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Formation of Deoxyfructosazine and Its 6-Isomer by the Browning Reaction between Fructose and Ammonium Formate

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2 - (D - *arabino* - 1′,2′,3′,4 - Tetrahydroxybutyl) - 5 - (D - *erythro* - 2″,3″,4″ - trihydroxybutyl)pyrazine ("Deoxyfructosazine") and 2-(D-*arabino*-1′,2′,3′,4′-tetrahydroxybutyl)-6-(D-*erythro*-2″,3″,4″-trihydroxybutyl)pyrazine (6-isomer) have been isolated from the browning mixture of fructose and ammonium formate. Especially, deoxyfructosazine was obtained in a considerable high yield, but its 6-isomer was formed only in a very low yield. In the reaction of glucose and ammonium formate, on the contrary, the 6-isomer was predominantly formed. Formation of these compounds was also dependent on the concentration of ammonium formate, and under appropriate conditions the yields of these compounds amounted to 0.38～0.42 g per g of the original sugars.

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The formation of 2,5-bis-(D-*arabino*-tetrahydroxybutyl)pyrazine ("Fructosazine") from the reaction of fructose or glucose with alcoholic ammonia,[1] and from self-condensation of glucosamine[2] or isoglucosamine,[3] has been known for a long time. On the other hand, Kuhn *et al.*[4] found that the main product of the self-condensation of glucosamine in acetic acid is not fructosazine but deoxyfructosazine. Although Jezo and Luzak[5] assumed formation of deoxyfructosazine via isoglucosamine (Amadori product)[6] or glucosamine (Heyns product[7]) by ammonolysis of sucrose, they could not prove its actual presence.

Recently, the authors[8] isolated deoxyfructosazine, its 6-isomer and fructosazine from the mixture obtained by the reaction of glucose with ammonia under the preparation-condition (pH 5.3～6.0) of ammonia-caramel,[10] and found that the amount of deoxyfructosazine and its 6-isomer was incomparably larger than that of fructosazine. Hence, it was inferred that deoxyfructosazine and its 6-isomer would be formed by condensation of 3-deoxyglucosone, isoglucosamine and ammonia. Consideration of the formation mechanism suggests that the fructose-ammonium formate reaction system also will give the two compounds by the condensation of glucosamine (Heyns product[7]), 3-deoxyglucosone[9] and ammonia.

This paper deals with isolation and identification of these compounds expected in fructose-ammonium salt reaction system and quantitative comparison of these compounds between the above reaction system and glucose-ammonium salt reaction system.

METHODS

Melting points are uncorrected. Ultraviolet spectra were recorded with a Hitachi recording spectrometer Model EPS–2. Nuclear magnetic resonance spectra were recroded with a JNM NMR spectrometer Model PS–100 in deuteriochloroform with tetramethylsilane as internal standard.

Elution pattern of fructose-ammonium formate and glucose-ammonium formate reaction mixture on Dowex 50 W×4 column (H^+ form, 200～400 mesh, 1.2×27.0 cm). Fructose (0.9 g, 5 mmoles) and ammonium formate (2.2 g, 35 mmoles) were dissolved in water (2.5 ml), and heated at 100°C for 30 min, and then evaporated to dryness under reduced pressure. Also, by the same procedure, a glucose-ammonium formate reaction mixture was prepared, and evaporated to dryness. The fructose-ammonium formate and glucose-ammonium formate reaction mixtures weighed 3.01 g and 3.17 g, respectively, when dried. The former (56 mg) and the latter (76 mg) were applied on Dowex 50×4 columns (H^+ form, 200～400 mesh, 1.2×27.0 cm), and eluted with deionized water (600 ml).

The eluates were fractionated into 5 ml portions, and the optical density of each portion was determined at 275 nm. The prepared elution patterns of these reaction mixtures are shown in Figs. 1 and 2. Each portion represented by a peak in Fig. 1 was also examined by paper chromatography.

Isolation of Compounds I and II from the fructose-ammonium formate reaction mixture. Fructose (9.0 g) and ammonium formate (15.75 g) were dissolved in 25 ml of water and heated at 100°C for 30 min. The reaction mixture was applied on an Amberlite IRC–50 column (H^+ form, 200 ml), and eluted with deionized water (4500 ml). The first 500 ml of the eluate was discarded, and the successive eluate (3800 ml) was evaporated to sirup. The sirup was applied on a Dowex 50 W×4 column (H^+ form, 100~200 mesh, 400 ml) and eluted with deionized water (8000 ml). The eluate was fractionated into 10 ml portions, and fractions Nos. 307~367 and Nos. 441~695 (respectively containing Compounds I and II) were separately combined and evaporated to sirup. The two compounds were crystallized from water-ethanol, and obtained in 24.7% and 1.9% yields, respectively. Each crystal was twice recrystallized from the same solvent.

Quantitative determination of deoxyfructosazine and its 6-isomer formed in reaction mixture. In accordence with the reaction condition (at 100°C, for 30 min) described above, the reaction mixtures differing in molar ratio of ammonium formate to fructose or glucose as shown in Table I were prepared, and diluted to 10 ml. Zero point two to one ml aliquots of each diluted solution were analyzed by column chromatography with the same ionexchanger as described in Fig. 1. The amounts of deoxyfructosazine and its 6-isomer in the reaction mixtures were calculated from both the obtained elution patterns and the calibration curve of authentic deoxyfructosazine (Fig. 3).

TABLE I. COMPOSITION RATIO OF FRUCTOSE OR GLUCOSE TO AMMONIUM FORMATE IN THE REACTION SYSTEM

Mole ratio	Fructose or glucose (g)	Ammonium formate (g)	H_2O (ml)
1/ 1	0.9	0.315	2.5
1/ 3	0.9	0.938	2.5
1/ 5	0.9	1.575	2.5
1/ 7	0.9	2.205	2.5
1/10	0.9	3.150	2.5
1/15	0.9	4.725	2.5

RESULTS AND DISSCUSION

Firstly, the elution pattern of the fructose-ammonium formate reaction mixture on a Dowex 50 W×4 column was compared with that of the glucose-ammonium formate reaction mixture (Figs. 1 and 2). Compounds I and II in the fructose-ammonium formate system (Fig. 1) were respectively eluted in equal elution volumes to those of deoxyfructosazine and its 6-isomer in the glucose-

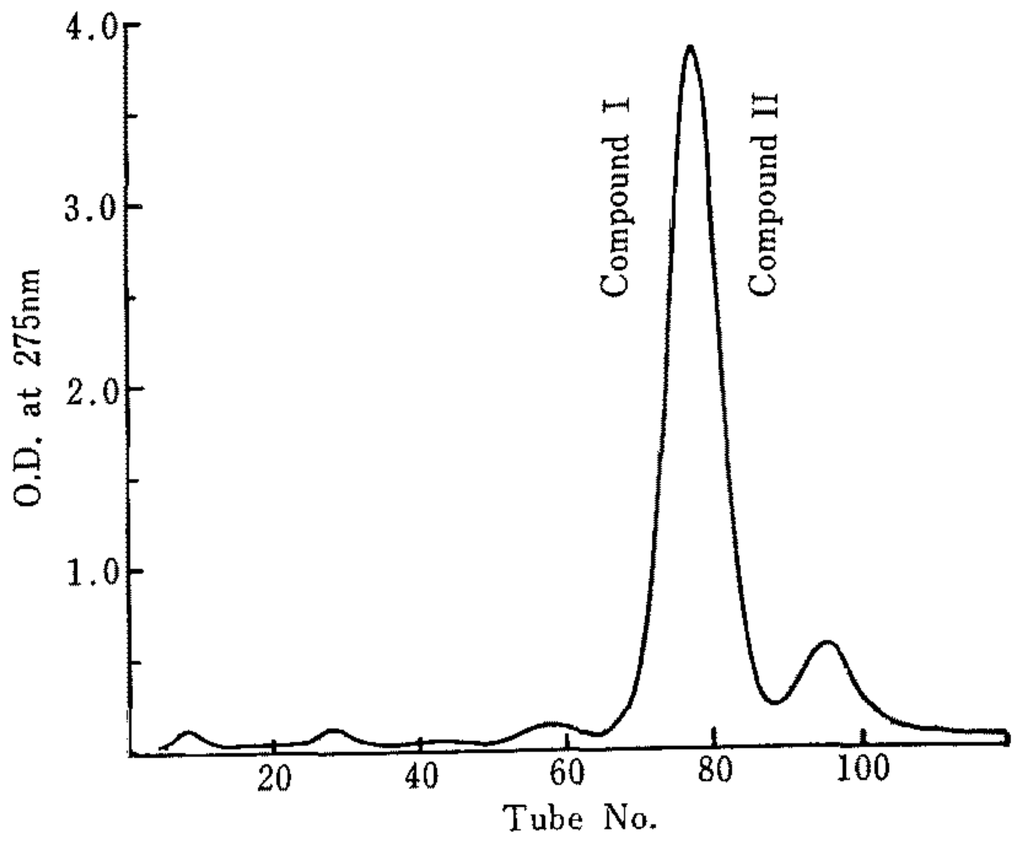

FIG. 1. Elution Pattern of Fructose-Ammonium Formate Reaction Mixture on Dowex 50 W×4 Column (H^+ form, 200~400 mesh, 1.2×27.0 cm)

The reaction mixture was evaporated to dryness, and 56 mg of the dried sample was dissolved in 0.5 ml of water. The solution was applied on the Dowex 50 W×4 column, and eluted with deionized water (600 ml). The eluate was fractionated into 5 ml portions.

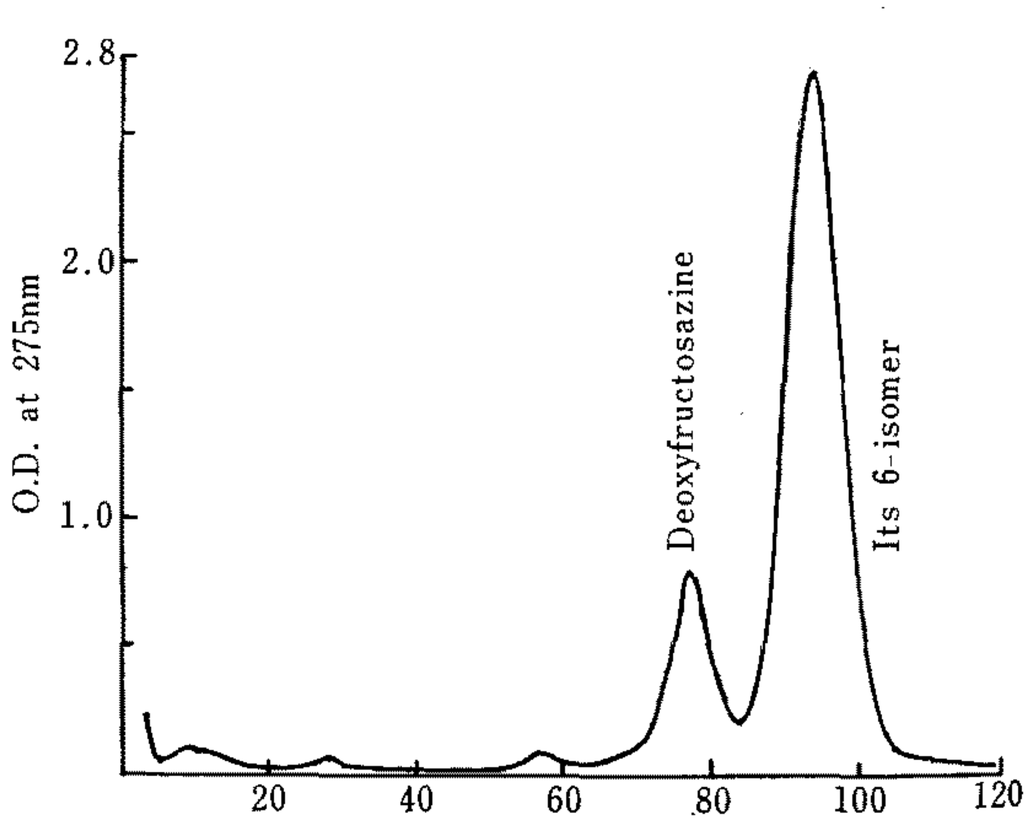

FIG. 2. Elution Pattern of Glucose-Ammonium Formate Reaction Mixture on Dowex 50 W×4 Column (H^+ form, 200~400 mesh, 1.2×27.0 cm)

The reaction mixture was evaporated to dryness, and 76 mg of the dried sample was dissolved in 0.5 ml of water. The solution was chromatographed by the same procedure as described in Fig. 1.

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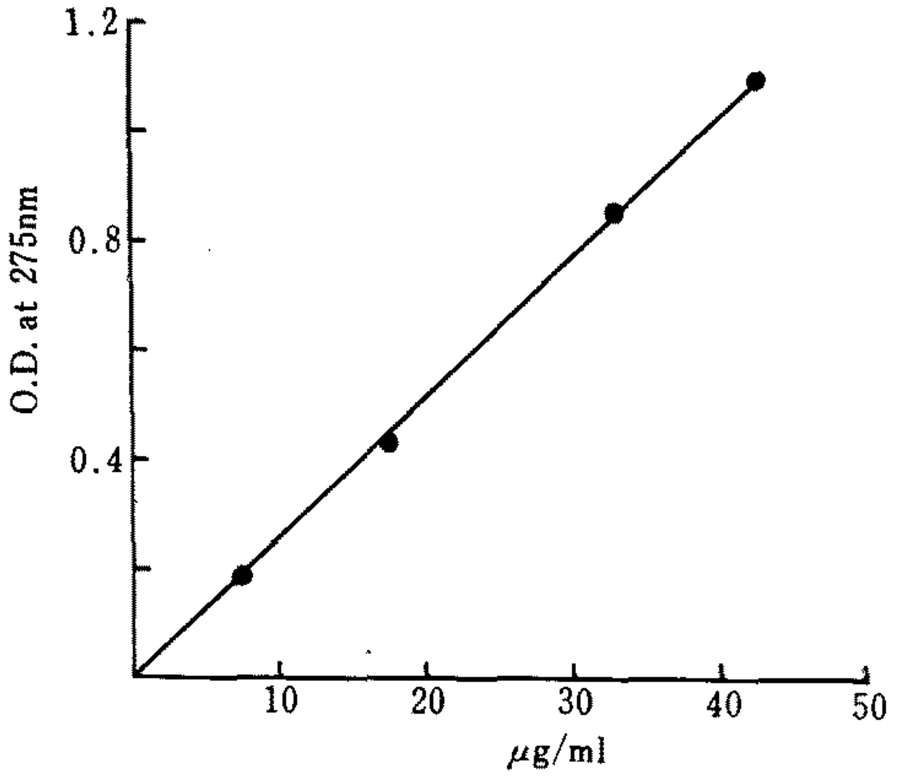

FIG. 3. The Calibration Curve of Authentic Deoxyfructosazine.

ammonium formate system (Fig. 2). However, as to the amount ratio of these compounds, there was a distinct difference between the fructose-ammonium formate system and the glucose-ammonium formate system: namely, the main product of the former was Compound I (deoxyfructosazine), while that of the latter was Compound II (its 6-isomer). Also, Compounds I and II formed in the former were examined by paper chromatography with *n*-butanol-acetic acid- water (4: 1: 1 v/v/v) as solvent and Tollens reagent as spraying reagent by the method described previously.[8] The *Rf* values of these compounds agreed respectively with those of deoxyfructosazine and its 6-isomer.

Subsequently, in order to confirm that Compounds I and II are undoubtedly deoxyfructosazine and its 6-isomer, respectively, these compounds were isolated by the procedure described above. A few properties of the two compounds isolated were compared with those of the authentic deoxyfructosazine and its 6-isomer. As shown in Table II, the properties of each compound were found to reasonably agree with those of the corresponding authentic compound. Besides, the NMR spectra of the peracetates of these compounds prepared by the procedure previously described[8] were respectively coincident with those of authentic deoxyfructosazine heptaacetate and 6-isomer heptaacetate. Consequently, Compounds I and II were respectively identified as deoxyfructosazine and its 6-isomer. As to the formation of deoxyfructosazine, the mechanism resulting from

TABLE II. PROPERTIES OF COMPOUND I AND COMPOUND II

	Melting point	$[\alpha]_D$ (in H_2O)	UV_{max}
Compound I	161.5~162.0°C	−79°	275.5 nm
Deoxyfructosazine	161.5~162.0°C	−81°	275.5 nm
Compound II	168.0~168.5°C	−83°	275.0 nm
6-Isomer of deoxyfructosazine	168.5~168.8°C	−84°	275.5 nm

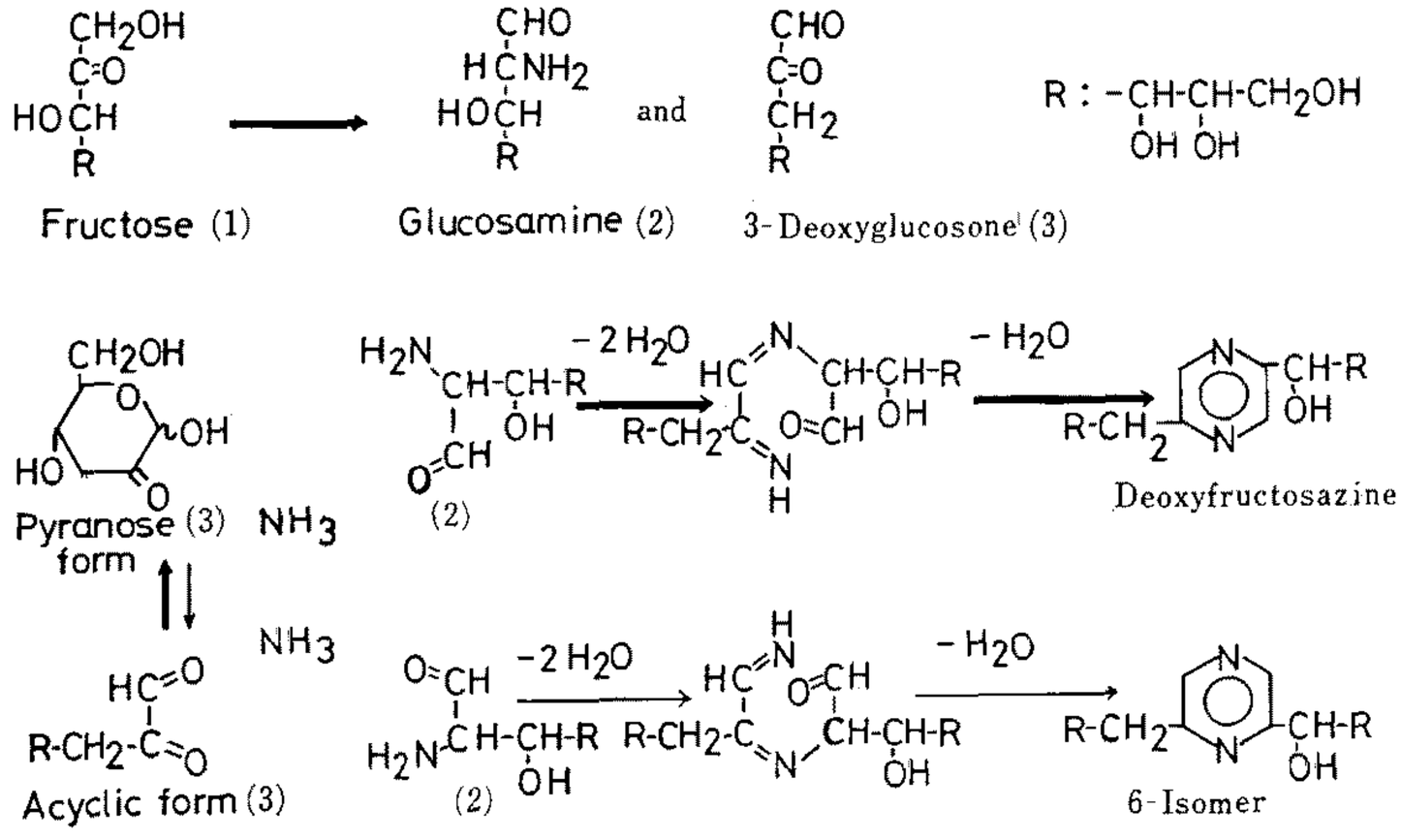

FIG. 4. Formation Mechanism of Deoxyfructosazine and Its 6-Isomer from Fructose-Ammonium Formate Reaction.

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the self-condensation of glucosamine produced by the Heyns rearrangement in the reaction of fructose and ammonia seems to be the most probable one (as described by Jezo and Luzak[5]). However, it cannot explain the formation of its 6-isomer under the same reaction conditions. Therefore, on the basis of the large proportion of deoxyfructosazine to its 6-isomer, condensation of glucosamine (Heyns product[7]), 3-deoxyglucosone[9] and ammonia as shown in Fig. 4 is considered to give a more suitable explanation for the formation of those products. This means that the reaction via pyranose form of 3-deoxyglucosone mainly proceeds, as 3-deoxyglucosone exists predominantly in pyranose form in aqueous solution according to Kato[11] and Anet,[12] and the smaller proportion of its 6-isomer probably owes to the reaction via acyclic form of 3-deoxyglucosone.

Finally, effects of ammonium formate concentration on the formation of deoxyfructosazine and its 6-isomer were examined: the amounts of these compounds formed from the reaction system differing in molar ratio of ammonium formate to fructose or glucose were determined by the procedure described above. The results are shown in Table III. As seen from the results, in case of fructose-ammonium formate reaction system, deoxyfructosazine was formed in an incomparably larger amount than its 6-isomer under all conditions shown above, and under an appropriate condition (fructose/ammonium formate: 1/5), deoxyfructosazine was formed in a yield of 424.9 mg per g of the original fructose. On the contrary, in the glucose-ammonium formate reaction system, the 6-isomer was incomparably abundant, and under an appropriate condition (glucose/ammonium formate: 1/10), its amount in-

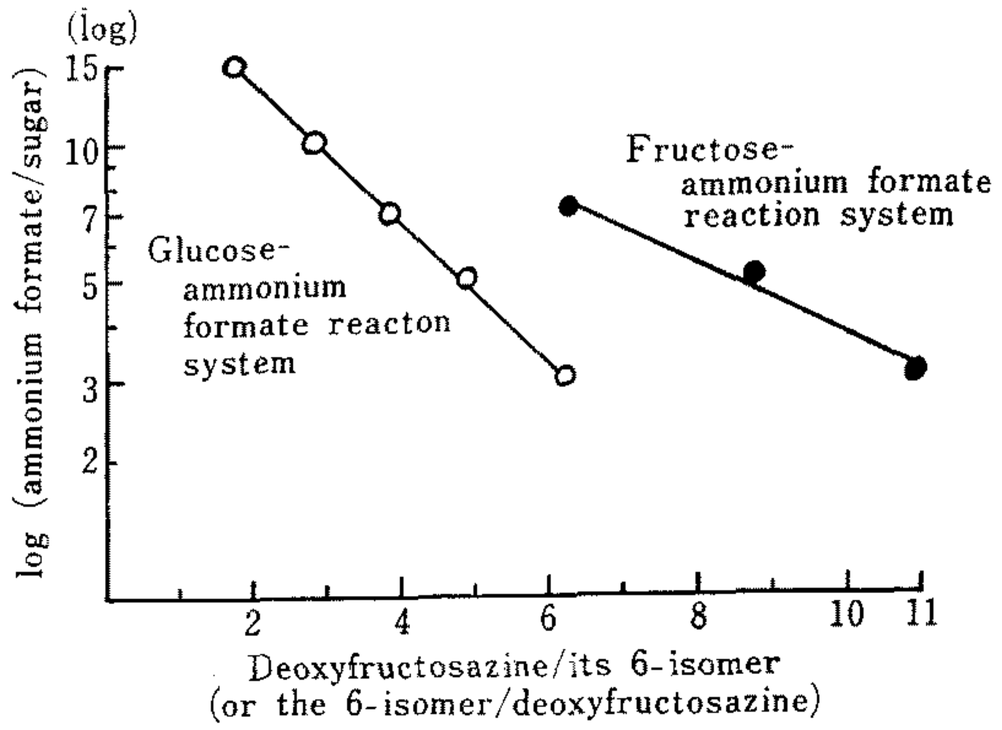

FIG. 5. Correlation between Mole Ratio of Ammonium Formate to Sugar and Amount Ratio of Deoxyfructosazine to Its 6-Isomer (or of the 6-Isomer to Deoxyfructosazine) Formed in the Reaction.

Deoxyfructosazine/its 6-isomer: For fructose-ammonium formate reaction system.
The 6-isomer/deoxyfructosazine: For glucose-ammonium formate reaction system.

TABLE IV. AMOUNTS OF DEOXYFRUCTOSAZINE AND ITS 6-ISOMER FORMED UNDER SEVERAL CONDITIONS DIFFERING IN MOLE RATIO OF AMMONIUM FORMATE TO SUGAR

Fructose/$HCOONH_4$ (mole ratio)	Initial pH	Deoxyfructosazine (mg/fructose 1 g)	Its 6-isomer (mg/fructose 1g)	Deoxyfructosazine/ Its 6-isomer
1/ 1	6.2	25.7	5.7	4.5/ 1
1/ 3	6.8	188.2	17.4	10.9/ 1
1/ 5	7.0	424.9	48.1	8.8/ 1
1/ 7	7.2	398.8	64.8	6.2/ 1
Glucose/$HCOONH_4$ (mole ratio)	Initial pH	Deoxyfructosazine (mg/glucose 1g)	Its 6-isomer (mg/glucose 1 g)	Deoxyfructosazine/ Its 6-isomer
1/ 1	5.8	0.4	4.4	1/11.0
1/ 3	6.0	11.0	68.6	1/ 6.2
1/ 5	6.3	31.0	151.0	1/ 4.9
1/ 7	6.7	62.1	234.6	1/ 3.8
1/10	7.3	135.7	381.7	1/ 2.8
1/15	7.3	178.9	297.7	1/ 1.7

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creased to 381.7 mg per g of the original glucose. However, as concentration of ammonium formate increases, the ratio of amounts of these compounds formed in the reaction systems gradually approached to 1/1; a plot of log (ammonium formate/the original sugar) vs. amount ratio of deoxyfructosazine to its 6-isomer (or of the 6-isomer to deoxyfructosazine) gives a straight line, except the equimolar ratio of ammonium formate to the original sugar (Fig. 5).

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