(3), 85 (7); 98 (13), 99 (11); 112 (19), 113 (6); 126 (100), 127 (14), 140 (23), 141 (5); 154 (3); *169* (15). 4-Ethyl-5-butylthiazole (V) had a ^{1}H NMR spectrum: 100 MHz ($CDCl_3$) δ 0.97 (t, 3, J = 7 Hz, $CH_2CH_2CH_2CH_3$), 1.27 (t, 3, J = 7 Hz, CH_2CH_3), 1.5–1.8 (m, 4, $CH_2CH_2CH_2CH_3$), 2.72 (q, 2, J = 7 Hz, CH_2CH_3), 2.77 (t, 2, J = 7 Hz, $CH_2CH_2CH_2CH_3$), 8.55 (s, 1, H 2 pos.).

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Volatile Heterocyclic Compounds in the Reaction of Glyoxal with Glycine

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The basic fraction of the volatiles from an equimolar glyoxal–glycine mixture heated under reflux for 4 hr was separated by gas chromatography. Carbonyls were converted to their 2,4-dinitrophenylhydrazones and separated by thin-layer chromatography. Individual components were isolated and analyzed by mass spectrometry, ultraviolet, visible, and infrared spectrophotometry. Six nitrogen-containing heterocycles (pyrazine, methyl-, 2,5-dimethyl-, 2,6-dimethyl-, and trimethylpyrazine, and 2-pyrrolaldehyde) were positively identified. Formaldehyde was found as the only carbonyl group containing reaction product. A possible precursor, aminoacetaldehyde, was synthesized and the formation mechanism of pyrazines and pyrrole was presented.

During the past few years numerous heterocyclic nitrogen-containing compounds, namely pyrazines and pyrroles, have been reportedly isolated from various food and model systems. Reviews on pyrazines, their chemistry, reactions, occurrences in foods, and importance in the roasted or cooked flavor of many foods, have been recently published by Cheeseman and Werstiuk (1972) and by Maga and Sizer (1973). Some important model systems employing simple carbonyls, carbohydrates, their derivatives, amino acids, and proteins are listed in Table III. The role of carbohydrate degradation or lipid decomposition on the formation of pyrazines has been well documented (Maga and Sizer, 1973; Koehler and Odell, 1970; Wang and Odell, 1972). The formation of pyrroles in foods as well as in model systems has been greatly neglected and most attempts at the formation of these compounds have taken the form of reporting their occurrence in the flavor of various heated and toasted foods.

In food chemistry, glyoxal is known as a degradation product of sugars (Hodge, 1967; Gotlieb and Markakis, 1968; Fagerson, 1969), ascorbic acid (Osthoff, 1966), and lipids (Enders et al., 1962; Cobb and Day, 1965; Bala et al., 1968; Wang and Odell, 1972). This α-dicarbonyl compound reacts with α-amino acids to produce carbon dioxide and aldehydes with one less carbon atom through Strecker degradation (Schönberg and Moubacher, 1952; Plechan and Mardašev, 1953; Fujimaki et al., 1971; Chuyen et al., 1972; Velíšek et al., 1972). Acetaldehyde was identified in the reaction of glyoxal with alanine (Neuberg and Kobel, 1927) and isovaleraldehyde in the reaction with leucine (Fujimaki et al., 1968). Glyoxal was also used as a model compound in the Strecker degradation of numerous α-amino acids. Carbon dioxide evolution was observed in its reaction with glycine (Fujimaki et al., 1971; Chuyen et al., 1972) and this compound was also detected as the only reaction product by Rizzi (1972). On the other hand, Bengelsdorf (1953) and Kamata and Sakurai (1957) observed only the formation of brown high molecular weight pigments.

Koehler and Odell (1970) have found that glyoxal produced pyrazine when heated with ammonium hydroxide or asparagine. Some methylpyrazine was also formed indicating that secondary recombinations and rearrangements occurred.

The present study was undertaken as a part of a series of studies of flavor-significant compounds produced in simple model systems and concerned the identification of

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volatile nitrogenous heterocyclic compounds obtained in glyoxal–glycine reactions. The mechanism of the formation of these compounds was also studied with a synthesized precursor, aminoacetaldehyde.

EXPERIMENTAL SECTION

Materials. Glyoxal, glycine, and pyrazine were obtained from reliable commercial sources. Methyl-, 2,5-dimethyl-, 2,6-dimethyl-, and trimethylpyrazine were gifts. 2-Pyrrolaldehyde was synthesized by the method of Silverstein et al. (1954); aminoacetaldehyde, in the form of its diethyl acetal, was synthesized according to the procedure described by Fischer (1908).

Nitrogenous Heterocycles. The model system used in these studies utilized 0.1 mol each of glyoxal and glycine in 100 ml of water. The reaction mixture was heated 4 hr under reflux and cooled to room temperature. The dark brown solution was adjusted to pH 9–10 with sodium carbonate, saturated with sodium chloride, and extracted with five portions, 20 ml each, of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to approximately 0.5 ml by careful distillation in a Kuderna-Danish concentrator equipped with a 150 × 10 mm i.d. Snyder column (7 plates). The concentrate was analyzed by gas chromatography. The individual fractions of the mixture were isolated in a melting point tube cooled with diethyl ether and rechromatographed using a stationary phase of different polarity. Individual components in the isolated fractions were identified by comparison of their mass, infrared, ultraviolet spectral, and gas chromatographic retention data with those of authentic compounds.

In another experiment, the reaction mixture was atmospherically steam distilled for 4 hr and the distillate (200 ml) was treated as described above.

Aminoacetaldehyde diethyl acetal (0.1 mol) was dissolved in water, the pH of this solution was adjusted to 6.1 (pH of the original glyoxal–glycine mixture) with hydrochloric acid, and the volume of the mixture was made up to 100 ml with water. Further procedure was the same as described above.

In another experiment, a solution (pH 6.1) containing 0.1 mol each of aminoacetaldehyde diethyl acetal and formaldehyde in 100 ml of water was used. The solution was treated in the same way as the solution of aminoacetaldehyde diethyl acetal.

Carbonyl Compounds. A reaction mixture again containing 0.1 mol each of glyoxal and glycine was heated under the above described conditions in an apparatus used by Pippen et al. (1958) for the isolation of volatile carbonyl compounds. 2,4-Dinitrophenylhydrazones produced were extracted with five 20-ml portions of chloroform and the combined extracts were concentrated to about 0.5 ml. 2,4-Dinitrophenylhydrazones in the concentrate were isolated by preparative thin-layer chromatography on silica gel CH (Lachema Brno) in benzene–chloroform (3:1, v/v). The spots corresponding to the individual components were extracted with ethanol–ethyl acetate (1:1, v/v), concentrated near to dryness, and purified again in the same way. Pure 2,4-dinitrophenylhydrazones were identified by comparison of their ultraviolet and visible spectra in chloroform and in 0.25 *N* sodium hydroxide and infrared spectra in potassium bromide with those of authentic compounds.

Gas Chromatography. The gas chromatographic analysis was performed on a Chrom 4 (Laboratorní přístroje Prague) gas chromatograph with a flame ionization detector, using a 2500 × 3 mm i.d. glass column containing 5% Carbowax 20M on 0.125–0.160 mm Chromaton N-AW-DMCS (w/w) (Lachema Brno) at a nitrogen flow rate of 30 ml/min. The temperatures were programmed from 100 to 220°C at a rate of 5°C/min. The injection port was held at 220°C.

Using a 10:1 stream-splitter and 1700 × 7 mm i.d. glass columns containing 15% Carbowax 20M or 15% Apiezon M on the same solid support as described above, aliquots of several peaks were collected and subjected to mass spectrometry and infrared and ultraviolet spectrophotometry.

Spectrometric and Spectrophotometric Measurements. Mass spectra were obtained using an LKB Model 9000 mass spectrometer at 70 eV, by using a direct sample introducing method. Infrared spectra were recorded using a Perkin-Elmer Model 325 spectrophotometer. Ultraviolet and visible spectra were measured using a spectrophotometer Unicam SP 1800.

Table I. Heterocyclic Nitrogenous Heterocycles Arising in Model Systems Containing Glyoxal

Compound	Model system	Ref
Imidazole	Glyoxal-ammonium hydroxide	*a*, *b*
	Glyoxal-formaldehyde-ammonium hydroxide	*c*
2,2′-Diimidazole	Glyoxal-ammonium hydroxide	*a*, *b*
2-Methylimidazole	Glyoxal-acetaldehyde-ammonium hydroxide	*d*, *e*
4(5)-Imidazole-formamide	Glyoxal-formaldehyde-ammonium hydroxide	*e*
2-Ethylimidazole	Glyoxal-ethyl glyoxal-ammonium hydroxide	*e*
Pyrazine	Glyoxal-ammonium hydroxide (asparagine)	*f*
Methylpyrazine	Glyoxal-ammonium hydroxide (asparagine)	*f*

[a] Debus (1858). [b] Wyss (1877). [c] Behrend and Schmitz (1893). [d] Radziszewski (1882). [e] Radziszewski (1883). [f] Koehler and Odell (1970).

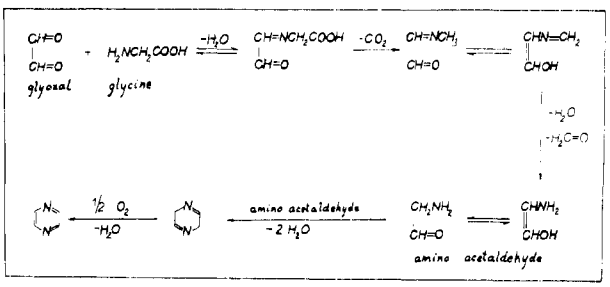

Figure 1. Formation of carbon dioxide, formaldehyde, aminoacetaldehyde, and pyrazine from glyoxal and glycine.

RESULTS AND DISCUSSION

Some nitrogen-containing heterocyclic compounds produced in glyoxal–ammonium hydroxide, glyoxal–asparagine, and in some other model systems are listed in Table I. We have found that glyoxal produced carbon dioxide (Velíšek et al., 1972) and formaldehyde when heated with glycine. The same reaction products have also been identified by other authors (Plechan and Mardasev, 1953; Bengelsdorf, 1953; Kamata and Sakurai, 1957; Fujimaki et al., 1971; Chuyen et al., 1972; Rizzi, 1972). However, from the reactions of alkylglyoxals with α-amino acids, glyoxal can be assumed to be a precursor of pyrazine. According to the general reaction mechanism suggested by Franke (1933) and Dawes and Edwards (1966), the following reactions leading to the formation of a reactive aminoreductone, aminoacetaldehyde, and pyrazine can be postulated (Figure 1).

We have identified pyrazine as the major component of

Table II. Identification of Volatile Compounds Isolated from Glyoxal-Glycine System

Peak no. (see Figure 2)	Compound	Evidence[a,b]
1	Pyrazine	MS, t_R, ir, uv
2	Methylpyrazine	MS, t_R
3	2,5-Dimethylpyrazine	MS, t_R, ir
3	2,6-Dimethylpyrazine	MS, t_R, ir
4	Trimethylpyrazine	MS, t_R
5	Alkylpyrrole (mol wt 137)	MS
6	Unknown (mol wt 149)	MS
7	Unknown (mol wt 158)	MS
8	2-Pyrrolaldehyde	MS, t_R, ir

[a] MS, t_R, ir, and uv: mass spectral, gas chromatographic retention time, infrared, and ultraviolet absorption evidence, respectively. [b] Evidence cited under this column is consistent with that of an authentic sample on the same instrument.

the basic fraction of the volatiles from glyoxal–glycine mixtures. Some other pyrazines and pyrroles were present in smaller quantities. Table II is a summary of the compounds identified and criteria for identity. The identifications were designated positive if mass, and in some cases infrared and ultraviolet spectral and retention time data, matched authentic compounds analyzed under identical instrument conditions. A gas chromatogram of the analyzed basic fraction can be seen in Figure 2a. The peak numbers refer to those of Table II. There were no qualitative differences between the two procedures used for the preparation of the basic fraction concentrate (direct extraction or steam distillation of the reaction mixture). Figures 2b and 2c compare the volatile reaction products produced either in aminoacetaldehyde or in aminoacetaldehyde–formaldehyde model systems. The identification of the six heterocycles, five pyrazines and 2-pyrrolaldehyde, in glyoxal–glycine systems was obtained for the first time. These heterocyclic compounds have been isolated from the volatiles produced in various other model systems containing simple carbonyls or their precursors (Table III).

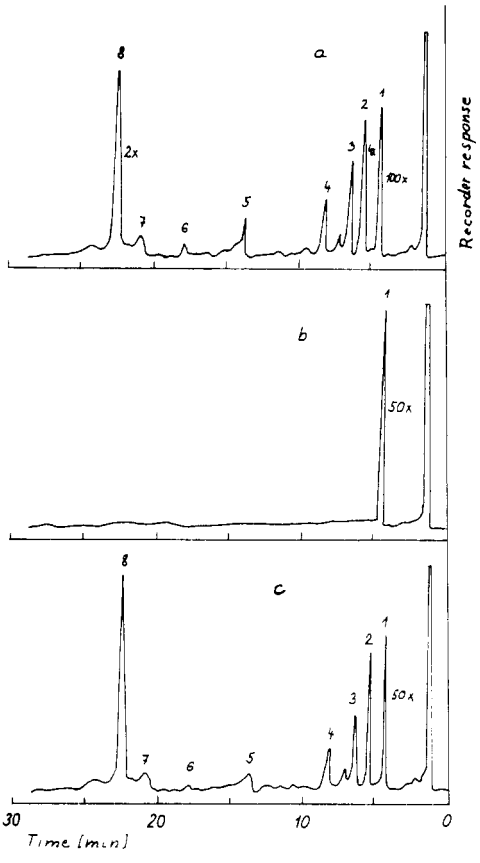

Figure 2. (a) Gas chromatogram of glyoxal–glycine reaction products; (b) gas chromatogram of aminoacetaldehyde reaction products, (c) gas chromatogram of aminoacetaldehyde–formaldehyde reaction products.

As it can be seen, 2,5-dimethyl-, 2,6-dimethyl-, and trimethylpyrazine and 2-pyrrolaldehyde have not been identified in any model systems utilizing glyoxal; 2-pyrrolaldehyde has not been identified in any model systems containing simple carbonyl compounds.

The formation of the major volatile component of

Table III. Formation of Pyrazine, Methyl-, 2,5-Dimethyl-, 2,6-Dimethyl-, and Trimethylpyrazine, and 2-Pyrrolaldehyde in Some Model Systems

Model system	Pyrazine	Methyl-pyrazine	2,5-Di-methyl-pyrazine	2,6-Di-methyl-pyrazine	Tri-methyl-pyrazine	2-Pyr-rolalde-hyde	Ref
Acetaldehyde–ammonium hydroxide	+						*b*
Acetaldehyde–asparagine	+	+	+				*b*
Glyoxal–ammonium hydroxide	+	+					*b*
Glyoxal–asparagine	+	+					*b*
Pyruvaldehyde–glycine			+	+	+		*c*
Glycerol–glycine		+	+	(+)	+		*a, d*
Glycerol–alanine		+	+	(+)	+		*a, c*
Glycerol–asparagine	+	+	+				*b*
Hydroxyacetone–ammonium hydroxide			+				*b*
Hydroxyacetone–asparagine			+				*b*
Amino acetone			+		+		*c, e*
Glucose–ammonium hydroxide	+	+	+	+	+		*f–j*
Glucose–ammonium chloride	+						*k*
Glucose–alanine	+	+	+		+		*l, m*
Glucose–asparagine			+		+		*k*
Glucose–glutamine		+					*k*
Glucosamine	+	+	+				*b*
Fructose–glycine			+		+		*n*
Fructose–phenylalanine			+				*n*
Rhamnose–ammonium hydroxide	+	+	+	+	+		*f*
Lactose–casein	+	+	+	+	+	+	*o, p*

[a] (+) = tentatively identified. [b] Koehler and Odell (1970). [c] Rizzi (1972). [d] Wang and Odell (1972). [e] Grimmett et al. (1968). [f] van Praag et al. (1968). [g] Brandes and Stoehr (1896). [h] Havre and Ender (1971). [i] Heyns and Koch (1971). [j] Scanlan and Libbey (1971). [k] Koehler et al. (1969). [l] Shigematsu et al. (1972). [m] Fujimaki et al. (1972). [n] Deck and Chang (1965). [o] Ferretti and Flanagan (1971). [p] Ferretti et al. (1970).

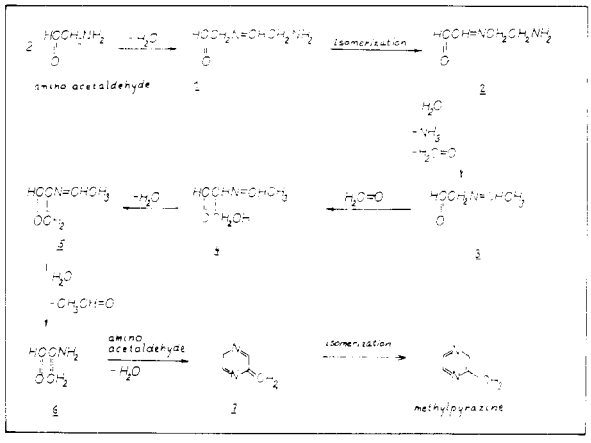

Figure 3. Formation of methylpyrazine from aminoacetaldehyde.

glyoxal–glycine mixtures, pyrazine, could be expected and possible reactions leading to its formation have been derived (Figure 1). However, much more significant is the fact that pyrazine derivatives were also identified. Apparently, the reaction scheme proposed in Figure 1 cannot be applied to the formation of these compounds. According to these equations (Wang et al., 1969), methylpyrazine would be the major product in reactions of α-amino acids with an equimolar glyoxal–pyruvaldehyde mixture, 2,5-dimethyl- and 2,6-dimethylpyrazine would arise in mixtures containing pyruvaldehyde alone, and trimethylpyrazine would arise in an equimolar mixture of pyruvaldehyde and 2,3-butanedione. The alkylpyrazines identified in glyoxal–glycine mixtures contained 5, 6, and 7 carbon atoms in their molecules. Apparently, reactions involving interactions of glyoxal or some other reaction intermediates, e.g. formaldehyde, aminoacetaldehyde, etc., can be expected. Recently, an interesting mechanism of the formation of trimethyl- and 2,5-dimethyl-3-ethylpyrazine from amino acetone was published by Rizzi (1972). A similar mechanism can be derived for the formation of methylpyrazine in glyoxal–glycine mixtures (Figure 3). Two molecules of the methylpyrazine precursor, aminoacetaldehyde, condense to form **1** which with isomerization leads to **2**. Elimination of formaldehyde and ammonia and subsequent condensation with formaldehyde yield **3** and then its hydroxymethyl derivative **4** which reacts with elimination of water to form the methylene derivative **5**. Acetaldehyde is then split off; **6** reacts with the second molecule of aminoacetaldehyde with elimination of water to form **7** and then methylpyrazine. Self-condensation of **6** can also yield 2,5-dimethylenepyrazine which can undergo hydrogenation to form 2,-5-dimethylpyrazine. The formation of other pyrazine derivatives is not possible. A more likely mechanism for the formation of these pyrazines could involve initial condensation of aminoacetaldehyde with formaldehyde to form **6**, followed by hydrolysis with or without elimination of ammonia to form pyruvaldehyde or its amino derivative, e.g. 1-amino-2-propanone. 1-Amino-2-propanone can form 2,5-dimethylpyrazine according to the reaction mechanism given in Figure 1. Its isomer, 2-aminopropanol, yields 2,6-dimethylpyrazine after condensation with 1-amino-2-propanone. It is also well known that formaldehyde alone, in an alkaline medium, can condense to form numerous carbonyl group containing products, e.g. glycolaldehyde, glyceraldehyde, etc. (Tambawala and Weiss, 1972). Pyruvaldehyde is produced by glyceraldehyde dehydration and by isomerization of the products (Fleury et al., 1968). Neither glycolaldehyde, glyceraldehyde, and

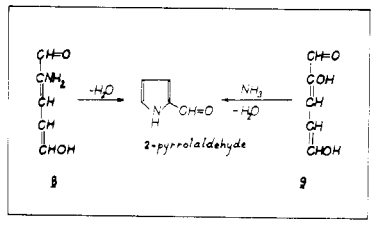

Figure 4. Formation of 2-pyrrolaldehyde in a glyoxal-glycine system.

pyruvaldehyde in the glyoxal–glycine system nor formaldehyde and acetaldehyde suggested by Rizzi (1972) as intermediates in the formation of pyrazines from amino acetone have been detected and the proposed reaction mechanism would therefore be further studied and verified. The above mentioned reactions involving autocondensation of formaldehyde or condensation of formaldehyde with aminoacetaldehyde are not very probable as the reaction mixture is almost neutral. However, according to Hodge (1953), the amino compounds in the reaction mixture could act as catalysts.

The formation of pyrazine from aminoacetaldehyde (Figure 2b) proves the reaction mechanism described in Figure 1. In this case, neither other pyrazines nor 2-pyrrolaldehyde was detected. If we suppose that the presence of formaldehyde is necessary for the formation of these compounds then formaldehyde arises from aminoacetaldehyde in small quantities or it does not arise at all. The reaction mechanism of the formation of methylpyrazine from amino acetone (Rizzi, 1972) is then unprobable. As can be seen in Figure 2c, the same nitrogenous heterocycles which arise in glyoxal–glycine mixtures form also in the reactions of aminoacetaldehyde with formaldehyde. It is highly probable that formaldehyde arises in glyoxal–glycine mixtures entirely by the Strecker degradation of glycine and does not arise from aminoacetaldehyde. The presence of formaldehyde is then necessary for the formation of pyrazine and pyrrole derivatives which arise by condensation reactions and by oxidations and reductions of numerous intermediates.

The formation of 2-pyrrolaldehyde also indicates that secondary reactions occur. Based on experimental results of other authors (Kato, 1966, 1967; Kato and Fujimaki, 1970; Kato et al., 1972; Wieland and Severin, 1973), the possible precursors of 2-pyrrolaldehyde are compounds **8** or **9** in Figure 4. 2-Pyrrolaldehyde is then formed by simple dehydration of **8** or by condensation with ammonia followed by dehydration of **9**. **8** can arise as a product of condensation of aminoacetaldehyde with glyceraldehyde, glycolaldehyde, and formaldehyde or formaldehyde alone and by elimination of water. **9** can arise by the same reactions of glycolaldehyde instead of aminoacetaldehyde. **8** can also arise by condensation of **9** with ammonia which is supposed to be an intermediate in the Strecker degradation of glycine (Schönberg and Moubacher, 1952; Fujimaki et al., 1971).

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Effect of Total Lipids and Phospholipids on Warmed-Over Flavor in Red and White Muscle from Several Species as Measured by Thiobarbituric Acid Analysis

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TBA (2-thiobarbituric acid) analysis demonstrated that turkey meat is most susceptible to WOF (warmed-over flavor) development, followed closely by chicken, then by pork, beef, and mutton in that order. Although freshly cooked muscle from all species except mutton had higher TBA numbers than fresh raw samples, the most dramatic change occurred during storage of cooked meat at refrigerated temperature (48 hr at 4°C). Red muscles had consistently higher TBA values than white muscles under these storage conditions, indicating that red muscles were more susceptible to oxidative deterioration. Correlation coefficients between TBA numbers and total lipid levels and between TBA values and phospholipids suggest that phospholipids play a major role in development of WOF in all cooked meats, except for pork, where total lipid levels seem to be the major contributor to WOF.

Warmed-over flavor (WOF) was first noted by Timms and Watts (1958), who coined the term to describe the rapid onset of rancidity in cooked meat during short-term refrigerated storage. Although WOF was assumed to be due to metmyoglobin-catalyzed lipid oxidation (Hirano and Olcott, 1971; Kendrick and Watts, 1969; Younathan and Watts, 1960), it has since been shown that nonheme iron is the major prooxidant in cooked meat (Love and Pearson, 1974; Sato and Hegarty, 1971). Even though earlier studies have utilized beef, pork, poultry, and fish, a comparison of the various species from the standpoint of susceptibility has not been previously made. It is well known that the lipid content of red fibers is appreciably higher than that of white fibers (Cassens and Cooper, 1971; George and

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