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Perception of Odors of Simple Pyrazines by Young and Elderly Subjects: A Multidimensional Analysis[1,2]

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SCHIFFMAN, S. S. AND J. C. LEFFINGWELL. *Perception of odors of simple pyrazines by young and elderly subjects: A multidimensional analysis.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 787–798, 1981.—Ten simple stimuli from the heterocyclic class of nitrogen chemicals known as pyrazines were arranged in a three-dimensional space by INDSCAL using similarity judgments by 12 young and 11 elderly subjects as input. Odor quality at two intensity levels, moderate and strong, was examined. At both concentrations, pyrazines with the highest intrinsic polarity were arranged separate from unsubstituted pyrazines as well as alkyl pyrazines with a single alkyl moiety or any substitution pattern wherein alkyl groups are geometrically opposite. Use of the pattern recognition procedure ADAPT in conjunction with PREFMAP and canonical correlation procedures pointed to the relationship of three variables to olfactory quality: (1) the presence of oxygen atoms, (2) the environment associated with a substructure of the pyrazines and (3) stimulus concentration. Six of the elderly subjects showed a possible loss in the ability to discriminate among the pyrazines at the moderate intensity level.

Odor Pyrazines Multidimensional scaling Aging

THE INTRODUCTION of sophisticated analytical techniques and equipment in the last two decades (e.g., gas chromatography and mass spectrometry) has led to the determination of the chemical constituents in many foods that are responsible for their odorous properties. One of the most important discoveries during this period was the detection, isolation, identification, and development of syntheses for a class of nitrogenous chemical compounds which have been found to contribute in an important manner to the complex sensory properties of many natural products such as cocoa, bell peppers (and other green vegetables), coffee, bread, popcorn, potatoes, and tobacco, to name a few.

Although the heterocyclic class of nitrogen chemicals known as *pyrazines* had long been known to organic chemists, it was not until the mid-1960's that their widespread occurrence (usually in minor concentrations) in foods and other natural products was noted by analytical flavor chemists [15,18]. They quickly grasped that reaction mechanisms by which pyrazines could be formed in nature from flavor precursors could answer many questions as to variations in flavor quality due to agrinomics and subsequent processing. For example, the fermentation of ripe cocoa beans prior to drying and roasting is required for the full development of the cocoa odor. During this process, polysaccharides and protein fractions are enzymatically hydrolyzed to reducing sugars and free amino acids which, during the cocoa bean roasting, are converted via the Maillard and Strecker reactions to the important cocoa volatile constituents, of which many are pyrazines. In fact, of the approximately 360 identified volatiles from cocoa, 64 were identified as pyrazines [13]. Table 1 provides an overview of some of the important natural products which derive an important part of their odor from pyrazines.

Discovery of this relatively simple class of chemicals has permitted their addition to foods to both simulate and enhance their sensory properties. Indeed, the odorous properties of this class of compounds are so important that nearly 40 individual pyrazines have been added to the so-called "GRAS" list of approved flavor additives in the last 10 years [11, 12, 20–25].

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Although many pyrazines have been identified as constituents of foods, there are considerable problems with vagueness, uniqueness, as well as discrepancies among the verbal descriptors used to define their odor qualities. In the case of 2-alkyl-3-methoxy pyrazines, for example, at least 15 different chemicals are reported to have "bell pepper-like" odors. The problem, however, remains that words do not accurately define their olfactory qualities. Although it is known that there is a rough physicochemical relationship between molecular structure of certain 2-alkyl-3-methoxy pyrazines and odor character, no quantitative measure of olfactory quality has been published.

The mathematical technique called *multidimensional scaling* (MDS), which can be used to arrange chemical stimuli in spatial maps on the basis of quantitative measures of similarity, has particular applicability to classes of chemicals whose importance as food additives is known, but where no finite definition as to olfactory quality in a class is available. MDS has previously been utilized to classify olfactory and taste stimuli [1, 6, 28–43, 48, 49].

The application of MDS to individual classes of odorants which can simulate or amplify foods (e.g., pyrazines) is useful for several reasons. First, it provides a map which permits exploration of the structure-activity relationships within a class of compounds. Second, it can provide a system for selection of alternative odorants for simulation of the sensory properties of foods and other natural products to maximize their palatability and acceptability. Third, it can provide information regarding the discriminability of odors among individual subjects. This third reason is especially important from the standpoint of the elderly for whom simulation and enhancement of foods with pyrazines have considerable potential value from the sensory point of view. Previous studies indicate that the elderly have diminished sensitivity to odors when compared with the young (see [31]); amplification of odor has been found to increase palatability of foods for the elderly [31].

The specific purpose of this work is to delineate the psychophysical quality space for a series of pyrazines and to determine if there are differences in discriminability between young and elderly subjects. The relationship to physicochemical parameters is explored as well as the implication of these data to theoretical considerations on the basic mechanism of the interaction of odor molecules at the olfactory receptor site.

TABLE 1

IMPORTANT NATURAL PRODUCTS IN WHICH PYRAZINES ARE CONTRIBUTING FLAVORANTS [7,18]

Cocoa products	Mushrooms
Coffee products	Pork liver (cooked)
Bread (American, white)	Cooked beef (and beef fat)
Rye bread (toasted)	Veal (fried)
Barley (roasted)	Macadamia nuts
Casein (milk protein)	Almonds (roasted)
Whey	Peanuts (roasted)
Skim milk powder	Filberts (roasted)
Popcorn	Tobacco (Virginia)
Potato chips	Tobacco (burley)
Potatoes (dehydrated)	Tobacco (Maryland)
Rum (Jamaican)	Asparagus
Whiskey (Scotch)	Broad beans
Soy sauce	Spinach
Soybeans (fried)	Sweet corn
Bell peppers (green)	Parsnips
Red peppers	Cucumbers
Peas (green)	Lettuce
Tomatoes	Cabbage
Sesame oil	Carrots
Sesame seeds (roasted)	Beets
Chicken (broth)	Pumpkin

TABLE 2

STIMULI

	Stimulus	Moderate Concentration	Strong Concentration
1	2,6 dimethyl	1/34,135	1/3200
2	2,5 dimethyl	1/36,435	1/1707
3	2,3,5,6 tetramethyl	1/1912	1/424
4	2,3,5 trimethyl	1/41,391	1/1940
5	2 methoxy, 3 isobutyl	1/50,000,000,000	1/16,922,666
6	2 methyl, 3 methoxy	1/7,454,000	1/372,700
7	2,3 dimethyl	1/6777	1/635
8	2 methyl	1/2000	1/500
9	2 acetyl	1/256,000	1/8000
10	pyrazine	1/271	1/67
11	2,3,5 trimethyl	1/41,391	1/1940

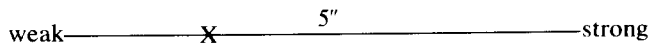
METHOD

Subjects

The subjects were 12 Duke University students, aged 20–24, and 11 healthy, elderly subjects, aged 73–77. The elderly subjects were residents of the Methodist Retirement Home in Durham, NC. All subjects were Caucasians, nonsmokers, and no more than 10% overweight according to Metropolitan Life Insurance tables. The young group consisted of 6 males and 6 females. The elderly group consisted of 2 males and 9 females. The young and elderly groups were approximately equal with regard to intelligence, cultural eating patterns, and socioeconomic status.

Stimuli

The stimuli were a series of pyrazines given in Table 2 which were presented at two different intensities, moderate and strong. The concentrations utilized were determined as follows. A series of 8 serial dilutions in water were made for each pyrazine which ranged subjectively from suprathreshold intensities to very strong. Each dilution for a given stimulus was presented to the blindfolded subjects in 4-oz wide-mouthed bottles at the level of the nostrils. An intensity judgment for each dilution was made, after removing the blindfold, by marking an X along a 5-inch line as shown below:



The positions of the marks were transcribed from 0 to 100 with 0 meaning "weak" and 100 meaning "strong." Geometric mean ratings for each dilution were determined over subjects. Concentrations which had a mean rating be-

tween 40 and 50 were designated to be of "moderate" intensity. Concentrations with a mean rating between 80 and 90 were considered to be of "strong" intensity.

Experimental Procedure

The similarity experiment was divided into two parts: (1) judging similarities among stimuli of moderate intensities and (2) judging similarities among stimuli of strong intensities. First, the subjects, while blindfolded, were presented with each of the 10 stimuli at the moderate intensity level by the experimenter in order to familiarize them with the overall range. Next, the subjects were presented with each of the possible combinations of pairs of stimuli in a randomized sequence. The subjects sniffed two stimuli in sequence from 4-oz wide-mouthed sniff bottles presented by the experimenter at the level of the nostrils. After removing their blindfolds, they made a rating of similarity along a 5-inch line as shown below:

same——————————————————————different

One stimulus (2,3,5 trimethyl pyrazine) was repeated as a control and became stimulus 11. Thus, the subjects made C_2^{11} or 55 judgments of similarity for the stimuli at moderate intensity. The time interval between presentation of two stimuli of a pair was approximately 10 sec. No more than 10 pairs were rated during a one-hour session. The order of presentation of two stimuli of a pair was counterbalanced so that each stimulus was presented first in a pair half of the time. The same procedure was followed for the stimuli at strong intensities. The entire experiment took place in a room well-ventilated with fans.

After completion of the similarity judgments, at both concentration levels, the subjects made hedonic judgments of each stimulus at both moderate and strong intensities by making ratings along a 5-inch line labeled good at one end and bad at the other. In addition, each subject was asked to generate as many adjectives as possible to describe each odor.

Analysis of Data

The positions of the similarity and hedonic judgments along the 5-inch line were transcribed from "0" to "100" in a manner similar to the intensity measurements. The young and elderly similarity data were analyzed by INDSCAL [4], which permits arrangement of the pyrazines in a multidimensional space such that compounds which smell similar to one another are located proximate to one another in the space. Compounds which smell different are arranged distant from one another. The INDSCAL model is a metric individual difference model which provides weights for individual subjects, both young and elderly, on each of the dimensions of a multidimensional space common to all subjects.

The ADAPT system [46,47] was used to generate molecular descriptors to relate to the multidimensional spaces achieved by INDSCAL. ADAPT is a computer software system which searches a molecular structure to determine if designated fragments and substructures are embedded within it. ADAPT also generates environment descriptors for substructures present within the molecular structure, as well as six variants for molecular connectivity and molecular volume. PREFMAP [3] and canonical correlation (see [5]) were used to determine the predictive value of the descriptors generated by ADAPT for describing the olfactory quality of the pyrazines.

RESULTS

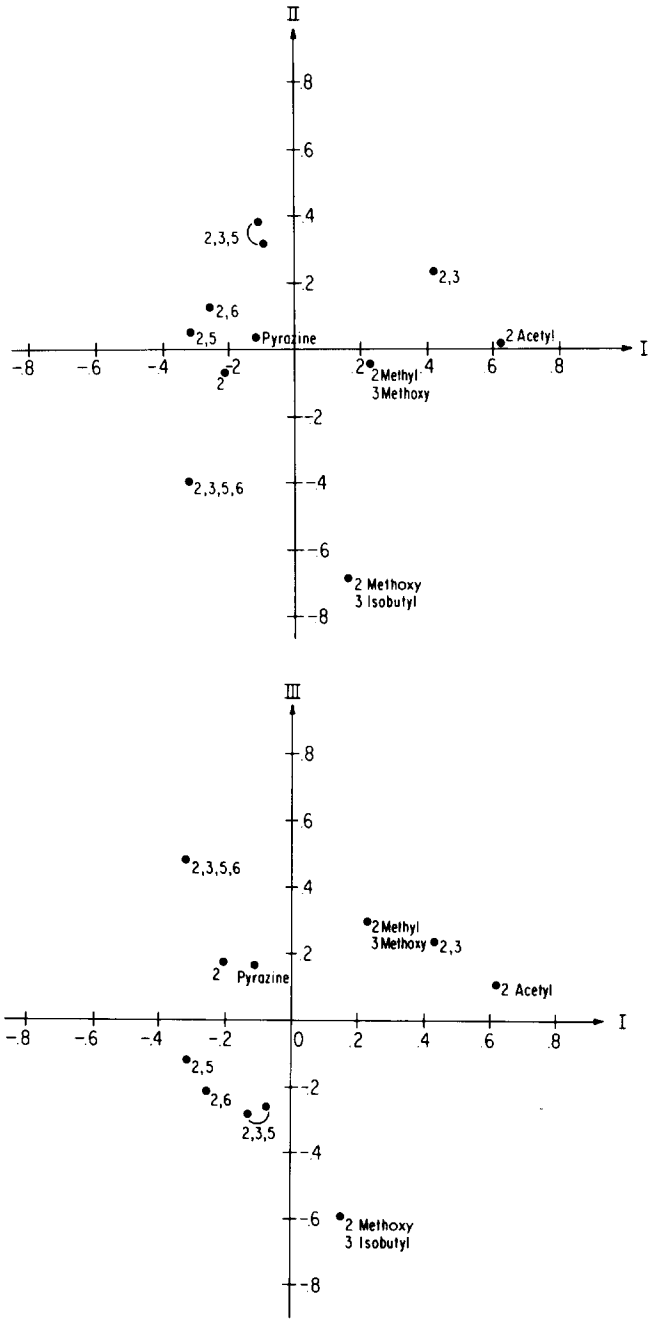
The INDSCAL procedure was applied to 23 similarity matrices (i.e., 12 young and 11 elderly) for the moderate concentrations, resulting in the common three-dimensional space shown in the cross-sections in Figs. 1a and 1b. The chemical structures associated with these stimuli are given in Figs. 2a and 2b. In the I vs II dimensional cross-section in Fig. 1a (also 2a), those pyrazines with the highest intrinsic polarity fall to the right in the arrangement. Thus, pyrazines with only a 2, 3-substitution pattern are found to the right as well as compounds with highly polar moieties (e.g., methoxy or acetyl). Unsubstituted pyrazines, as well as alkyl pyrazines with a single alkyl moiety or any substitution pattern wherein alkyl groups are geometrically opposite, tend to fall to the left. In the I vs III dimensional cross-section, Fig. 1b (also 2b), it can be seen that the third dimension basically separates 2, 3, 5, 6 tetramethyl pyrazine from 2 methoxy 3 isobutyl pyrazine with slight refinements in the arrangement of the other stimuli. The replicates, 2, 3, 5 trimethyl pyrazine, fall closer together than to any other stimuli.

The INDSCAL model yielded weight spaces which indicated the salience an individual subject placed on each of three dimensions of the stimulus space for moderate concentrations in Figs. 1a and 1b. The plot of these weights for dimensions I vs II are shown in Fig. 3. The weights indicate that six of the eleven elderly subjects weighted dimension II much more than dimension I. There were no differences between young and elderly subjects in their relative weightings of dimension III. The differential weighting of dimensions in Fig. 3 implies that for the majority of the elderly subjects, the arrangement in Fig. 1a is stretched out in an elliptical fashion along the second dimension according to the square root of the subject weights. Thus, they perceived 2 methoxy 3 isobutyl pyrazine as relatively more distant from the alkyl pyrazines than the remaining elderly and young subjects.

The INDSCAL procedure was also applied to the 23 similarity matrices for the strong concentrations. The derived space which was common for young and elderly subjects was virtually identical to that in Fig. 1. However, the weight spaces showed no differences between young and elderly subjects. This indicates that for strong concentrations of pyrazines the performance of young and elderly subjects in this similarity experiment was comparable.

The spatial arrangement in Fig. 1 was examined with regard to a number of reported physicochemical correlates of odor quality: the molecular weight, boiling point, molecular shape, water solubility, infrared spectra, u.v. absorption maxima, nuclear magnetic resonance shift value for ring protons and refractive index. For these measurements no strong predictive relationship for an individual variable was found. However, several molecular descriptors generated by ADAPT [46,47], including fragments, connectivities, as well as substructures with related environment values, when weighted mathematically were found to be helpful in providing some predictive relationship to the arrangement in Fig. 1.

The 30 descriptors generated for the 10 pyrazines by ADAPT are given in Table 3. The substructures associated with descriptors 15–30 are given in Fig. 4. These 30 descriptors plus an additional descriptor, the concentrations of pyrazines used at moderate intensity, were correlated with one another using a Pearson product moment correlation



FIGS. 1a and 1b. The I vs II and I vs III cross-sections of the three-dimensional space achieved by INDSCAL. It is based on the similarity judgments for all subjects, both young and elderly, for pyrazines at moderate intensity.

coefficient. The correlation matrix was analyzed by MINISSA [10,17], a nonmetric multidimensional scaling procedure which can be applied to a single matrix of similarity judgments. The resultant arrangement of descriptors in two dimensions is given in Fig. 5 to illustrate the similarity of descriptors pictorially.

It can be seen in Fig. 5 that the molecular connectivity,

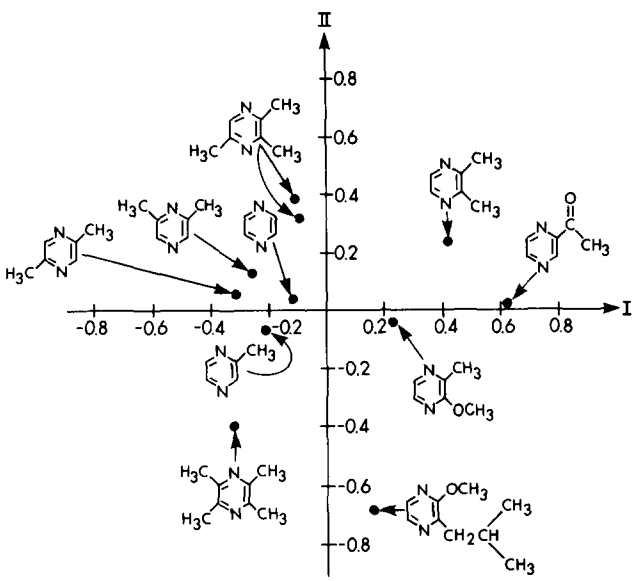
TABLE 3

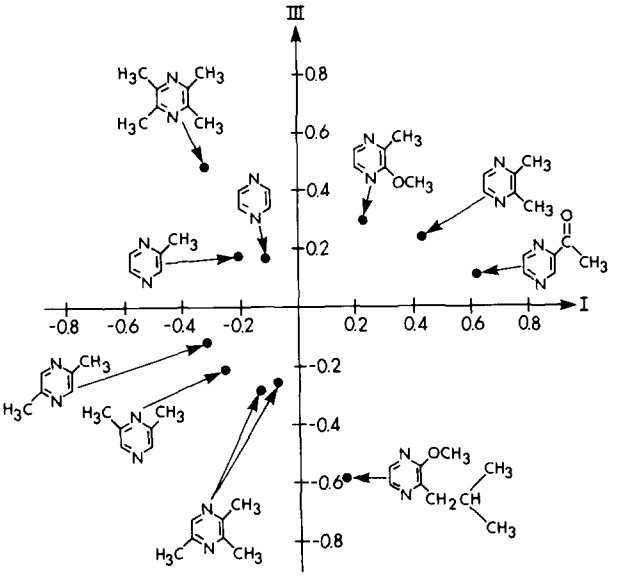
DESCRIPTORS OF THE PYRAZINES GENERATED BY ADAPT [46,47]

		Abbreviation
1	Number of atoms except hydrogen	No. of Atoms
2	Number of carbon atoms	Carbon
3	Number of oxygen atoms	Oxygen
4	Number of bonds	No. of Bonds
5	Number of single bonds	No. of Single Bonds
6	Number of double bonds	No. of Double Bonds
7	Molecular weight	MW
8	Path 1 molecular connectivity for all bonds in the structure	MC(1a)
9	Path 1 molecular connectivity corrected for rings	MC(1b)
10	Path 1 molecular connectivity calculated using the valences of heteroatoms and corrected for rings	MC(1c)
11	Path 2 molecular connectivity	MC(2)
12	Path 3 molecular connectivity	MC(3)
13	Path 4 molecular connectivity	MC(4)
14	Molecular volume	MV
15	Number of substructure 1 (see Fig. 4)	SSS(1)
16	Environment-substructure 1 (calculates connectivity for substructure 1 and nearest neighbors)	ENVR(1)
17	Number of substructure 2	SSS(2)
18	Environment-substructure 2	ENVR(2)
19	Number of substructure 3	SSS(3)
20	Environment-substructure 3	ENVR(3)
21	Number of substructure 4	SSS(4)
22	Environment-substructure 4	ENVR(4)
23	Number of substructure 5	SSS(5)
24	Environment-substructure 5	ENVR(5)
25	Number of substructure 6	SSS(6)
26	Environment-substructure 6	ENVR(6)
27	Number of substructure 7	SSS(7)
28	Environment-substructure 7	ENVR(7)
29	Number of substructure 8	SSS(8)
30	Environment-substructure 8	ENVR(8)
31	Concentration	CONC

volume, and weight parameters are highly related to one another for this set of compounds. Molecular connectivity is essentially a branching index which was originally developed by Randic [26] to characterize the amount of branching in hydrocarbon molecules. Its use has been extended by Kier and coworkers (see [14]) to studies of structure-activity relations. The substructures and their associated environments (the connectivity for the substructures plus their first nearest neighbors (x)) are correlated with one another but, in general, are less correlated with the group molecular connectivity, volume, and weight parameters.

The PREFMAP procedure [3] was used here to attempt to relate the chemical descriptors in Table 3 to the multidimensional arrangement of stimuli derived by INDSCAL given in Figs. 1a and 1b. The PREFMAP procedure has a hierarchy





FIGS. 2a and 2b. Chemical structures associated with the arrangements in Figs. 1a and 1b.

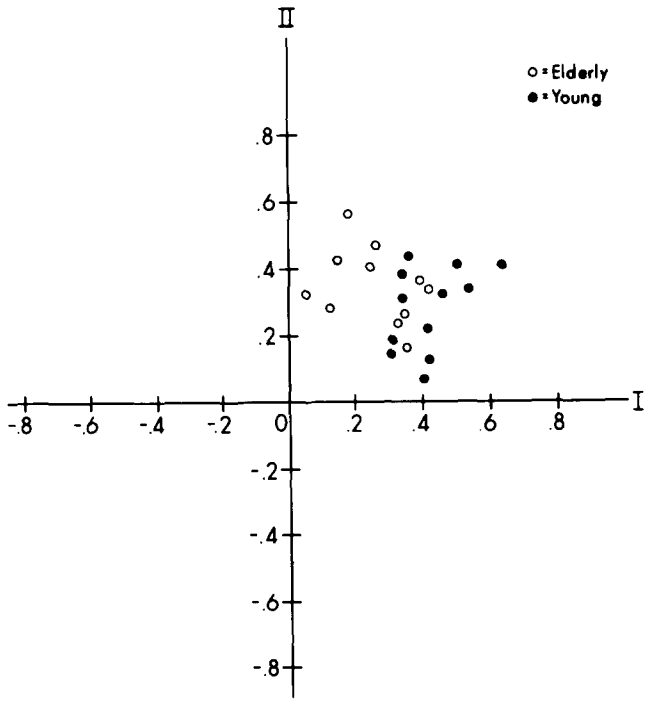


FIG. 3. The weight space associated with Fig. 1a which indicates the salience an individual subject placed on dimensions I and II. Six of the elderly subjects weighted dimension II considerably more than dimension I.

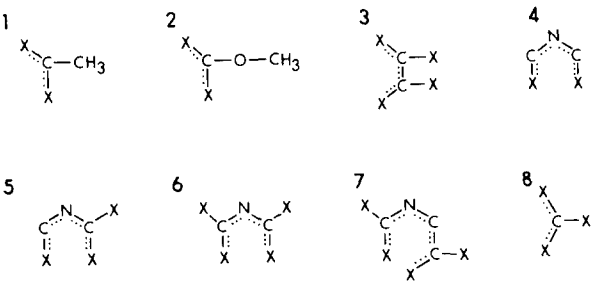


FIG. 4. The substructures utilized by ADAPT [46,47] to generate environment descriptors.

of models, the simplest of which was employed in this case. This model, called "phase 4," is a vector model in which the chemical descriptors are represented as scale values along vectors in the multidimensional space. The direction cosines for each of the descriptors (in Table 3) in the three-dimensional arrangement (shown in Figs. 1a and 1b) are given in Table 4 along with the correlations between the scale values and actual values for the chemical descriptor; the F values are given as well. It can be seen that the correlations for only two descriptors, 3 (number of oxygen atoms) and 31 (concentration) are statistically significant.

Since individual vectors for single variables generated by ADAPT could not be found to span the three-dimensional space in Fig. 1, a multivariate technique called "canonical correlation" (see [5]) was used to investigate whether a *group* of these variables could be used to predict the arrangement. Canonical correlation is used here to investigate patterns of interdependency between two groups of variables; the first group contains the dependent or criterion variables, which are the coordinates of the stimulus points in Fig. 1, while the second group consists of the predictor or independent variables in Table 3. The procedure of canonical correlation finds a linear combination of variables (called a "canonical" variable) for each of the two groups such that the correlation between the two canonical variables has the

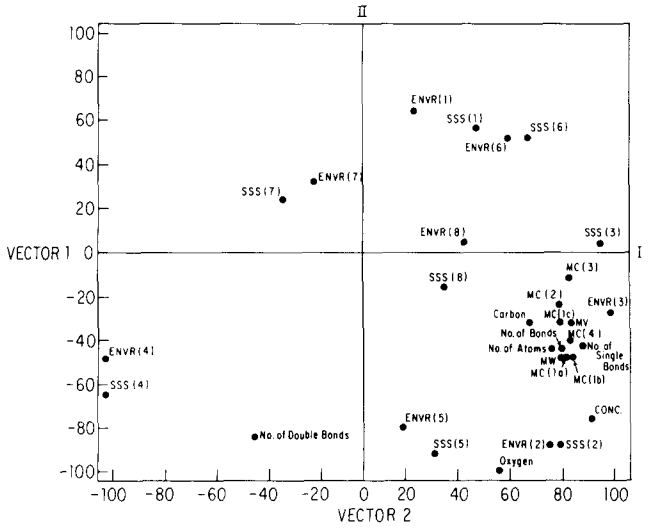


FIG. 5. Multidimensional arrangement achieved by MINISSA [10,17] of the descriptors generated by ADAPT [46,47]. Descriptors located near one another in the spatial arrangement are highly correlated with one another.

TABLE 4

DIRECTION COSINES OF FITTED DESCRIPTOR VECTORS

Descriptor				Correlation	F Value (3,6)
	1	2	3		
1	0.29	−0.85	−0.42	.78	3.04
2	0.04	−0.86	−0.49	.69	1.83
3	0.79	−0.58	−0.16	.85	5.22
4	0.29	−0.85	−0.42	.78	3.04
5	0.13	−0.87	−0.46	.76	2.72
6	0.99	0.00	0.13	.66	1.52
7	0.30	−0.85	−0.42	.78	3.19
8	0.22	−0.85	−0.46	.81	3.86
9	0.21	−0.86	−0.45	.80	3.54
10	0.08	−0.86	−0.49	.74	2.38
11	−0.04	−0.82	−0.55	.72	2.11
12	0.04	−0.98	−0.17	.50	0.67
13	0.17	−0.89	−0.39	.77	2.82
14	0.12	−0.87	−0.46	.73	2.24
15	−0.83	0.32	0.43	.61	1.16
16	−0.57	0.58	0.56	.62	1.27
17	0.42	−0.84	−0.33	.69	1.80
18	0.42	−0.84	−0.33	.69	1.80
19	0.06	−0.89	0.44	.43	0.44
20	0.51	−0.79	−0.32	.44	0.47
21	−0.19	0.52	0.82	.27	0.15
22	−0.01	0.53	0.84	.29	0.19
23	0.47	0.32	−0.82	.45	0.51
24	0.60	0.25	−0.75	.77	2.99
25	−0.79	−0.23	0.56	.60	1.09
26	−0.94	0.20	0.26	.53	0.78
27	−0.51	0.57	−0.63	.71	2.06
28	−0.40	0.64	−0.64	.83	4.53
29	0.75	−0.42	−0.49	.18	0.06
30	0.06	−0.92	0.38	.13	0.03
31	0.14	−0.73	−0.66	.96	25.03

TABLE 5

RESULTS OF THE APPLICATION OF CANONICAL CORRELATION

(a)

Canonical Variable	Canonical Correlation r_c	r_c^2	χ^2	df	Prob χ^2
1	0.98	0.96	25.82	9	0.002
2	0.82	0.67	7.52	4	0.111
3	0.48	0.23	1.42	1	0.233

(b)

CORRELATION COEFFICIENTS BETWEEN EACH CANONICAL VARIABLE OF THE STIMULUS SPACE COORDINATE SET WITH THE ORIGINAL VARIABLES OF THE SET

Variable	Original Coordinate I	Original Coordinate II	Original Coordinate III	Percent Trace
1	−0.35	0.77	0.58	0.35
2	0.69	−0.14	0.71	0.33
3	0.63	0.62	−0.38	0.32

(c)

CORRELATION COEFFICIENTS BETWEEN EACH CANONICAL VARIABLE OF THE DESCRIPTOR SET WITH THE ORIGINAL VARIABLES OF THE SET

	Descriptor			
	3	28	31	
Variable	Oxygen	ENVR(7)	CONC	Percent of of Trace
1	−0.72	0.30	−0.96	0.51
2	0.51	−0.76	−0.25	0.30
3	0.47	0.58	−0.11	0.19

(d)

REDUNDANCY COEFFICIENTS

Solution	Stimulus Space Coordinates	Descriptors
1	0.34	0.49
2	0.22	0.20
3	0.07	0.04
Total	0.63	0.73

TABLE 6

FREQUENCY OF ADJECTIVES GENERATED FOR STRONG CONCENTRATIONS

Pyrazine		2 methyl		2,3 dimethyl		2,5 dimethyl		2,6 dimethyl	
sweet	7	solvent	4	bitter	2	chemical, solvents	3	alcohol	4
bland, not offensive	2	meat	3	leafy, green	2	alcohol	2	fruity, citrus	4
chocolate	2	faint, weak	2	rubber	2	ammonia	2	aromatic	3
solvents	2	acidic	1	distinct	1	weak	2	clean, antiseptic	3
strong, penetrating	2	alcoholic	1	dry	1	acidic	1	ether	3
bug spray	1	ammonia	1	epoxy resin	1	antiseptic	1	neutral, bland	2
dirt	1	animal-like	1	faint	1	aromatic	1	chemical	1
flower	1	appetizing	1	gravel	1	distinct	1	flowery	1
glue	1	aromatic	1	in food	1	dust	1	nut-like food	1
nauseating	1	bitter	1	light	1	ether	1	sharp	1
non-edible	1	buttery	1	mice	1	formaldehyde	1	solvent	1
paint	1	cool	1	moldy	1	green	1	stale	1
pleasant	1	ether	1	mushrooms	1	light	1	strong	1
rich	1	food	1	mushy	1	moldy	1	sweet	1
sharp fragrance	1	moldy	1	non-living	1	musk	1	white	1
sour	1	onion/garlic	1	nutty	1	salty	1		
stale	1	resinous	1	old paper	1	sharp	1		
sticky	1	semi-sweet	1	organic	1	smelling salts	1		
thick	1	soft	1	plastic	1	sour	1		
		sweet	1	powdery	1	strong	1		
				rich	1	sulfur	1		
				shrimp	1	sweet	1		
				spicy	1	unpleasant	1		
				stale	1	yellow	1		
				strong	1				
				sweet	1				

2,3,5 trimethyl		2,3,5,6 tetramethyl		2 acetyl		2 methyl 3 methoxy		2 methoxy 3 isobutyl	
faint	3	sweet	5	decay, dirt, dirty sweat socks	3	popcorn	3	other vegetable	8
fruity	3	sour	3	burnt	2	peanuts	2	green pepper	5
alcohol	2	citrusy	2	greasy	2	sweet	2	fresh, clean	3
bitter	2	leafy, green	2	heavy, strong	2	banana	1	aromatic	2
moldy	2	rotten, rancid	2	mice	2	burnt	1	leafy, green	2
pungent	2	vanilla	2	nutty	2	cocoa beans	1	animal	1
acidic	1	acidic	1	popcorn	2	cool	1	heavy	1
celery seed	1	bitter	1	salty	2	epoxy resin	1	pungent	1
dry	1	downtown	1	brown	1	flowery	1	spicy pickle	1
dusty	1	edible	1	food	1	fresh	1	weak	1
ether	1	esters in butter	1	fried onions	1	fritos	1		
fresh	1	fattening	1	musty	1	heavy	1		
peppermint	1	flower-like	1	resinous	1	meat-like	1		
rotting	1	penetrating	1	semi-sweet	1	mice	1		
scotch	1	prunes, raisins	1	sharp	1	musty	1		
sharp	1	pungent	1	spicy	1	salty	1		
sour	1	strong	1			sharp	1		
spicy	1	thick	1			spoiled food	1		
stale food	1	unpleasant	1			weak	1		
		weak	1						

maximum possible value. In order to achieve this, weights are selected for the coordinates and chemical descriptors such that the correlation (called the "canonical correlation") is maximized. After finding the first set of canonical variables, subsequent sets which are uncorrelated with previous pairs are found. This process continues until the number of pairs of canonical variables equals the number of variables in the smaller group—in this case, three.

Numerous possible combinations of chemical descriptors which spanned the spaces in Fig. 5 were chosen for input to the canonical correlation procedure. Only one variable was chosen from a cluster of highly intercorrelated descriptors. The highest canonical correlations were found when only three descriptors were related to the coordinates in the three-dimensional space. The three descriptors were 3, 28, and 31 in Table 3 which also had the highest correlations when PREFMAP was utilized to find vectors through the stimulus space.

TABLE 7

FREQUENCY OF ADJECTIVES GENERATED FOR MODERATE CONCENTRATIONS

Pyrazine		2 methyl		2,3 dimethyl		2,5 dimethyl		2,6 dimethyl	
solvent	3	fruit	2	nuts	4	bland, neutral		alcohol	3
sweet	3	green peppers	2	sweet	3	flat	4	weak	3
aromatic	2	magnolia,		dusty	2	weak, faint	4	light	2
alcohol	1	flowery	2	mouse	2	alcohol-like,		sweet	2
anise	1	sweet	2	musky	2	astringent	2	acetone	1
bloody	1	acetone	1	weak	2	green vegetable-		aromatic	1
chocolate cake	1	acid	1	alcohol	1	like	2	dusty	1
clean	1	ammonia	1	ammonia	1	acetone	1	ether	1
deodorizer	1	aromatic	1	bird feces	1	aromatic	1	fresh	1
dirty	1	brown	1	biting	1	ether	1	fruity	1
food	1	chemical	1	brown	1	gasoline	1	mold-like	1
fresh	1	dark	1	chocolate	1	lightly salty	1	not irritating	1
heavy	1	flat	1	dry	1	mold-like	1	not like food	1
meaty	1	formaldehyde	1	harsh	1	nutty	1	sharp	1
mild	1	harsh	1	oil	1	rubber, plastic	1	slightly vinegary	1
putrid	1	heavy	1	popcorn	1	sour	1	sour	1
raw	1	musk	1	resinous	1	sterilized	1	stale	1
red	1	not sweet	1	shrimp	1	vinegary	1		
refreshing	1	pungent	1			woody	1		
semi-sweet	1	smokey	1						
spicy	1	sour	1						
vanilla	1	vegetable	1						
		weak	1						

2,3,5 trimethyl		2,3,5,6 tetramethyl		2 acetyl		2 methyl 3 methoxy		2 methoxy 3 isobutyl	
ammonia-like	3	sweet	5	rodents	3	bland, mild, weak		other vege-	
flat, neutral	2	weak, faint	3	popcorn	2	tasteless	6	table	7
sulphurous	2	flat, neutral	2	sweet	2	sweet	5	green pepper,	
weak	2	airy	1	warm	2	baked product	2	bell pepper	6
alcoholic	1	almost irritant	1	ammonia	1	food	2	rotten, spoiled	2
bitter	1	cooked vege-		bitter	1	vegetable	2	acidy	1
brown	1	table	1	bitter-		alcohol	1	alcohol	1
cocoa beans	1	freshly cut		chocolate	1	bitter	1	ammonia-like	1
damp	1	grass	1	brown	1	grain	1	bitter	1
dry	1	mild, flower-		dirt	1	lemon	1	flat	1
moss	1	like	1	epoxy resin	1	moldy orange	1	pleasant	1
moldy	1	moldy	1	fritos	1	musky	1	red	1
mushrooms	1	old, musty,		green pepper	1	pecans	1	rubber	1
mushy	1	dusty prunes	1	heavy	1	pepper	1	sour	1
not clean or		paint thinner	1	meaty	1	popcorn	1	sweet	1
fresh	1	pleasing	1	mild	1	spices	1	weak	1
salty	1	pungent	1	musk	1	vanilla	1		
slightly sour	1	rich	1	nutty	1	wisteria	1		
sweat	1	sour	1	pungent	1				
thick	1	slightly fruity	1	resinous	1				
vanilla	1	vanilla	1	rubbery	1				
warm	1	vinegar	1	salty	1				
weak benzene		wheat-like	1	sour	1				
solution	1			spoiled vege-					
				table	1				
				wood-like	1				

The first pair of canonical correlates were strongly correlated, i.e., $r_{c1}=0.98$, as can be seen in Table 5(a). The χ^2 test indicates that the first solution is statistically significant beyond the 0.01 level. However, the second and third solutions could have arisen by chance.

Weights were found for both the stimulus space coordinates and the descriptors which were used to calculate the three pairs of canonical variates from the original variables. The original variables were correlated with the canonical variates and these results are reported in Table 5(b) and 5(c). It can be seen in Table 5(c) that the correlations of descriptors 3, 28, and 31 with the first canonical variate are −0.72, +0.30, and −0.96, respectively. Several other calculations are reported as well. Percent of trace indicates roughly the proportion of a set's variance associated with each solution; thus 35% of the stimulus space trace and 51% of the descriptor trace are achieved by their respective variates in the first solution. The percent of trace is calculated by squaring the correlation coefficients for a solution. Redundancy coefficients, shown in Table 5(d), are calculated by multiplying the percent of trace times the squared canonical correlation. Thus, 34% of the stimulus space set trace is accounted for by the first descriptor set variate; and 49% of the descriptor set trace is accounted for by the first stimulus space variate. Over the three solutions, 63% of the stimulus space set trace is accounted for by the three descriptors.

The results for the generation of adjective descriptors for 12 young subjects are given in Tables 6 and 7 for the moderate and strong intensity pyrazines, respectively. The adjective labels generated, as well as the number of subjects who used the label. indicate wide disagreement among subjects for verbal descriptors, although their individual similarity spaces, which are not based on words, reveal more agreement.

The verbal descriptors generated by the elderly subjects are not shown. The elderly subjects on the average generated only 20% of the descriptors given by the young subjects at both levels of concentration. Numerous aged subjects were unable to generate verbal descriptors at all. However, the descriptors which were generated by the elderly were just as variable as those provided by young subjects. There were no statistically significant differences among the hedonic ratings of the stimuli for young or elderly subjects.

DISCUSSION

Relationship of Present Data to Previous Studies

The three-dimensional space derived from the application of INDSCAL to these data point to the importance of several physicochemical variables which relate to the olfactory quality of the pyrazines tested here. First, a simple examination of the space in Fig. 1 reveals that those pyrazines with the highest intrinsic polarity had different odors from unsubstituted pyrazine and alkyl pyrazines with a single alkyl moiety or any substitution pattern wherein alkyl groups are geometrically opposite. Use of PREFMAP and the pattern recognition procedure ADAPT in conjunction with a canonical correlation technique confirmed these observations in that they found the presence of oxygen atoms, the environment associated with substructure 7, and the concentration to be mathematically related to the multidimensional arrangement. No single variable could be found, however, which corresponded to each of the three dimensions of the configuration in Fig. 1.

The importance of concentration as a variable is not unexpected since previous studies have pointed to a relationship between pyrazine odor thresholds and molecular structure (see Tables 8, 9, and 10 as examples; from [2, 7, 9]). Extreme care must be taken in comparing odor threshold data from different groups of workers since as much as 10^3 concentration differences in thresholds have been reported for some pyrazines among different workers. With this caution in mind, the following generalities appear to hold:

1. Pyrazine and 2 methyl pyrazine have higher thresholds than other substituted pyrazines.
2. Incorporation of a highly polar group (e.g., methoxy) tends to lower odor threshold values.
3. Incorporation of a bulky alkyl side chain tends to decrease odor thresholds.

These observations on thresholds are consistent, in general, with the concentrations of pyrazines used here at both moderate and strong intensities.

As may be emphasized, the introduction of the powerful electron inductive group (−ÖR) as an osmophoric group has a powerful influence both on relative threshold perception as well as olfactory quality. We know that, in the case of the pyrazines as a class, the influence of powerful electron donating or withdrawing groups provides a system in which the electron density at one of the nitrogens in the pyrazine ring becomes sufficiently different from the other ring nitrogen so as to cause a marked distortion in the shape of the nitrogen lone pair orbitals relative to each other. This is indicated in the case of reported acetyl pyrazines by the NMR shift of hydrogens adjacent to such nitrogens [19,27] as shown in Fig. 6.

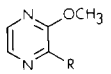
TABLE 8

ODOR THRESHOLDS OF MONOALKYL PYRAZINES

R	Odor Threshold (PPB in water)
Methyl	60,000
Ethyl	6,000
Isobutyl	400

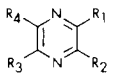
TABLE 9

ODOR THRESHOLDS OF 2-METHOXY-3-ALKYL PYRAZINES



R	Odor Threshold (PBB in water)
Methyl	4
Ethyl	0.4
Propyl	0.006
i-Propyl	0.002
i-Butyl	0.002
Hexyl	0.001

TABLE 10
ODOR THRESHOLDS IN POLYAKYL PYRAZINES



R_1	R_2	R_3	R_4	Odor Threshold (PPB in water) (Calabretta [2])	(Guadagani *et al.* [9])
H	H	H	H	500,000	175,000
Methyl	H	H	H	100,000	60,000
Methyl	Methyl	Methyl	Methyl	1,000	—
Methyl	Methyl	Methyl	H	400	—
Methyl	Methyl	H	H	400	2,500
Methyl	H	Methyl	H	—	1,800
Methyl	H	H	Methyl	—	1,500

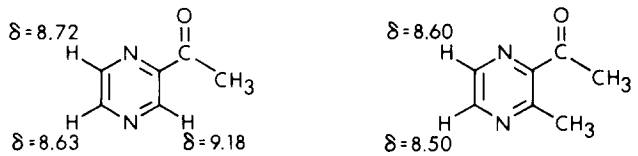


FIG. 6. NMR shift of hydrogens.

Thus, in addition to geometric distortion which provides an orientation factor at the olfactory receptor site, we also have a shift in electron density within a system which is *biologically and chemically ideal for disrupting hydrogen bonding in a coiled protein receptor site.*

In 1974, one of us presented a hypothetical model for odorant message transfer across a highly organized bimolecular leaflet lipoprotein membrane in which odorant interaction with a helical protein ion pump spanning the biomembrane causes conformational deformation due to disruption of intramolecular hydrogen bonds. This conformational alteration of the protein structure composing the bimolecular transmembrane channel by complexing via hydrogen bonding could thus alter the rate and pulsation order of the "ion pump" resulting in a change of the transmembrane code and electrical potential. The new code and pulsation order of the modified conformation was postulated as resulting in the specific odorant message [16]. The model recently postulated by Getchell *et al.* [8] is in general accord with this hypothesis.

Implication of Subject Weights for Age-Related Changes in Perception

Examination of (1) the subject weights for the I vs II dimensional cross-section of the spatial arrangement for moderate intensities, as well as (2) the raw similarity data, suggests a loss in olfactory acuity for the six elderly subjects who weighted dimension II more than I. These subjects tended to consider 2 methoxy 3 isobutyl pyrazine as relatively more dissimilar from the other pyrazines when compared with the remaining subjects. Schiffman *et al.* [40] have proposed a model for sensory discrimination which suggests that a critical difference in neural patterning is required for discrimination between two compounds. A difference in neural patterns would be expected to decline with the loss of olfactory neurons during aging; thus there would be a concomitant loss of discrimination ability in the elderly.

In the present case, it would appear that only a very few receptors, and thus neurons, are required to perceive a substance such as 2 methoxy 3 isobutyl pyrazine because of its low threshold; thus age-related neural loss would be less likely to affect its recognition than substances with higher thresholds which may require more neurons for their perception. For this reason, the difference in neural patterning between 2 methoxy 3 isobutyl pyrazine and other stimuli may be more readily perceived by the elderly than differences among pyrazines with higher thresholds which may require more neural mass. This explanation is consistent with the fact that both young and elderly showed equal abilities to discriminate the pyrazines at the strong concentrations. In this case, enough neurons were activated so that the differences in neural patterns were detectable by both groups of subjects in spite of potential neural loss suffered by the elderly.

Multidimensional Scaling (MDS) as an Aid for Finding Physicochemical Dimensions

Before analytical chemists working in flavor laboratories discovered that the class of pyrazines was widespread in natural products and potent odorants, there was little to suggest that these dinitrogen analogs of the benzene series should possess such an astounding array of odor characteristics in conjunction with remarkably low odor threshold values. This fact, however, is not unique just to the class of pyrazines. To date, investigators have not yet developed any accurate theoretical technique to predict, outside a narrow range of molecular structure, what odor, taste or other biological function will be induced by specific molecular structure. Thus, fundamental research in areas of drug design, as well as the chemical design of fragrance and flavor molecules, is still limited to minor variations around a known

natural product of potency or a molecular structure of chance discovery.

Even with the vast literature reviews [2, 15, 18, 44] on pyrazines as flavorants, a search has indicated considerable discrepancy among flavor scientists in using verbal flavor descriptors to define the specific odor character of well-defined pyrazines. The vagueness of verbal descriptors and discrepancies in uniform odor descriptors in different groups of professional flavorists often makes it difficult for one to more than generally "group" a flavor chemical even though its published flavor characteristics have been described. The practical implications of the inability of "words" to describe odors is immense (and costly). The wide variability of descriptors used by subjects in this study illustrates this point.

In the case of molecular drug design, quantitative mechanisms for efficacy and potency measurements have been developed and adopted. Unfortunately, the degree of sophistication and uniform techniques in the field of flavor and fragrance chemical evaluation leaves much to be desired. MDS, however, provides a quantitative tool from which a considerably more accurate data base can be developed. As in the case of drug design based on physicochemical relationships to drug efficacy and potency, progress in design for odorants cannot be made until workers in the field agree on methods of measurements relative to a standard. We now propose that MDS should be the method. The method presented both here and previously provides a system for developing quantitative olfactory data. While controversy may arise about conclusions based on MDS data as they relate to physicochemical dimensions, the MDS technique itself provides a standard method in an area that previously has had no standards.

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