Organoleptic Properties of Some Alkyl-Substituted Alkoxy- and Alkylthiopyrazines

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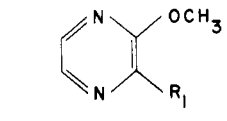
2-Methoxy-3-isobutylpyrazine has previously been reported to be an extremely potent flavoring material with a characteristic aroma of bell peppers. Twenty-one compounds structurally related to this molecule have been synthesized in an attempt to determine structural variations of the molecule which may be made and still retain the intense bell pepper note. For this flavor effect, the alkyl chain can vary from C_3 to at least C_6; the effect is reduced by C_9. Replacing the methoxy group by ethoxy markedly reduces the effect, and it disappears with butoxy substitution. Adding a second alkyl function on the ring reduces the bell pepper character. The effect is not limited to the 2,3 isomer, since the 2,6 isomer possesses a similar note. A most surprising result is the fact that the oxygen of the methoxy group may be replaced by sulfur with little change in character.

The compound having the characteristic aroma of bell peppers was identified as 2-methoxy-3-isobutylpyrazine (structure I, R_1 = isobutyl) and was shown to possess an extremely low odor threshold (Buttery *et al.*, 1969). Subsequently this compound was also reported in peas (Murray *et al.*, 1970), in galbanum oil (Bramwell *et al.*, 1969), and in coffee (Friedel *et al.*, 1971). Due to the potency and unusual character of this compound, several reports have been published which describe the synthesis and odor properties of structurally related compounds (Seifert *et al.*, 1970, 1972). This report extends the previous work for the purpose of further defining the basic chemical structure which is responsible for the intense bell pepper note.

There are a number of ways of modifying the basic 2-methoxy-3-isobutylpyrazine molecule. The effect of varying alkyl chain length of R_1 in structure I is one obvious choice. Other ways of varying the molecule include: investigating other positional isomers (structures II and VI); preparing the dialkyl-substituted methoxy compounds (III); replacing the methoxy by higher alkoxy (IV); and replacing the oxygen by sulfur (V and VI).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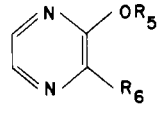
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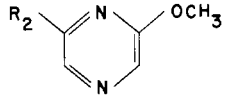
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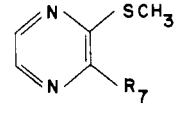
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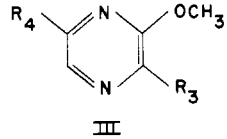
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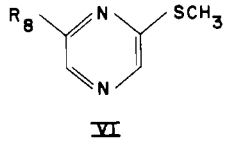
VI

EXPERIMENTAL SECTION

Gas–Liquid Chromatography. Since the synthetic procedures described below result in a mixture of the 2,3- and 2,6-chloroalkylpyrazine isomers (Lutz *et al.*, 1964), final purification of the desired isomer was achieved by gas–liquid chromatography. The samples were purified on a ⅛ in. × 6 ft column packed with 10% DEGS on 60/80 mesh Anakrom ABS in a Perkin-Elmer Model 900 gas chromatograph. Helium carrier gas flow was 30 ml/min and detection was by flame ionization. Temperature programming was utilized and the purified compound was trapped by condensation in a Dry Ice-cooled melting point capillary tube.

General Synthetic Procedure. 2-Chloro-3-methylpyrazine containing a small amount of 2-chloro-6-methylpyrazine was prepared as described in the literature (Hirschberg and Spoerri, 1961; Lutz *et al.*, 1964). This mixture was converted to the corresponding alkoxymethylpyrazine or thioalkoxy-methylpyrazine by reaction with the appropriate sodium alkoxide or sodium thioalkoxide.

Structures I and II: 2-Methoxy-3 or 6-alkylpyrazines. The 2-methoxy-3-alkylpyrazines (compounds 1–12, Table I) were prepared from the 2,3- and 2,6-methoxymethylpyrazine isomer mixture by alkylation with the appropriate alkyl bromide in liquid ammonia containing sodium amide (Behun and Levine, 1961). 2-Methoxy-6-isobutylpyrazine (13) was prepared in a similar fashion to compound 6 and was separated from the 2,3 isomer by glc.

Structure III: 2-Methoxy-3,6-dialkylpyrazines. 2-Methoxy-3,6-dimethylpyrazine (14) was prepared by the reaction of 2-chloro-3,6-dimethylpyrazine (Aldrich Chemical Company) with sodium methoxide in methanol. Alkylation of 14 with an excess of isopropyl bromide in liquid ammonia containing sodium amide afforded 2-methoxy-3,6-diisobutylpyrazine (15).

Structure IV: 2-Alkoxy-3-alkylpyrazines. The chloromethylpyrazine isomer mixture was converted to 2-ethoxy-3-methylpyrazine (16) and 2-butoxy-3-methylpyrazine (18) by reaction with sodium ethoxide in ethanol and sodium butoxide in butanol, respectively. 2-Ethoxy-3-isobutylpyrazine (17) was prepared by alkylation of 16 with isopropyl bromide; 2-butoxy-3-propylpyrazine (19) was prepared by alkylation of 18 with ethyl iodide.

Structures V and VI: 2-Methylthio-3 or 6-alkylpyrazine. 2-Methylthio-3-methylpyrazine (20) was prepared from the chloromethylpyrazine isomer mixture by reaction with sodium methylmercaptide in methanol. 2-Methylthio-3-isobutylpyrazine (21) was prepared by alkylation of 20 with isopropyl bromide. 2-Methylthio-6-isobutylpyrazine (22) was prepared in the same way as compound 21 and was separated from the 2,3 isomer by glc.

Mass Spectra. The mass spectra of the compounds purified by glc were recorded on a CEC 21-110 double-focusing high-resolution mass spectrometer using an ionizing voltage of 70 eV.

Infrared Spectra. The infrared spectra of the purified

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Table I. Organoleptic Properties of Substituted Pyrazines

Compound number	Compound	Arbitrary intensity	Flavor description
	2-Methoxy-3-alkylpyrazines (structure I)		
1	2-Methoxy-3-methylpyrazine	Moderate	Nutty, earthy
2	2-Methoxy-3-ethylpyrazine	High	Earthy, bell pepper
3	2-Methoxy-3-propylpyrazine	High	Bell pepper
4	2-Methoxy-3-isopropylpyrazine	High	Bell pepper, earthy
5	2-Methoxy-3-butylpyrazine	High	Bell pepper
6	2-Methoxy-3-isobutylpyrazine	High	Bell pepper
7	2-Methoxy-3-(1-methylpropyl)pyrazine	High	Bell pepper
8	2-Methoxy-3-isopentylpyrazine	High	Bell pepper
9	2-Methoxy-3-(1-methylbutyl)pyrazine	High	Bell pepper
10	2-Methoxy-3-hexylpyrazine	High	Bell pepper
11	2-Methoxy-3-(2-methyloctyl)pyrazine	Moderate	Earthy, bell pepper
12	2-Methoxy-3-isononylpyrazine	Low	Bell pepper
	2-Methoxy-6-alkylpyrazines (structure II)		
13	2-Methoxy-6-isobutylpyrazine	High	Bell pepper
	2-Methoxy-3,6-dialkylpyrazines (structure III)		
14	2-Methoxy-3,6-dimethylpyrazine	Moderate	Medicinal, earthy
15	2-Methoxy-3,6-diisobutylpyrazine	Low	Earthy, low bell pepper
	2-Alkoxy-3-alkylpyrazines (structure IV)		
16	2-Ethoxy-3-methylpyrazine	Moderate	Earthy, nutty
17	2-Ethoxy-3-isobutylpyrazine	Moderate	Earthy, bell pepper
18	2-Butoxy-3-methylpyrazine	Low	Floral, medicinal
19	2-Butoxy-3-propylpyrazine	Low	Medicinal, earthy
	2-Methylthio-3-alkylpyrazines (structure V)		
20	2-Methylthio-3-methylpyrazine	High	Nutty, cracker
21	2-Methylthio-3-isobutylpyrazine	Moderate	Bell pepper
	2-Methylthio-6-alkylpyrazine (structure VI)		
22	2-Methylthio-6-isobutylpyrazine	Low	Bell pepper

components were run as films between sodium chloride plates in a Perkin-Elmer 521 spectrophotometer.

Organoleptic Evaluation. In order to simplify the evaluation and to make the results more comparable, two constraints were placed on the evaluation technique. First, all samples were evaluated initially by aroma and then by taste at the same level of 0.1 ppm in spring water. This level was chosen since preliminary results indicated that the subject compounds show a range of intensities at this level. The expert panel of seven members was requested to indicate whether the level was undetectable, low, moderate, or high, on an arbitrary scale where 0.1 ppm of 2-methoxy-3-isobutylpyrazine was defined as a high level. Secondly, since we were attempting to define the structure responsible for a strong bell pepper note, the panel was requested to use the following descriptors: bell pepper, earthy, potato, cracker, nutty, fatty, medicinal, floral, none of above. Remarks of infrequent occurrence have been omitted and odor descriptors have been simplified (Bedoukian, 1971).

A maximum of five chemicals, each in 50 ml of spring water, was evaluated in a random order at one time, and 2-methoxy-3-isobutylpyrazine was always included as one of the five chemicals in each test. In every case, 2-methoxy-3-isobutylpyrazine was reported to have a high bell pepper note under the conditions described.

RESULTS AND DISCUSSION

The organoleptic properties of the various substituted pyrazines are shown in Table I.

From Table I it can be seen that a strong bell pepper character occurs when the 3-alkyl substituent is normal propyl or higher. When this group is methyl, ethyl, or isopropyl, then nutty, earthy, and/or green notes predominate, as was indicated by Seifert *et al.* (1970), and the threshold is higher. The strong bell pepper character persists as the alkyl chain is extended up to at least six carbons; when the alkyl chain has a total of nine carbons, the intensity is decreased and the character becomes less bell pepper and more earthy.

Seifert *et al.* (1972) have indicated that the 5- or 6-isomeric methyl-2-isobutyl-3-methoxypyrazines have reduced thresholds with minty, slight bell pepper notes. When we evaluated 3,6-dimethyl or 3,6-diisobutyl-2-methoxypyrazines (compounds **14** and **15**, respectively) we found earthy notes becoming apparent and only a slight bell pepper note in the diisobutyl compound. This indicates that increasing ring substitution reduces odor intensity and bell pepper character. The high bell pepper note of 2-methoxy-6-isobutylpyrazine (**13**) demonstrates that the characteristic note is not restricted to the 2,3 isomer, but also occurs when the substituent groups are on opposite sides of the molecule.

When the methoxy group is replaced by a higher alkoxy group (compounds **16** to **19** inclusive), the bell pepper note is markedly reduced as is the odor intensity. The ethoxy methyl isomer is nutty and earthy; Seifert *et al.* (1970) have indicated the ethoxy ethyl compound is raw-potato like. 2-Ethoxy-3-isobutylpyrazine was found to possess a moderate earthy green character, not nearly as clean or intense as the methoxy compound. As a further indication of the importance of the methoxy group, 2-butoxy-3-methylpyrazine (**18**) possesses only a low floral note. This compound is isomeric with compound **5**, which

possessed the characteristic note. In a similar fashion, 2-butoxy-3-propylpyrazine (**19**) exhibits only a low medicinal character.

Based on three compounds, it appears that the oxygen of the methoxy group can be replaced by sulfur with a quantitative but not qualitative change in character. Thus, it can be seen that while the intensity of 2-methylthio-3-isobutylpyrazine (**21**) may be lower than the oxygen analog (**6**), the bell pepper note remains similar. Analogously, 2-methylthio-6-isobutylpyrazine (**22**) possesses this note, but with lower intensity than the 2,3 isomer (**21**).

Infrared and mass spectral data of previously unpublished compounds have been deposited in the microfilm edition of this volume of the journal.

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Quantitative Isolation and Partial Characterization of Elastin in Bovine Muscle Tissue

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Three tissues (muscle, aorta, and *ligamentum nuchae*) from one young animal and one old animal were utilized as sources of connective tissue to compare staining properties and amino acid composition to ascertain the purity of elastin isolated from bovine muscle. Elastin preparations from the *triceps brachii* and *biceps femoris* muscles were essentially identical in amino acid composition to elastin from *ligamentum nuchae* in both young and old animals. The ease with which elastin can be purified varies considerably with the source, but by the use of extraction procedures of increasing severity, the end product from tissues of different kinds and from animals of different ages approaches constancy of composition. It can be concluded that elastin isolated as the residue remaining after extraction with 0.1 *N* NaOH at 98° for 45 min yields a material that is relatively homogenous in composition and varies little with tissue source or animal age.

Although considerable research has been reported concerning the role of connective tissue in determining the tenderness of meat, the majority of the related literature has dealt with collagen (Cross *et al.*, 1972; Goll *et al.*, 1963; McClain, 1969; McClain *et al.*, 1965; Wilson *et al.*, 1954). Collagen has been extensively investigated, both with regard to its physical and chemical properties and to determine its relationship to tenderness. Elastin has been less extensively researched, perhaps because it forms a lesser proportion of connective tissue than collagen and because of the inherent difficulty in studying a protein which is characteristically insoluble during heating (Partridge *et al.*, 1955).

The historical assumption that elastin is present in muscle tissue in only small amounts has been challenged by Partridge (1966), who found considerable amounts of elastin in the muscles of the beef rump. The necessity to consider the singular effects of elastin, rather than total connective tissue, on muscle tenderness suggests that a quantitative method for determining small quantities of elastin in muscle tissue needs to be developed. The methods presently available for the isolation of elastin fibers are based on the insolubility of elastin and its resistance to hydrolytic reagents. The ease with which elastin can be purified varies considerably with the connective tissue source. Most of the purification methods reported in the literature have been conducted on an elastin-rich tissue such as aorta and *ligamentum nuchae* (Gotte *et al.*, 1963; Lansing *et al.*, 1952; Partridge, 1962). Bendall (1967) isolated elastin from muscle tissue using numerous extractions with NaOH and various fat solvents. Contrary to results reported by Partridge (1966), Bendall found significant amounts of elastin only in the *semitendinosus* muscle of the bovine. Little additional work has been reported on the isolation of elastin in muscle tissue; thus, procedures for isolating elastin without undue hydrolytic damage appear to be needed. More specifically, the objective of this study was to develop a quantitative assay for elastin derived from muscle tissue and to document age-associated changes in bovine elastin.

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EXPERIMENTAL PROCEDURE

Four tissues (*ligamentum nuchae*, aorta, *triceps brachii*, and *biceps femoris*) were utilized from a young Hereford female (388 days of age) and an old Hereford female (3660 days of age) as sources of bovine elastin.

Purification of Elastin. Purified elastic-fiber prepara-