Synthesis of Some 2-Methoxy-3-Alkylpyrazines with Strong Bell Pepper-Like Odors

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The synthesis, mass spectra, and infrared spectra are described for four new 2-methoxy-3-alkylpyrazines with alkyl groups hexyl, propyl, isopropyl, and ethyl. The odor thresholds of the hexyl, propyl, isopropyl, and ethyl compounds were found to be 1, 6, 2, and 425 parts of compound per 10^{12} parts of water, respectively. The odor character of these four compounds compared to that of the known bell pepper component 2-methoxy-3-isobutylpyrazine are described as very similar for the hexyl and propyl, moderately similar for the isopropyl, and not similar for the ethyl. The related compounds 2-isobutylpyrazine, 2-methoxypyrazine, and 2,5-dimethylpyrazine were considerably weaker odorants with odor thresholds of 4×10^5, 7×10^5, and 1.8×10^6 parts of compound per 10^{12} parts of water, respectively.

In a recent publication (Buttery *et al.*, 1969a) we reported the isolation, identification, and synthesis of the characteristic highly potent aroma component of bell peppers 2-methoxy-3-isobutylpyrazine (I, R = isobutyl). Because of the unusually high odor potency and characteristic aroma of this compound, we decided to investigate the odor threshold and character of other 2-methoxy-3-alkylpyrazines.

EXPERIMENTAL

Synthesis of Amino Acid Amides. Three general methods were used. The first method (Greenstein and Winitz, 1961), which was used for *dl*-norvaline, involved treatment of its methyl ester hydrochloride for 60 hours at 25° C. with a saturated methanolic solution of anhydrous ammonia (previously saturated at 0° C.). This gave the amino acid amide hydrochloride.

The second method, which was used for both leucine and valine, involved conversion of the amino acid ester hydrochlorides to the free amino acid ester by treatment with triethylamine (Chambers and Carpenter, 1955) before treatment with the methanolic ammonia. This gave the free amino acid amide which gave better yields in the reaction with glyoxal in the next step. Using the synthesis of leucine amide as an example, L-leucine methyl ester hydrochloride (14.5 grams) gave 11.2 grams of free amino acid ester, which when added to methanol (175 ml.) previously saturated at 0° C. with anhydrous ammonia and kept for three days at 25° C. in a stoppered flask, yielded 8.75 grams of L-leucine amide (recrystallized from a methanol–ether solution).

The third method, which was used for the synthesis of α-aminobutyroamide hydrobromide, involved treating ethyl α-bromobutyrate with aqueous ammonia (saturated at 0° C.) at −5° C. for 10 days according to the procedure of Karmus and Spoerri (1952). 2-Aminooctanamide hydrobromide was prepared using a modification of this method by treating 2-bromooctanamide with methanolic ammonia (saturated at 0° C.) for two days at 25° C. This method has the advantage of being more flexible and does not require an available amino acid.

Synthesis of 2-Hydroxy-3-Alkylpyrazines. Several different methods (Jones, 1949; Karmus and Spoerri, 1952) were tried for the condensation of the amino acid amide with glyoxal. The following procedure using the synthesis of 2-hydroxy-3-isobutylpyrazine (II, R′ = isobutyl) as an example proved to be the most satisfactory. L-Leucine amide (3.9 grams) in methanol (30 ml.) and water (30 ml.) was cooled below −20° C. Glyoxal di(sodium bisulfite) (8 grams) followed by sodium hydroxide (3 grams) dissolved in water (10 ml.) was then added. After stirring for one hour, water (120 ml.) was added to dissolve all of the glyoxal di(sodium bisulfite). Stirring was continued for 3 hours and the temperature maintained at −10° C. before the solution was neutralized with 12*N* hydrochloric acid (3.75 ml.) and then concentrated to dryness under vacuum at 50° C. The solid residue was extracted with boiling chloroform (4 × 75 ml.) to give 3.2 grams of crystalline product. Infrared absorption (IR) spectra and mass spectra (MS) were consistent with the structure of 2-hydroxy-3-isobutylpyrazine. A portion of the crude product recrystallized from ethyl acetate melted at 90 to 92.5° C. The yield calculated from L-leucine methyl ester was 60%.

2-Hydroxy-3-isopropylpyrazine (II, R′ = isopropyl) was prepared in a similar way. A sample recrystallized from ethyl acetate had m.p. 74.5 to 76.5° C. (Karmus and Spoerri, 1952, reported 76 to 77° C.).

2-Hydroxy-3-propylpyrazine (II, R′ = propyl) was prepared by condensing the norvaline amide hydrochloride in methanol with glyoxal (a commercially obtained 40% aqueous solution) at −20° C. During a 3-hour period, the mixture was allowed to reach 25° C. and was then stored 18 hours at −20° C. The reaction mixture after acidification with hydrochloric acid followed by treatment with excess sodium bicarbonate was concentrated *in vacuo* at 45° C. leaving a dark gummy residue. A portion of the residue recrystallized from ethyl acetate gave a m.p. of 86 to 86.5° C. (Karmus and Spoerri, 1952, reported 79 to 80° C.).

2-Hydroxy-3-ethylpyrazine (II, R′ = ethyl) and 2-hydroxy-3-hexylpyrazine (II, R′ = hexyl) were prepared from the amide hydrobromides using conditions similar to that used for the isobutyl compound except adding sufficient additional sodium hydroxide to neutralize the hydrobromide. 2-Hydroxy-3-ethylpyrazine recrystallized from benzene:pentane (5:1) had m.p. 105 to 106° C. (Karmus and Spoerri, 1952, reported 96 to 97° C.). 2-Hydroxy-3-hexylpyrazine recrystallized from benzene:pentane (4:1) had m.p. 83.5 to 84° C.

Methylation of 2-Hydroxy-3-Alkylpyrazines. Methylation of the 2-hydroxy-3-alkylpyrazines was easily effected with diazomethane. The methylation of 2-hydroxy-3-isobutylpyrazine is typical of the reaction. The diazomethane (from 3.6 grams of nitrosomethylurea) in ether (75 ml.) was added to the ice cooled solution of 2-hydroxy-3-iso-

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butylpyrazine (1.52 grams) in ether. The mixture was kept at 5° C. for 20 minutes and at 25° C. for a further 20 minutes before the excess diazomethane and ether were removed on a steam bath to give 1.58 grams of crude product. A modification used for 2-hydroxy-3-propylpyrazine was to dissolve it in methanol.

Purification of 2-Methoxy-3-Alkylpyrazines. The samples were purified by gas–liquid chromatography (GLC) on a 10-foot × $^1/_4$-inch aluminum column packed with Chromosorb P coated with 10% silicone SF 96-350 containing 5% of the silicone as Igepal CO-880 and 0.1% as Ionox. Detection was by thermal conductivity. In the case of 2-methoxy-3-isobutylpyrazine (I, R = isobutyl), for example, the column was maintained at 160° C. and the helium carrier gas at a flow rate of 53 ml. per minute for 40 minutes, then raised to 200° C. and a flow rate of 100 ml. per minute. Injection of the mixture gave 2-methoxy-3-isobutylpyrazine at 9.5 minutes (31%), 1,2-dihydro-3-isobutyl-1-methyl-2-pyrazinone (III, R″ = isobutyl) at 35 minutes (58%), and unreacted 2-hydroxy-3-isobutylpyrazine at 44 minutes (9%). The collected peaks were identified by their MS and infrared absorption spectra.

Methylation of the other 2-hydroxy-3-alkylpyrazines (II, R″ = propyl, isopropyl, ethyl, or hexyl) gave the corresponding 2-methoxy-3-alkylpyrazines (I) and the corresponding 1,2-dihydro-3-alkyl-1-methyl-2-pyrazinone (III) in approximately the same ratios as found for the isobutyl homologs.

2-Methoxypyrazine. This was prepared by refluxing 2-chloropyrazine with methanolic potassium hydroxide according to the procedure of Klein *et al.* (1964).

2-Methoxy-3-Methylpyrazine. This was prepared by the method outlined by Firmenich & Co. (1965).

2-Isobutylpyrazine. This was prepared from 2-methylpyrazine and 2-bromopropane using the sodium amide–liquid ammonia method of Behun and Levine (1961). MS and IR spectra of the GLC purified product were consistent with that expected for 2-isobutylpyrazine.

2,5-Dimethylpyrazine. This was obtained commercially and purified by GLC. The MS and IR spectra of the purified product were consistent with published data.

Mass Spectra. The MS of the GLC purified compounds were recorded using a modified Consolidated 21-620 cycloidal type mass spectrometer. High resolution mass spectral measurement of molecular weight was carried out using a Consolidated 21-110 B double focusing instrument.

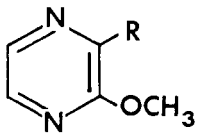
Infrared Absorption Spectra. The IR spectra of the GLC purified compounds were recorded using a Perkin-Elmer 237 double beam grating instrument. The spectra of the pure material as a film or in KBr were recorded.

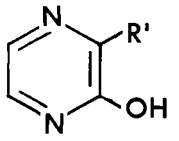
Odor Thresholds. Thresholds of the GLC purified compounds were determined as described previously (Guadagni *et al.*, 1963) except that Teflon, instead of polyethylene, bottles and tubing were used as containers for the odor solutions.

RESULTS AND DISCUSSION

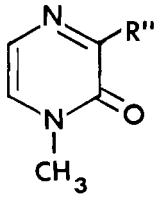
The bell pepper aroma compound 2-methoxy-3-isobutylpyrazine (I, R = isobutyl) and the previously undescribed compounds 2-methoxy-3-hexylpyrazine (I, R = hexyl), 2-methoxy-3-propylpyrazine (I, R = propyl), 2-methoxy-3-isopropylpyrazine (I, R = isopropyl), and 2-methoxy-3-ethylpyrazine (I, R = ethyl) were synthesized from the amides of the amino acids leucine, 2-amino-octanoic acid, norvaline, valine, and α-aminobutyric acid, respectively, by condensing each with glyoxal (Jones, 1949) to give the corresponding 2-hydroxy-3-alkylpyrazine and converting this to the methoxy compound by methylation with diazomethane.

High resolution mass spectrometry of the synthetic 2-methoxy-3-propylpyrazine showed a molecular weight of 152.0951 ($C_8H_{12}N_2O$ requires 152.0949). The synthetic 2-methoxy-3-isopropylpyrazine showed a molecular weight of 152.0950 ($C_8H_{12}N_2O$ requires 152.0949). The synthetic 2-methoxy-3-ethylpyrazine showed a molecular weight of 138.07930 ($C_7H_{10}N_2O$ requires 138.07933). The synthetic 2-methoxy-3-hexylpyrazine showed a molecular weight of 194.1425 ($C_{11}H_{18}N_2O$ requires 194.1419).





II



III

2-Hydroxy-3-propylpyrazine (II, R′ = propyl), 2-hydroxy-3-isopropylpyrazine (II, R′ = isopropyl), and 2-hydroxy-3-ethylpyrazine (II, R′ = ethyl) had been previously reported by other workers (Karmus and Spoerri, 1952). The synthesis of 2-hydroxy-3-isobutylpyrazine (II, R = isobutyl) had not been reported until the authors' recent publications (Buttery *et al.*, 1969a,b). The parent compound of this series 2-methoxypyrazine was synthesized according to the method of Klein *et al.* (1964) by treating 2-chloropyrazine with methanolic potassium hydroxide. 2-Methoxy-3-methylpyrazine (I, R = methyl) was synthesized in several steps by the method described by Firmenich & Co. (1965).

The condensation of the amino acid amide with glyoxal generally proceeded smoothly and in reasonable yield. However, because hydroxypyrazines exist largely in the pyrazinone form (Cox and Bothner-By, 1968; Katritzky, 1963), methylation of hydroxyalkylpyrazines with diazomethane actually gives larger yields of 1,2-dihydro-3-alkyl-1-methyl-2-pyrazinones (III) than of the desired 2-methoxy-3-alkylpyrazines (I). These compounds were formed in the present work in the ratio of about 2 to 1, respectively. An alternate method of converting a hydroxypyrazine to the methoxy compound is to treat the hydroxypyrazine with $POCl_3$ to give the chloropyrazine and then treat the chloropyrazine with sodium methoxide (Karmus and Spoerri, 1952). The authors, however, preferred the diazomethane method because the yield of methoxypyrazine was sufficient for the purpose and could be readily separated from the much less volatile pyrazinone either by gas chromatography or distillation.

Odor Properties. Table I lists odor thresholds found for the methoxyalkylpyrazines. The isopropyl and hexyl compounds had about the same odor thresholds as found previously (Buttery *et al.*, 1969a,b) for the isobutyl compound.

Table I. Odor Thresholds in Water Solution

Compound	Odor Threshold in Parts of Compound per 10^{12} Parts of Water
2-Methoxy-3-isobutyl-pyrazine	2
2-Methoxy-3-hexyl-pyrazine	1
2-Methoxy-3-pro-pylpyrazine	6
2-Methoxy-3-iso-propylpyrazine	2
2-Methoxy-3-ethyl-pyrazine	425
2-Methoxy-3-methylpyrazine	4,000
2-Methoxypyrazine	700,000
2-Isobutylpyrazine	400,000
2,5-Dimethyl-pyrazine	1,800,000

The normal propyl compound was slightly weaker but is still considered an extremely potent odorant. Shortening the chain to the ethyl compound weakened the odor strength considerably although its threshold of 425 parts per 10^{12} is still of the same order as many common flavoring materials. Shortening the chain still further to the methyl compound results in a further weakening of odor strength. The complete absence of the alkyl side chain in the case of 2-methoxypyrazine gave an odor threshold approximately 3.5×10^5 times higher than the isopropyl and isobutyl compounds. This obviously represents a great decrease in odor potency.

The odor threshold of the nonmethoxypyrazines 2-isobutylpyrazine and 2,5-dimethylpyrazine, were also determined and are listed in Table I. In comparison with the methoxyalkylpyrazines, they are rather weak odorants in water solution.

In the authors' opinion, the odor characters of the isobutyl, propyl, and hexyl methoxypyrazines were very similar to that of bell peppers. The odor character of the isopropyl compound was moderately similar to bell pepper and under certain conditions slightly similar to raw potato. The odor character of the ethyl compound was more like that of raw potatoes and unlike that of bell pepper. The authors recently tentatively identified this compound in potatoes. The odor character of the methyl compound was like that of roasted peanuts. The odor of the unsubstituted 2-methoxypyrazine was not very characteristic. A more detailed evaluation of the odor characteristics of these compounds will be reported later. The pyrazinone compounds and hydroxyalkylpyrazines possessed essentially no odor. The reason for this is probably due to their low volatility and highly polar nature.

Mass spectra data found for 2-methoxy-3-alkylpyrazines (I) and 2-hydroxy-3-alkylpyrazines (II) were as follows (molecular ion and major ions above *m/e* 40, intensities in parentheses with base peak taken as 100):

2-Methoxy-3-isobutylpyrazine (see Buttery *et al.*, 1969b).

2-Methoxy-3-hexylpyrazine, mol. ion 194(1.6), major ions 124(100), 137(24), 41(21), 94(21), 95(12), 42(10), 53(9), 43(8), 81(8), 54(7).

2-Methoxy-3-propylpyrazine, mol. ion 152(5), major ions, 124(100), 137(25), 94(24), 41(20), 95(16), 53(16), 93(15), 42(13), 54(12), 81(10).

2-Methoxy-3-isopropylpyrazine, mol. ion 152(38), major ions 137(100), 41(25), 124(21), 43(14), 105(13), 68(12), 52(12), 95(11), 54(10), 53(9).

2-Methoxy-3-ethylpyrazine, mol. ion 138(100), major ions 123(65), 137(35), 41(27), 68(25), 56(23), 107(22), 54(20), 53 (19), 52(18), 42(18).

2-Hydroxy-3-isobutylpyrazine, mol. ion 152(4), major ions 110(100), 81(38), 41(34), 123(30), 82(23), 54(20), 42(17), 138(17), 137(15), 43(13).

2-Hydroxy-3-hexylpyrazine, mol. ion 180(2.7), major ions 124(100), 110(67), 95(67), 81(54), 41(46), 123(46), 54(28), 42(26), 68(21), 96(18).

2-Hydroxy-3-propylpyrazine, mol. ion 138(14), major ions 110(100), 41(35), 81(29), 123(29), 54(22), 42(21), 82(17), 60 (11), 43(10), 55(10).

2-Hydroxy-3-isopropylpyrazine, mol. ion 138(51), major ions 123(100), 110(67), 95(46), 41(43), 42(36), 54(16), 68(15), 43(14), 81(10), 82(10).

2-Hydroxy-3-ethylpyrazine, mol. ion 124(100), major ions 41(78), 95(71), 123(54), 81(47), 42(46), 110(30), 96(25), 54(24), 68(21), 69(14).

Mass spectral data found for 1,2-dihydro-3-alkyl-1-methyl-2-pyrazinones (III) were as follows:

1,2-Dihydro-3-isobutyl-1-methyl-2-pyrazinone, mol. ion 166(21), major ions 124(100), 151(48), 95(39), 42(35), 41(24), 54(22), 96(17), 123(13), 43(11), 55(10).

1,2-Dihydro-3-hexyl-1-methyl-2-pyrazinone, mol. ion 194 (7.5), major ions 124(100), 42(28), 41(24), 137(19), 95(17), 54(17.5), 96(12), 55(10), 11(9), 179(8).

1,2-Dihydro-3-propyl-1-methyl-2-pyrazinone, mol. ion 152 (37), major ions 124(100), 137(58), 95(52), 42(46), 54(31), 41(23), 96(20), 123(13), 55(13).

1,2-Dihydro-3-isopropyl-1-methyl-2-pyrazinone, mol. ion 152(60), major ions 137(100), 124(77), 109(74), 42(51), 54(21), 56(19), 55(13), 123(13), 96(11), 68(11).

1,2-Dihydro-3-ethyl-1-methyl-2-pyrazinone, mol. ion 138 (100), major ions 109(67), 95(60), 123(43), 41(30), 54(28), 56(12), 55(12), 52(11), 53(7).

Infrared absorption spectra found for the 2-methoxy-3-alkylpyrazines (I) and the 2-hydroxyalkylpyrazines (II) were as follows (in the region of 5 to 15 microns, absorption maxima in microns, S means strong, M medium, W weak, VW very weak).

2-Methoxy-3-isobutylpyrazine (see Buttery *et al.*, 1969a).

2-Methoxy-3-hexylpyrazine, as a film, S(6.5, 6.8, 6.9, 7.2, 8.5, 9.7), M(7.4, 7.8, 8.4, 8.7, 11.9), W(6.3, 7.9, 9.2, 9.3, 11.3, 13.8), VW(5.3, 5.6, 6.0, 6.2, 7.7, 12.6, 13.1).

2-Methoxy-3-propylpyrazine, as a film, S(6.5, 6.85, 6.9, 7.2, 8.6, 9.9), M(7.4, 7.6, 8.4, 9.4, 11.9), W(5.9, 6.3, 7.9, 8.2, 9.2, 11.1, 11.4, 13.7), VW(5.3, 5.6, 9.1, 10.6, 13.2).

2-Methoxy-3-isopropylpyrazine, as a film, S(6.5, 6.85, 6.9, 7.16, 7.2, 7.5, 8.6, 8.9, 9.9), M(7.7, 7.9, 8.4, 9.1, 9.2, 11.9), W(6.3, 9.4, 9.6, 11.2, 13.7), VW(5.3, 5.6, 10.5, 10.8, 12.5).

2-Methoxy-3-ethylpyrazine, as a film, S(6.5, 6.85, 6.9, 7.2, 7.5, 8.4, 8.6, 8.7, 9.9), M(7.6, 9.6, 11.9), W(5.2 to 5.3, 5.6 to 5.7, 7.9, 10.3, 11.5, 13.4), VW(8.0, 9.3, 9.4).

2-Hydroxy-3-isobutylpyrazine, in KBr, S(5.99, 6.04, 6.09, 6.2, 12.3), M(6.6, 6.8, 7.5, 7.7, 10.3), W(5.1 to 5.4, 7.2, 7.3, 8.1, 8.2, 9.2, 10.9, 11.0, 12.8), VW(6.7, 7.0, 7.1, 7.8, 8.6, 8.7, 9.0, 9.6, 10.6, 10.7, 11.3, 13.5).

2-Hydroxy-3-hexylpyrazine, in KBr, S(6.04, 6.08, 6.2, 12.4, 12.5), M(6.6, 6.9, 8.2, 10.4), W(7.5, 7.8, 8.1, 8.15, 9.2, 10.9, 13.9), VW(5.25–5.4, 6.8, 7.1, 7.4, 7.6, 7.7, 8.7, 9.0, 9.6, 13.4).

2-Hydroxy-3-propylpyrazine, in KBr, S(6.01, 6.04, 6.09, 6.2, 12.4), M(6.6, 6.8, 8.3, 8.9, 9.2, 10.4, 11.4), W(5.3 to 5.5, 7.1, 7.3, 7.6, 7.9, 8.2, 11.0, 12.7, 13.9), VW(6.83, 7.0, 7.2, 7.7, 8.0, 9.5).

2-Hydroxy-3-isopropylpyrazine, in KBr, S(6.0, 6.3, 9.3, 12.4, 12.6), M(6.6, 6.8, 7.35, 7.9, 8.3, 10.4, 11.0), W(5.2 to 5.5, 6.87, 6.92, 7.0, 7.2, 7.4, 8.1, 8.2, 8.6, 8.9, 9.1, 9.5, 11.4, 11.5, 13.9), VW(7.0, 10.7).

2-Hydroxy-3-ethylpyrazine, in KBr, S(6.1, 6.2, 12.4), M(6.0, 8.4, 8.9, 11.2), W(6.56, 6.86, 7.1, 7.4, 7.8, 8.2, 9.3, 10.5, 13.6), VW(6.59, 6.89, 7.3, 9.7, 10.2, 10.8).

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