Origin of Alkyl-Substituted Pyridines in Food Flavor: Formation of the Pyridines from the Reaction of Alkanals with Amino Acids

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The condensation reaction of amino acids with simple aldehydes to quarternary pyridinium betaines and the thermal decomposition of the pyridinium betaines to volatile pyridines were investigated. When products from the glycine–propanal system were heated at 180 °C, 3,5-dimethyl-2-ethylpyridine, 3,5-dimethyl-4-ethylpyridine, and 3,5-dimethylpyridine were immediately formed. An analogous thermal decomposition occurred with the condensation products from the glycine–propanal–crotonal system to afford 2,5-dimethylpyridine and 3,4-dimethylpyridine and many other unidentified compounds. The products obtained in these reactions had an undesirable grassy flavor and the composition of the products were dependent on the species of amino acids. The formation pathways of these pyridines were discussed.

Nowadays the importance of some alkyl-substituted pyridines in food flavors is generally recognized. The presence of alkylpyridines in foods has been investigated by many researchers (e.g., Ferritti and Flanagan, 1971; MacLoed and Coppock, 1976; Watanabe and Sato, 1971; Yajima et al., 1978). Recently, Buttery et al. (1977) reported that 12 pyridines were separated from roasted lamb and presumed that the condensation of deca-2,4-dienal with ammonia could occur to form an aldimine, followed successively by ring closure and oxidation to pyridines through the 1,2-dihydropyridines. The condensation of aldehydes, ketones, α,β-unsaturated carbonyl compounds, or various derivatives of such compounds with ammonia to form substituted pyridines, the "Chichibabine pyridine condensation", is one of the oldest organic reactions which has been extensively investigated (e.g., Elderfield, 1950; Eliel et al., 1953; Frank and Seven, 1949; Smith, 1976). However, no attention has been given to the pyridines formed by the reaction between amino acids and carbonyl compounds.

In our previous article dealing with the reaction of alkanals with amino acids under neutral conditions at room temperature, it was reported that quarternary pyridinium betaines with four substituents located at the 1,2,3,5 and 1,3,4,5 positions were obtained (Suyama and Adachi, 1979). The chemical change that quarternary pyridinium salts are easily decomposed to pyrines by heat treatment is well known as the "Hofmann degradation" and/or the "Ledenburg rearrangement". Therefore, it is further suggested that the reaction of carbonyl compounds with ammonia and primary amines including amino acids may be an important reaction for the formation of alkylpyridines in various foods. The objective of the present study was to condense aldehydes with amino acids in a model system, to isolate as many volatile pyridines as possible, and to determine the approximate molar ratios of the products. In addition, it was hoped that some correlation could be found between the properties of the volatile pyridines and those of the aldehydes and amino acids.

EXPERIMENTAL SECTION

General. UV spectra were obtained by a Hitachi-Perkin-Elmer 139 UV-vis spectrophotometer. IR spectra were recorded on a Hitachi 260-10 infrared spectrophotometer. ^{1}H-NMR spectra were taken on a JEOL JNM-ML-60 spectrometer operated at 60 MHz using tetramethylsilane as the internal standard in $CDCl_3$. Mass spectrum were obtained on a JEOL MS-12 gas chromatograph–mass spectrometer operated at 70 eV and the spectra were given as m/e. All melting points were determined with a Yanagimoto melting point aparatus and were not corrected; boiling points also were uncorrected.

Reagents. Glycine and other amino acids used were of commercial G.R. grade. Commercial propanal, 1-butanal, and crotonal of G.R. grade were purified by redistillation, bp 46–48, 74–75, and 104–105 °C, respectively. The 3,5-, 3,4-, and 2,5-dimethylpyridine which were used as a standard in the GLC analysis were obtained commercially.

Chromatography. Thin-layer chromatography (TLC) was run on aluminum sheets precoated with silica gel $60F_{245}$ (E. Merck) by using a mixture of chloroform–methanol (50:50, v/v) as a solvent system: Dragendorff reagent was used as a spray reagent. Analytical gas–liquid chromatography (GLC) was carried out on a Hitachi 063 instrument (flame ionization detector, inlet and detector, 200 °C), using the following stainless steel columns: column A, 200 × 0.3 cm column packed with 10% DEGS on 80–100 mesh Diasolid L at 130 °C; column B, 200 × 0.3 cm column packed with 5% SE-30 on 80–100 mesh Chromosorb W at 120 °C.

Reaction of Glycine with Propanal. The mixture of glycine (14 g, 0.2 mol) suspended in 20 mL of water and excess propanal (100 g, 1.6 mol) was stirred at room temperature. The reaction was initiated exothermically after a short induction period and completed in ca. 3 h. The mixture was then treated with hexane (3 × 50 mL), and the extracts were discarded. The residual fraction was extracted with chloroform (2 × 50 mL). The combined chloroform fraction and residual water fraction were evaporated to dryness under reduced pressure to afford viscous syrups, 25 and 18 g, respectively. From 15 g of the water-soluble reaction products, ca. 10 g of the hygroscopic colorless needles of 1-(1-carboxymethyl)-2-ethyl-3,5-dimethylpyridinium betaine (2) was separated from methanol–ethyl acetate by cooling; mp 135–136 °C dec, identical with that described previously (Suyama and Adachi, 1979).

The condensation reactions of glycine with propanal in the presence of 20 mL of aqueous 10% sodium carbonate or 10% acetic acid were also carried out at room temperature; after the simultaneous exothermic reaction, both reaction products were treated with hexane (2 × 100 mL). The viscous products were obtained by evaporation and these were subjected to further experiments. In a similar fashion, the condensation reaction of glycine (0.2 mol) with a mixture of crotonal and propanal (each 1 mol) carried out at room temperature. Similarly the other various amino acids (0.2 mol, see Table I) were also treated with

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Table I. Relative Concentration of Pyridines Formed by the Reaction of Amino Acids with Propanal

amino acid	pyridine 8c	8a	8b	unk[a]
Gly	3	100	4	10
Gly (+AcH)	3	100	5	8
Gly (+Na_2CO_3)	4	100	4	10
Ala	4	100	10	10
Leu	6	100	49	20
Lys	7	100	35	32
Tyr	6	100	30	22
Glu-Na	4	100	22	10
Ser	2	100	9	35

[a] Sum of unknown basic compounds.

propanal (2 mol). From the reacted α-L-leucine–propanal system, 1-(1-carboxy-3-methylbutyl)-4-ethyl-3,5-dimethylpyridinium betaine was separated by treating with ethyl acetate as needles, about a 4-g yield, mp 129–130 °C dec; the spectrum data were consistent with the structure of the compounds as described previously (Suyama and Adachi, 1979).

Thermal Decomposition of the Reaction Products. Pyridinium betaines were heated at 180 °C for 2 min in the test tube: the products formed were extracted with ethyl ether. On the other hand, the thermolysis of hexane-insoluble fraction obtained from the glycine–propanal system was carried out by heating successively at 120 to 180 °C in a flask; volatile products formed were distilled immediately under reduced pressure. The distillate obtained was acidified with concentrated HCl and washed with ethyl ether three times. The ether fraction contains a neutral and an acid compound. The residual water-soluble fraction was treated subsequently with 10% NaOH and was extracted with ethyl ether and then dried with anhydrous potassium carbonate. Each product obtained from the reaction mixture of the other amino acids and aldehydes was also treated in the same manner.

RESULTS AND DISCUSSION

Reaction Products of the Glycine–Propanal System. The hexane-soluble and -insoluble products obtained from the glycine–propanal system were analyzed. The hexane-soluble fraction contained mainly 2-methyl-2-pentenal which is the aldol condensation reaction product of propanal catalyzed by the amino group of glycine; bp 136–138 °C; IR (neat) 1685 (C=O). The water-soluble fraction from the hexane-insoluble product gave a major spot (R_f 0.30) and three other spots (R_f 0.45, 0.68, and 0.95) on the TLC. The major spot on the TLC coincided in R_f with that of 1-(1-carboxymethyl)-2-ethyl-3,5-dimethylpyridinium betaine (**2**). On the TLC of the chloroform-soluble fraction from the hexane-insoluble product, a major spot (R_f 0.68) and four other spots (R_f 0.30, 0.45, 0.75, and 0.95) were detected. An identification of these compounds was not done in this experiment.

Thermal Decomposition Products of Pyridinium Betaines. Five grams of **2** was heated at 180 °C for 2 min: **2** was melted immediately and carbon dioxide gas was formed. The pale-yellow products which have an undesirable grassy odor were distilled under reduced pressure to afford ca. 3.5 mL of distillate, giving a major peak (t_R 5.7 min) and three additional minor peaks on the GLC (column A). The t_R value of the major peak was similar to that of authentic 2-ethyl-3,5-dimethylpyridine; bp 182–184 °C at normal pressure. The structure of the compound (**8a**) was further confirmed spectrometrically. Its IR spectrum exhibit bands at 1635 and 1595 cm^{-1}, suggesting the presence of pyridine nucleus. ^{1}H-NMR spectrum showed two olefinic singlets at 8.04 ppm (1 H) and 7.01 ppm (1 H) which were attributed to ring protons attached to the 4 and 6 positions, respectively, of the pyridine ring. A six proton singlet at 2.20 ppm was attributed to the methyl at the 3 and 5 positions. The melting point of **8a** picrate, 157–158 °C, was identical with that of the picrate recorded previously (Chichibabin, 1924b).

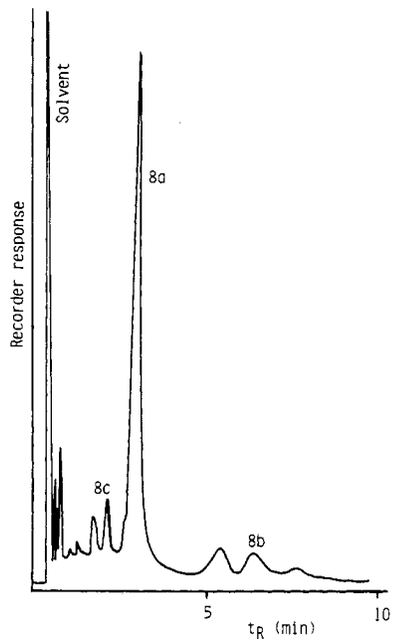


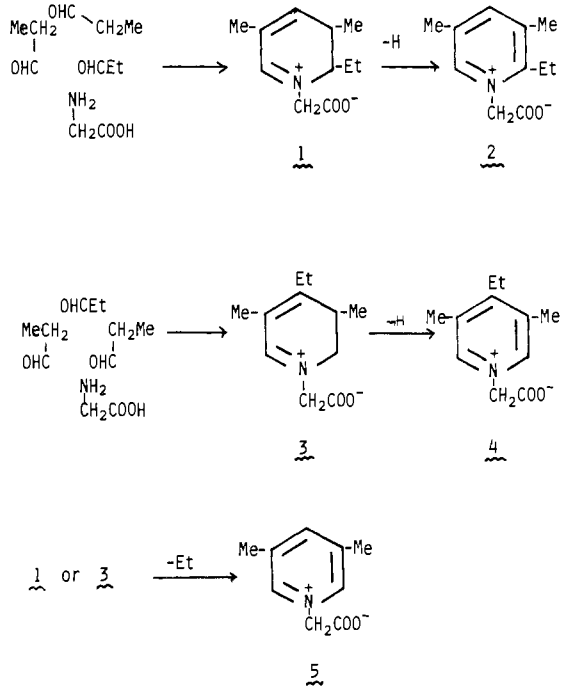
Figure 1. GLC of thermal decomposition products of glycine–propanal system. Pyridines **8a–8c**; see Scheme III.

In the same manner, 1-(1-carboxy-3-methylbutyl)-4-ethyl-3,5-dimethylpyridinium betaine (0.5 g) gave off an undesirable grassy smelling brown syrup, giving a major peak (t_R 7.80) and two additional minor peaks on the GLC (column A). The compound giving rise to the major peak was identified as 4-ethyl-3,5-dimethylpyridine (**8b**). **8b** was also isolated as picrate: mp 155–156 °C, which is identical with that of the picrate recorded previously (Chichibabin, 1924a,b); IR (KBr) 1640, 1620, 1485, 1335, 1270, 1160, 1085, 740, 710 cm^{-1}; ^{1}H NMR δ 1.3 (3 H, t, J = 7 Hz, CH_2CH_3), 2.5 (6 H, s, CH_3 × 2), 2.9 (2 H, q, J = 7 Hz, CH_2CH_3), 8.3 (2 H, s, picric acid), 8.7 (2 H, s, pyridine-H at the 3 and 5 positions).

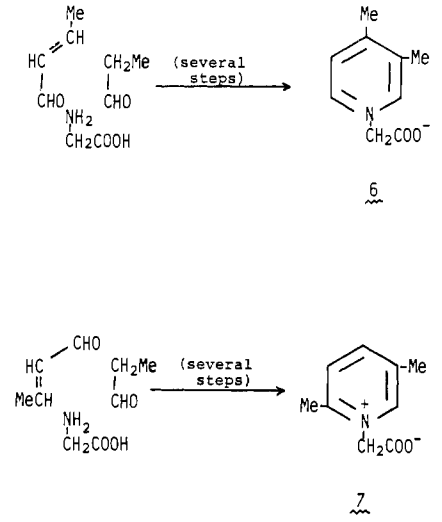
Anal. Calcd for $C_{15}H_{16}N_4O_7$: C, 49.45; H, 4.43; N, 15.38. Found: C, 49.42; H, 4.49; N, 15.33.

Thermal Decomposition of the Reaction Products in the Amino Acid–Propanal System. When the hexane-insoluble reaction products (20 g) of the glycine–propanal system were heated at 120 °C, thermal decomposition occurred immediately with the evolution of carbon dioxide gas and about 3 mL of distillate with a low boiling point (bp 60–95 °C). The temperature was then elevated gradually at 180 °C, and 13 mL of undesirable grassy smelling distillate was collected by distillation under reduced pressure. The latter distillate using columns A and B gave 11 peaks on the GLC, three of them were assigned to the peaks of 3,5-dimethyl- (**8c**), 2-ethyl-3,5-dimethyl- (**8a**), and 4-ethyl-3,5-dimethylpyridine (**8b**) as shown in Figure 1. Both pyridines **8a** and **8b** gave M^+ as m/e 104. The t_R's of the pyridines **8a**, **8b**, and **8c** on the GLC using columns A and B agree very closely with those of the authentic samples. The picrates of **8a** and **8b** were separated by fractional crystallization from the methanol. The melting point, IR spectra, and ^{1}H NMR spectra of these pyridine picrates were also identical with those of the picrates described above.

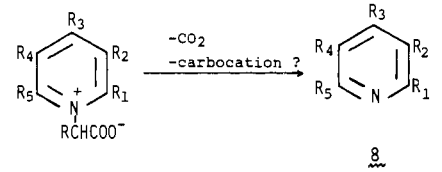
Scheme I



Scheme II



Scheme III



8a; R_1 = Et, R_2 = R_4 = Me, R_3 = R_5 = H.
8b; R_1 = R_5 = H, R_2 = R_4 = Me, R_3 = Et.
8c; R_1 = R_3 = R_5 = H, R_2 = R_4 = Me.
8d; R_1 = R_4 = R_5 = H, R_2 = R_3 = Me.
8e; R_1 = R_4 = Me, R_2 = R_3 = R_5 = H

When the reaction products of various amino acids–propanal systems were treated in the same way described above, analogous volatile basic distillates were obtained. The composition of the distillates was calculated from the peak areas on the GLC, as summerized in Table I. No difference of the pyridine distributions was observed for the thermal decomposition products from the glycine–propanal systems with or without the additives. On the other hand, a quite different distribution of pyridines were observed for the products from the various amino acids–propanal systems. The concentration values of **8b** were significantly higher in the leucine–propanal and lysine–propanal systems than in the glycine–propanal system. The amount of unidentified basic compounds in the lysine–propanal system was much higher than that in the glycine–propanal system.

Condensation of Glycine with the Mixture of Crotonal and Propanal and the Thermal Decomposition of the Products. An analogous exothermic reaction occurred between the glycine (0.2 mol) and crotonal–propanal (each 1 mol mixture) and produced a deep-brown syrup (52 g). By the thermal decomposition of the syrup (20 g), very undesirable smelling basic products [bp 90–210 °C (1 atm), yielding ca. 4 mL] were obtained. Figure 2 shows the GLC on column A of the products. Considerable amounts of 3,4-dimethyl- (**8d**, M^+; 75 *m/e*) and 2,5-dimethylpyridine (**8e**, M^+; 75 *m/e*) with **8a** and **8b** were identified with other unknown basic compounds (see Figure 2). It would be concluded, therefore, that these pyridines were formed from one molecule each of the glycine crotonal, and propanal by the condensation and subsequently thermal decomposition.

Thermal Decomposition of the Products of the Glycine–Propanal–1-Butanal System. Ten grams of resultant products from the mixture of glycine (0.2 mol), propanal, and 1-butanal mixture (each 1 mol) were heated at 180 °C. The volatile basic products obtained (ca. 7 g) gave **8a** plus 13 and 12 peaks on the GLC using columns A and B, respectively.

Mechanistic Consideration on the Pyridine Formation. Formation of pyridinium betaines **2** and **4** was explained in the terms of Scheme I, in which three molecules of propanal condense with one molecule of glycine to give dehydro **1** and **3** as intermediates. It was already reported that *N*-phenyl-3,5-diethyl-2-propyl-1,4-dihydropyridine with the same substitution pattern as **1** is prepared by the reaction of 1-butanal with aniline in the presence of acetic acids (Craig et al., 1948). The dihydro derivatives **1** and **3** undergo dehydrogenation (oxidation) to give **2** and **4**, or dealkylation to give **5**, though the separation of **5** is still unsuccessful. As an example of the dehydrogenation of the dihydro derivative of pyridinium salt moiety, it could be shown that nicotinamide adenine dinucleotide and its phosphate, also known as di- and triphosphopyridine nucleotide, respectively, are involved in the oxidation–reduction processes in living organisms. The dealkylation of dihydropyridine moiety to pyridine moiety is supported by a report by Farley and Eliel (1956), who have obtained 3,5-diphenylpyridine with the same substitution pattern as **8c** in a reaction of phenylacetaldehyde with ammonia.

On the other hand, crotonal can condense with the primary amine of glycine at both positions of carbonyl positive and β positive carbons (Andrews and Mosbo, 1977; Boiko et al., 1963; Suyama et al., 1979). Thus, one molecule each of glycine, crotonal, and propanal condensed as shown in Scheme II to give 1-(1-carboxymethyl)-3,4-dimethyl- (**6**) and 1-(1-carboxymethyl)-2,5-dimethylpyridinium betaine (**7**) (these were not separated) as intermediates. Scheme III showed the last step of thermal decomposition of pyridinium betaines to give pyridines via the Hofmann degradation reaction. The Hofmann degradation of these pyridinium betaines has not been studied. A feature of the thermal decomposition of these pyridinium betaines is accompanied by a loss of carbon dioxide. It is generally recognized that rearrangement of pyridinium

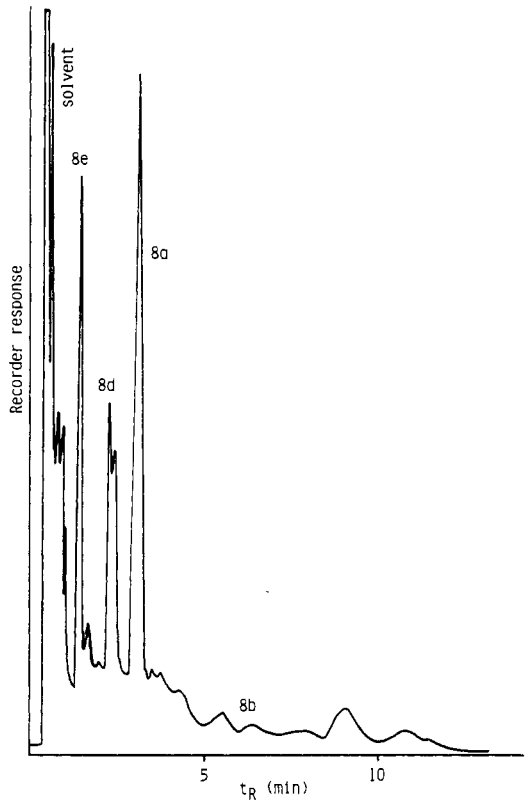


Figure 2. GLC of thermal decomposition products of glycine–propanal–crotonal system. Pyridines **8a–8e**; see Scheme III.

salt may be brought about by heating at a high temperature (Ledenburg rearrangement). In these rearrangements, the N-substituent migrates to a ring carbon, generally to the 2 or 4 position (Elderfield, 1950; Smith, 1976). Although we could not obtain the rearrangement products, the possibility that some of the rearrangement products are formed from pyridinium betaine cannot be excluded.

It can be considered from the reaction scheme that the formation of the cabocation may be involved in the course of the change of alkylpyridinium betaine to alkylpyridine since the carbocation is known as an amino alkyl agent (Olah and Donovan, 1978).

In connection with food chemistry, it would be necessary to do further study on the condensation of amino acids with alkanals or alkenals and the thermal decomposition of these products.

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Analysis of Carrot Volatiles Collected on Porous Polymer Traps

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An accurate and precise porous polymer trapping method was adapted for the gas–liquid chromatography analysis of volatiles from raw carrots. Small samples (25 g) can be employed, 25–30 samples can be collected per day, and the resulting chromatograms are similar to those obtained through conventional Likens–Nickerson distillations. Evaluation of the method revealed that relative to the selected blending procedure in sample preparation, slicing carrot roots enhanced the levels of caryophyllene and α-bisabolene while grating roots resulted in a reduction in total levels of volatiles. More volatile terpenes were found in the crown of roots than in midsection or tip sections, but exceptions were noted. Terpinolene and carophyllene levels were higher in the phloem than in the xylem of roots.

The ideal system for the analysis of volatile flavor components from large numbers of samples should be able to quantitatively capture pertinent compounds in a short time from a small sample (Heatherbell et al., 1971a,b). In addition, it is necessary to capture both high- and low-boiling compounds free of water to make it possible to relate these results to conventional distillation extractions.

This paper details a method that meets these requirements using porous polymer (Tenax GC) traps. Samples from a range of genetically and anatomically diverse raw carrot materials were examined.

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EXPERIMENTAL SECTION

Plant Materials. Eight carrot lines were analyzed 2 weeks after harvest. These lines were grown at four lo-

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