Mechanisms of Browning Degradation of D-Fructose in Special Comparison with D-Glucose-Glycine Reaction*

By Hiromichi KATO, Mitsuyoshi YAMAMOTO and Masao FUJIMAKI

Department of Agricultural Chemistry, The University of Tokyo, Tokyo
Received November 7, 1968

Degradation mechanisms of D-fructose by the interaction with amino acids or organic acids in aqueous solution at initial pH 5.5 heated at 100°C were investigated and a substantial difference in mechanisms between fructose degradation and glucose-glycine reaction was presented. D-Fructose browned more intensely than did D-glucose in lower concentration of glycine and/or in earlier stage of reaction period. By catalytic action of carboxylate anions without any condensation with amino groups, D-fructose was decomposed to 3-deoxy-D-erythrohexosulose, 5-(hydroxymethyl)-2-furaldehyde, and a less amount of pyruvaldehyde through caramelization. It was considered that the main path of fructose degradation was 1,2-enolization but 2,3-enolization would also occur to a little extent.

It is well known that reducing sugars, both aldoses and ketoses, easily decompose under strong acidic or alkaline condition. Kato and Sakurai previously described that, under moderately acidic condition at pH 2, D-fructose was degraded to 3-deoxy-D-glucosone (3-DG, 3-deoxy-D-erythrohexosulose) and further to 5-(hydroxymethyl)-2-furaldehyde (HMF) by caramelization, whereas D-glucose was considerably stable at pH 2.

At weakly acidic or neutral pH, the range of pH 3–7, reducing sugars are rather stable, but under the coexistence of amino compounds, aldoses cause nonenzymic browning, amino-carbonyl, or Maillard reaction. Amino-carbonyl reaction occurs in many foods and foodstuffs, because most of these materials encounter with the pH range of 3–7. Reaction of aldoses with amines or amino compounds has been widely investigated and much knowledge has been accumulated.

In the main course of the reaction, aldosylamines and then Amadori rearrangement products, 1-amino-1-deoxyketoses, are formed, and the latters decompose to 3-deoxyosones and other reactive carbonyl compounds, which cause browning through the reaction with amino compounds and polimerization.

In the reaction of ketoses with amines, ketosylamines and Heyns rearrangement products are formed. The reaction between D-fructose and amino acids gives 2-(N-amino acid)-2-deoxy-D-glucoses and -D-mannoses (glucose- and mannoseamino acids) and less amounts of 1-(N-amino acid)-1-deoxy-D-fruc-

---

* Presented in part at the Annual Meeting of the Agricultural Chemical Society of Japan, Nagoya, April 1–4, 1968.
5) idem., ibid., 15, 503 (1962).
toses (fructoseamino acids). However, aldoseamino acids are considered to be more stable than fructoseamino acids. For instance, the reducing properties of aldoseamino acids correspond to those of glucose, whereas fructoseamino acids show much stronger reducing properties. Various workers observed that the browning reactivity of D-fructose with amino acids was somewhat stronger than that of D-glucose, nevertheless the yields of aldoseamino acids and also of fructoseamino acids from D-fructose and amino acids in aqueous system were considerably less than those of fructoseamino acids from D-glucose and amino acids. These facts suggest that both aldoseamino acids and fructoseamino acids are not the main intermediates in the browning reaction of D-fructose. In other words, the browning mechanisms of fructose-amino acid system are difficult to be fully explained.

In the present work, browning or degradation mechanisms of D-fructose with or without amino acids in aqueous solution are investigated in special comparison with the case of D-glucose.

EXPERIMENTAL

Materials and reacting condition. Reducing sugars, amino acids, organic acids, and other reagents used were commercially available guaranteed reagents. 3-DG was prepared according to the method of Kato. These were dissolved in distilled and deionized water and heated in boiling water in flasks or testing tubes with glass stoppers. Buffer solution was not used to avoid its effect on browning reaction. Mixed solution of a sugar and glycine or L-valine showed initial pH 5.5. Accordingly, comparison experiments of effects on sugar degradation between amino acids and other compounds were made at initial pH 5.5 adjusted with sodium hydroxide. Browning rate was expressed by optical density (O.D.) at 470 mµ.

Isolation and identification of carbonyl compounds as their 2,4-dinitrophenylhydrazones (2,4–DNPs). After heating as above, the resulting browned solutions were cooled to room temperature and then excess 2,4-dinitrophenylhydrazine reagent was added. After 15 min, the precipitates of 2,4-DNPs formed were filtered with suction, washed with 2 N hydrochloric acid, water, and 50% ethanol successively, then dried.

The mixture of 2,4-DNPs obtained above was extracted with hot ethyl acetate and the insoluble substances were filtered off. The filtrate was adjusted to pH 5.5 with sodium hydroxide. The eluate was concentrated under reduced pressure. During concentration, 95% ethanol was added, then monohydrated crystals of 3-DG bis-2,4-DNP appeared. After repeating twice or thrice the addition of 95% ethanol, concentration was stopped at a volume of about 20 ml. The resulted crystalline precipitates were filtered, washed with ethanol, and dried, obtaining pure 3-DG bis-2,4-DNP monohydrate.

In case of the reaction between L-valine and reducing sugars or 3-DG, unadsorbed fraction of the above column chromatography was also investigated. The effluents and washings flowed out from the column were combined, concentrated, and dried up. The residue was dissolved in a small amount of tetrahydrofuran, mixed with acid-treated alumina (6 g), and dried. The resulted powder was placed on the top of a column which was packed in benzene. The column was developed with benzene, 2, 5, 10, and 30% ethyl acetate-containing benzene successively. The first eluted main yellow band gave isobutyraldehyde 2,4-DNP, the forth orange band gave pyruvaldehyde bis-2,4-DNP, and the sixth orange band gave HMF 2,4-DNP after evaporation, respectively.

Identification of mono- or bis-2,4-DNP isolated as their 2,4-dinitrophenylhydrazones (2,4–DNPs) by comparison with authentic samples. Side-products formed in the reaction of fructose with amino acids were identified by thin-layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS).

Mechanisms of Browning Degradation of D-Fructose 941

described above was accomplished by comparison of its infrared spectrum with that of authentic specimen, respectively. 3-DG bis-2,4-DNP monohydrate isolated from the reaction mixture of D-fructose with acetic acid at initial pH 5.5 (Table IV) was submitted to elementary analyses: Anal. Found: C, 40.35; H, 3.95; N, 20.25. Calcd. for C₁₆H₁₈N₈O₁₁·H₂O: C, 40.00; H, 3.73; N, 20.74%.

Approximate estimation of HMF. An approximate amount of HMF produced in the reacted solution was calculated from the difference between O.D. at \( \lambda_{\text{max}} \) and O.D. at \( \lambda_{\min} \) in the ultraviolet region. The browning solutions obtained in the present investigation revealed \( \lambda_{\text{max}} \) at 284–300 and \( \lambda_{\min} \) at 244–260 m\( \mu \).

RESULTS AND DISCUSSION

Effect of pH on browning reaction of D-fructose or D-glucose with glycine

Equimolar solutions of D-fructose or D-glucose and glycine (0.5 M each) adjusted to initial pH of range 2–8 were heated at 100°C for 1 hr. Optical densities (O.D.) at 470 m\( \mu \) of the resulted solutions were as shown in Fig. 1. In the case of glucose, browning was limited to a little extent at pH 2, although it favorably occurred over pH 3.5. It is considered that, in acidic solution, formation of N-glucoside, which is the first step of the reaction, is inhibited to a low level, on the other hand, in weakly acidic or neutral solution, both formation of N-glucoside and its Amadori rearrangement readily proceed to cause browning.14,15

In the case of fructose, differences in O.D. at pH range of 2–6.5 were not so large. As Kato and Sakurai13 previously reported, at pH 2, D-fructose was more unstable than D-glucose and decomposed to 3-DG and HMF by the effect of acidity, then caused browning. However, in weakly acidic or neutral solution (pH 3–7), as already mentioned, browning mechanisms of fructose-amino acid system remained to be solved, and the following several experiments were carried out at initial pH 5.5.

Browning rate of D-fructose or D-glucose by the interaction with glycine in function of concentration and reaction period

Equimolar solutions of D-fructose or D-glucose and glycine in concentration range of 0.05–2.0 M were heated at 100°C for 5 hr. The results were as shown in Fig. 2. In concentration of over 0.25 M each of sugar and glycine, O.D. at 470 m\( \mu \) increased linearly with concentration. In case of glucose, the rate of the increase was approximately proportional to the cube of concentration, whereas, in case of fructose, the rate was approximately proportional to the square of concentration. Reactivity of fructose was higher than that of glucose in lower concentration, while, in higher concentration, the relation became reverse.

To analyze such difference between fructose

FIG. 2. Browning Rate of Various Concentration of D-Glucose or D-Fructose by the Reaction with Equimolar Amount of Glycine.

Reacting condition: 100°C for 5 hr, at initial pH 5.5.

FIG. 3. Browning Rate of Various Concentration of D-Glucose or D-Fructose under the Presence of Large Excess Glycine (2 M).

Reacting condition: 100°C for 4 hr, at initial pH 5.5.

FIG. 4. Browning Rate of Reacting Solutions between Various Concentration of Glycine and Large Excess D-Glucose or D-Fructose (2 M).

Reacting condition: 100°C for 4 hr, at initial pH 5.5.

and glucose as described above, the following two experiments were carried out. Under the presence of large excess glycine (2.0 M), browning rate of fructose in concentration range of 0.02~1.0 M was measured, in which case the effect of glycine concentration would be able to be negated. As shown in Fig. 3, any obvious difference between the two reducing sugars was not observed and O.D. at 470 mµ increased approximately in proportion to sugar concentration in both cases.

Next, contrary to the above experiment, under the presence of large excess fructose or glucose (2.0 M), browning rate by its reaction with various concentrations of glycine (0.02~1.0 M) was determined, in which case the effect of sugar concentration would be eliminated. The result of Fig. 4 indicated a clear difference between the two reducing sugars: that is, in case of glucose, O.D. at 470 mµ increased approximately in proportion to the square of glycine concentration when it was over 0.1 M, whereas, in case of fructose,
Mechanisms of Browning Degradation of D-Fructose

O.D. increased in proportion to glycine concentration. Reactivity of fructose was stronger than that of glucose when glycine concentration was below 0.4 M, on the contrary, in higher concentration of glycine, glucose browned more intensely than did fructose similarly as the result in Fig. 2. Thus, the difference between fructose and glucose observed in Fig. 2 was shown to be caused by the concentration of glycine. However, when glycine concentration was below 0.1 M, O.D. of glucose-glycine system was proportional to glycine concentration similarly as O.D. of fructose-glycine system (Fig. 4).

Another important factor which causes difference of browning rates between fructose and glucose is reaction period. Some investigators compared the browning reactivities of D-fructose and D-glucose with amino acids and described complicated results. In general, during earlier period of reaction, the color values for fructose were more intense than those for glucose, but after longer period, the values for glucose exceeded those for fructose.

Equimolar solutions of glucose or fructose and glycine (1 M each) were heated at 100°C at various intervals and the results were as shown in Fig. 5. Fructose browned more intensely than did glucose until 2 hr, but after 2 hr, glucose exceeded fructose in accordance with the results of other workers.

As observed in Fig. 5, in case of glucose-glycine system, an induction period was clear and O.D. increased approximately in proportion to the square of reaction time, whereas O.D. of fructose-glycine system increased almost linearly with reaction time without any induction period.

These results described above indicated an important and substantial difference between browning mechanism of glucose and that of fructose. Haugaard et al. studied the kinetics of browning reaction between D-glucose and glycine and showed that melanoidin concentration, $C_M$, could be written as

$$ C_M = k \times C_A^3 \times C_G \times t^2 \quad (1) $$

where $C_A$ was amino acid or glycine concentration, $C_G$ was glucose concentration, and $t$ was reaction time. The results obtained in the present investigation were also in a close agreement with the formula (1). On the other hand, from the results in Figs. 2~5, the concentration of melanoids produced by the reaction of fructose with glycine, $C_M'$, can be written as

$$ C_M' = k' \times C_A \times C_F \times t \quad (2) $$

where $C_F$ is fructose concentration.

The formula (1) would express that a rate-limiting intermediate such as glucosylamino acid or fructoseamino acid, of which formation

---

18) H. S. Burton, D. J. McWeeny and D. O. Biltcliffe, Chem & Ind., 1963, 693.
should depend on the concentrations of both glucose and amino acid, should be required for melanoidin formation, and that reactive compounds such as carbonyls formed by degradation of the above intermediate should react again with amino acid to produce melanoidins. In case of fructose, the formula (2), which is simpler than formula (1), would express that any rate-limiting intermediate should not be required. Accordingly, fructosylglycine, glucose-, mannose- and fructose-glycine, of which formations depend on the concentrations of both fructose and glycine, are not considered to be important intermediates for melanoidin formation. The relations described above would be simply expressed as

$$\begin{align*}
C_G & \rightarrow \text{[Rate-limiting]} \rightarrow C_A \\
& \downarrow \text{intermediate} \\
C_A & \rightarrow C_M
\end{align*}$$

for the reaction of glucose with amino acids, and

$$\begin{align*}
C_A & \rightarrow C_M' \\
C_F & \rightarrow C_M
\end{align*}$$

for the reaction of fructose with amino acids.

Generally speaking, the results in Figs. 2-5 and also formulas (1) and (2) would show that fructose should brown more intensively than glucose in lower concentration of amino acids and/or in earlier stage of reaction period, but glucose should be more reactive than fructose in higher concentration of amino acids and/or after longer reaction period. From this point of view, the important roles of fructose in early stage of discoloration in foods should be considered.

Effects of carboxylate anions on the browning degradation of D-fructose or D-glucose with or without glycine

The formula (2) described above suggested that fructose would by itself decompose to such reactive compounds as carbonyls without any direct combination or condensation with amino groups. Accordingly, effects of various acids on the browning of fructose were examined. Fructose or glucose solution (2M) was heated at 100°C under the presence of acid (2N) partly neutralized to initial pH 5.5 with sodium hydroxide. O.D. at 470 mμ and precipitates of 2,4-dinitrophenylhydrazones (2,4-DNPs) obtained from the reacted solutions were determined (Table I). Hydrochloric acid partly neutralized to pH 5.5 was almost uneffective for degradations of both glucose and fructose. However, organic acids such as acetic, citric and malic, and phosphoric acid adjusted to pH 5.5 were effective especially for fructose degradation. Their effects on glucose were observed only to a little extent. The result of Table I showed that carboxylate and phosphate anions catalyzed the decomposition of fructose into carbonyls and that amino groups were not essential for it.

Next, the effects of various anions on the browning of sugar-glycine system were examined (Table II). Similar effects of carboxylate and phosphate anions were observed in fructose-glycine system, and also stronger effects of them were revealed in glucose

<table>
<thead>
<tr>
<th>Acid</th>
<th>D-Glucose*</th>
<th>D-Fructose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>0.032</td>
<td>0.052</td>
</tr>
<tr>
<td>AcOH</td>
<td>0.64</td>
<td>16.3</td>
</tr>
<tr>
<td>H₂PO₄</td>
<td>1.11</td>
<td>14.2</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.72</td>
<td>7.2</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.45</td>
<td>10.5</td>
</tr>
<tr>
<td>Glycine (2M)</td>
<td>180</td>
<td>54</td>
</tr>
</tbody>
</table>

* Aqueous solution of sugar (18 g, 2 M) and acid adjusted to initial pH 5.5 with NaOH was heated at 100°C for 4 hr.

** Resulted solution after heating was added to a reagent of 2,4-dinitrophenylhydrazine, stirred, centrifuged, washed, and dried.
glycine system. In case of the latter, it would be considered that Amadori rearrangement of glucosylglycine and decomposition of fructoseglycine were accelerated by catalytic action of the anions. As obvious in Table II, the effects of these anions were much stronger than the effect of the same molar amount of glycine of which carboxyl group would act in same way. Any synergistic effect among these carboxylate and phosphate anions was not observed.

Effects of acetic acid on the browning of sugar-glycine systems were compared at initial pH 4.0 and 5.5. As shown in Table III, the effect of acetic acid was stronger at pH 5.5 than at pH 4.0, suggesting that acetate anion would be an active form as catalyst, and further the effect of 0.2 M of acetic acid almost corresponded to that of 1 M of glycine.

Many workers mentioned the accelerating effect of phosphates and other buffers containing carboxylate anions on amino-carbonyl reaction or on sugar decomposition. Hodge, in his review, concluded that organic acids and their salts accelerated the caramelization of sugars by promoting enolization of the sugar. Isbell and Frush suggested the mechanisms accounted for the effectiveness of carboxylic acids and methylenic compounds in promoting Amadori rearrangement, which involved the interaction between imonium ion of N-glycoside and carboxylate anion. The quite analogous mechanism can be applied for enolization of fructose as written in Fig. 6. A similar scheme can be written also for enolization of glucose. This mechanism requires the formation of acyclic cation or carbonyl form of the reducing sugar. The amount of al-

Table II. Effects of Various Acids on the Browning of Sugar-Glycine System

<table>
<thead>
<tr>
<th>Acids used and their concentration</th>
<th>D-Glucose</th>
<th>D-Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.430</td>
<td>0.838</td>
</tr>
<tr>
<td>HCl 0.1 N</td>
<td>0.407</td>
<td>0.865</td>
</tr>
<tr>
<td>Glycine 0.1 M</td>
<td>0.459</td>
<td>1.25</td>
</tr>
<tr>
<td>AcOH 0.1 M</td>
<td>1.50</td>
<td>1.61</td>
</tr>
<tr>
<td>Citric acid 0.1 N</td>
<td>2.06</td>
<td>1.81</td>
</tr>
<tr>
<td>AcOH 0.05 M + Citric acid 0.05 N</td>
<td>1.82</td>
<td>1.61</td>
</tr>
<tr>
<td>H2PO4 0.1 N</td>
<td>0.961</td>
<td>1.39</td>
</tr>
<tr>
<td>AcOH 0.05 M + H2PO4 0.05 N</td>
<td>1.21</td>
<td>1.62</td>
</tr>
<tr>
<td>HCl 0.5 N</td>
<td>0.349</td>
<td>0.927</td>
</tr>
<tr>
<td>Glycine 0.5 M</td>
<td>1.62</td>
<td>2.32</td>
</tr>
<tr>
<td>AcOH 0.5 M</td>
<td>6.67</td>
<td>3.32</td>
</tr>
</tbody>
</table>

a) Solution of glucose or fructose and glycine (0.5 M each) with the indicated acids adjusted to initial pH 5.5 with NaOH was heated at 100°C for 2 hr.
b) For the comparison with acids, additional glycine was added to the solution of sugar and glycine (0.5 M each).

table III. Effects of Acetic Acid at Different pH on the Browning of Sugar-Glycine System

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Catalyst added</th>
<th>D-Glucose</th>
<th>D-Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>HCl b</td>
<td>5.77</td>
<td>7.71</td>
</tr>
<tr>
<td></td>
<td>Acetic acid, 0.2 M c</td>
<td>11.2</td>
<td>8.54</td>
</tr>
<tr>
<td>5.5</td>
<td>None</td>
<td>13.1</td>
<td>8.61</td>
</tr>
<tr>
<td></td>
<td>Acetic acid, 0.2 M c</td>
<td>49.3</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>Glycine, 1 M</td>
<td>52.2</td>
<td>26.4</td>
</tr>
</tbody>
</table>

a) Solution of D-glucose or D-fructose and glycine (1 M each) with catalyst was heated at 100°C for 4 hr.
b) Acidified to pH 4.0 with hydrochloric acid for comparison with acetic acid.
c) Addition of acetic acid (0.2 M) to the above sugar-glycine solution resulted to pH 4.0. The mixture was partly neutralized to pH 5.5 with NaOH.

Fig. 6. Proposed Mechanism for Effect of Carboxylic Acids in Enolization of Reducing Sugars.

dehyde form in glucose solution is very small\(^{24}\) owing to the high stability of glucopyranose structure,\(^{25}\) whereas the amount of keto form in fructose solution is considered to be larger because of the rapid mutarotation of D-fructose\(^{26}\) and also because of the low stability of fructopyranose or -furanose structure. Accordingly, as shown in Table I, the effect of carboxylic acids is weak on glucose, but is much stronger on fructose. Browning reaction between fructose and malic acid reported by Livingston\(^{27}\) is considered to involve a similar mechanism. On the other hand, Mednick\(^{28}\) reported that, at high temperature over 170°C, D-glucose was decomposed to HMF as well as sucrose by acidic and/or basic catalysts. Under such drastic condition, considerable amounts of glucose would change into aldehyde form and then glucose degradation would also readily proceed. The effect of phosphates on sugars is also considered to be partly explained by the same mechanisms as described above.

**Formation of 3-DG and HMF from D-fructose or D-glucose by the reaction with glycine or acetic acid**

In order to obtain some chemical evidences in fructose degradation, carbonyl compounds formed by various browning reactions were investigated by conversion into their 2,4-DNPs followed by separation on column chromatography. HMF was approximately estimated from ultraviolet absorption.

Fructose or glucose was heated with acetic acid at initial pH 5.5 in 2M concentration of each reactant and formation rate of 3-DG and HMF was compared with the case of sugar-glycine reaction. The result in Table IV showed that fructose was considerably degraded to 3-DG and HMF by the action of acetate to cause browning. In case of fructose-glycine system, less amounts of 3-DG and HMF were detected (Table IV). It would be considered that these carbonyls reacted with glycine and caused more intense browning to reduce their amounts. The formation of 3-DG and HMF from fructose indicated that 1,2-enolization was the main path of fructose degradation under the condition given above, because both 3-DG and HMF had been considered to be formed from 1,2-enediol of hexoses (Fig. 7).\(^{1,29,30}\)

**Formation of isobutyraldehyde, pyruvaldehyde, 3-DG and HMF by the interaction between reducing sugars and L-valine**

Two molar concentration of D-glucose, D-fructose, or L-sorbose was reacted with 0.5M of L-valine in a 100ml volume at 100°C for 3hr. The yields of isobutyraldehyde, the

---


---

**Table IV. Optical Density and Formation of 3-DG and HMF during Browning Reaction of D-Glucose or D-Fructose with Glycine or Acetic Acid**

<table>
<thead>
<tr>
<th>Reacting substances*</th>
<th>O.D. at 470 m(\mu)</th>
<th>2,4-DNPs of carbonyls (mg)</th>
<th>HMFb (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc (36 g)+Gly (15 g)</td>
<td>25.6</td>
<td>654</td>
<td>292</td>
</tr>
<tr>
<td>Fru (36 g)+Gly (15 g)</td>
<td>10.8</td>
<td>162</td>
<td>48</td>
</tr>
<tr>
<td>Glc (36 g)+AcOH (12 g)</td>
<td>0.04</td>
<td>33</td>
<td>1.9</td>
</tr>
<tr>
<td>Fru (36 g)+AcOH (12 g)</td>
<td>3.71</td>
<td>570</td>
<td>298</td>
</tr>
</tbody>
</table>

a Sugar and glycine or acetic acid were dissolved in water and, in case of acetic acid, the solution was adjusted to pH 5.5 with NaOH, and then filled up to 100ml (2M each) and heated in boiling water for 1 hr in case of glycine and for 2 hr in case of acetic acid.

b Approximate estimation calculated from ultraviolet absorption.
Mechanisms of Browning Degradation of D-Fructose

TABLE V. YIELDS OF CARBONYL COMPOUNDS PRODUCED BY BROWNING REACTION OF REDUCING SUGAR OR 3-DG WITH L-VALINE

<table>
<thead>
<tr>
<th>Reacting substancesa</th>
<th>Filled up to O.D. at 470 m(\mu)</th>
<th>2,4-DNPs of carbyonyls (mg)</th>
<th>HMFb</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose (36 g)+L-Val (5.86 g)</td>
<td>100 ml 8.33</td>
<td>193 (3)c 905</td>
<td>73.9</td>
</tr>
<tr>
<td>D-Fructose (36 g)+</td>
<td>&quot; 3.87</td>
<td>92 12 210</td>
<td>32.7</td>
</tr>
<tr>
<td>L-Sorbose (36 g)+</td>
<td>&quot; 7.61</td>
<td>152 6 224</td>
<td>68.1</td>
</tr>
<tr>
<td>3-DG (2 g)+L-Val (2.93 g)</td>
<td>50 ml 23.6</td>
<td>158 0 102</td>
<td>60.7</td>
</tr>
</tbody>
</table>

a Aqueous solution (initial pH 5.5) of reducing sugar (2 M) or 3-DG and L-valine (0.5 M) was heated in boiling water for 3 hr.
b Approximate estimation calculated from ultraviolet absorption.
c This fraction contained impurities, although the thin layer chromatogram showed a spot of pyruvaldehyde bis-2,4-DNP.

Fig. 7. Degradation of D-Fructose Heated at 100°C and at Initial pH 5.5.

One-less carbon aldehyde from valine by Strecker degradation, pyruvaldehyde, 3-DG and HMF were as shown in Table V. Two ketoses, fructose and sorbose, produced a little amount of pyruvaldehyde. However, glucose produced only lesser amount of it in spite of formation of a larger amount of 3-DG. In relation to formation mechanism of pyruvaldehyde from sugars, 3-DG was reacted with L-valine in aqueous solution (pH 5.5) at 100°C, but any formation of pyruvaldehyde was not observed (Table V). The facility of pyruvaldehyde formation from ketoses and the lack of the formation from 3-DG suggest that pyruvaldehyde is formed via 2,3-enolization or 2,3-enediol of ketoses, although the detail path is not known. As already mentioned, the main path of fructose degradation is considered to be 1,2-enolization, but it is natural that 2,3-enolization also occurs in some extent. The relations described above are summarized in Fig. 7. In case of the reaction of glucose with valine (Table V), it is speculated that 2,3-enolization of 1-deoxy-l-valino-fructose may cause pyruvaldehyde formation. However, the lack of pyruvaldehyde formation from 3-DG under such condition as heating at 100°C and at initial pH 5.5 could not deny the possibility of aldol cleavage of 3-DG into pyruvaldehyde at
higher temperature or at larger pH than 5.5.\textsuperscript{31}

Reactivity of pyruvaldehyde in Strecker degradation was known to be much stronger than that of 3-DG,\textsuperscript{32} because of hemiacetal ring formation of 3-DG\textsuperscript{29} which would reduce the reactivity with amino groups. Casey \textit{et al.}\textsuperscript{33} reported that fructose (0.1 M) produced larger amounts of low-boiling aldehydes from amino acids (0.01 M) than did glucose when heated for 30 min at 110°C. This stronger reactivity of fructose might depend on 2,3-enolization and pyruvaldehyde formation.