Synthesis of some substituted pyrazines and their olfactive properties

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There are three possible ways in which two nitrogen atoms may be combined with four carbon atoms to form the system of six-membered heterocyclic rings known as diazines. The most familiar class of this system is that in which the two nitrogen atoms are meta (viz. 1,3) with respect to each other, namely the pyrimidines.

These are of enormous importance because of their occurrence in such diverse natural products as the purines (caffeine, uric acid, etc.); vitamins (thiamin, riboflavin); the synthetic barbiturates; and the nucleic acids which are the governing chemical agents of life.

The ortho-diazines (viz. 1,2) named the pyridazines, are not as well known as their other two counterparts.

Their chemical significance has surfaced mainly in the last twenty years, particularly in the area of synthetic medicinal chemistry.

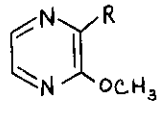
The final class of diazines are the pyrazines, in which the ring nitrogens are situated para to each other (viz. 1,4).

Stoehr and Wolff were the first to work intensively in this field.[1,2] Their pioneering research was published almost one hundred years ago. The organic chemist has had a long acquaintance with pyrazine compounds, but their occurrence in foods was not widely reported until the mid-1960s. Since then pyrazines have been characterized as significantly contributing to the unique flavor and aroma associated with the roasting or toasting of numerous foods, in addition to foods which had not been exposed to a heating process.

Deck and Chang isolated and structurally identified 2,5-dimethyl pyrazine in the volatile flavor portion of potato chips.[3] Substituted pyrazines were being increasingly identified in foods, particularly those foods where a roasting or baking process was involved. For example, Goldman and coworkers observed that a portion of the volatile aroma of coffee was attributed to the presence of a number of alkylated pyrazines.[4] Marion and coworkers researched the constituents of cocca, and Bondarovik and coworkers with coffee and Mason and coworkers with roasted peanuts confirmed this observation.[5-7] Recently Vitzthum and Werkhoff researched the volatile components of roasted coffee and separated 17 alkylated five- and six-membered alicyclic pyrazines for the first time in roasted coffee.[8]

Furthermore, pyrazines have been identified in various meats by von Sydow and Anjou, and Walradt and coworkers.[9-11] The latter actually reported (with scientific substantiation) 47 new constituents of peanuts which included 23 pyrazine derivatives.

However, naturally-occuring pyrazines have also been isolated from food systems that have not undergone heat treatment, thus demonstrating that various biological pathways for pyrazine formation exist. Murray and Whitfield discovered the occurrence of 3-alkyl-2-methoxy pyrazines



where the alkyl group equals 3-isopropyl or 3-sec. butyl or 3-isobutyl, in 27 raw vegetables.[12] In addition, the pyrazines have been isolated and identified in naturally-occurring oils, namely, galbanum oil, petitgrain oil, camphor oil (white), fenugreek extract, and most recently, lovage root oil.

It has been suggested that alkoxylpyrazines may be widely distributed in the plant kingdom. Thus it is apparent that not all pyrazines result from heat treatments but some, especially the potent alkoxypyrazines, may be present in raw

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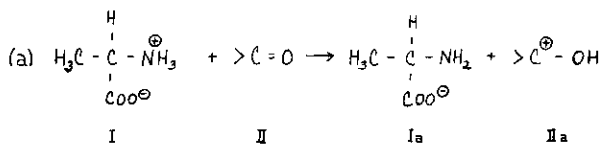
products. Maga and Sizer have presented a complete treatise on the isolation and structural identification of pyrazines in different products (roasted, baked or nonheated systems).[13]

As a result of model system studies, various possible pathways for the formation of pyrazines have been investigated. Dawes and Edwards and van Praag and coworkers have suggested that free ammonia was the primary intermediate and that the composition of the resulting pyrazine mixture was not dependent upon the amino acid source. They concluded that, regardless of the amino acid present, a similar series of pyrazines was always formed. However, Koehler and coworkers reported that the pyrazines were formed via carbohydrate decomposition followed by interaction of these materials with nitrogen-containing molecules, for example alpha-amino acids or ammonia per se.[16] Basically this is the Maillard reaction, which involves the reaction of amino acids in general with the hydroxyl group (—OH) and/or the carbonyl group (> c= o) of sugar molecules. Specifically the Maillard reaction ("Browning reaction") has been described as the reaction of amino groups of amino acids, peptides or proteins with the "glycosidic" hydroxyl group of sugars, ultimately resulting in the formation of brown pigments.

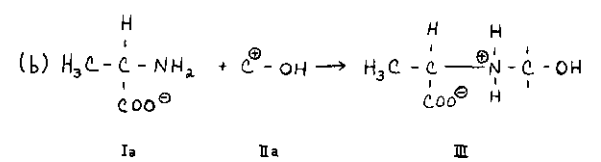
Koehler's studies clearly demonstrated that the reactive ammonia unit was attached to the amino acid and that the different pyrazine product profiles were the result of the differences in the ease of the nucleophilic attack of the amino group in the amino acid molecules on the sugar. Thus, it would appear that in a food system where both sugars and amino acids are present, bound amino acid nitrogen is the "primary contributor" to the nitrogen found in the ring structure of pyrazines.

It is apparent that both mechanisms can proceed in a synchronous manner. For example it should be noted that at a specific pH various amino acids can break down to yield ammonia gas, thus an additional source of nucleophilic attack (nitrogen in ammonia) on a sugar molecule in addition to bound nitrogen to an amino acid. For example, consider the amino acid alanine which could easily react with a carbonyl moiety by nucleophilic attack (viz. a, b)



(a) $H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-\overset{\oplus}{N}H_3 + >C=O \longrightarrow H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-NH_2 + >C^{\oplus}-OH$

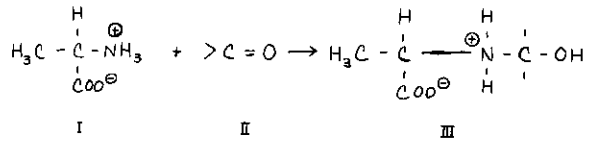
I II Ia IIa



(b) $H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-NH_2 + C^{\oplus}-OH \longrightarrow H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-\overset{\oplus}{N}\underset{H}{\overset{H}{\overset{|}{C}}}-OH$

Ia IIa III

Therefore, algebraically the following occurred:

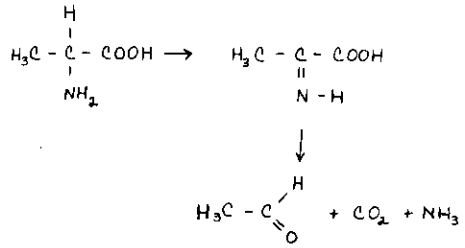


$H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-\overset{\oplus}{N}H_3 + >C=O \longrightarrow H_3C-\underset{\underset{COO^{\ominus}}{|}}{\overset{H}{\overset{|}{C}}}-\overset{\oplus}{N}\underset{H}{\overset{H}{\overset{|}{C}}}-OH$

I II III

This precursor (III) undergoes a series of reactions to yield pyrazine derivatives.

However, as this reaction is occurring it is possible that simultaneously another competing reaction can occur. For example, a molecule of alanine can undergo the Strecker reaction (degradation).



$H_3C-\underset{\underset{NH_2}{|}}{\overset{H}{\overset{|}{C}}}-COOH \longrightarrow H_3C-\underset{\underset{N-H}{\|}}{C}-COOH$

$\downarrow$

$H_3C-\overset{H}{\overset{|}{C}}=O + CO_2 + NH_3$

Alternatively the ammonia can attack a carbonyl and thus we can have a situation where an amino acid can both generate a nitrogen atom as bound or as ammonia gas via degradation, therefore accounting for a variety of substituted products being formed during reaction.

A variety of pyrazines have been formed by various modifications of the Maillard reaction, viz., in lieu of sugar many other hydroxy containing reactants have been employed, for example glycerin. Recently Schibamoto and Bernhard reported the significance of time, temperature, and reactant ratios or pyrazine formation in model systems.[17] Furthermore, various carbonyl containing molecules were used, for example pyruvic aldehyde, levulenic aldehyde, acetaldehyde, biacetyl, or acetoin. These reaction products displayed a "baked bread" odor. Again, for a complete review, Maga and Sizer treat this study in detail.[13]

A British patent was issued to Reichstein and Standinger in 1928. They claimed that pyrazines could be synthesized and that pyrazines in combination with other chemicals yielded an excellent aroma in the preparation of synthetic coffee oil.[18] Thirty years passed before the synthesis of pyrazine began to surface. It still was relatively unknown to the flavorist and perfumer.

However, during this same period Krems and Spoerri, at the Polytechnic Institute of Brooklyn, published extensively the synthesis of a wide variety of pyrazines.[19]

In our study we concentrated on synthesizing a series of substituted pyrazines with the highest purity, which were structurally identified using MS, infrared, and nuclear magnetic resonance.[20] Furthermore, we proceeded to characterize the odors and quantitate the odor thresholds.

The specific pyrazine (structure) method of synthesis, odor characteristic, and odor threshold was integrated for simplicity in the following tables.

In the synthetic sequences our starting material was an ultra-pure grade of 2-methyl pyrazine. All the corresponding amines and aldehydes were carefully redistilled prior to using them.

The complete analytical procedure and the detailed structural assignments and identifications have been reported in the literature.[13]

Following the structural assignments the aroma of each of these pyrazines was fully evaluated and defined for odor character by a group of six, including four flavorists and two perfumers. Each member defined the specific pyrazine for odor character on an individual basis. In most cases the odor character was unanimously defined. In some cases the "more popular" definition was assigned.

We then evaluated and measured the odor threshold for each of these pyrazines. Extreme care was exercised in the handling of the material to ensure purity. The materials were dissolved and diluted in boiled (odor free) water at 22°C to the desired threshold concentration just before the odor was measured.

The panel consisted of 15 persons who repeatedly demonstrated sensitivity and reproducibility in odor perception. All threshold determinations were based on the judgments of the full panel.

In order to acquaint the panel with the characteristic odor of each pyrazine, the first series of concentrations was selected so that one or two concentrations definitely exhibited the characteristic odor of the compound. This series was used to train the panel for the new pyrazine and was not used for threshold concentration. The threshold study took exactly four months to complete.

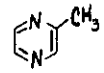
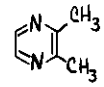
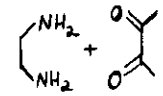
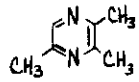
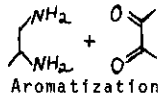
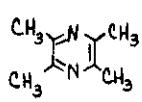
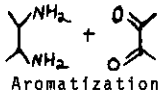
To determine thresholds, two or more series of at least three concentrations were judged. Sometimes several replications were made to assure reproducibility. Each concentration was paired with a sample of boiled (odor free) water and the panel was asked which sample contained the odorous materials. The position of the water sample was randomized in all pairs, and the order of presentation of the different concentration was also randomized. Actually, we were correlating % correct response vs. concentrations (expressed in parts per billion). These values are not intended to be absolute threshold values but in actuality, relative values which do possess some meaning.

We used a modified squeeze bottle technique developed by Guadagni for the U.S. Department of Agriculture.[25] Instead of glass covers we used teflon lids (the normal lids proved to be inadequate).

Tables I and II list the odor characteristics and the odor thresholds. Most of these threshold values are in accord with those reported by Takken and coworkers.[26]

It is interesting to note in these tables that when you have substituents in the 1 and 2 position relative to each other, a marked low threshold is achieved. For example Seifert and coworkers stated in 1972 that the most potent odors were formed when an alkyl substitutent stands in the ortho position to a methoxyl function.[27] This fact is substantiated by observing that in Table II, both the 3-methoxy and the 3-thiomethoxy exhibited the lowest threshold values. This could be attributed to hydrogen bond formation resulting from these groups being

Table I. Alkylated Pyrazines

Name	Structure	Synthesis	Characteristic Odor	Odor Threshold ppb/water
Pyrazine		Purchased (Aldrich Chemical)	Strong sweet odor (slightly ammonical)	500,000
2-Methyl Pyrazine		Purchased (Pyrazine Specialties)	Strong basic role; in dilution chocolate character	100,000
2,3-Dimethyl Pyrazine		a)  b) Aromatization	Pungent; in dilution chocolate type	400
2,3,4-Trimethyl Pyrazine		a)  b) Aromatization	Very similar to 2,3-dimethyl pyrazine, however, slightly heavier	400
2,3,5,6-Tetramethyl Pyrazine		a)  b) Aromatization	Similar to TMP but does not possess the odor intensity	1,000

ortho to one another.

Pyrazine and 2-methyl pyrazine were obviously the least potent of the pyrazines, suggesting that alkyl and/or alkoxyl substitution does enhance the odor intensity of the pyrazine molecule. This was proven in Table I by the fact that the di- , the tri- , and the tetra-substituted pyrazines possessed lower odor thresholds than the corresponding mono-substituted and the nonsubstituted pyrazine. Furthermore, it was demonstrated that replacing the pyrazine ring by a pyridine ring resulted in a marked decrease in potency and complete change of character, concluding that the pyrazines were far superior in odor potency and unique in desirable character.[27]

Also, the isobutyl and the isopropyl series are considerably stronger in odor potency than the di-alkylated and tri-alkylated series. Again, hydrogen bonding does not partake in the latter but is certainly a factor in the alkoxyl series. Hyper conjugation effects is also a theoretical consideration that one must not overlook in the explanation of odor thresholds.

One of the most interesting facts evolved from this study (Table III) was observed when the 3-alkoxyl pyrazine derivative was evaluated vs. the corresponding alcohol at a given concentration. Furthermore, comparing the citronellol adduct* vs. the geraniol adduct the latter is five times as strong in odor. The reason is probably the presence of a double bond alpha to the pyrazine moiety.

*Synthesized in the same manner as the geraniol adduct.

Table II. Alkoxylated and Thioalkylated Pyrazines

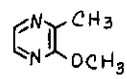
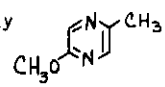
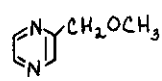
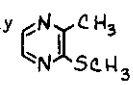
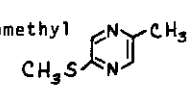
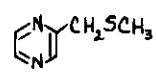
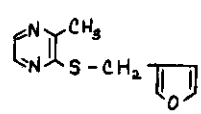
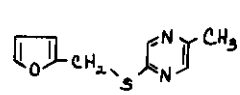
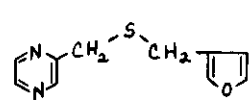
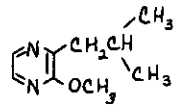
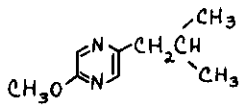
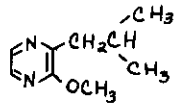
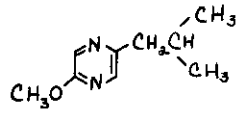
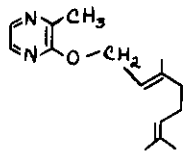
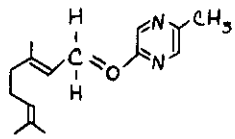
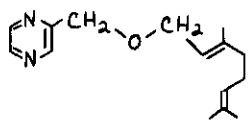
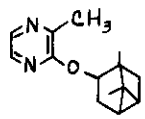
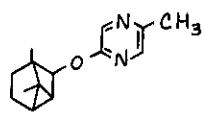
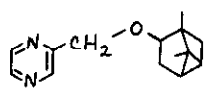
Pyrazine	Structure	Synthesis	Characteristic Odor	Odor Threshold ppb/water
2-Methyl-3-Methoxy		a) Chlorination of 2-Methyl Pyrazine, Reference 21 b) Na + CH_3OH (Anhydrous), Reference 22	In concentration vegetably; dilution popcorn/potato	3
2-Methyl-5-Methoxy		a) Chlorination of 2-Methyl Pyrazine b) Na + CH_3OH (Anhydrous)	Green vegetably character	15
2-Methoxymethyl		a) Chlorination of 2-Methyl Pyrazine b) Na + CH_3OH (Anhydrous)	Ethereal character	150
2-Methyl-3-Thiomethoxy		a) Chlorination of 2-Methyl Pyrazine b) Na + CH_3OH (Anhydrous)	Cooked meat; vegetably	1
2-Methyl-5-Thiomethyl		a) Reference 21 b) Reference 22	Meaty; vegetably	4
2-Thiomethoxymethyl		a) Reference 21 b) Reference 22	Weak sulfide note	20
2-Methyl-3-Thiofurfuryl		a) Reference 21 b) Reference 22	Powerful coffee cooked meat note	<1
2-Methyl-5-Thiofurfuryl		a) Reference 21 b) Reference 22	As above	<1
2-Thiofurfurylmethoxy		a) Reference 21 b) Reference 22	As above; also strong chocolate character	<1

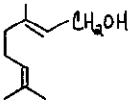
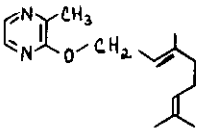
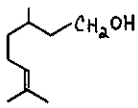
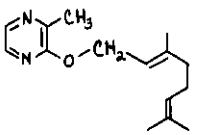
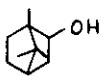
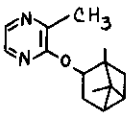
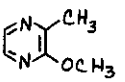
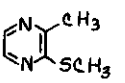
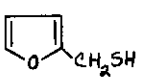
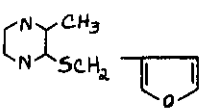
Table II. (Continued)

2-Isopropyl-3-Methoxy		a) 2-Methyl-3-Methoxy Pyrazine with Metallic Lithum (References 23,24) b) Isopropyl Iodide (Reference 24)	Strong galbanum note	10
2-Isopropyl-5-Methoxy		a) Reference 23 b) Reference 24	Strong galbanum note	10
2-Isobutyl-3-Methoxy		a) Reference 23 b) Reference 24	Green bell pepper note	10
2-Isobutyl-5-Methoxy		a) Reference 23 b) Reference 24	Strong green bell note	10
2-Methyl-3-Geranyloxy		a) Reference 21 b) Reference 22 (Using Anhydrous Geraniol)	Earthy-floral (weak)	100
2-Methyl-5-Geranyloxy		a) Reference 21 b) Reference 22	Green floral	100
2-Geranyloxymethyl		a) Reference 21 b) Reference 22	Strong earthy floral note	>500
2-Methyl-3-Camphoroxy		a) Reference 21 b) Reference 22	Weakly minty	300
2-Methyl-5-Camphoroxy		a) Reference 21 b) Reference 22	Woody-champhor note	300
2-Camphoroxymethyl		a) Reference 21 b) Reference 22	Strong woody-camphor note	>500

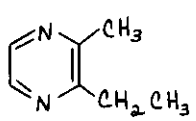
It was observed that considering molecular weights and molecular effects one would expect that the alcohol would be more odorous than the corresponding adduct with increasing molecular weight at a given concentration.

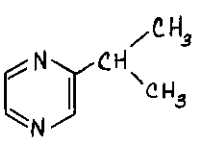
However, this was not observed. This again indicated the odor potency induced by the pyrazine moiety. Only in the case of the thio-alcohols were the alcohols stronger than the corresponding pyrazine moiety. This could be explained from the lock-key theory for enzymes which tend to hydrogen bond with thiol groups (—SH) or mercapto groups (RSH), since enzymes have a strong chemical and physical affinity for these groups. Furthermore, the alkoxyl methyl series was not as strong in odor intensity

Table III

Alcohol used	Pyrazine derivative	Concentration (10^9)	Comment
 (Geraniol)		100	Alcohol not detected
 (Citronellol)		500	Alcohol not detected
 (Borneol)		300	Alcohol not detected
CH_3OH (Methanol)		3	Alcohol not detected
CH_3SH (Thiomethanol)		1	Thioalcohol detected
 (Furfuryl Mercaptan)		<1	Thioalcohol detected

as the corresponding 3- and/or 5-isomer. A theoretical consideration may be attributed to planarity of the molecule vs. the pluggardness of the molecule. Planar molecules do not have the odor strength as the corresponding pluggard isomer.


(130 ppb)


(100 ppb)

What we did was to synthesize these interesting compounds; separate and purify each isomer; and characterize each structure using GLC, MS, IR techniques, and NMR spectroscopy. The most significant aspect of this investigation was to demonstrate the interesting character they possess and to demonstrate their low odor threshold. These chemicals, although expensive, are reasonable in cost vs. level used because of their very low odor threshold. Furthermore, they possess the unique property of synergism.

Most assuredly, these materials are gaining favor in the food and flavor industries and also room on the perfumer's shelf, and will be most vital in the future.

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