Formation of Methoxypyrazines in Reactions of 2(1*H*)-Pyrazinones with Naturally Occurring Methylating Agents

George P. Rizzi

Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, Ohio 45239-8707

Methoxypyrazines are formed in low yield when 2(1*H*)-pyrazinones are heated with fruit pectin, betaine, trigonellin, *S*-methylmethionine, choline, and lecithin. The methylation reactions provide a chemical link to nonenzymic methoxypyrazine formation in heat-processed foods.

Alkoxypyrazines are of great interest to flavorists and flavor chemists because of their unique flavors and extremely low flavor thresholds (Shibamoto, 1986). Methoxypyrazines are found most frequently in living organisms, where they appear to be formed as minor products of amino acid metabolism (Gallois et al., 1988; McIver and Reineccius, 1986). The observation of methoxypyrazines in heat-processed foods like coffee (Becker et al., 1987), cocoa (Baigrie et al., 1987), and roasted pulse (Vasundhara and Parihar, 1981) and in model systems (Arnoldi et al., 1988) also suggests the existence of nonenzymic formation pathways.

A mechanism was proposed for the biological formation of methoxypyrazines in foodstuffs based on successful laboratory chemistry (Murray et al., 1970) involving 2(1*H*)-pyrazinones **1** as penultimate intermediates. 2(1*H*)-Pyrazinones are reasonable precursors since members of this class and structurally similar methoxypyrazines have been found in nature (Sammes, 1975; Takken et al., 1975). Methylation of **1** in vivo is probably mediated by a methyltransferase and *S*-adenosylmethionine similar to the biological methylation of phenols (Wallenfels and Diekmann, 1967).

It occurred to us that other electron-deficient methyl compounds in foods might simply react thermally with **1** to provide a nonenzymic source of methoxypyrazines during processing. 2(1*H*)-Pyrazinones undergo both O- and N-methylation with powerful electrophiles like diazomethane, dimethyl sulfate, and methyl iodide, but reactions with weaker electrophiles have not been described (Barlin, 1982). Certain food materials are capable of inter- and intramolecular methylation to produce important flavor compounds. Pectin (methyl ester) reacts with methionine to form dimethyl sulfide (Casey et al., 1963) apparently via S-methylation and hydrolysis of an intermediate sulfonium salt. Trigonellin (the inner salt of 3-carboxy-1-methylpyridinium hydroxide), a 1% component of coffee beans, decomposes at 180–230 °C to produce 21 methyl-containing volatiles (Viani and Horman, 1974).

The disposition of **1** toward alkylation, the natural occurrence of **1**, and the availability of natural methylating agents prompted us to investigate thermal generation of methoxypyrazines in model systems.

EXPERIMENTAL PROCEDURES

Materials. Reagents were purchased as follows: 3-isobutyl-2-methoxypyrazine (**2a**), 3-isopropyl-2-methoxypyrazine (**2b**), methyl laurate, methyl benzoate, *S*(−)-methyl lactate, *S*(+)-methyl 3-hydroxy-2-methylpropionate, and choline chloride (Aldrich Chemical Co.); fruit pectin [8.9% methoxy content], alginic acid, trigonellin hydrochloride, and *dl*-*S*-methylmethionine hydrochloride (Sigma Chemical Co.); methyl glycolate (Eastman Kodak Co.); betaine hydrate (Matheson Coleman & Bell Corp.); D-sorbitol (Fisher Scientific Co.); soy lecithin, Epikuron 145V, 44–47% phosphatidylcholine (Lucas Meyer Inc.). Acidic CMC was Hercules cellulose gum 12M8 that was acidified with HCl, dialyzed to remove inorganic salts, and freeze-dried. Literature procedures were used to synthesize 2(1*H*)-pyrazinones **1a–c** (Karmas and Spoerri, 1952) and **1d** (Dunn et al., 1949). Inorganic reagents, common chemicals, and solvents were also commercial products and of analytical grade purity. Compounds **2c**, **3c**, **2d**, and **3d** were prepared by methylation of **1c** and **1d** (see below).

Methods of Analysis. Extracts of reaction mixtures were analyzed by gas chromatography (GC) using a Hewlett-Packard 5880A chromatograph and a 60 m × 0.25 mm fused silica column containing DB-5 (1-μm film thickness) (J & W Scientific) programmed for 10-min hold time at 50 °C and 4 °C/min to 250 °C. Injector temperature was 250 °C. Injection (2 μL) was splitless, and column effluent was split 1:1 between the FID and a sniff port. Methoxypyrazines were quantitated by using external standards, and detector response factors for **2c** and **2d** were assumed for yield calculations to be equal to the response factor of **1a**. GC–MS was done with a Finnegan Model 800 (ion trap detector) mass spectrometer interfaced to a Hewlett-Packard 5880A GC. For GC, a 30 m × 0.25 mm fused silica column containing RTX-5 (0.5-μm film thickness) (Restek Corp.) was used with a heated injector at 250 °C and programmed for zero hold time at 50 °C and 4 °C/min to 130 °C. Injection split ratio was 10:1. Mass spectra were obtained in the electron ionization mode (70 eV) at a scan acquisition of 26–200 amu spectrum^{-1} s^{-1}. Reaction products were identified by comparing GC retention time, mass spectra, and (in some cases) olfactory sniff port response with similar information from authentic compounds.

Proton NMR spectra were obtained in $CDCl_3$ solution on a Varian T-60 (60 MHz) spectrometer using tetramethylsilane (TMS) as internal reference standard. Data are in parts per million downfield from TMS (δ = 0.00 ppm).

HPLC was done on a Spectra Physics SP8800 system using a 15 cm × 0.46 cm Supelcosil LC-18 column (3-μm film thickness) (Supelco Inc.) under isocratic conditions at room temperature. Solvent was 1:1 MeOH–water at a flow rate of 1.00 mL/min. The UV detector was set at 320 nm.

IR spectra were recorded in thin films on a Perkin-Elmer Model 298 spectrophotometer.

Reaction Procedures. Summary results of 2(1*H*)-pyrazinone methylations are presented in Tables I and II, and a general equation depicting reactants and products is shown in Figure 1. Reactions without solvent were done by heating mixtures of reactants (generally 0.1–0.3 mmol of limiting reagent) in flame-sealed 1-mL glass ampules in a thermostated oil bath. Reaction products were triturated with methylene chloride and filtered through plugs of glass wool, and the filtrates were adjusted to definite volumes (usually 2.00 mL) before GC analysis. Reactions without solvent, but with large amounts of added polyols, were worked up by adding water (1.00 mL), sonicated to obtain solution, and stirred with methylene chloride (1.00 mL) to extract volatile products. Reactions run in methanol sol-

0021-8561/90/1438-1941$02.50/0

Table I. Methoxypyrazine Formation in 2(1*H*)-Pyrazinone/Pectin Reactions

2(1*H*)-pyrazinone	mole ratio[a] pyrazinone/Me-X	reaction conditions		methoxypyrazine, % yield
		temp, °C	time, h	
1a	0, control	180	1	**2a**, ND[b]
1b	0, control	180	1	**2b**, ND
	pectin control	180	1	ND
1a	1.44	100	1.0	**2a**, ND
1a	1.96	100	15.0	**2a**, ND
1a	1.44	187	0.25	**2a**, 0.089
1a	1.77	185	1.0	**2a**, 0.62
1a	1.14	187	2.5	**2a**, 0.17
1b	2.40	180	1.0	**2b**, 0.27
1d	1.42	187	1.0	**2d**, 0.36

[a] Me-X equals moles of methyl galacturonate in pectin based on methoxy analysis. [b] ND, none detected at the limit of measurement, ca. <0.001% yield.

Table II. Pyrazinone Reactions with Various Methylating Agents

2(1*H*)-pyrazinone	methylating agent Me-X	mole ratio pyrazinone/Me-X	reaction conditions[a]	methoxypyrazine, % yield[b]
1a	methanol		A	**2a**, ND
1a	methanol		B	**2a**, trace
1b	methyl laurate	1.05	C	**2b**, ND
1b	methyl benzoate	0.54	C	**2b**, trace
1b	methyl laurate	1.29	D	**2b**, trace
1b	methyl laurate	1.14	E	**2b**, trace
1b	methyl glycolate	0.74	C	**2b**, 0.11
1b	methyl glycolate	0.93	F	**2b**, 0.085
1b	methyl glycolate	0.66	G	**2b**, 0.10
1b	methyl glycolate	0.84	H	**2b**, 0.12
1b	methyl lactate	0.91	C	**2b**, 0.10
1b	methyl 3-hydroxy-2-methylpropionate	1.05	C	**2b**, 0.019
1b	betaine hydrate	1.07	C	**2b**, 0.41
1b	trigonellin hydrochloride	0.76	C	**2b**, 0.21
1b	*S*-methylmethionine chloride	0.90	C	**2b**, 0.057
1b	choline chloride	0.74	C	**2b**, 0.52
1c	choline chloride	0.31	C	**2c**, 0.091
1b	lecithin	2.8	C	**2b**, 0.14
none	lecithin control		C	**2b**, ND

[a] Key: A, refluxed **1a** in excess methanol 17 h; B, like A, but with the addition of 4.2 mol of citric acid/mol of **1a** and refluxed 7 h; C, reaction in sealed tube/180 °C for 1 h; D, like C, but with the addition of 78 wt % alginic acid based on methyl laurate; E, like C, but with the addition of 36 wt % acidic CMC (ds 1.2) based on methyl laurate; F, like C, but with the addition of 430 wt % D-sorbitol based on methyl glycolate; G, like C, but with the addition of 456 wt % mannitol based on methyl glycolate; H, like C, but with the addition of 418 wt % sucrose based on methyl glycolate. [b] ND, none detected within error of measurement, <0.001% yield; trace, between 0.001 and 0.01% yield. Yields are percent of theoretical on the basis of limiting reagent present.

vent were adjusted to definite volumes and sampled directly for analysis. Aqueous buffer reactions were subjected to solid-phase extraction (Waters Associates C-18 SepPak cartridges) and eluted with methylene chloride to obtain volatiles.

Methylation of 5,6-Dimethyl-3-isopropyl-2(1*H*)-pyrazinone (1c). A mixture containing 0.173 g of **1c** and 0.414 g of anhydrous potassium carbonate in acetone (5 mL) was treated with 0.099 mL of dimethyl sulfate and heated under reflux for 16 h. The cooled mixture was treated with water (1 mL), and after 16 h at 22 °C, acetone was removed under vacuum. Extraction of the residue with methylene chloride and concentration gave 0.151 g of an oil with a pleasant nutty aroma, which according to GC contained 50.6% **2c** (sniff port, weak camphoraceous) and 44.6% **3c** (sniff port, odorless). HPLC indicated 0.25% unchanged **1c**, but did not resolve **2c** and **3c**. NMR data were consistent for a mixture of methylated products: 1.22 (d, J = 7 Hz, isopropyl methyls), 2.25, 2.30, 2.40 (s, ring methyls),

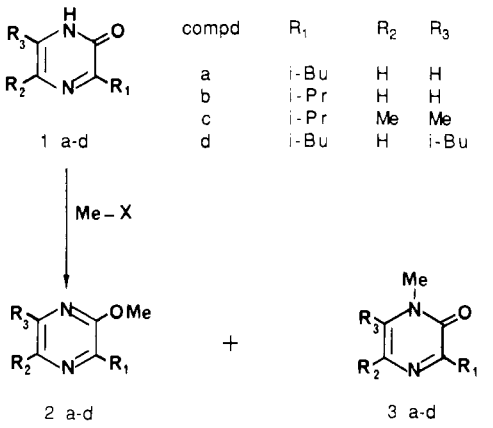

Figure 1. Methylation of 2(1*H*)-pyrazinones.

3.23 (m, J = 6 Hz, isopropyl methine), 3.50 (s, *N*-methyl), 3.90 (s, *O*-methyl). IR analysis showed pyrazinone C=O absorption at 1640 cm^{-1}. Partial vacuum distillation of the oil (15 mmHg) gave a fraction enriched in **2c** (68.3% by GC) as evidenced by an enhanced NMR signal at 3.90 ppm. Greater volatility of **2c** was also indicated by its shorter GC retention time (44.08 min) vs that of **3c** at 57.20 min. GC/MS: m/z (% of base peak), **2c** 180 [M$^+$] (42), 165 (100), 152 (79), 133 (27), 122 (16), 80 (20), 68 (20), 53 (46), 42 (64), and 27 (51); **3c** 180 [M$^+$] (28), 165 (60), 152 (45), 137 (100), 123 (9), 84 (31), 56 (77), 42 (65), and 28 (78).

Methylation of 3,6-Diisobutyl-2(1*H*)-pyrazinone (1d). A mixture containing 0.086 of **1d**, 0.5 g of anhydrous potassium carbonate, and acetone (5 mL) was treated with 0.1 mL of dimethyl sulfate and heated at reflux for 3 h. After the acetone was removed under vacuum, the residue was basified with NaOH and extracted with ether. Evaporation of ether gave 0.0946 g of oil mixed with crystals, which was shown by GC and NMR analyses to contain ca. 78% **2d**, 19% **3d**, and a little unreacted **1d**. Methoxypyrazine **2d** (R_t = 57.47 min) was indicated by unique NMR signals at δ 3.92 (MeO) and 7.92 (pyrazine ring CH=), while the *N*-methylpyrazinone **3d** (R_t = 69.00 min) exhibited characteristic signals at δ 3.48 (*N*-Me) and 7.17 (pyrazinone ring CH=). GC-MS: m/z (% of base peak), **2d** 223 [M$^+$ + 1] (9), 222 [M$^+$] (0.6), 207 (9), 181 (10), 180 (92), 138 (13), 137 (100), 109 (6), 80 (7), 67 (5), and 53 (10); **3d** 223 [M$^+$ + 1] (5), 222 [M$^+$] (13), 207 (43), 180 (72), 179 (19), 165 (6), 138 (9), 137 (100), 109 (48), 95 (8), 68 (10), and 56 (17).

RESULTS AND DISCUSSION

Reactions of 2(1*H*)-pyrazinones with naturally occurring methylating agents produced up to 0.62% yield of methoxypyrazines under roasting conditions (Tables I and II).

Fruit pectin containing methyl galacturonate units was investigated as a possible methyl source since an earlier study (Casey et al., 1963) showed that methionine was apparently methylated by pectin in dilute aqueous buffer solution (pH 6.5) at 100 °C. Substitution of the 2(1*H*)-pyrazinones **1a** or **1b** for methionine under these conditions did not, however, produce detectable amounts (<0.001% yield) of methoxypyrazines **2a** or **2b** after 1 h.

Since pectin is known to undergo numerous hydrolytic side reactions (Kratchanov et al., 1989), we decided to investigate water-free systems. Dry mixtures of 2(1*H*)-pyrazinones and pectin [with methyl galacturonate (in pectin) as the limiting reagent] were heated in sealed glass ampules (Table I). With **1a** and pectin no methoxypyrazine was detected after 1 or 15 h at 100 °C. However, at 187 °C a strong bell pepper aroma was produced within 15 min, and GC analysis indicated 3-isobutyl-2-methoxypyrazine (**2a**) in 0.089% yield. The yield of **2a** increased to 0.62% after 1 h and decreased to 0.17% at 2.5 h. The reaction was shown to be general by using 2(1*H*)-pyrazi-

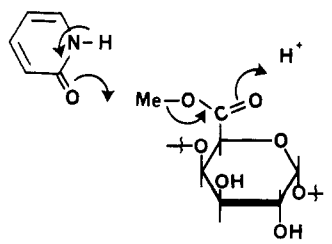

Figure 2. 2(1*H*)-Pyrazinone O-methylation by pectin.

nones **1b** and **1d**, which subsequently generated 3-isopropyl-2-methoxypyrazine (**2b**) and 3,6-diisobutyl-2-methoxypyrazine (**2d**) in 0.27 and 0.36% yields, respectively. In the case of **1d** no GC–MS evidence was found for the isomeric *N*-methyl product, **3d**. N-Methylation may have occurred to a minor extent with **1a** and **1b**, but authentic samples of **3a** and **3b** were not available to permit GC–MS identification among the many trace products. Methoxypyrazine **2d** was of special interest since its precursor pyrazinone, **1d**, is a natural product produced by the mold *Aspergillus flavus* (Dunn et al., 1949).

Control experiments in which **1a**, **1b**, and pectin were each heated alone did not produce methoxypyrazines, although GC and HPLC analyses showed evidence for many volatile and nonvolatile decomposition products. Also, no **2b** was detected if the product obtained by heating **1b** for 1 h at 185 °C (89% unchanged **1b**) was boiled in methanol for 3 h. These data suggest that methoxypyrazines are formed by straightforward nucleophilic attack of the pyrazinone oxygen on the methyl group in pectin (Figure 2) and not by reaction of methanol with pyrazinone decomposition products. Low yields are predictable in this reaction since it is well-known that pectin methyl esters undergo mainly nucleophilic reactions at the ester carbonyl, i.e., hydrolysis and aminolysis (Kratchanov et al., 1989).

The mechanism of the pectin methylation reaction was studied indirectly by observing methoxypyrazine formation in related reactions of 2(1*H*)-pyrazinones with structurally simpler methyl esters (Table II). Methyl laurate and **1b** produced no trace of 3-isopropyl-2-methoxypyrazine (**2b**) after 1 h at 180 °C, but a similar reaction done in the presence of alginic acid (a linear copolymer of D-mannuronic and L-guluronic acids) gave a trace of **2b**. Presumably then, uronic acid groups in pectin could assist methylation by protonating adjacent methyl ester groups. A similar effect was noted when alginic acid was replaced by acidic (carboxymethyl)cellulose (acid CMC). The reaction of methyl benzoate and **1b** also produced a trace of **2b**, consistent with better leaving group properties predicted for benzoate versus laurate.

Reaction of methyl glycolate with **1b** produced 0.11% yield of **2b** compared with <0.001% **2b** from methyl laurate under similar conditions and demonstrated the importance to methylation of an oxygen atom situated adjacent, i.e., α, to the methyl ester group. Electronegative substituents like the α-oxygen in methyl glycolate can enhance the methylation reaction relative to methyl laurate by inductive stabilization of an incipient carboxylate ion. A similar result was obtained by using methyl lactate wherein a 0.10% yield of **2b** was obtained. Reaction of a β-hydroxy ester, i.e., methyl 3-hydroxy-2-methylpropionate, with **1b** led to a significantly diminished yield of **2b** (0.019%) relative to the yield of **2b** from methyl glycolate. The lower yield is consistent with a reduced inductive effect expected for the more distant oxygen substituent. Our experiments suggest that methyl esters in pectin are activated for methyl transfer by an inductive effect of the C-5–oxygen substituent in the galactopyranosyl ring combined with acid catalysis by nearby uronic acid moieties (Figure 2).

Since polar reactions are known to be enhanced in media of high dielectric constant, we attempted to enhance the yield of **2b** in **1b**/methyl glycolate reactions by adding excess polyols. Contrary to expectation, addition of sorbitol, mannitol, or sucrose in large excess failed to increase yields of **2b** beyond a control value. Thus, media effects, if they exist, must be small and are probably offset by dilution of reacting species.

In view of the apparent electrophilic nature of the pectin/pyrazinone methylation reaction, we decided to test other natural products as possible methylating agents (Table II). Quaternary ammonium salts containing methyl groups were selected since similar compounds are known to produce O-methylation on heteroaromatic nuclei, i.e., 2-methyl-3-pyridol → 2-methyl-3-methoxypyridine via trimethylphenylammonium salt decomposition (Baker and McEvoy, 1955). Reaction of betaine (trimethylglycine hydroxide, inner salt) with **1b** at 180 °C produced 0.41% yield of **2b** in 1 h comparable with 0.62% **2b** produced by pectin under similar conditions. The low yield of **2b** may have resulted from competitive isomerization of betaine to form a less active methylating agent, methyl dimethylaminoacetate. Trigonellin (3-carboxy-1-methylpyridinium hydroxide, inner salt) was reacted as its hydrochloride salt in an attempt to offset possible competitive rearrangement to form methyl nicotinate. Trigonellin hydrochloride reacted with **1b** to generate **2b** in 0.21% yield. Under similar conditions *S*-methylmethionine chloride and **1b** generated a 0.057% yield of **2b**. However, the dominant odor in the latter reaction was that of dimethyl sulfide apparently produced by a concomitant nucleophilic displacement at the γ-carbon atom of *S*-methylmethionine.

Choline [(2-hydroxyethyl)trimethylammonium hydroxide] was investigated because of its wide occurrence in plants and animal organs and because it is a basic moiety in lecithin phospholipids. Reaction of excess choline (chloride) with **1b** at 180 °C produced **2b** in 0.52% yield after 1 h. A major volatile byproduct was trimethylamine, which was easily recognized by GC–MS and by its characteristic sniff port aroma. In a similar way choline (chloride) reacted with 5,6-dimethyl-3-isopropyl-2(1*H*)-pyrazinone (**1c**) to generate a previously unreported methoxypyrazine **2c** in 0.091% yield. Unlike other methoxypyrazines mentioned in this study compound **2c** had almost no sniff port aroma. An increase in odor threshold was noted previously in the case of more highly substituted methoxypyrazines (Takken et al., 1975). The related *N*-methyl isomer, **3c**, could not be detected by GC–MS.

On the basis of our results with choline it seemed logical to expect that the choline moiety in lecithin would also serve as an active methylating agent. Reaction of commercial soybean-derived lecithin (ca. 46% phosphatidylcholine) with **1b** at 180 °C generated 0.14% **2b** within 1 h, comparable with the results of choline (chloride) itself. The odor of the former reaction mixture was predominantly that of the methoxypyrazine, but a significant amount of trimethylamine was also formed as evidenced by GC and sniff port analysis.

ACKNOWLEDGMENT

I thank D. R. Patton for quantitative GC analyses and A. R. Steimle for assistance in obtaining GC/MS data.

LITERATURE CITED

Arnoldi, A.; Arnoldi, C.; Baldi, O.; Griffini, A. Flavor Components in the Maillard Reaction of Different Amino Acids with Fructose in Cocoa Butter-Water. Qualitative and Quantitative Analysis of Pyrazines. *J. Agric. Food Chem.* **1988**, *36*, 988–992.

Baigrie, B. D.; Rumbelow, S. J.; McHale, D. Flavor Defects of Cocoa. In *Flavour Science and Technology*; Martens, M., Dalen, G. A., Russwurm, H., Jr., Eds.; Wiley: New York, 1987; pp 133–141.

Baker, B. R.; McEvoy, F. J. An Antimalarial Alkaloid from Hydrangea. XXIII. Synthesis by the Pyridine Approach II. *J. Org. Chem.* **1955**, *20*, 136–142.

Barlin, G. B. The Pyrazines. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Taylor, E. C., Eds.; Wiley: New York, 1982; Vol. 41, pp 175–179.

Becker, R.; Dohla, B.; Nitz, S.; Vitzthum, O. G. Identification of The Peasy Off-flavour Note in Central African Coffees. *Colloq. Sci. Int. Cafe, [C. R.]* **1987**, *12*, 203–215.

Casey, J. C.; Self, R.; Swain, T. Origin of Methanol and Dimethyl Sulphide From Cooked Foods. *Nature (London)* **1963**, *200*, 885.

Dunn, G.; Newbold, G. T.; Spring, F. S. Synthesis of Flavacol, a Metabolic Product of Aspergillus flavus. *J. Chem. Soc.* **1949**, 2586–2587.

Gallois, A.; Kergomard, A.; Adda, J. Study of the Biosynthesis of 3-Isopropyl-2-methoxypyrazine Produced by Pseudomonas taetrolens. *Food Chem.* **1988**, *28*, 299–309.

Karmas, G.; Spoerri, P. E. The Preparation of Hydroxypyrazines and Derived Chloropyrazines. *J. Am. Chem. Soc.* **1952**, *74*, 1580–1584.

Kratchanov, Chr.; Denev, P.; Kratchanova, M. Reaction of Apple Pectin With Ammonia. *Int. J. Food Sci. Technol.* **1989**, *24*, 261–267.

McIver, R. C.; Reineccius, G. A. *Synthesis of 2-Methoxy-3-alkylpyrazines by Pseudomonas perolens*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986; pp 266–274.

Murray, K. E.; Shipton, J.; Whitfield, F. B. 2-Methoxypyrazines and the Flavour of Green Peas (Pisum sativum). *Chem. Ind.* **1970**, 897–898.

Sammes, P. G. Naturally Occurring 2,5-Dioxopyrazines and Related Compounds. *Fortschr. Chem. Org. Naturst.* **1975**, *32*, 51–118.

Shibamoto, T. Odor Threshold of Some Pyrazines. *J. Food Sci.* **1986**, *51*, 1098–1099.

Takken, H. J.; van der Linde, L. M.; Boelens, M.; van Dort, J. M. Olfactive Properties of a Number of Polysubstituted Pyrazines. *J. Agric. Food Chem.* **1975**, *23*, 638–642.

Vasundhara, T. S.; Parihar, D. B. Heterocyclic Flavour Volatiles Formed in the Basic Fraction of Roasted Phaseolus Aureus (Mung Dal). *Z. Lebensm. Unters. Forsch.* **1981**, *172*, 460–465.

Viani, R.; Horman, I. Thermal Behavior of Trigonellin. *J. Food Sci.* **1974**, *39*, 1216–1217.

Wallenfels, K.; Diekmann, H. Biological Formations and Reactions. In *The Chemistry of the Ether Linkage*; Patai, S., Ed.; Wiley Interscience: New York, 1967; Chapter 5, pp 207–242.

Received for review March 20, 1990. Accepted April 30, 1990.

Registry No. **1a**, 25680-53-9; **1b**, 25680-59-5; **1c**, 113139-68-7; **1d**, 495-98-7; **2a**, 24683-00-9; **2b**, 25773-40-4; **2c**, 128600-00-0; **2d**, 36329-97-2; **3c**, 128600-01-1; **3d**, 128600-02-2; methyl laurate, 111-82-0; alginic acid, 9005-32-7; CM-cellulose, 9000-11-7; methyl benzoate, 93-58-3; methyl glycolate, 96-35-5; *S*-(−)-methyl lactate, 27871-49-4; *S*-(+)-methyl 3-hydroxy-2-methylpropionate, 80657-57-4; low-methoxylpectin, 9049-34-7; betaine hydrate, 590-47-6; trigonellin HCl, 6138-41-6; *dl*-*S*-methylmethionine chloride, 3493-12-7; choline chloride, 67-48-1; trimethylamine, 75-50-3; dimethyl sulfide, 75-18-3.