Investigation of Pyrazine Formation Pathways in Sugar–Ammonia Model Systems

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Model systems containing ammonium hydroxide and glucose, mannose, galactose, fructose, rhamnose, xylose, arabinose, 2-deoxyglucose, glyceraldehyde, 1,3-dihydroxyacetone, sorbitol, and glycerol were reacted at 100 °C (solution temperature) for 2 h to investigate the effect of the nature of the sugar on pyrazine formation pathways. Pyrazines identified in these model systems were the unsubstituted, 2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl-, 2,3-dimethyl-, 2-ethyl-5-methyl-, 2-ethyl-6-methyl-, 2,3,5-trimethyl-, 2-ethyl-3-methyl-, 2-ethyl-3,6-dimethyl-, and 2-ethyl-3,5-dimethylpyrazine. Total yields of pyrazines from mannose and fructose are about equal with galactose giving slightly lower yields. The distribution patterns of pyrazines from all three sugars were essentially similar. Pentoses gave slightly larger yields than the hexoses, but their pyrazine distribution patterns were similar in most respects to those of the hexoses. Distribution patterns obtained from aldoses and ketoses were similar with the exception that aldoses gave more unsubstituted pyrazines. Regiospecific effects do not appear to be significant, for epimers, diastereomers, and enantiomers gave identical pyrazine distribution patterns. Deoxy sugars gave unique distribution patterns depending upon their specific deoxy sites.

Recently, the authors suggested pyrazine formation pathways for a D-glucose–ammonia model system (Shibamoto and Bernhard, 1976a). We proposed ten α-amino carbonyl compounds as the intermediates of pyrazine formation. van Praag et al. (1968) studied the glucose–ammonia and rhamnose–ammonia model systems and obtained six alkylpyrazines from a glucose–ammonia system and ten alkylpyrazines from a rhamnose–ammonia system. Their results indicated that the kinds of pyrazines obtained from the two systems were quite different. Rhamnose gave large alkylpyrazines (e.g., ethylmethylpyrazines) in greater amounts than did glucose. Koehler and Odell (1970) reported the formation of methylpyrazines and dimethylpyrazines from their sugar–(fructose, glucose, sucrose, and arabinose) asparagine model system. They found that fructose gave the greatest and that arabinose gave the least yields of the above pyrazines. These experiments indicate that the yields and distribution patterns of pyrazines depend upon the kind of sugars used. Using isotopic techniques, Koehler et al. (1969) found that the carbon atoms in pyrazines came only from sugars. Thus the manner of sugar fragmentation might control the kinds of pyrazines formed. In order to investigate the pyrazine formation pathways further, we studied the pyrazines formed from model systems which consisted of various sugars and ammonia.

EXPERIMENTAL SECTION

The sugars and authentic pyrazine compounds were obtained commercially.

Reaction conditions for each experiment are listed in Table I. Reaction media consisted of a solution of 8 M ammonium hydroxide and 1 M carbohydrate in distilled water. Solution temperature was maintained at 100 °C for 2 h for both open systems (experiments conducted at atmospheric pressure) and closed systems (experiments conducted in sealed ampules). Each experiment gave a dark-brown reaction mixture. The method of analysis has been described previously (Shibamoto and Bernhard, 1976b).

RESULTS AND DISCUSSION

The results of the quantitative analyses of pyrazines obtained from this study are shown in Table II. The sugar alcohols (experiments 1 and 2) gave negligible yields of

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Table I. Reaction Conditions of Experiments

Experiment	Carbohydrate	System	Replicates
1	D-Sorbitol	open	1
2	Glycerol	open	2
3	D(+)-Mannose	open	1
4	D(+)-Galactose	open	2
5	D(−)-Fructose	open	2
6	L(−)-Galactose	open	1
7	D(+)-Xylose	open	2
8	L(+)-Arabinose	open	2
9	L(+)-Rhamnose (monohydrate)	open	3
10	L(+)-Rhamnose (monohydrate)	closed	2
11	2-Deoxy-D-glucose	open	1
12	2-Deoxy-D-glucose	closed	2
13	D L-Glyceraldehyde	open	2
14	1,3-Dihydroxyacetone	open	2
15	D-Glucose	open	8

pyrazines (0.0006%). Even though the gas chromatographic system we used would permit imidazoles and piperazines to be readily chromatographed, we were unable to isolate any of those compounds or other less volatile heterocyclic compounds from our reaction mixtures. The chromatogram of our extract showed some very small peaks eluted after the pyrazines; their total peak area was, however, less than 1% of the total peak area for all pyrazines. We felt those latter peaks on the chromatogram of the extracts were small enough to be ignored since they were well within the limits of error in our quantitative study of pyrazine recovery. These peaks may be imidazoles or other less volatile heterocyclic compounds. To recover these compounds, one apparently needs a continuous extraction (Tsuchida and Komoto, 1967). We used a discontinuous procedure (separatory funnel) and were unable to recover or detect these materials. However, we were able to recover over 98% of the pyrazines (Shibamoto and Bernhard, 1976a).

Hexoses (Table II, Experiments 3, 4, 5, and 6). The total yields of pyrazines from mannose and fructose were approximately equal. Galactose gave slightly lower overall yields, but the pyrazine distribution pattern was quite similar to the other hexoses.

Marked differences were observed in the percentage of unsubstituted pyrazine formed; mannose gave the largest

Table II. Yield (% w/w) of Each Pyrazine Relative to Total Pyrazines Isolated[a,b]

Experiment no.	A	B	C	D	E	F	G	H	I	J	K	L	Total yield %[c] of pyrazines rel to carbohydrate used
1	3.27	64.4	7.42	19.1	+	5.86	+	+	+	−	−	−	0.001
2	71.0	29.0	−	−	−	−	−	−	−	−	−	−	0.001
3	6.54	79.6	3.48	7.79	0.21	1.27	0.27	0.17	0.66	+	+	+	0.886
4	4.01	82.1	4.56	6.81	0.36	2.01	0.19	0.13	1.01	+	+	+	0.850
5	2.70	81.0	3.57	9.31	0.24	2.00	0.20	0.12	1.03	+	+	+	0.945
6	4.46	82.7	4.41	6.91	0.35	1.98	0.14	0.11	0.93	+	+	+	0.855
7	6.15	81.4	3.08	6.26	0.38	1.77	0.16	0.11	0.88	+	+	+	1.25
8	9.48	80.5	2.08	4.67	0.53	1.70	0.60	0.24	0.61	+	+	+	1.10
9	0.81	21.1	12.9	21.9	5.16	3.75	6.93	3.25	12.13	1.53	5.81	4.68	12.5
10	0.79	20.2	15.0	22.4	5.16	3.79	6.18	3.26	11.83	1.53	5.85	4.35	12.6
11	3.55	26.7	1.23	1.86	53.0	4.34	3.11	2.71	3.31	+	+	+	0.052
12	3.51	28.2	1.28	1.73	51.3	4.41	3.41	2.81	3.13	+	+	+	0.058
13	4.38	72.2	8.03	12.6	−	1.38	−	−	1.40	−	−	−	0.642
14	0.84	73.1	10.0	14.0	−	0.38	−	−	1.13	−	−	−	1.21
15	3.86	80.4	3.75	8.16	0.32	1.89	0.34	0.19	1.04	+	+	+	0.940

[a] A, unsubstituted-; B, 2-methyl-; C, 2,5-dimethyl-; D, 2,6-dimethyl-; E, 2-ethyl-; F, 2,3-dimethyl-; G, 2-ethyl-6-methyl; H, 2-ethyl-5-methyl-; I, 2,3,5-trimethyl-; J, 2-ethyl-3-methyl-; K, 2-ethyl-3,6-dimethyl-; L, 2-ethyl-3,5-dimethyl-. [b] (+) present in amount less than 0.01%; (−) not detected. [c] Yield % = (wt of pyrazines obtained)/(wt of carbohydrate used) × 100.

Table III. Type of Reactions in Figures 1, 2, 3, and 4

Code on reaction scheme	Type of reaction	References
A	Amadori rearrangement type reaction	Kuhn and Weygand (1937)
B₁	Keto-enol tautomerism	Lea and Hannan (1950)
B₂	Enolization	Lobry de Bruyn and Ekenstein (1895); Speck (1958)
C	Aldol and reverse aldol reaction	Budnitskaya (1941); Fischer and Marschall (1931)
D	Dehydration reaction	Isbell (1944)
E	Enediol cleavage	West et al. (1966); Hodge (1967)
F	Addition reaction	Cohen et al. (1964); Jencks (1969)
G	Splitting of a-dicarbonyl sugars	Hayami (1961)
H	Hodge's reductone formation	Hodge (1967)

and fructose the least. The yields of the remaining substituted pyrazines were similar. They are listed in order of decreasing percentage as follows: 2-methyl-, 2,6-dimethyl, unsubstituted or 2,5-dimethyl-, 2,3-dimethyl-, 2,3,5-trimethyl-, 2-ethyl-, 2-ethyl-6-methyl-, 2-ethyl-5-methylpyrazine. This order did not change throughout the series of experiments.

Factors affecting the total yield and distribution patterns will be discussed below, but these results indicate that the stereoisomers examined give identical results, and that ketoses and aldoses give almost identical results.

Pentoses (Table II, Experiments 7 and 8). The two pentoses gave larger yields than the hexoses. Their distribution patterns were similar to those of hexoses, but arabinose uniquely yielded more unsubstituted pyrazine than 2,6-dimethylpyrazine.

Heyns and Paulsen (1960) list the reactivities of sugars in sugar–amine model systems in order of decreasing reactivity: xylose, arabinose, galactose, mannose, and glucose. The amount of pyrazines obtained in our studies from different sugars corresponded to browning reactivities, except for the case of galactose.

Pyrazine Formation Pathway. Pyrazine formation is closely related to the classic browning reactions. Throughout these experiments, the color of the reaction mixture became darker as the reaction progressed; and as the pyrazine products increased, the color of the reaction mixture changed from colorless to yellow, then brown, and finally dark brown.

Shibamoto and Bernhard (1976a) have previously reported the possible formation pathways of pyrazines and

Scheme I
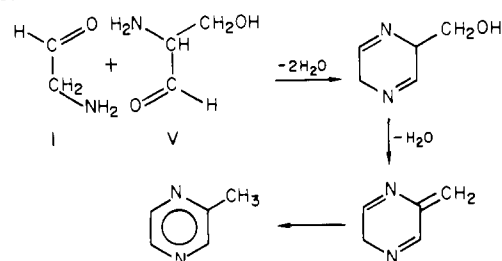
suggested ten α-amino carbonyl intermediates (Figure 1), for example, see Scheme I. For the present investigation the formation pathways of the α-amino carbonyl intermediates from the reaction of various sugars with ammonia are postulated and shown in Figures 2, 3, 4, and 5 [see Shibamoto and Bernhard (1976a) for the formation pathways of α-amino carbonyl intermediates from the reaction of D-glucose with ammonia]. The type of reactions involved in these schemes are listed in Table III.

Glucose is readily transformed to fructose and a lesser amount of mannose through an enediol reaction intermediate in alkaline solution (Speck, 1958). The pathway of pyrazine formation from the above three sugars might therefore be considered to be similar.

Relations Between the Fragments and Pyrazine Distribution Patterns. The yield of each pyrazine must depend upon the amount of each fragment produced (Shibamoto and Bernhard, 1976a). The fragments which require dehydration of a sugar moiety for their formation
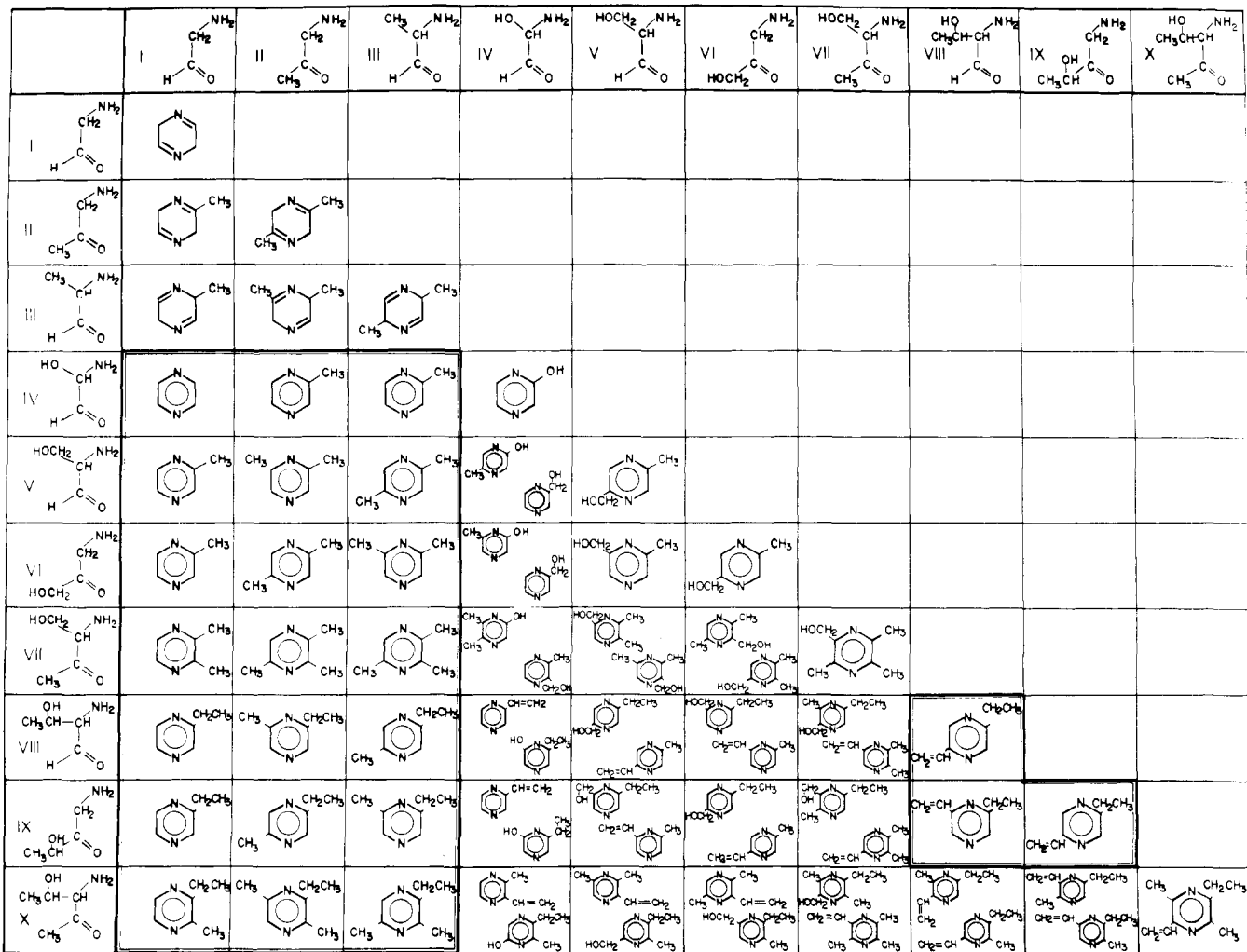
Figure 1. The proposed α-amino carbonyl fragments and pyrazines formed from these fragments. Pyrazines framed by the double lines were identified as being present in our sugar–ammonia model systems. The others are suggested compounds from the proposed α-amino carbonyl fragments, but have not been isolated or identified with certainty.

are more difficult to form in aqueous solutions (fragments II and III). For example, Hodge's reductones are obtained from nonaqueous or nearly anhydrous systems (Hodge, 1953). The amount of these fragments (II, III, or those from Hodge's reductones) may increase at higher temperatures when dehydration is favored. Pyrazines (e.g., 2,3,5-trimethylpyrazine) which are formed from fragments II, III, and VII increased in yield at higher temperatures. We conclude that the fragments which do not require a dehydration step such as I, V, and VI must be easier to form than others, and that fragment I predominates. Assuming that formaldehyde condenses with fragment I, more fragment V must be produced than fragment VI. Consequently, the pyrazine compound from the combination of fragments I and V gives the greatest yield of 2-methylpyrazine.

The presence of the ammonia adduct of glyoxal, fragment IV, is reasonable (Connors, 1973), but this adduct easily loses water to form a Schiff base; therefore, the unsubstituted pyrazine formation must compete with an intramolecular dehydration reaction of these fragments [Scheme II, according to Cohen et al. (1964) and Jencks (1969)]. This is indicated by the relatively low yield of unsubstituted pyrazine as compared with 2-methyl-pyrazine. It is possible that the carbonyl group of the α-amino carbonyl fragment adds ammonia to form an intermediate of ammonia adduct. This is, however, no longer an α-amino carbonyl fragment. We excluded these

Scheme II
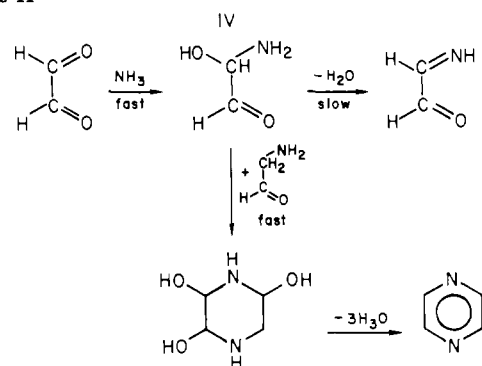
fragments from our postulation of pyrazine formation pathway.

The rhamnose–ammonia model system (experiments 9 and 10; Figure 1) gave quite different results from that of glucose. All fragments except fragment IV can be formed without dehydration and fission of the sugar moiety; therefore, the pyrazines from this system were distributed more equally than the pyrazines from glucose. Additionally, the total yield of pyrazines was considerably larger than that from a glucose system.

The low yield of pyrazines (0.05% relative to sugar used) from the 2-deoxyglucose–ammonia system (experiments
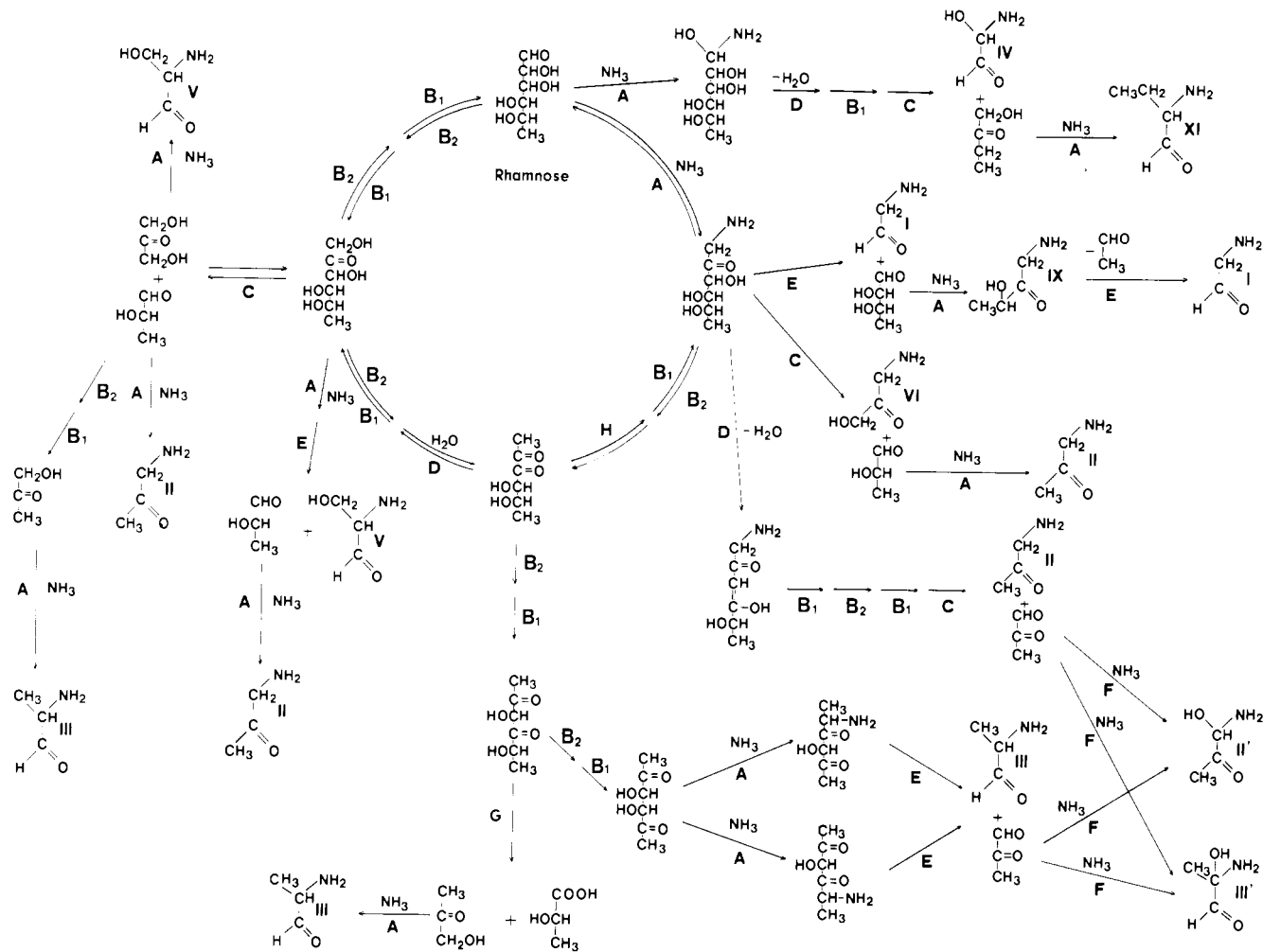
Figure 2. Proposed formation pathways of α-carbonyl fragments from rhamnose. Roman numerals indicate the α-amino carbonyl fragment number (see Figure 1) and capital letters indicate the type of reaction (Table III).

Figure 3. Proposed formation pathways of α-carbonyl fragments from 2-deoxyglucose. Roman numerals indicate the α-amino carbonyl fragment number (see Figure 1) and capital letters indicate the type of reaction (Table III). Superscript a indicates pyrazine formation pathways in D-glucose–ammonia model system (Shibamoto and Bernhard, 1976a).

11 and 12) was due to the blocking of the Amadori rearrangement by a C-2 deoxy site. Hodge and Rist (1952) obtained 30 to 50% yields of Amadori products when the

C-2 hydroxyl was present in a crystalline N-substituted glycosylamine. On the other hand, they did not obtain Amadori products from a C-2-O-substituted glycosylamine

Figure 4. Proposed formation pathways of α-carbonyl fragments from xylose and arabinose. Roman numerals indicate the α-amino carbonyl fragment number (see Figure 1) an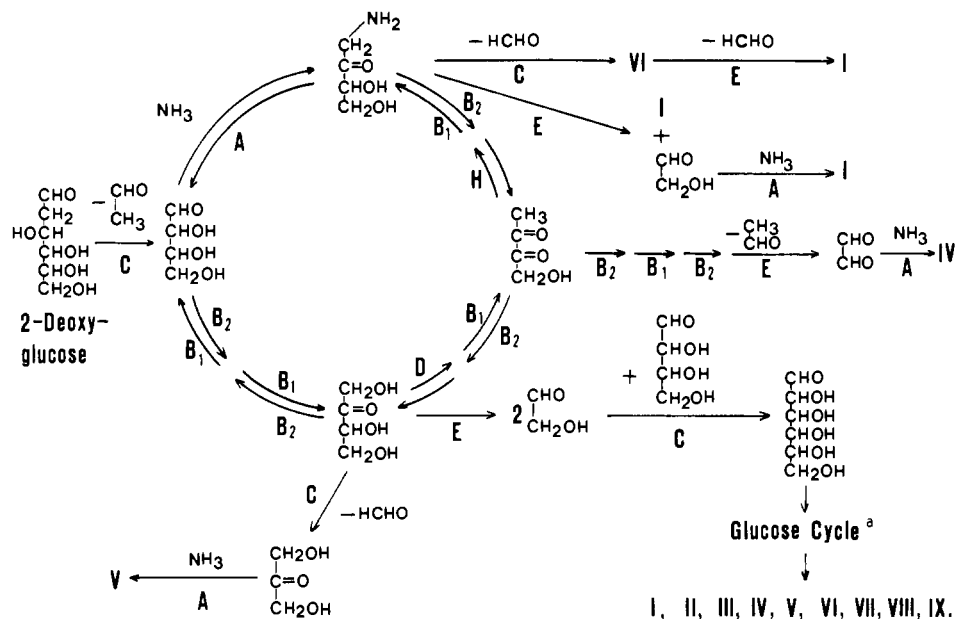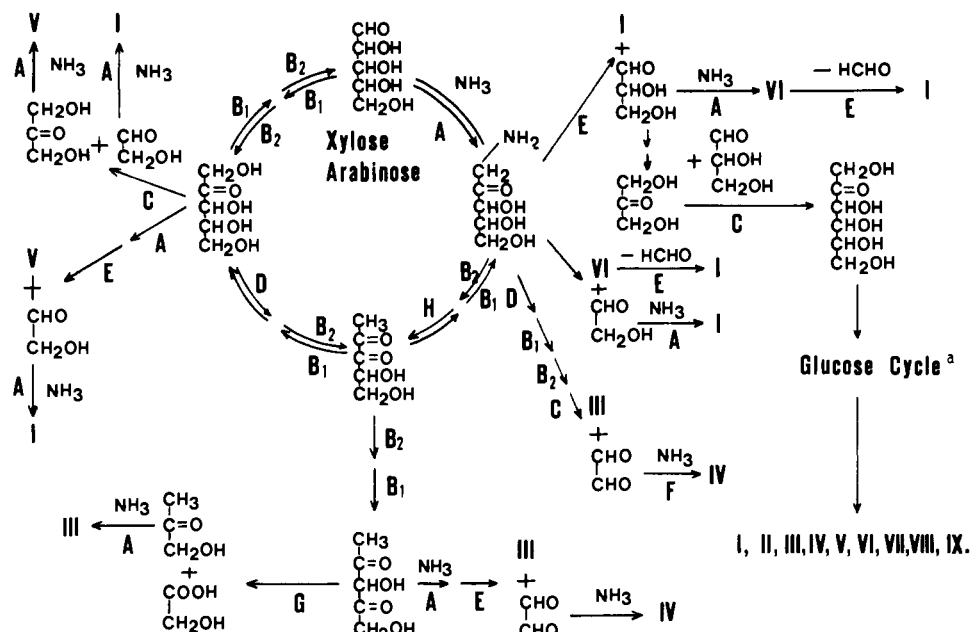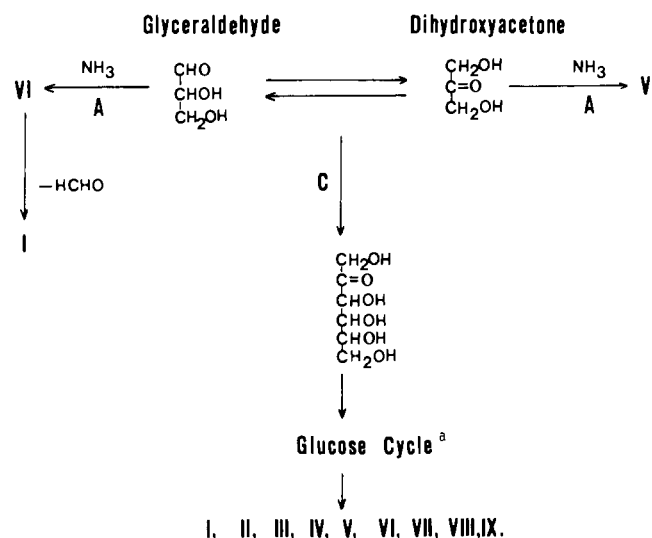d capital letters indicate the type of reaction (Table III). Superscript a indicates pyrazine formation pathways in D-glucose–ammonia model system (Shibamoto and Bernhard, 1976a).

Figure 5. Proposed formation pathways of α-carbonyl fragments from glyceraldehyde and dihydroxyacetone. Roman numerals indicate the α-amino carbonyl fragment number (see Figure 1) and capital letters indicate the type of reaction (Table III). Superscript a indicates pyrazine formation pathways in D-glucose–ammonia model system (Shibamoto and Bernhard, 1976a).

even after storage for 2 years at 25 °C. They concluded that blocking the Amadori rearrangement of an N-substituted glycosylamine blocked the browning reaction (which includes pyrazine formation). Several other investigators showed that the C-2 hydroxyl of an aldose is important for normal browning reactions using model systems composed of 2-deoxy or 2-O-substituted aldoses and amino acids (Hurd and Kelso, 1948; Beacham and Dull, 1951; Haugard et al., 1951).

In order to initiate pyrazine formation, a carbonyl group must be generated. Probably 2-deoxyglucose is cleaved into two compounds via a reverse aldol reaction to give acetaldehyde and a tetrose (Figure 3). This tetrose would combine with ammonia to produce the pyrazines. The 2-deoxyglucose–ammonia model system produced large

amounts of 2-ethyl-5-vinylpyrazine (47% of total pyrazines produced). The significant difference between this system and the glucose system is the high percentage of 2-ethylpyrazine and ethylvinylpyrazine (28 and 47% relative to the total pyrazines) which comprised less than 0.5 and 0.01%, respectively, of the total pyrazines in the glucose system. The distribution pattern excepting these two compounds is very similar to that of the glucose system.

Because the 2-deoxyglucose system gave large amounts of 2-ethyl- and 2-ethyl-5-vinylpyrazine, the formation of fragment VIII, an essential intermediate of the above two pyrazines, can be rationalized. According to Figure 3, 2-deoxyglucose produces acetaldehyde which condenses with fragment I to give fragment VIII.

The distribution patterns from xylose–ammonia and arabinose–ammonia (Figure 4) were fairly similar to those of a hexose. The noticeable difference from the glucose system was the low yield of 2,6-dimethylpyrazine. The arabinose system gave a lower yield of total pyrazines than the xylose system, and in particular, the yield of 2,6-dimethylpyrazine from arabinose was low compared with xylose. Thus the same sort of relationship can be seen between arabinose and xylose and glucose and galactose.

Glyceraldehyde and 1,3-dihydroxyacetone (Experiments 13 and 14) are interconvertible through the enediol. The aldol condensation of D-glyceraldehyde and 1,3-dihydroxyacetone proceeds rapidly in alkaline solution with the formation of D-fructose and D-sorbose (90 to 95% yield) (Fischer and Marschall, 1931). This indicates that C-3 sugar units produce C-4 fragments (fragment VII for 2,3-dimethyl- and 2,3,5-trimethylpyrazines) through aldol condensation to hexoses which subsequently produce the larger alkylpyrazines.

Figure 4 shows the proposed pyrazine formation pathways in glyceraldehyde–ammonia and 1,3-dihydroxyacetone–ammonia model systems. Experimental results (Table II) indicate an absence of the larger alkylpyrazines (ethylmethyl- or ethyldimethylpyrazines). In order to produce the larger alkyl pyrazines, fragments VIII and/or X should be present (Figure 4). Thus there should be competition between the reactions of fragments II and III with fragments V and VI to form methyl- and di-

methylpyrazines and reactions of fragments II and III with acetaldehyde to form fragments VIII and X, respectively, which subsequently produce larger alkylpyrazines (Figure 1). Fragments II and III, therefore, must react with fragments I, V, or VI to form methyl- or dimethylpyrazines before being condensed with acetaldehyde to give fragments VIII or X.

There is a greater yield of unsubstituted pyrazines from the glyceraldehyde system than from the dihydroxyacetone system. This may well depend upon the ease of formation of fragment I which can form directly from glyceraldehyde.

Stereochemical Problem of Dehydration Reaction. It is assumed that fragments II and V are essential fragments for formation of 2,6-dimethylpyrazine. In any case, fragment V should form as long as fragment I forms, and the facile formation of fragment I is obvious because of the high yield of 2-methylpyrazine. Therefore, diminished production of fragment II causes this difference in yield.

One of the differences between glucose and galactose, and xylose and arabinose is that the hydroxy groups on carbons 3 and 4 are in the trans and cis configurations, respectively. If there is a possibility of neighboring group participation in these dehydration reactions in addition to a β-elimination reaction, the trans form must be favored to eliminate water (Cram, 1952). On the other hand, these differences may be due simply to the different reactivity of the sugars. Further investigation is necessary to clarify this point.

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Gas–Liquid Chromatographic Analysis of Amino Acids in Food Samples

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The gas chromatographic method of amino acid analysis requires that the sample is hydrolyzed to release the amino acids, which are then cleaned in an ion-exchange column, converted to volatile derivatives, and then separated on a polyester as well as on a silicone column. These different steps contribute to certain amounts of variation in the recovery of the individual amino acids. In addition, the various components of the food samples, especially carbohydrates, could affect the recovery of some amino acids. In this paper certain quantitative aspects of amino acid analysis of food samples by gas chromatography are investigated. The recovery of amino acids as affected by duration of hydrolysis and presence of various carbohydrates during hydrolysis is estimated. Furthermore, the amino acid patterns determined by GLC of a series of diet samples collected under a nutrition survey program are compared with the values obtained by ion-exchange chromatography.

Proteins are one of the most important dietary components of human food. The nutritive value of the protein depends mainly on its amino acid composition. As a result of the scarcity of proteins and increasing consciousness of the aspects of protein nutrition and health, there is a great demand for a routine method for amino acid analysis for the evaluation of the proteins in raw materials and estimation of the effect of various steps of manufacturing on the nutritional quality of protein in the finished product.

Ion-exchange chromatography (Moore and Stein, 1951) is so far the most widely used method of amino acid analysis. Nevertheless, due to speed, sensitivity, and versatility, gas chromatography could be a cheaper alternative. The experimental conditions for the quantitative derivatization and chromatographic requirements for their separation are detailed in a series of publications

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