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Formation of Pyrazines from Thermal Treatment of Some Amino-Hydroxy Compounds

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Some nitrogenous organic compounds were heated individually under air flow and the volatiles produced were qualitatively investigated by gc-ms. Pyrazines were obtained from heating certain amino-hydroxy compounds, such as ethanolam-

ine, glucosamine, serine, threonine, 4-amino-3-hydroxybutyric acid, and alanylserine. This type of compound may be considered as one of the important precursors of pyrazines in foods.

To date, it is well known that pyrazines are present in a wide variety of heated foodstuffs, notably in those of fried or roasted, such as potato chips (Buttery *et al.*, 1971; Deck and Chang, 1965), coffee (Bondarovich *et al.*, 1967; Friedel *et al.*, 1971; Goldman *et al.*, 1967), cocoa (Marion *et al.*, 1967; Rizzi, 1967; van Praag *et al.*, 1968; van der Wal *et al.*, 1971), popcorn (Walradt *et al.*, 1970), deep fat-fried soybeans (Wilkens and Lin, 1970), roasted peanuts (Johnson *et al.*, 1971; Mason *et al.*, 1966; Walradt *et al.*,

1971), roasted barley (Collins, 1971; Wang *et al.*, 1969), and roasted pecans (Wang and Odell, 1972). The aroma of this class of compounds has been described as "roasted," "roasted nutty" or "cooked." Their precursors in foods have been considered as sugars with amino acids (Dawes and Edwards, 1966; Koehler and Odell, 1970; Koehler *et al.*, 1969; Mason *et al.*, 1966; van Praag *et al.*, 1968; Wang *et al.*, 1969), and a further mechanistic study of alkylpyrazine formation in model systems of α -dicarbonyls with amino acids was reported recently (Rizzi, 1972). In the previous paper, we obtained a series of pyrazines by heating a mixture of glycerol and amino acids without the presence of sugars. The present study was directed toward the identification of the pyrazines produced by thermal

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Table I. Pyrazine Compounds Produced from Thermal Treatment of Some Amino-Hydroxy Compounds

Compound ^a	Serine ^b	Threonine ^b	Ethanolamine ^b	Glucosamine ^b	4-Amino-3-hydroxy-butyric acid ^b	Alanyl-serine ^b
Pyrazine	L		VL	S		M
2-Methylpyrazine	M	S	L	VL		M
2,5-Dimethylpyrazine		VL	S	L	L	
2-Ethylpyrazine	VL		M			L
2,3-Dimethylpyrazine	S			M		
2-Ethyl-6-methylpyrazine	S					M
2-Ethyl-5-methylpyrazine				S		
Trimethylpyrazine		L		M	S	
2,6-Diethylpyrazine	M					L
2,5-Dimethyl-3-ethylpyrazine	S	L	M		M	
Pyrazine with mol wt 136		M	S			
Second pyrazine with mol wt 136		S				
Pyrazine with mol wt 150	VS	M	VS			VS
Second pyrazine with mol wt 150	VS	S	VS			VS
Pyrazine with mol wt 164	VS	VS				VS
Pyrazine with mol wt 178		VS				

^a Identified by gc-ms. ^b Size of peaks was roughly estimated: VS = very small; S = small; M = medium; L = large; VL = very large.

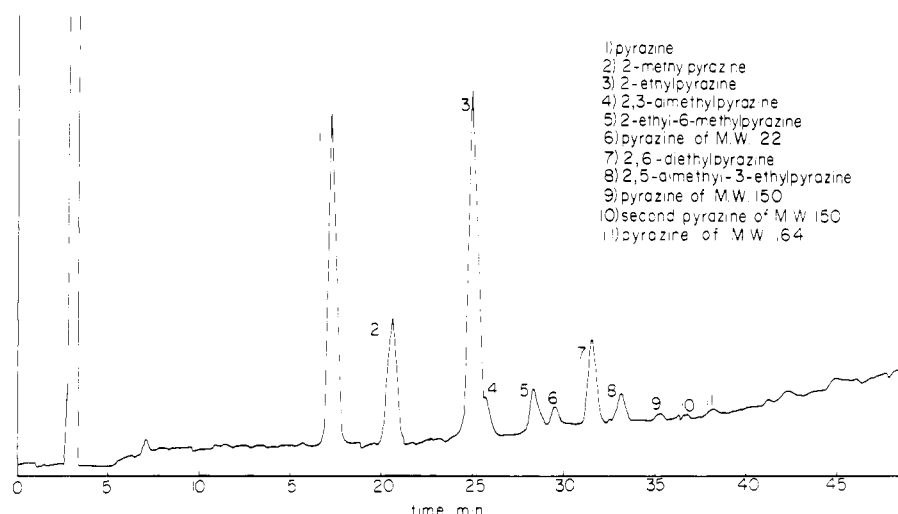


Figure 1. Gas chromatogram of the basic fraction from the volatiles of pyrolysis of serine. 10% Carbowax 20M KOH on Gas Chrom Q, 20 ft \times $\frac{1}{4}$ in. o.d., 80° for 7 min, then programmed at 2°/min from 80° to 180°, and held at 180°, with a flow of 30 ml of He/min.

treatment of some individual nitrogenous organic compounds with the expectation of obtaining further information about their formation.

EXPERIMENTAL SECTION

Materials. Glycine, DL-alanine, DL-phenylalanine, β -alanine, L-leucine, L-isoleucine, DL-valine, DL-methionine, L-cystine, L-tyrosine, L-histidine, L-proline, L-hydroxyproline, L-tryptophan, L-lysine, L-hydroxylysine, DL-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, DL-serine, DL-threonine, DL-alanyl-DL-serine, 4-amino-3-hydroxybutyric acid, ethanolamine, glucosamine, adenine, adenosine, egg albumin, bovine albumin, and the authentic reference pyrazines were obtained from reliable commercial sources or were gifts.

Generation and Separation of Pyrazines from a Model System. About 2 g of each compound listed above was heated individually under 50–60 ml/min of air flow at $200 \pm 5^\circ$ for 4 hr (except ethanolamine, which was heated at 160°, a little lower than its bp, for 6 hr) in a glass tube of 20 in. \times 1 in. i.d. electrically heated by a nichrome wire coiled around the tube (Wang *et al.*, 1968). The volatiles produced were collected in two traps pre-cooled in Dry Ice-acetone and then extracted three times each with 30-ml portions of ether. The combined ether ex-

tract was treated three times each with 20-ml portions of 5% HCl and the resultant aqueous layer was concentrated below 50° *in vacuo* to near dryness. Ten milliliters of 12 N NaOH was added and extracted three times each with 20-ml portions of ether. The combined ether extract was concentrated to about 20 μ l, and 8 μ l of the concentrate was analyzed by glc and gc-ms.

Gas-Liquid Chromatography (glc). A modified Barber-Coleman Model 8000 gas chromatograph (Waller, 1968) with a flame ionization detector was utilized for preliminary gas chromatographic investigation. The column used was a 20-ft \times 0.25-in. o.d. coiled glass column packed with 10% Carbowax 20M [containing 1% (w/w) KOH additive] on 100–120 Mesh Gas Chrom Q (w/w). Helium flow rate was 30 ml/min, and the temperature was held at 80° for 7 min, then programmed at 2°/min to 180° and held at 180°. The injection port and flame ionization detector were held at 220 and 270°, respectively.

Gas Chromatography-Mass Spectrometry (gc-ms). The column used in glc was connected to a prototype of the LKB 9000 gc-ms (Waller, 1968) and operated under the following conditions: ionization voltage, 70 eV; ion source temperature, 250°; accelerating voltage, 3.5 kV; trap current, 60 μ A; electron multiplier voltage, 1.7 to 2.1 kV; separator temperature, 220°; scan speed, from m/e 6 to m/e 250 in 5 sec.

RESULTS AND DISCUSSION

Table I is a summary of the pyrazine compounds identified and the amino-hydroxy containing compounds which produced pyrazines upon heating. Identification was made by matching the mass spectra and the retention time data of unknown compounds with those of reference compounds analyzed under identical instrument conditions.

As shown in Table I, various pyrazine compounds were obtained by heating serine, threonine, ethanolamine, glucosamine, 4-amino-3-hydroxy-butyric acid, and alanylserine individually. The composition of pyrazine mixtures and the relative amounts of each pyrazine produced varied from one amino-hydroxy compound to another. For example, pyrazine, 2-methyl-, 2,5-dimethyl-, 2,3-dimethyl-, and trimethylpyrazine (with 2-methylpyrazine for the largest peak) were found from glucosamine while only 2,5-dimethyl-, trimethyl-, and 2,5-dimethyl-3-ethylpyrazine (with 2,5-dimethylpyrazine for the largest peak) were obtained from 4-amino-3-hydroxy-butyric acid. Ten and eight pyrazine compounds were produced from serine and alanylserine, respectively, but no pyrazine was detected while egg albumin and bovine albumin were heated under the conditions described above. Pyrazines also could not be found when glycine, alanine, phenylalanine, β -alanine, leucine, isoleucine, valine, methionine, cystine, tyrosine, histidine, proline, hydroxyproline, tryptophan, lysine, hydroxylysine, aspartic acid, asparagine, glutamic acid, glutamine, adenine, and adenosine were heated individually under the same conditions. A typical gas chromatogram of pyrazines obtained from serine is shown in Figure 1.

Koehler and Odell (1970) reported the production of pyrazine, methyl- and dimethylpyrazine from heated glucosamine in a diethylene glycol-water (10:1) system ($120 \pm 1^\circ$ for 24 hr). In the present study, glucosamine was dry heated. In addition to these findings, pyridine, 2-ethyl-5-methyl-, and trimethylpyrazine were obtained.

The occurrence of pyrazines from heated serine was first reported by Hornstein (1966). Kato *et al.* (1970) heated serine (at 280°) and threonine (at 360°) under a nitrogen stream, and six pyrazines were found from each of the amino acids. In the present study, serine and threonine were heated at 200° under air flow, and more pyrazines (ten pyrazines from each of the amino acids) were obtained. The presence of oxygen might facilitate the formation of pyrazines even though the heating temperature was lower. Thus, different compositions of pyrazine mixtures could be obtained from heating the same compound at the different conditions.

The formation of pyrazines was previously studied by the model system of sugars (or the breakdown products of sugars, such as dicarbonyls, hydroxyketones) with amino acids (or ammonia) (Dawes and Edwards, 1966; Koehler and Odell, 1970; Koehler *et al.*, 1968; van Praag *et al.*, 1968; Wang *et al.*, 1969). As a result of these studies, the major carbon and nitrogen sources of pyrazines have been suggested as sugars and amino acids (or ammonia), respectively; hence, sugars were considered as the important precursors for pyrazines in foods. In the present study,

however, pyrazines were obtained by heating individual amino-hydroxy compounds, notably those having amino and hydroxy groups in adjacent carbon positions, without the participation of sugars. It is apparent that the amino-hydroxy compounds themselves could serve as a source both for pyrazinic carbon and nitrogen. In the formation of pyrazines, the participation of sugars is obviously favorable but not necessary. Accordingly, these types of compounds may be considered as other important precursors for pyrazines in foods.

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