A Study of Pyrazine Formation

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The volatiles isolated from the reaction mixture of a D-glucose and ammonia model system were identified using the gas chromatographic retention indices and the gas chromatographic–mass spectrometric technique. The principal constituents of the dichloromethane extract of the above reaction mixture were imidazoles, pyrazines, and pyrroles. The identification of 5-imino-2-methyl-1-cyclopentenol is further evidence that 2-methyl-1-cyclopentenol-5-one could be a precursor of cyclopentapyrazines. The dehydrogenation of 2,3,5-trimethyl-5,6-dihydropyrazine was conducted in ethanol–KOH solution under acidic and basic conditions. When the reaction mixture was treated with acid, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl-3,6-dimethyl-, 2-ethyl-3,5-dimethyl-, tetramethyl-, and 2-ethyl-2,3,5-trimethylpyrazine were obtained in addition to 2,3,5-trimethylpyrazine and unreacted dihydropyrazine. This indicated the presence of dihydropyrazine carbanions, which participated in introducing ethyl groups of solvent ethanol to pyrazine rings, in the reaction system.

It is a well-known fact that sugars react with aqueous ammonia to produce nitrogen-containing heterocyclic compounds (imidazoles, pyrazines, piperazines, and pyridines; Kort, 1970). Pyrazines form in many cooked foods (Bondarovich et al., 1967; Stoffelsma et al., 1968; Walradt et al., 1971; Mussinan et al., 1973) and are believed to be the volatiles which give roasted or smoky aromas to cooked foods. Since the discovery of the flavor characteristics of these heterocyclic compounds, their formation in foods has also been studied by many investigators using sugar–ammonia model systems (van Praag et al., 1968; Koehler and Odell, 1970; Shibamoto and Bernhard, 1977). Shibamoto and Russell (1977) reported the formation of sulfur- and nitrogen-containing compounds from the D-glucose–hydrogen sulfide–ammonia model system. They identified thiophenes, furans, pyrazines, and thiazoles, which could be produced from the reaction of D-glucose and hydrogen sulfide; decomposition of D-glucose; D-glucose and ammonia; and D-glucose, hydrogen sulfide, and ammonia, respectively. Sakaguchi and Shibamoto (1978) studied the compounds produced from D-glucose and hydrogen sulfide. They identified thiophenes and furans, some of which had been found in a D-glucose–hydrogen sulfide–ammonia model system. In the present study, the compounds produced from D-glucose and ammonia, which is another combination of the above three reactants (D-glucose, hydrogen sulfide, and ammonia), are identified in order to be able to investigate the formation pathways of heterocyclic flavor compounds in foods.

The formation of pyrazines is a quite complicated process. A very simple model system produces a large number of alkylpyrazines (Shibamoto and Bernhard, 1978). Kato et al. (1970) reported formation of nine alkylpyrazines from the pyrolysis of α-hydroxy amino acids. Most recently, Shibamoto and Bernhard (1978) identified 39 pyrazines, which included cyclopentapyrazines and quinoxalines, in their L-rhamnose–ammonia reaction mixture. It has been found that other alkylpyrazines form when the one desired alkylpyrazine is synthesized from the corresponding dihydropyrazine by dehydrogenation (Akiyama et al., 1978). Mager and Berends (1957) reported that a dihydropyrazine is hydrolyzed into a carbonyl and amine by the action of hydrogen peroxide. Akiyama et al. (1978) found that many byproducts were produced when an alkyldihydropyrazine was treated with acid. In this study, 2,3,5-trimethylpyrazine was synthesized from 2,3,5-trimethyl-5,6-dihydropyrazine using the method reported by Ishiguro and Matumura (1958). The reaction mixture was then treated with acid to investigate the byproducts of this reaction and their formation mechanisms.

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EXPERIMENTAL SECTION

Reaction of D-Glucose and Ammonia. A Kjeldahl flask (100 mL) containing an aqueous solution of 1 M D-glucose and 5 M ammonium hydroxide was cooled in an ice bath for 10 min. The neck of the flask was flame sealed and the ampule placed in an oven at 100 °C for 2 h. Reaction products were isolated from the reaction mixture with 200 mL of dichloromethane using a continuous liquid–liquid extractor for 16 h. The dichloromethane extract was dried over anhydrous magnesium sulfate and solvent was removed using a rotary flash evaporator. Approximately 0.2 g of a brown viscous material was obtained. This material was subjected to gas chromatographic mass stectrometric analysis.

Synthesis of 2,3,5-Trimethyl-5,6-dihydropyrazine. An ethyl ether solution (170 mL of ether, 21 g of diacetyl) was dripped into a second solution (200 mL of ethyl ether containing 20 g of 1,2-propanediamine) and then stirred for 1.5 h. The solution temperature was maintained at below 10 °C. After the addition of 1,2-propanediamine was completed, stirring was continued until the white cloudy material disappeared. The solution was then refluxed using a water bath for 30 min. After the reaction was finished, the water layer was removed using a separatory funnel. The ethyl ether layer was dried over anhydrous sodium sulfate for 12 h. After the ethyl ether was removed by distillation, pure 2,3,5-trimethyl-5,6-dihydropyrazine (22 g) was isolated by means of fractional distillation under reduced pressure (bp, 53–55 °C at 15 mmHg). The structure of the above compound was confirmed by IR, NMR, and MS.

Synthesis of Trimethylpyrazine and Acid Treatment. The dehydrogenation of 2,3,5-trimethyl-5,6-dihydropyrazine was conducted by the method reported by Ishiguro and Matumura (1958). 2,3,5-Trimethyl-5,6-dihydropyrazine (40 g) was dissolved in 500 mL of ethanol solution containing 17 g of KOH. After the above solution was refluxed for 5 h, its pH was adjusted to 3 by the addition of 20% HCl solution, and ethanol was removed using a rotary flash evaporator. Approximately 70 g of brown oily residue (chloride salts) was neutralized by 25 mL of 10% NaOH solution. This solution was extracted with two 100-mL portions of ethyl ether. After the ethyl

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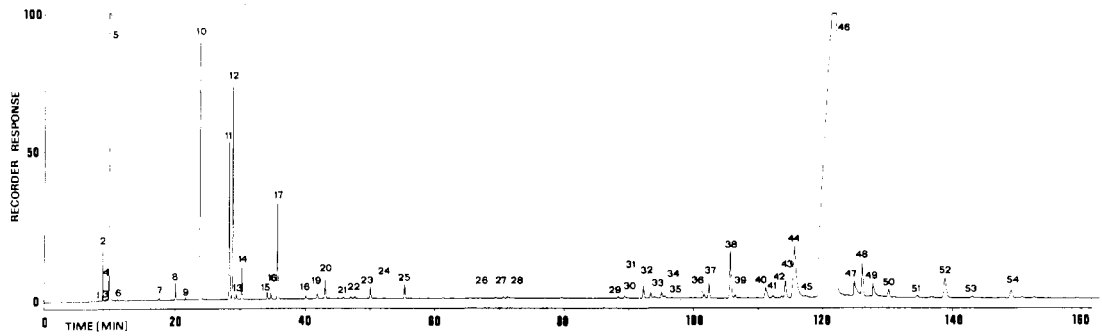


Figure 1. Gas chromatogram of compounds isolated from the reaction of D-glucose with ammonia. A Hewlett Packard Model 5710 A gas chromatograph equipped with a flame ionization detector and a 50 m × 0.25 mm i.d. glass capillary column coated with Carbowax 20M was used. The oven temperature was programmed from 80 to 200 °C at 1 °C/min. See Table I for peak identification.

ether was removed by distillation, a brown oil material (9 g) was obtained by fractional distillation under reduced pressure (60–63 °C at 20 mmHg).

Method of Analysis. The qualitative and quantitative analyses of the reaction products of the above experiments were conducted following the gas chromatographic–mass spectrometric methods described by Shibamoto and Russell (1976a). Unknowns were identified by comparison of their mass spectra and Kovats indices with those of authentic samples. Identities of compounds which were deduced from mass spectra, but for which no authentic samples are available, are indicated as tentatively identified.

RESULTS AND DISCUSSION

The compounds identified in the D-glucose–ammonia model system and their occurrence in foods and presence in D-glucose–hydrogen sulfide and D-glucose–hydrogen sulfide–ammonia model systems are shown in Table I. The compounds are listed in order of elution from the gas chromatographic column (Carbowax 20 M), and a typical gas chromatogram is shown in Figure 1. The main constituents of this extract are imidazoles, pyrazines, and pyrroles. The percentages of their total gas chromatographic peak areas are 81.0, 14.7, and 3.0, respectively (excluding compounds only tentatively identified).

Comparison of Results between the D-Glucose–Ammonia Model System and D-Glucose–Hydrogen Sulfide or D-Glucose–Hydrogen Sulfide–Ammonia Model Systems. The difference between the results obtained in the present study (D-glucose–ammonia) and the previous study (D-glucose–hydrogen sulfide–ammonia; Shibamoto and Russell, 1977) is that the present study recovered imidazoles in large quantities. This is due to a difference in the extraction time used in the two experiments. When continuous extraction is carried over 6 h, the more polar compounds (e.g., imidazoles) begin to be recovered (Shibamoto and Russell, 1976b). Extraction was, therefore, continued for 16 h in this study in contrast to 6 h in the previous study. Some cyclopentapyrazines, which were not found in the D-glucose–hydrogen sulfide–ammonia model system, were also recovered. More pyrroles were recovered from this study than from the D-glucose–hydrogen sulfide–ammonia model system. Furans were not recovered from this study, but it is obvious that a furan ring was formed in this reaction because some pyrroles which contained a furan moiety were recovered: 1-furfurylpyrrole, 1-(5-methylfurfuryl)pyrrole and 1-furyfurylpyrrole-2-carboxaldehyde. The D-glucose–ammonia model system produced pyrroles in contrast to the D-glucose–hydrogen sulfide model system, which produced thiophenes. It is possible that neither the formation of pyrroles nor that of thiophenes requires a high degree of sugar fragmentation Scheme I. The D-glucose–ammonia

Scheme I

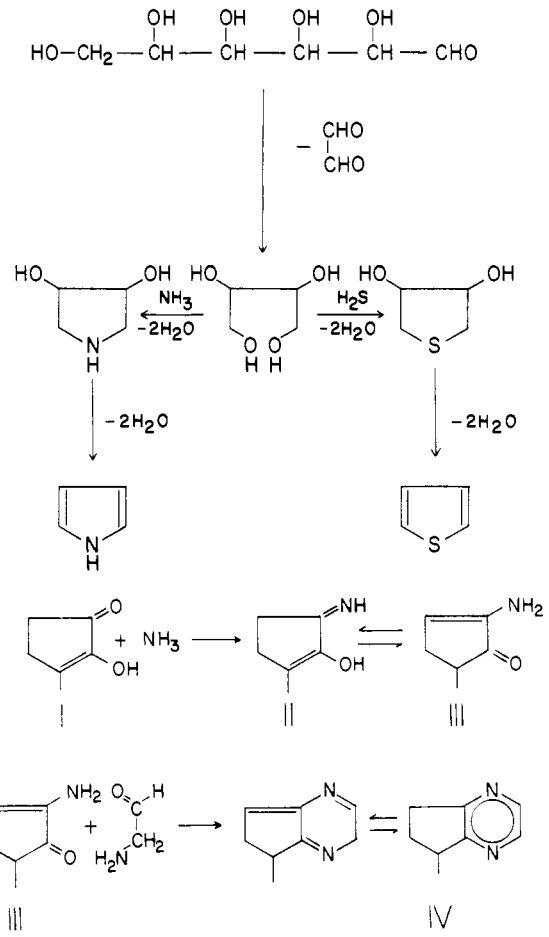


Figure 2. The proposed formation pathway of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine.

model system produced pyrazines and imidazoles in contrast to the D-glucose–hydrogen sulfide model system, which produced cyclic methylene polysulfides (1,2,4-trithiolane, *s*-trithiane). The formation of pyrazines, imidazoles, and cyclic methylene polysulfides requires a high degree of sugar fragmentation, as does the formation of thiazoles such as were produced in the D-glucose–hydrogen sulfide–ammonia model system.

Formation of Cyclopentapyrazine. A proposed formation pathway for 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine is shown in Figure 2. Shibamoto and Bernhard (1978) postulated the formation pathway of 5-methyl-5,6-dihydro-5*H*-cyclopentapyrazine (IV). They suggested that this compound is formed from 2-methyl-1-cyclopentenol-5-one (I), which is a sugar degradation product (Hodge, 1967), through 2-amino-5-methyl-2-

Table I. Compounds Isolated from D-Glucose-Ammonia Model System

peak no.	compd	area, %	occurrence in foods	presence in model systems[h] A	B	MS ref
1	acetaldehyde	a	peanuts[c]			Kinlin et al. (1972)
2	acetone	0.5	peanuts[c]	b		Kinlin et al. (1972)
3	solvent impurity					
4	2-butanone	0.1	coffee[d]	b		Kinlin et al. (1972)
5	solvent (dichloromethane)					
6	3-pentanone	a	coffee[d]			Mussinan and Walradt (1974)
7	2-methyloxazole (tentative)	a				
8	pyrazine	0.3	beef[e]		b	Mussinan and Walradt (1974)
9	1-methylpyrroline (tentative)	a				
10	2-methylpyrazine	4.3	beef[e]		b	Mussinan and Walradt (1974)
11	2,5-dimethylpyrazine	2.9	beef[e]		b	Mussinan and Walradt (1974)
12	2,6-dimethylpyrazine	4.0	beef[e]		b	Mussinan and Walradt (1974)
13	2-ethylpyrazine	a	beef[e]		b	Mussinan and Walradt (1974)
14	2,3-dimethylpyrazine	0.6	beef[e]		b	Mussinan and Walradt (1974)
15	2-ethyl-6-methylpyrazine	0.1	beef[e]		b	Mussinan and Walradt (1974)
16	2-ethyl-5-methylpyrazine	0.1	pork[f]		b	Mussinan and Walradt (1974)
17	trimethylpyrazine	2.0	pork[f]		b	Mussinan and Walradt (1974)
18	2,6-diethylpyrazine	a	pork[f]			Bondarovich et al. (1967)
19	2-ethyl-3,5-dimethylpyrazine	a	pork[f]		b	Friedel et al. (1971)
20	tetramethylpyrazine	0.4	pork[f]		b	Mussinan and Walradt (1974)
21	pyrrole	a	peanuts[c]		b	Budzikiewicz et al. (1964)
22	2-ethyl-3,5,6-trimethylpyrazine	a			b	Friedel et al. (1971)
23	unknown	a				
24	2-methylpyrrole	0.2	peanuts[c]			Budzikiewicz et al. (1964)
25	pyrrole-1-carboxaldehyde	0.3	peanuts[c]			Walradt et al. (1971)
26	5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	a	peanuts[c]			Mussinan and Walradt (1974)
27	2,5-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	a	pork[f]			Mussinan and Walradt (1974)
28	3,5-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	a	pork[f]			Vitzthum and Werkhoff (1975)
29	acetamide	a			b	Ferretti et al. (1970)
30	propionamide	a				Ferretti et al. (1970)
31	5-imino-2-methyl-1-cyclopentenol	0.4				Flament et al. (1976)
32	2-ethyl-4,5-dimethyloxazole (tentative)	0.2				
33	2-acetylpyrrole	a	filberts[g]			Kinlin et al. (1972)
34	unknown	0.3				
35	6-methylquinoxaline	a	pork[f]			Mussinan and Walradt (1974)
36	unknown	0.1				
37	2-acetylpyrrole (tentative)	0.4				
38	5-ethylfurfural (tentative)	1.5				
39	5-methylpyrrole-2-carboxaldehyde	0.1	filberts[g]			Kinlin et al. (1972)
40	5-methyl-2-acetylpyrrole	0.5				
41	imidazole-1-carboxaldehyde (tentative)	0.1				Bowie et al. (1967)
42	unknown	a				
43	unknown	0.6				
44	1,2-dimethylimidazole	3.6				Bowie et al. (1967)
45	1,4-dimethylimidazole (tentative)	a				
46	2-methylimidazole	72.5				Bowie et al. (1967)
47	2-ethylimidazole	0.7				
48	1-acetyl-4-methylimidazole	1.2				Fuchs and Sundell (1975)
49	imidazole-2-carboxaldehyde	0.5				Bowie et al. (1967)
50	unknown	0.3				
51	imidazole derivate (mol wt 138)	0.1				
52	1-furfurylpyrrole	1.1				Stoll et al. (1967)
53	1-(5-methylfurfuryl)pyrrole	a				Stoll et al. (1967)
54	1-furfurylpyrrole-2-carboxaldehyde	0.4				Stoll et al. (1967)

[a] Area % less than 0.1. [b] Present. [c] Walradt et al. (1971). [d] Stoll et al. (1967). [e] Mussinan et al. (1973). [f] Mussinan and Walradt (1974). [g] Kinlin et al. (1972). [h] A, D-glucose-hydrogen sulfide model system; B, D-glucose-hydrogen sulfide-ammonia model system.

cyclopentenone (III). The present study found 5-imino-2-methyl-1-cyclopentenol (II, peak no. 31 in Figure 1), which is an isomer of the above α-amino carbonyl compound (III), in the D-glucose–ammonia model system.

Imidazoles. Imidazoles were the main products of this reaction. Their total area of the gas chromatogram is over 80% and 2-methylimidazole was recovered in the largest quantity (area %: 72). Imidazoles have been found in many sugar–amine model systems (Tsuchida and Komoto, 1967; Tsuchida et al., 1975; Shibamoto and Bernhard, 1978). They have not, however, been found in common foods. This may be due to the fact that imidazoles are less volatile compounds and hard to pass through the stainless steel gas chromatographic column. Unlike pyrazines, which are widely used as flavor ingredients, imidazoles are not used as flavor ingredients at the present time.

Dehydrogenation of 2,3,5-Trimethyl-5,6-dihydropyrazine. The compounds produced from the dehydrogenation reaction of 2,3,5-trimethyl-5,6-dihydropyrazine are listed in Table II. In addition to trimethylpyrazine, which is a dehydrogenated product of 2,3,5-trimethyl-5,6-dihydropyrazine, other alkylpyrazines formed from this reaction. This may be due to the unreacted dihydropyrazine, which is very unstable under acidic conditions

Table II. Compounds Produced from the Dehydrogenation Reaction of 2,3,5-Trimethyl-5,6-dihydropyrazine

pyrazine	GC peak area %
2,5-dimethylpyrazine	0.95
2,6-dimethylpyrazine	*a*
2,3,5-trimethyl-5,6-dihydropyrazine	1.59
trimethylpyrazine	80.55
2-ethyl-3,6-dimethylpyrazine	0.58
2-ethyl-3,5-dimethylpyrazine	*a*
tetramethylpyrazine	11.69
2-ethyl-3,5,6-trimethylpyrazine	4.70

[a] Area % less than 0.01.

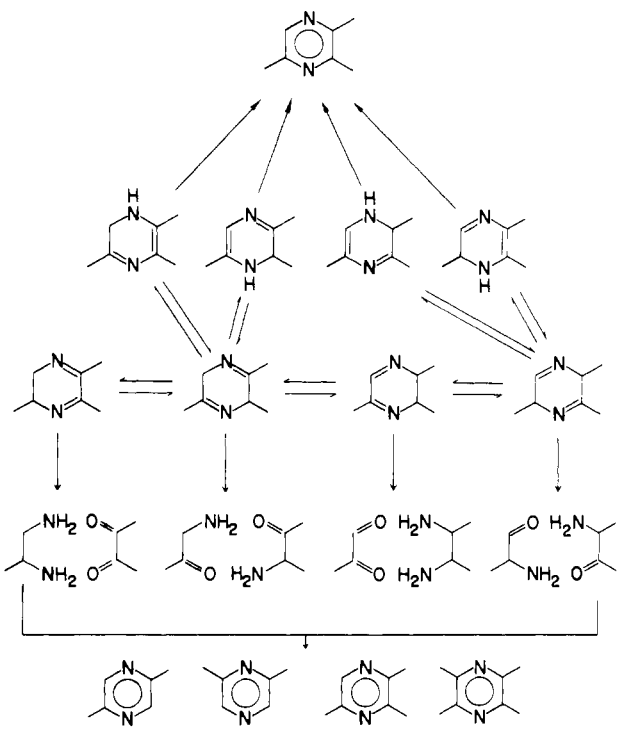


Figure 3. The proposed formation pathways of alkylpyrazines from 2,3,5-trimethyl-5,6-dihydropyrazine.

(Bondarovich et al., 1967). These alkylpyrazines were not found when extraction was carried out before the acid treatment. These results indicate that byproducts of alkylpyrazines form only when a solution is treated by acid. This helps to explain the complexity of pyrazine formation in the sugar–amine model system, because the pH of the above reaction solution decreases following the progress of the reaction and the final pH is less than 4 (Hayami, 1961). The dehydrogenation of dihydropyrazine does not occur under acidic or neutral conditions (Akiyama et al., 1978). This fact explains the presence of dihydropyrazine carbanion intermediates under acidic conditions.

Figure 3 shows the proposed formation pathways of alkylpyrazines from 2,3,5-trimethyl-5,6-dihydropyrazine. The rearrangement to different dihydropyrazine isomers would occur under basic conditions. The major rearrangement product is likely to be a mixture of the four possible trimethyl-1,2-dihydropyrazines, which then undergo rapid oxidation to the major final product trimethylpyrazine (Rizzi, 1968). Other isomeric dihydropyrazines are less susceptible to oxidation and they would remain in the crude reaction mixture along with the trimethylpyrazine. The dihydropyrazines would be hydrolyzed to yield α-amino carbonyl compounds, dicarbonyl compounds, and diamino compounds by the addition of hydrogen chloride solution. Finally, neutralization of the

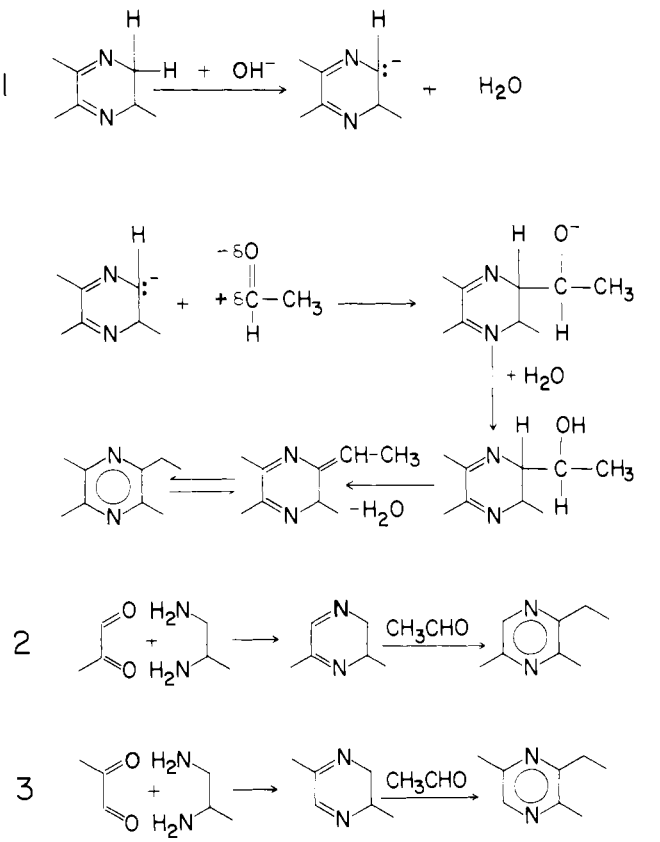
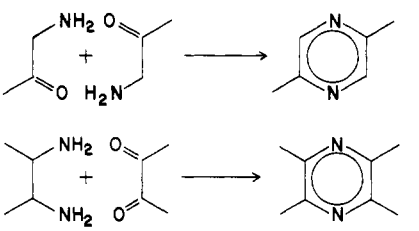


Figure 4. The postulated reaction pathways for ethylpyrazines.

hydrochlorides could lead those compounds to the alkylpyrazines shown in Figure 3; for example:



2-Ethyl-3,5,6-trimethylpyrazine. In addition to these methyl-substituted pyrazines, ethyl-substituted pyrazines were also obtained. Figure 4 shows the postulated reaction pathways for ethylpyrazines (1, 2-ethyl-3,5,6-trimethylpyrazine; 2, 2-ethyl-3,5-dimethylpyrazine; 3, 2-ethyl-3,6-dimethylpyrazine). The small amount of ethanol from the solvent was oxidized into acetaldehyde under basic conditions (House, 1972) and this acetaldehyde reacted with dihydropyrazine carbanions to give ethylpyrazines. When acetone was used instead of ethanol as a solvent, 2-isopropyl-3,5,6-trimethylpyrazine was obtained in yields of 75%. The results obtained from this study indicate that various alkylpyrazines formed from an α-amino carbonyl compound, a diketone, and a diamine. Effects of solvents on pyrazine formation is under investigation at the present time.

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Factors Affecting the Stability of SIR-8514 (2-Chloro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide) under Laboratory and Field Conditions

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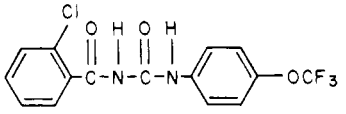
SIR-8514 (2-chloro-*N*-[[[-4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide) is a potent inhibitor of mosquito larval development. Its stability in water is greatly reduced as temperature and pH both become relatively high. Its persistence is not greatly affected by sunlight. In water having a high organic matter content, its persistence appears to be affected by adsorption and by microbial degradation. SIR-8514 is stable on vegetation under field conditions; residues on vegetation can be minimized by application of sand granule formulations. The biological and chemical properties of SIR-8514 are similar to those of diflubenzuron (2,6-difluoro-*N*-[[[4-chlorophenyl]amino]carbonyl]benzamide).

SIR-8514 (2-chloro-*N*-[[[4-(trifluoromethoxy)phenyl]-amino]carbonyl]benzamide) is a highly effective inhibitor of mosquito larval development in the laboratory and field (Schaefer et al., 1978). SIR-8514, like diflubenzuron



(2,6-difluoro-*N*-[[[4-chlorophenyl]amino]carbonyl]benzamide) is a potent inhibitor of the terminal polymerization step in chitin formation (Hajjar and Casida, 1978).

Because extensive field testing of SIR-8514 is anticipated, investigations of methods for quantitative analyses and of factors which affects its persistence were conducted in laboratory and small-scale field trials during 1977 and 1978.

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MATERIALS AND METHODS

High-Performance Liquid Chromatograph (LC). A Varian Model 8500 LC having a μV photometer (254 nm) was utilized for all quantitative analyses. A Micro Pak-CH column (octadecylsilane bonded on 10-μm particles) 8 mm × 25 cm, provided reverse-phase separations using 70% acetonitrile–30% water as the mobile phase at a rate of 70 mL/h. At an ambient temperature of 27 °C, SIR-8514 has a retention time of 12 min.

Extraction of SIR-8514 from Water. Duplicate 600-mL samples of water were partitioned against 3 × 200-mL aliquots of dichloromethane (technical grade, redistilled in all glass); these were dried over anhydrous Na_2SO_4 and the combined aliquots were reduced to dryness in a rotary vacuum evaporator. The residues were dissolved in 1 mL of the mobile phase, and 30-μL samples were subjected to LC. Tap water and field waters collected from mosquito breeding habitats (pasture, pond, and sewage) were fortified with ethanolic solutions of SIR-8514 to give 0.02 (the maximum water solubility as determined

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