared to the open-chain form. The open-chain form of Amadori compounds might lead to 1,2-diaminosugar (Kitaoka and Onodera, 1962). In this study, DDMP was, in fact, preferentially generated, while the other compound was hardly detected.

DDMP formation was observed from the early stages of the heating of fructose and β-alanine, immediately after FAC-1 and FAC-2 formation. Furthermore, the formation

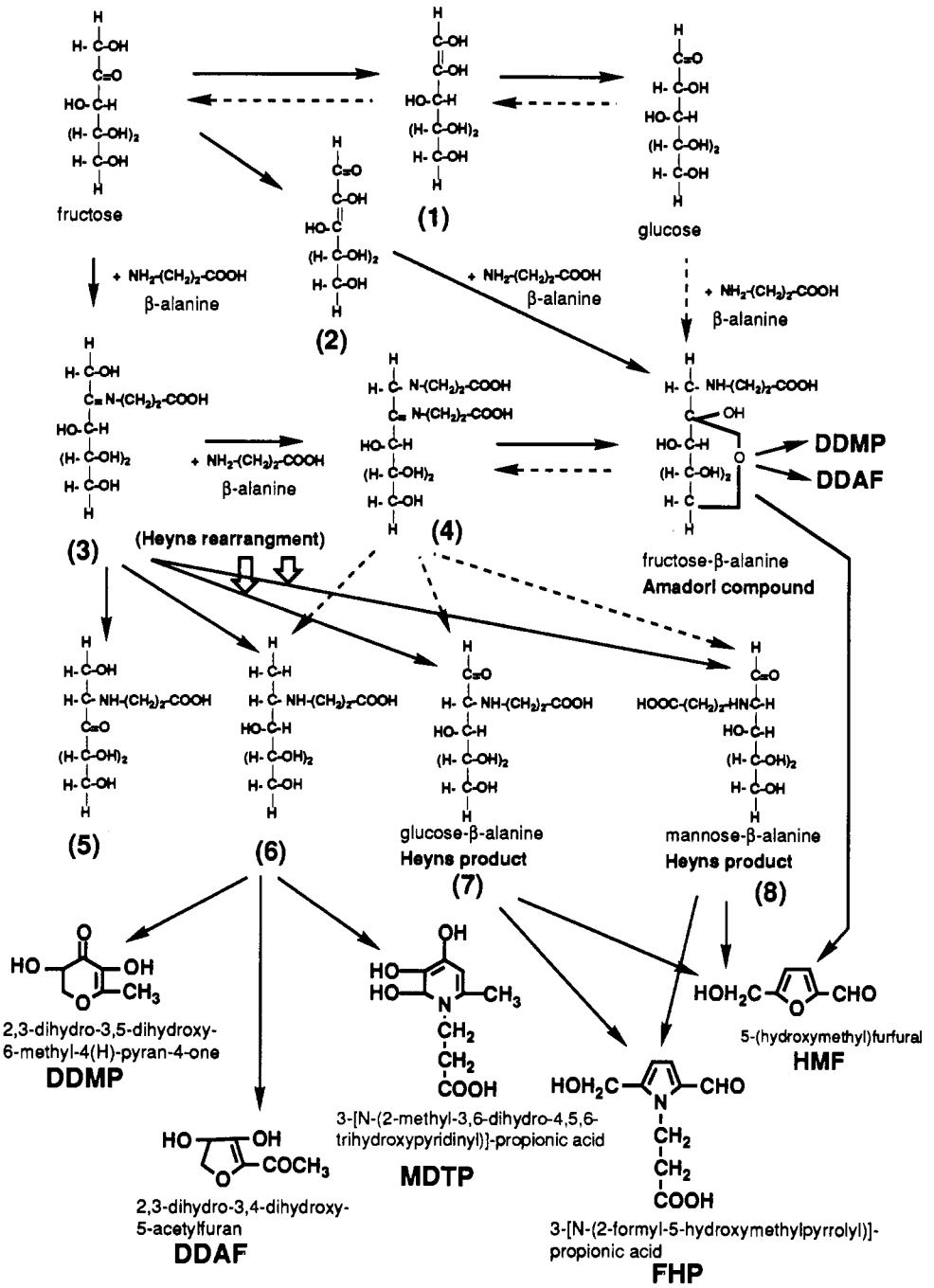

Figure 6. Possible formation pathway of five compounds via *N*-substituted compounds in the reaction of fructose and β-alanine.

of 2,3-dihydro-3,4-dihydroxy-5-acetylfuran (DDAF) (Nishibori and Kawakishi, 1991) also occurred from fructose and β-alanine in the initial stage of the reaction, *i.e.*, at 150 °C for 2 min. In this system, the formation of 3-[*N*-(2-methyl-3,6-dihydro-4,5,6-trihydroxylpyridinyl)]propionic acid (MDTP) by the heating of fructose and β-alanine for 4 min (Nishibori and Kawakishi, 1993) was confirmed. These results support the existence of compounds **6**.

Compounds **7** and **8** will lead to 3-[*N*-(2-formyl-5-(hydroxymethyl)pyrrolyl]propionic acid (FHP). Shaw and Berry (1977) attribute the formation of pyrroles and pyridinols in the Maillard reaction to a hypothetical path from the Amadori compounds via 3-deoxyosones. However, Nyhammer et al. (1983) demonstrate that pyrrole and pyridinols are formed more easily from 3-deoxyosone than from glucose by using [^{13}C]glucose. In this system, FHP was formed in large quantities from fructose heated with β-alanine over 4 min (Nishibori and Kawakishi, 1993). Compounds **7** and **8** may give FHP easily, rapidly, and directly via Heyns products (compound **3**).

DDMP was formed from the Amadori compounds by heating at 150 °C for 10 min as shown in Figure 3. The amount of DDMP formed from Amadori compounds was much larger than that of HMF. DDMP and HMF increased gradually as fructose–β-alanine was heated over a period of 10 min (Figure 4). The amount of DDMP was about 8 times that of HMF, that is to say, the amounts of DDMP and HMF formed were 16 and 2 μmol, respectively, from 1 mmol of fructose–β-alanine over the 10 min. However, DDMP was first detected 2 min after the heating of fructose–β-alanine was begun, while HMF was detected after 4 min. Beck et al. (1988) show that Amadori compounds are predominantly degraded to three deoxyosones which have the methyl group, hydroxymethyl group, and methylene group connected with the amino acid in C_1. The preferential formation of DDMP in this study supported the existence of the pathway via these deoxyosones.

HMF is also generated from hexose in the absence of protein, amino acids, and amines. Therefore, there was

a much greater generation of HMF from the reaction of fructose or glucose with β-alanine than from fructose–β-alanine. This result indicates that the HMF formation from the mixture of sugars and amino acids might be generated through the various pathways of sugar dehydration, Amadori compounds, and Heyns products:

[**pathway 1**] fructose → compound **2** → 3-deoxyglucoson (3-DG) → HMF

[**pathway 2**] fructose → compound **2** → Amadori compounds → 3-DG → HMF

[**pathway 3**] fructose → compound **3** → Heyns products → HMF

[**pathway 4**] fructose → compound **3** → compound **4** → Heyns products → HMF

[**pathway 5**] fructose → compound **1** → glucose → Amadori compounds → 3-DG → HMF

[**pathway 6**] glucose → Amadori compounds → 3-DG → HMF

Kato (1960) reported the existence of 3-DG in the pathway of HMF formation.

The effects of temperature on the DDMP formation are shown in Figure 5. In the reaction of fructose and β-alanine, DDMP produced by heating at 150 °C was much more than DDMP produced at 100, 200, and 250 °C. DDMP and HMF were scarce with heating at 100 °C, because fructose does not yet dissolve at 100 °C. The amounts of DDMP and HMF produced by heating at 200 and 250 °C were less than those of DDMP and HMF produced at 150 °C because the reaction proceeds rapidly as the heating temperature rises. DDMP is difficult to form by heating at 200 and 250 °C. Fructose may perfectly decompose to lower molecular weight compounds at the high temperature (Suarez, 1989) before Heyns or Amadori rearrangements arise, and then the small substance may form N compounds (Grandhee and Monnier, 1991) and melanoidins.

On the basis of these results, it has been proved that dry heating of fructose and β-alanine at 150 °C gives an Amadori compound and four N compounds (**5–8**) in which amino acids are attached to the C_2 of fructose. DDMP, MDTP, DDAF, and FHP may be formed easily via these N compounds. This condition, with its high temperature (150 °C) and dry state, is also the best for the formation of DDMP and DDAF with their desirable cookie flavor.

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