Olfactive Properties of 3-Substituted 5-Alkyl-2-methylpyrazines

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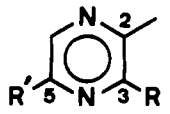
Seven 3-substituted 5-alkyl-2-methylpyrazines (1–7) have been synthesized. Their olfactive properties are discussed. 2,5-Dimethyl-3-methoxypyrazine (1) had the lowest odor threshold value (T = 0.1 ppb) of all the compounds that have the nutty odor. It has been suggested that there is an affinity between certain functional groups of a compound having a nutty or green odor and special affinity sites on the olfactive receptor.

In a study for the structure–odor relationship of alkylpyrazines and 3-substituted 2-alkylpyrazines, we have reported that the alkyl substituents play an important role in the tonalities of the pyrazines (Masuda and Mihara, 1988). In this paper, we describe the effects of varying the alkyl chain length of 3-substituted 5-alkyl-2-methylpyrazines (I) on their olfactive properties.



(I)

R = CH_3, OCH_3, OC_2H_5, SCH_3, SC_2H_5

R′ = H, CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)C_2H_5$, $CH_2CH(CH_3)C_3H_7$

EXPERIMENTAL PROCEDURES

2,3-Dimethyl-, 2-methoxy-3-methyl-, 2-ethoxy-3-methyl-, 2-methyl-3-(methylthio)-, and trimethylpyrazine were purchased from Pyrazine Specialties. Among them, 2,3-dimethylpyrazine was distilled by the Perkin-Elmer spinning band distillation system, using a Model 251 Auto Annular still. The purities of all pyrazines were checked by GLC to be more than 99.9%. All other starting chemicals were obtained from reliable commercial sources and used without further purification. IR, NMR, and mass spectral data were obtained by using Hitachi 260-10, Bruker AM-400, and Hitachi M-80B instruments, respectively. GC analyses were carried out on a Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column coated with Carbowax 20M or OV-101. The preparative HPLC was performed on a Japan Analytical Industry LC-08 instrument equipped with a refractive index detector and a gel permeation chromatography (GPC) column using chloroform as eluent.

Synthesis of 3-Substituted 2,5-Dimethylpyrazines (1–4). According to the reported procedure (Hirschberg and Spoerri, 1961), treatment of 2,5-dimethylpyrazine with chlorine afforded a 3-chloro-2,5-dimethylpyrazine, which was subsequently reacted with sodium methoxide, sodium methanethiolate, sodium ethoxide, and sodium ethanethiolate to obtain the desired 1, 2, 3, and 4, respectively (see Table I).

Synthesis of 5-Ethyl-2,3-dimethylpyrazine (5). The procedure used for the synthesis of this compound was the same as that previously described (Masuda et al., 1980; see Table I).

Synthesis of 5-Ethyl-3-methoxy-2-methyl- and 5-Ethyl-2-methyl-3-(methylthio)pyrazine (6 and 7). According to the reported procedure (Kaiser et al., 1973), 2-ethyl-5-methylpyrazine was obtained from 2,5-dimethylpyrazine (5.4 g, 0.05 mol). Treating 2-ethyl-5-methylpyrazine with chlorine afforded 3-chloro-5-ethyl-2-methyl- and 3-chloro-2-ethyl-5-methylpyrazine (Hirschberg and Spoerri, 1961). These compounds were subsequently reacted with sodium methoxide or sodium methanethiolate. The solution was extracted with chloroform, and the extract was dried over anhydrous sodium sulfate. The solvent was removed, and the reaction mixture was subjected to preparative HPLC (GPC; solvent, chloroform) followed by preparative TLC (Macherey-Nagel silica gel; solvent, hexane–ethyl acetate, 5:1 v/v) to give the two isomers, 5-ethyl-3-methoxy-2-methylpyrazine (6; 1.51 g, total yield = 20%, R_f 0.53) and 2-ethyl-3-methoxy-5-methylpyrazine (6′; 1.53 g, 20%, R_f 0.58), or 5-ethyl-2-methyl-3-(methylthio)pyrazine (7; 1.34 g, 16%, R_f 0.57) and 2-ethyl-5-methyl-3-(methylthio)pyrazine (7′; 1.26 g, 15%, R_f 0.61), respectively (see Table I). The relative stereochemistries of 6, 6′, 7, and 7′ were confirmed by their nuclear Overhauser effect (NOE) difference experiments. Irradiations at δ 2.72, 2.42, 2.77, and 2.47 (CH_3-Ar or CH_2-Ar) resulted in 8, 9, 4, and 17% NOEs at δ 7.86, 7.88, 7.96, and 8.00 (aromatic proton), respectively. These NOE interactions established that the spacial proximity among aromatic proton (H6) and CH_3-C5 or CH_2-C5. The spectral data of these compounds are shown in Table II.

Table I. Yields, Boiling Points, Odor Descriptions, and Odor Thresholds of Pyrazines (I)

no.	R	R′	yield, %	bp, °C/mmHg	odor descriptions	T, ppb
1[a]	OCH_3	CH_3	72[e]	73/18	nutty, roasted, chocolate-like	0.1
2[b]	SCH_3	CH_3	91[e]	80/4	nutty, roasted, brown	1.3
3[c]	OC_2H_5	CH_3	82[e]		nutty, roasted, chocolate-like	11
4[b]	SC_2H_5	CH_3	85[e]	107/11	nutty, brown	21
5[d]	CH_3	C_2H_5	14[f]	92/5	nutty, brown, chocolate-like	530
6	OCH_3	C_2H_5	20[e]		nutty, roasted, sweet	0.2
7	SCH_3	C_2H_5	16[e]		nutty, roasted	2.0

[a] Parliment and Epstein (1973). [b] Winter et al. (1976). [c] Hirschberg and Spoerri (1961). [d] Tressl (1979). [e] Overall yields based on 2,5-dimethylpyrazine. [f] Overall yields based on 2,3-dihydro-5,6-dimethylpyrazine.

Sensory Evaluation. Threshold values for the pyrazines (>99.9% purity) were determined by the "2/5 test" (selecting the same two of five samples) employed by Amoore (1970). The panelists (seven males) ranged in age from approximately 22 to 29 years of age. They had extensive experience and proven reliability in odor judgments. The odor characteristics of the pyrazines were evaluated at 10–100 times higher concentrations than the thresholds. The sessions were conducted in midmorning of every day.

RESULTS AND DISCUSSION

The yields, boiling points, odor descriptions, and odor thresholds (detection threshold, T) of I obtained in this work are shown in Table I. Figure 1 shows the relation between log $1/T$ and the number of carbons [n (Figure 1a) and m (Figure 1b)] in the alkyl side chain at the 5-position

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Table II. Spectral Data of New Pyrazines

no.	IR (neat), cm^{-1}	NMR ($CDCl_3$), δ	MS, m/z (%)	HRMS, m/z found	HRMS, m/z calcd
6	2960, 2940, 1580, 1550, 1450, 1390, 1370, 1180, 1030, 1000, 880	1.29 (3 H, t, J = 7.6 Hz, CH_2CH_3), 2.47 (3 H, m, CH_3), 2.72 (2 H, br q, J = 7.6 Hz, CH_2CH_3), 3.99 (3 H, s, OCH_3), 7.86 (1 H, m, ring H)	152 (M^+, 100), 151 (37), 137 (60), 134 (14), 123 (18), 109 (31), 107 (14), 68 (17)	152.0944	152.0950 ($C_8H_{12}N_2O$)
6′	2970, 2940, 1580, 1540, 1450, 1370, 1350, 1310, 1180, 1150, 1050, 1040, 1000, 880	1.25 (3 H, t, J = 7.5 Hz, CH_2CH_3), 2.42 (3 H, m, CH_3), 2.80 (2 H, br q, J = 7.5 Hz, CH_2CH_3), 3.96 (3 H, s, OCH_3), 7.88 (1 H, m, ring H)	152 (M^+, 100), 151 (28), 137 (66), 123 (16), 121 (20), 109 (29), 107 (19), 56 (30), 54 (28)	152.0940	152.0950 ($C_8H_{12}N_2O$)
7	2960, 2920, 1520, 1460, 1430, 1370, 1330, 1300, 1170, 1090, 970, 890	1.31 (3 H, t, J = 7.6 Hz, CH_2CH_3), 2.48 (3 H, br s, CH_3), 2.57 (3 H, s, SCH_3), 2.77 (2 H, br q, J = 7.6 Hz, CH_2CH_3), 7.96 (1 H, br s, ring H)	169 (13), 168 (M^+, 100), 153 (19), 135 (96), 134 (14), 107 (20), 53 (24)	168.0735	168.0721 ($C_8H_{12}N_2S$)
7′	2970, 2920, 1550, 1515, 1460, 1420, 1330, 1285, 1240, 1155, 1095, 1050, 910	1.30 (3 H, t, J = 7.5 Hz, CH_2CH_3), 2.47 (3 H, m, CH_3), 2.54 (3 H, s, SCH_3), 2.77 (2 H, br q, J = 7.5 Hz, CH_2CH_3), 8.00 (1 H, br s, ring H)	169 (12), 168 (M^+, 100), 153 (53), 135 (81), 133 (20), 121 (12), 119 (12)	168.0741	168.0721 ($C_8H_{12}N_2S$)

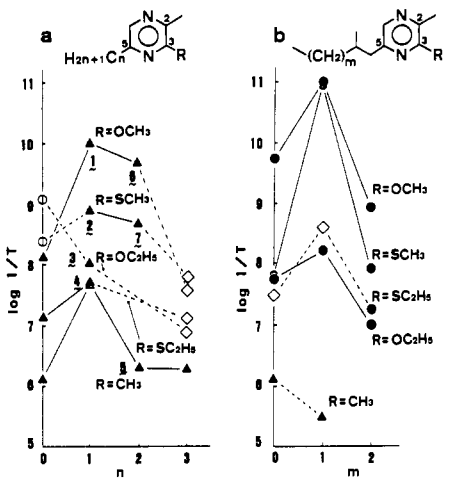

Figure 1. Relation between log $1/T$, where T is the odor threshold, and the number of carbons n (a) and m (b) in the alkyl group for 3-substituted 5-alkyl-2-methylpyrazines (I). n = 3, R′ = $CH(CH_3)_2$. Symbols: (▲) nutty; (●) green; (Φ) nutty and green; (◇) other.

in the pyrazine ring for I obtained in both this work (1–7) and a previous paper (Masuda and Mihara, 1986).

Although 5-alkyl-3-methyl-2(1*H*)-pyrazinones prepared from the reaction of 3-methyl-5,6-dihydro-2(1*H*)-pyrazinones and ketones or aldehydes have been reported (Masuda et al., 1981; Masuda and Mihara, 1986), we reported that NOE difference experiments and X-ray crystallography demonstrate that these compounds are actually 6-alkyl-3-methyl-2(1*H*)-pyrazinones (Mihara and Masuda, 1990). The structures of pyrazines derived from 6-alkyl-3-methyl-2(1*H*)-pyrazinones have to be revised to 3-substituted 5-alkyl-2-methylpyrazines (I).

As shown in Figure 1a, in all pyrazines having a nutty odor (▲), except the ones having both nutty and green odors (Φ), log $1/T$ has a maximum value at n = 1. That is to say, the odor thresholds are at a minimum when the number of carbons is 1, namely 3-substituted 2,5-dimethylpyrazine. Particularly, 2,5-dimethyl-3-methoxypyrazine (1) has the lowest odor threshold value (T = 0.1 ppb) of all the compounds that have a nutty odor (Fors, 1983).

The stereochemical and electronic structural features of 1 are close to those of 2-methoxy-3-methylpyrazine (8; nutty, roasted, sweet; T = 7 ppb) (Masuda and Mihara, 1988) or 5-acetyl-4-methylthiazole [9; roasted, nutty, sulfury (Pittet and Hruza, 1974); T = 50 ppb (Vernin, 1979)]. These observations, together with the report that the nutty aroma compounds defined consist of planar, unsaturated, heterocyclic compounds with either one or two nitrogen atoms in the ring (Hodge et al., 1972), suggest that a nitrogen atom of a heterocyclic ring, hydrophobic methyl groups, and an electronegative oxygen atom are required at the proper positions on the receptor to produce an intense nutty odor, as shown in Figure 2.

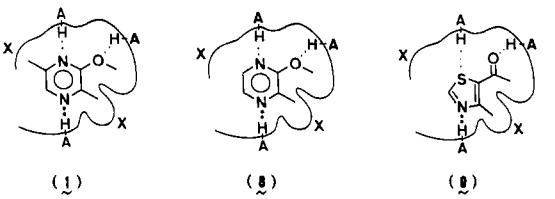

Figure 2. Specific receptor interaction of nutty odorants (1, 8, and 9). A–H is the receptor site corresponding to the electronegative group; X is the receptor site corresponding to the hydrophobic group.

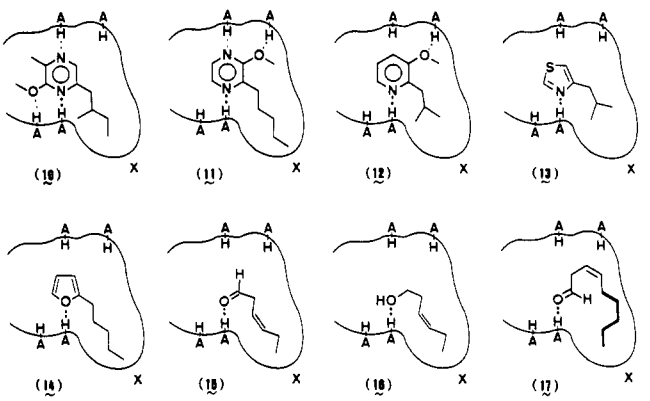

Figure 3. Specific receptor interaction of green odorants (10–17). A–H is the receptor site corresponding to the electronegative group; X is the receptor site corresponding to the hydrophobic group.

Many of the pyrazines shown in Figure 1b have green odor (Masuda and Mihara, 1986). Irrespective of the substituents at the 3-position except for R = CH_3, their odor thresholds are at a minimum when the number of carbons is five, namely m = 1. It appears that the alkyl substituents play an important role in the tonalities of the trisubstituted pyrazines in the same manner as the mono- and disubstituted pyrazines (Masuda and Mihara, 1988). Figure 3 shows the interactions between eight typical green odorants and the receptor model responsible for the green odor sensation. 3-Methoxy-2-methyl-5-(2-methylbutyl)-pyrazine (10; burdock-like, weakly green; T = 0.01 ppb)

(Masuda and Mihara, 1986) and 2-methoxy-3-pentylpyrazine (11; green, earthy, ginseng-like, burdock-like; T = 0.02 ppb) (Masuda and Mihara, 1988) have very low odor threshold values. The odors of 3-isobutyl-2-methoxy- and 2-isobutyl-3-methoxypyridine (12) have been described as being camphoraceous and strong green pepper like, respectively (Pittet and Hruza, 1974). The fact suggested that the interaction of the N atom located in the α-position of the alkyl group having around five carbons with the electropositive group AH on the receptor might play an important role to produce a green odor. 4-Isobutylthiazole [13; strong green (Reymond and Egli, 1969); T = 3.5 ppb (Buttery et al., 1971)] and 2-pentylfuran [14; green, sweet; T = 6 ppb (Buttery et al., 1971)] have isobutyl and pentyl groups at the α-position of heterocyclic atoms (—O— and —N=) of heteroaromatic compounds, respectively. It is well-known that (Z)-3-hexenal [15; green beans, tomato green (Meijboom and Jongenotter, 1981); T = 0.25 ppb (Buttery et al., 1971)] and (Z)-3-hexenol [16; green, grassy (Arctander, 1969); T = 70 ppb (Buttery et al., 1971)] are the character impact compounds in the green leaves. These compounds were made up of six carbon atoms, but the number of carbon atoms of the alkyl group corresponding to those of heteroaromatic compounds 10–14 may be regarded as five. The stereochemical and electronic structural similarity between 2-pentylfuran (14) and (Z)-3-nonenal (17; green, cucumber-like; T = 0.25 ppb) (Meijboom and Jongenotter, 1981) was shown in Figure 3. The green odorants with more purely simple molecules (15 and 16) may have higher odor purities than those with heteroaromatic molecules (10–14). These results suggested that the basic functional groups responsible for an intense green odor are the electronegative group such as a nitrogen or oxygen atom and the hydrophobic alkyl or alkenyl group having a chain length of around five methylene units at the α-position.

All 5-alkyl-2,3-dimethylpyrazines, which have the highest odor threshold of all the corresponding trisubstituted pyrazines, possess nutty and/or brown odors as shown in Figure 1. These dimethylpyrazine derivatives may be more liable to fit the receptor site of nutty odorants than that of green odorants.

LITERATURE CITED

Amoore, J. E. *Molecular Basis of Odor*; Thomas; Springfield, IL, 1970; pp 16–25.

Arctander, S. *Perfume and Flavor Chemicals (Aroma Chemicals)*; published by the author; Montclair, NJ, 1969.

Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of Additional Volatile Components of Tomato. *J. Agric. Food Chem.* **1971**, *19*, 524.

Fors, S. *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; p 185.

Hirschberg, A.; Spoerri, P. E. Chlorination of Some Alkylpyrazines. *J. Org. Chem.* **1961**, *26*, 2356.

Hodge, J. E.; Mills, F. D.; Fisher, B. E. Compounds of Browned Flavor Derived from Sugar-Amine Reactions. *Cereal Sci. Today* **1972**, *17*, 34.

Kaiser, E. M.; Bartling, G. J.; Thomas, W. R.; Nichols, S. B.; Nash, D. R. Selective Metalations of Methylated Pyridines and Quinolines. Condensation Reactions. *J. Org. Chem.* **1973**, *38*, 71.

Masuda, H.; Mihara, S. Synthesis of Alkoxy-, (Alkylthio)-, Phenoxy-, and (Phenylthio)pyrazines and Their Olfactive Properties. *J. Agric. Food Chem.* **1986**, *34*, 377.

Masuda, H.; Mihara, S. Olfactive Properties of Alkylpyrazines and 3-Substituted 2-Alkylpyrazines. *J. Agric. Food Chem.* **1988**, *36*, 584.

Masuda, H.; Tanaka, M.; Akiyama, T.; Shibamoto, T. Preparation of 5-Substituted 2,3-Dimethylpyrazines from the Reaction of 2,3-Dimethyl-5,6-dihydropyrazine and Aldehydes or Ketones. *J. Agric. Food Chem.* **1980**, *28*, 244.

Masuda, H.; Yoshida, M.; Shibamoto, T. Synthesis of New Pyrazines for Flavor Use. *J. Agric. Food Chem.* **1981**, *29*, 944.

Meijboom, P. W.; Jongenotter, G. A. Flavor Perceptibility of Straight Chain, Unsaturated Aldehydes As a Function of Double-Bond Position and Geometry. *J. Am. Oil Chem. Soc.* **1981**, *58*, 680.

Mihara, S.; Masuda, H. Structure Elucidation of the Product Prepared from the Reaction of 3-Methyl-5,6-dihdyro-2(1*H*)-pyrazinone and Ketones or Aldehydes. *J. Agric. Food Chem.* **1990**, *38*, 1032.

Parliment, T. H.; Epstein, M. F. Organoleptic Properties of Some Alkyl-Substituted Alkoxy- and Alkylthiopyrazines. *J. Agric. Food Chem.* **1973**, *21*, 714.

Pittet, A. O.; Hruza, D. E. Comparative Study of Flavor Properties of Thiazole Derivatives. *J. Agric. Food Chem.* **1974**, *22*, 264.

Reymond, D.; Egli, R. H. Composition of Tomato Aroma. *Helv. Chim. Acta* **1969**, *52*, 887.

Tressl, R. The Formation of Aroma Substances by Means of the Maillard Reaction. *Monatsschr. Brau.* **1979**, *32*, 240.

Vernin, G. Heterocycles in Food Aromas. I. Structure and Organoleptic Properties. *Parfums, Cosmet., Aromes* **1979**, *27*, 77.

Winter, M.; Gautschi, F.; Flament, I.; Stoll, M. U.S. Patent 3 931 245, 1976.

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