ISOLATION AND IDENTIFICATION OF SOME VOLATILE CONSTITUENTS OF ROASTED PEANUTS

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Basic flavor research contributes significantly to man's understanding of flavor and his ability to measure and control this biological property of foods. Benefits obtained directly from basic flavor research include: facilitation in quality control of foods and food products, aid in the identification of the flavor precursors, complementation of the biologist's investigation of olfaction and taste and of the mechanism of action of odor and taste receptors, and control of "off-flavors."

Foods share to a large extent a number of the same volatile constituents. Lea (1) observed,

> Often there seems to be a rather disconcerting superficial resemblance between the mixture of volatile carbonyls, sulphides and alcohols recovered from food products of the most varied types, and one finds volatile carbonyl content, for example, being used as a measure of desirable flavor production in one product and of "off" flavor development in another.

Casey et al. (2) suggested that part of the difference in the aroma of certain foods is dependent on the observed relative quantative variations in the low boiling compounds.

Volatile constituents present in flavors consist of a wide array of compounds, each contributing to the overall

flavor in varying degrees. Jennings and Sevenants (3) divided flavor components into two classes: "character-impact compounds" and "contributory flavor compounds." The former are unique to a particular flavor, while the latter contribute to the "fruiteness," "bouquet," or "fullness." A number of character-impact compounds have been identified with certain flavors, for example; phthalides in celery flavor by Gold and Wilson (4), trans:2-cis:4-decadienate esters in pear flavor by Jennings and Creveling (5), and trans-non-2-enal in cucumber flavor by Forss et al. (6). On the other hand there exists the philosophy that, in some foods, characteristic flavor may not be due to one or two compounds but instead an integrated response to a wide spectrum of compounds whose individual aromas are not similar to the typical flavor. Peach flavor, for example, may be included in the latter classification (7).

The term "taste" is sometimes confused with flavor when referring to the sensory quality of food. Taste is described as that sensation produced when food is taken into the mouth and stimulates the receptors of the taste buds (8). Whereas flavor, as defined by Beidler (9), helps clarify the matter

> Flavor is the sensation derived when a material which is placed in the mouth stimulates the temperature, pain, tactile, and taste receptors of the tongue, and the odors released during mastication find their way to the olfactory region of the nose to produce various aromas.

Also Moncrieff (10) defined flavor:

> Flavor is a complex sensation. It comprises taste, odor, roughness or smoothness, hotness or coldness, and pungency or blandness. The factor which has the greatest influence is odor. If the odor is lacking then the food loses its flavor and becomes chiefly bitter, sweet, sour, or saline.

Thus, odor or aroma was implicated as the predominate but not the single factor of flavor. The terms odor, volatile constituents, and aroma have been used synonymously when referring to sensory evaluation of food, but are not synonymous with flavor. Since the study reported in this thesis is concerned with the volatile constituents of roasted peanuts, the term "aroma" or "volatile constituents" are used, rather than "flavor" when referring to the reported constituents.

Two general categories of flavor production are: those produced enzymatically and those produced non-enzymatically, the latter includes pyrolytic reactions. Roasted peanut flavor, produced by a pyrolytic process, fits into the latter category.

At the time of this study relatively little was known of the identities of the volatile constituents of roasted peanuts. The purpose of the study reported in this thesis was to achieve isolation, separation, and identification of the volatile constituents of roasted peanuts. The identification of a number of these constituents is presented.

After several methods were attempted to achieve isolation of the volatile constituents, the common technique of vacuum degassing was employed with some modifications (11).

The procedures for separation and collection of the individual components involved use of preparative gas chromatography and cryogenic trapping at low pressure. Identifications of the collected fractions were made by a combination of physical measurements including ultraviolet-, nuclear magnetic resonance- and mass spectrometry.

The amount of the most abundant individual aroma constituents from a two pound batch of roasted peanuts was estimated to be about 1 μl. Classical "wet chemical" methods of identification would have required processing of several hundred pounds of peanuts. Since the facilities for processing this amount of material were not available, the use of sensitive instrumentation was the only alternative means to identify the volatile constituents of roasted peanuts.

The constituents identified as a result of this study included a number of carbonyls (Chapter 3), a series of alkylated pyrazines, a pyrrole, and a few miscellaneous compounds (Chapter 4).

CHAPTER II

LITERATURE REVIEW

Identification of Volatiles in Roasted Peanut Flavor

The literature pertaining to roasted peanut flavor is noticeably lacking, being confined to only a few reports. The earliest work reported (1952) was that of Pickett and Holley (12) and a review (1953) by Hoffpauir (13). Findings of Pickett and Holley implicated sugars (sucrose principally) and free amino acids as participating in peanut roasting reactions. They were able to characterize a number of volatile constituents of roasted peanuts. These included: relatively large amounts of carbon dioxide, aldehydes, sulfur compounds, ammonia, and 2,3-butanedione. Hoffpauir noted that most of the volatile constituents of roasted peanuts identified at that time had also been found in roasted coffee volatiles.

More recently Mason (14) initiated work to reveal the chemical identities of the components of roasted peanut flavor and its precursors. 2,5-Dimethylpyrazine and benzaldehyde were identified as major components of a steam distillate of roasted peanuts. Also tentatively identified were acetylene, hydrogen cyanide, propiolonitrite,

tetrahydrofuran, methyl pyrrole, methane thiol, butanal, allyl ethyl ether, and 2-methylpropanol. Both steam distillation and degassing techniques were employed to obtain the flavor condensate. Three fractions were obtained from the condensate by bulb-to-bulb distillations (11) at room temperature, -80° C. and -196° C. Preparative gas chromatography and mass spectrometry made possible the identification of the reported constituents.

Studies of Young and Holley (15) on peanuts in storage and roasted peanuts interrelated a number of components of the peanut such as reducing sugars, total carbonyls, total nitrogen, and carbonyls as diacetyl. One conclusion was that total volatiles from roasting tended to increase as quality declined; the increase of volatiles being usually more evident in the total carbonyl fraction. Pattee et al. (16) recently separated twenty-one volatile components of high temperature-cured off-flavor peanuts. Eleven of these were identified, nine of which were carbonyls.

Flavors Produced by Pyrolytic Processes

In addition to being found in peanuts (14, Chapter 4), pyrazine derivatives are known to be present in two other pyrolytically processed foods, namely potato chips and coffee. Staudinger and Reichstein (17) reported pyrazine and N-methylpyrrole in coffee volatiles in 1939. A recent review by Reymond et al. (18) reports that coffee aroma contains four different pyrazines and N-methylpyrrole.

Deck and Chang (19) reported the identification of 2,5-dimethylpyrazine in potato chip volatiles.

Other than these three, only one other food is known to contain pyrazine derivatives: Kosuge et al. (20) identified tetramethylpyrazine produced enzymatically from "Natto," a food prepared from fermented soybeans. Later they isolated it from the fermenting organism Bacillus natto (21).

Volatile carbonyls were some of the first compounds to be identified from roasted peanuts (12). Carbonyl compounds occupy an important role in the flavor of a number of pyrolytically processed foods such as: potato chips, Dornsiefer and Powers (22); cooked lamb, Jacobson and Koehler (23); coffee, Reymond et al. (18); cocoa, Reymond et al. (18), Boyd et al. (24); cooked chicken, Pippen and Nonaka (25); "chickeney aroma," Minor et al. (26); and cooked potatoes, Self (27,28).

Isolation and Separation Techniques

Many techniques have been employed for removing the volatile flavor constituents from the substance being investigated. The majority of these can be classified into one of three general methods; head space analysis, steam distillation, or vacuum degassing (or film evaporation).

Steam distillation as employed by Dornseifer and Powers (22) was avoided for fear of the possible side

reactions caused by introducing steam into peanuts which represent a non-aqueous system.

Head space analysis, as introduced by Mackay et al. (29), modified by Bassette et al. (30) and reviewed by Jennings (31), was not successful when applied to peanuts because of the low volatility of the higher molecular weight constituents making identification of them impossible with this technique.

Vacuum degassing as proposed by Bazinet and Merritt (11) and later modified by Mason (14) was found best of the three methods of isolation of peanut flavor constituents. This method permitted isolation of the constituents with low vapor pressures and minimized the possibility of side reactions.

Gas liquid chromatography (GLC) is widely used to separate and identify flavor components by virtue of their retention on a column. Preparative gas chromatography which was employed in accomplishing the purposes of this investigation, involves the scaling up of the GLC system by use of longer columns and/or larger column diameters making possible analysis of larger samples. Thus the separation and isolation of the individual flavor components by trapping the effluent gas as each component came off the column was possible.

CHAPTER III

CARBONYL COMPOUNDS OF ROASTED PEANUTS

Apparatus

Roasting of the peanuts was performed in an electrically heated rotisserie fitted with a cylindrical wire basket designed specifically for this purpose. The basket was constructed of wire mesh and was approximately 6 inches in diameter and 15 inches long.

Homogenation of the peanuts was accomplished with a Sorvall Omnimixer in the quart jar attachment.

A vacuum degassing apparatus similar to the one used by Mason (14) was employed in the isolation of the volatile flavor components from roasted peanuts. This apparatus consisted of a Welch dual stage vacuum pump rated at 0.1 micron and 140 liters per minute, a three stage oil diffusion pump to obtain high vacuum, and a manifold with two small U-tube traps and a large cold finger trap. Degassing was accomplished by placing the sample in a 12 liter flask situated at the end of the manifold.

Relative retention times were obtained by gas chromatography on a Perkin-Elmer Model 800 gas chromatograph equipped with a dual hydrogen flame.

Preparative separations of the individual components were made on an F and M Model 500 gas chromatograph equipped with a four filament hotwire detector.

Mass spectral analyses were obtained with a Consolidated Electrodynamics Corporation (CEC) model 21-1036 mass spectrometer and a combination gas chromatogram-mass spectrometer (GC-MS). The latter instrument was a prototype of the LKB 9000 mass spectromter constructed in the laboratories of Dr. Ragnar Ryhage, Karolinska Institute, Stockholm, Sweden.

Reagents

Mineral oil. U.S.P., heavy-grade. Proctor and Gamble Co., Cincinnati, Ohio.

2,4-Dinitrophenylhydrazine. Reagent Grade, Eastman Kodak Company, Rochester, New York.

Methylene Chloride. N_D^{20} 1.4238, Aldrich Chemical Co., Milwaukee 10, Wisconsin, redistilled at 40°C.

α-Ketoglutaric acid. California Foundation for Biochemical Research, 3408 Fowler Street, Los Angeles, California.

Gas-Chrom Q. 100/120 mesh, Applied Science Laboratories, Inc., P. O. Box 40, State College, Pennsylvania.

Carbowax 20-M, Apiezon L, and Chromasorb W, 60/80 mesh. Wilkins Instrument and Research, Inc. Box 313, Walnut Creek, California.

Phenylacetaldehyde, benzaldehyde, acetaldehyde, isovaleraldehyde, 2-methylbutanal. Practical grade. K and K Laboratories, Plainview, New York.

Water. Distilled and de-ionized.

Procedures

Preparation of Condensate from Roasted Peanuts

Spanish peanuts of uniform quality were roasted in 2 1/2 pound batches and the testa and germ removed mechanically after roasting.

Two-pound batches of roasted peanut cotyledons were homogenized in glycerol and water as follows: 225 ml of glycerol and 25 ml of de-ionized water were added to 125 gm quantities of cotyledons and the mixture was homogenized approximately three minutes in a quart jar attachment on a Sorvall Omnimixer. The combined homogenates were placed in a 12-liter round bottomed flask attached to the vacuum manifold.

Two small U-traps and a large cold finger were situated in series between the diffusion pump and the sample, the small U-trap nearest the diffusion pump was positioned to prevent diffusion of silicones into the manifold. Volatiles were collected in the other two traps located between the manifold and diffusion pump. All three were cooled with liquid nitrogen.

The 12-liter flask containing the homogenized peanuts was degassed at room temperature under a vacuum of

approximately 10^{-4} mm Hg until the foaming ceased. The temperature was slowly raised over an 8 hour period to 60°C, at which time degassing was discontinued. The condensate in the large cold finger trap, mostly water with volatile aroma compounds entrained, was thawed and placed in a stoppered vessel. The small U-trap collected only traces of condensate and required only occasional emptying.

Extraction of Flavor Components

Preparative gas chromatography was used to separate the condensate into individual components. Since water behaves anomalously on most gas chromatographic columns giving broad diffuse peaks, a water free extract was obtained by extracting the thawed condensate three times with 0.1 volumes of redistilled methylene chloride. Gas chromatographic analysis of the aqueous phase showed that three extractions were sufficient to remove all but traces of organic constituents from the aqueous condensate. Extracts were combined, reduced to about 1 ml volume on a rotary evaporator and 200 μl quantities were separated by preparative gas chromatography.

Preparation of 2,4-Dinitrophenylhydrazone (2,4-DNPH) Derivatives

Ten volumes of a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl were added to the aqueous condensate freshly removed from the liquid nitrogen trap.

The resulting suspension, contained in a stoppered Erlenmeyer flask, was heated in a water bath at 55 to 60°C for 6 hours to accelerate formation and flocculation of precipitate. The resulting precipitate was collected on a sintered glass funnel of medium porosity, washed a number of times with de-ionized water, and dried over anhydrous calcium sulfate in a vacuum desiccator.

Regeneration of Carbonyls From Their 2,4-DNPH Derivatives

Regeneration was performed as described by Ralls (32) with some modification of the regeneration tubes (33). For GC-MS analysis 6 to 40 mg of 2,4-DNPH derivatives were weighed and mixed thoroughly with three times their weight of α-ketoglutaric acid and transferred to regenerating tubes.

Gas Chromatography

Retention times were obtained on a Perkin-Elmer Model 800 gas chromatograph equipped with dual hydrogen flame detectors. A 20-foot x 1/8 inch o.d. (.062 inch i.d.) stainless steel column packed with 20% W./W. Apiezon L on Chromasorb W 60-80 mesh was used. Nitrogen was the carrier gas and flow rates of 35 to 40 ml per minute were used. Initial column temperature was set at 100°C and after two minutes the temperature was programmed at 6° per minute to 200°C.

Relative retention times were determined by setting the retention time of butanal (internal standard) equal to 1.00. Variations occasionally caused some question of the accuracy of the retention time data. This problem was eliminated by calculating relative retention values normalized also on the retention of benzaldehyde and phenylacetaldehyde. If the values obtained for these two carbonyls were not in agreement with those of a standard mixture of n-butanal, benzaldehyde, and phenylacetaldehyde within $\pm$ .05 unit, the sample was rechromatographed and values recalculated.

Preparative gas chromatography was performed on a 20 foot column packed with Apiezon L prepared the same as the 1/8 inch column previously mentioned except it was 1/4-inch (0.25-inch o.d., 0.16-inch i.d.). Collection of individual eluting components was accomplished with simple U-tubes (6 mm o.d.) constructed of borosilicate glass and containing a small steel wool plug to prevent aerosol formation as suggested by Teranishi *et al.* (34). The inlet of the traps was constructed of 3.8 mm (o.d.) glass tubing so that it could be inserted through silicone rubber septums on the exit port of the gas chromatograph and so that it would fit the 300^{o} glass inlet of the CEC-103C mass spectrometer. Ethanol-dry ice baths were used to cool the traps as the components were being collected.

Mass Spectrometry

Mass spectral data were obtained by inserting the U-tube containing the sample into the heated inlet valve of the CEC model 21-103C mass spectrometer. The union was sealed by means of an "O" ring backed by a 1/4-inch Swage-lock nut and an inverted rear ferrule. The nut was tightened onto corresponding threads at the terminus of the inlet valve until an air tight seal was obtained. The U-tube was then cooled in liquid nitrogen and evacuated by opening the inlet valve to the vacuum system of the mass spectrometer. The coolant was removed and the tube allowed to warm until approximately 50 microns of pressure was obtained in the three liter glass expansion chamber of the inlet system to the mass spectrometer. Finally, the expanded gaseous sample was allowed to enter the ion source of the mass spectrometer through a glass leak. A normal 70 ev spectrum was obtained and recorded as has been described (35).

Certain precautions were taken to prevent samples, loaded as described above, from being appreciably diluted with carbon dioxide and water from the atmosphere. These included sealing of the traps with silicone rubber plugs immediately after collecting samples from the exit port of the gas chromatograph, and removing the tubes from liquid nitrogen baths and allowing them to warm slightly so that

any carbon dioxide would bleed off before opening the trap inlet for attachment to the mass spectrometer.

Combination Gas Chromatography-Mass Spectrometry

Regenerated carbonyls were immediately taken from the hot tube with a gas-tight syringe and injected into the combination gas chromatogram-mass spectrometer (GC-MS) instrument to obtain separation of the gaseous components and mass spectra of each. The chromatographic column used was a 0.25 inch o.d. x 24 foot glass column packed with 5% Carbowax 20 M on Gas-Chrom Q W./W. Helium was the carrier gas at a rate of 35 ml per minute. Column temperatures are indicated elsewhere.

Determination of the homogeneity or heterogeneity of the major peaks was possible by using the GC-MS instrument equipped with a rapid scanning device. A mass spectrum could be obtained every 1.6 seconds if needed, thus allowing spectra to be taken at successive points along each peak.

Results and Discussion

Figure 1 shows the typical chromatogram of the aqueous condensate of roasted peanut volatiles obtained in the manner already described.

The methylene chloride extract of the condensate was chromatographed on the preparative column using a hot wire detector. The effluent gas of each peak was bubbled

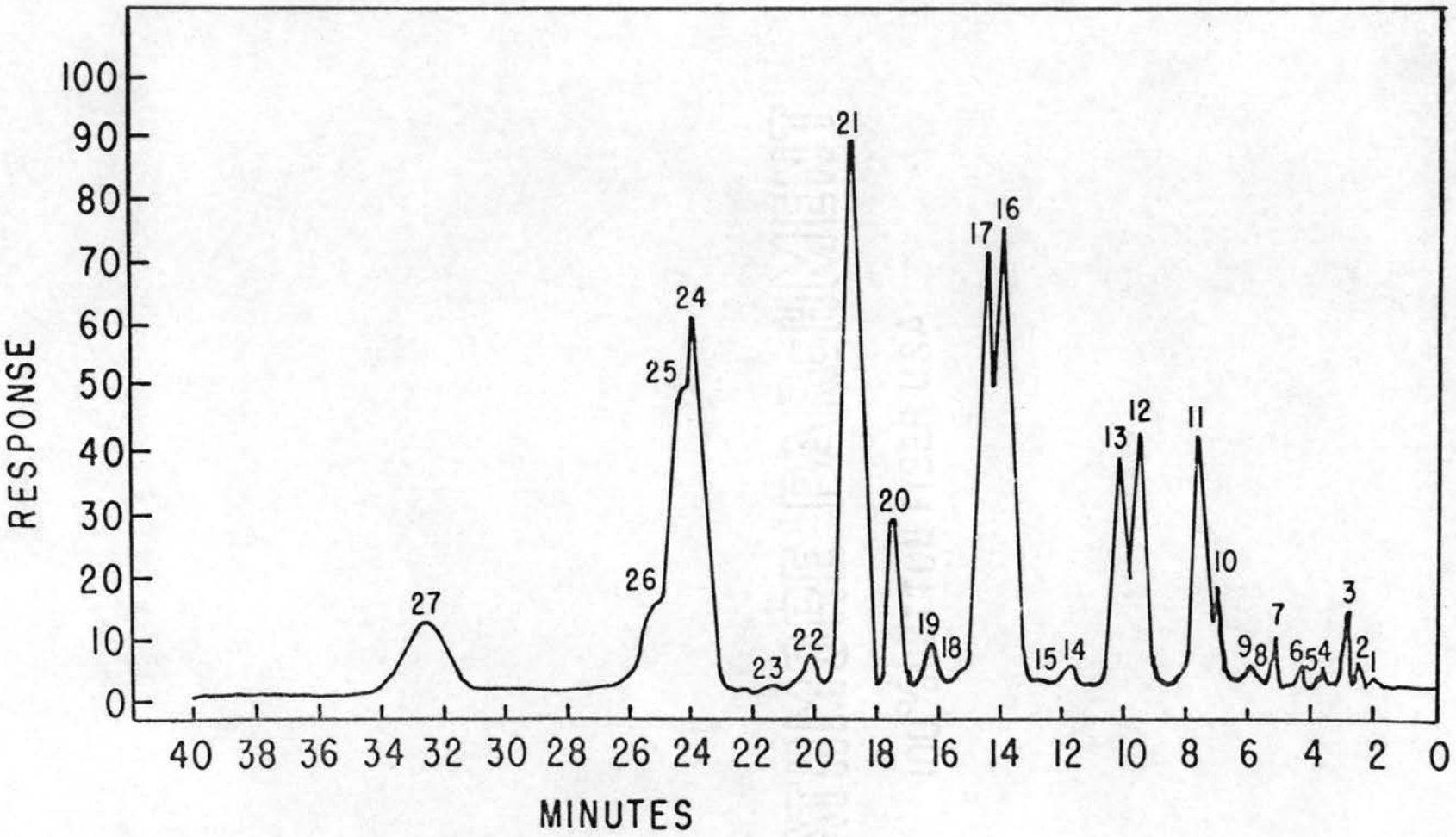

Figure 1. Gas Chromatogram of the Aqueous Condensate Removed from Roasted Peanuts by Vacuum Distillation

through a saturated solution of 2,4-dinitophenylhydrazone in 2N HCl which allowed the detection of the carbonyl components. Components labeled 2, 3, 4, 5, 18 and 21 formed precipitates. Components 2-5 possessed odors similar to aliphatic aldehydes while component 18 had a "cinnamon-like" odor. Component 21 had an odor reminiscent of "oil of roses."

Subsequently, relative retentions (retention of n-butanal = 1.00) of some of the common aliphatic aldehydes and of benzaldehyde (cinnamon-like) and phenylacetaldehyde (oil of roses) were determined. Component 3 corresponded to isobutyraldehyde which had an average relative retention (RR_t) of 0.86 whereas component 4 corresponded to 3-methylbutanal, (RR_t) of 1.37. Standard 2-methylbutanal was not available therefore the compound was made <u>in situ</u> from a heated mixture of isoleucine and ninhydrin in citrate buffer. A sample of the vapor above the reaction mixture was gas chromatogramed using headspace sampling techniques. The average RR_t obtained (RR_t = 1.41) was slightly higher than that of 3-methylbutanal but the same as that for component 5. Authentic benzaldehyde had an average RR_t of 5.56 and that of phenylacetaldehyde was 6.40. These corresponded to the average RR_t of components 18 and 21 respectively (Figure 1).

Collection of small quantities of these individual components in U-tube traps by preparative gas chromatography seemed most expedient, but the mechanics of sample collection

followed by mass spectrometry were troublesome. The presence of water prohibited preparative chromatography of large volumes of condensate. Methylene chloride extraction removed the water, but when large volumes (200 μl) of these extracts were chromatographed the solvent peak obliterated the peaks representing the low molecular weight aldehydes. The two aromatic aldehydes were collected in U-tube traps successfully, and a satisfactory mass spectrum was obtained for component 21 (phenylacetaldehyde). The spectrum of component 18 (benzaldehyde) contained enough background from bleeding column substrate and from overlapping adjacent peaks that only the major ion intensities agreed well with standard spectra. Spectra were obtained using a Consolidated Electrodynamics Corporation (CEC) model 121-103C mass spectrometer as described previously.

Access to a newly installed GC-MS instrument provided the solution of this problem. Monocarbonyl compounds were separated from other compounds present, including large amounts of nitrogen containing compounds (Chapter 4), by forming the 2,4-DNPH derivatives and regenerating the original monocarbonyl compounds as already described. Figure 2 shows a chromatogram from the GC-MS instrument obtained by injecting 250 μl of gas from above 9 mg of regenerated 2,4-DNPH derivatives; the column was operated at 70^{o}C. Three peaks other than air were obtained at the attenuations used and one of these, component 2, was very small. Thus, as determined by this procedure, components

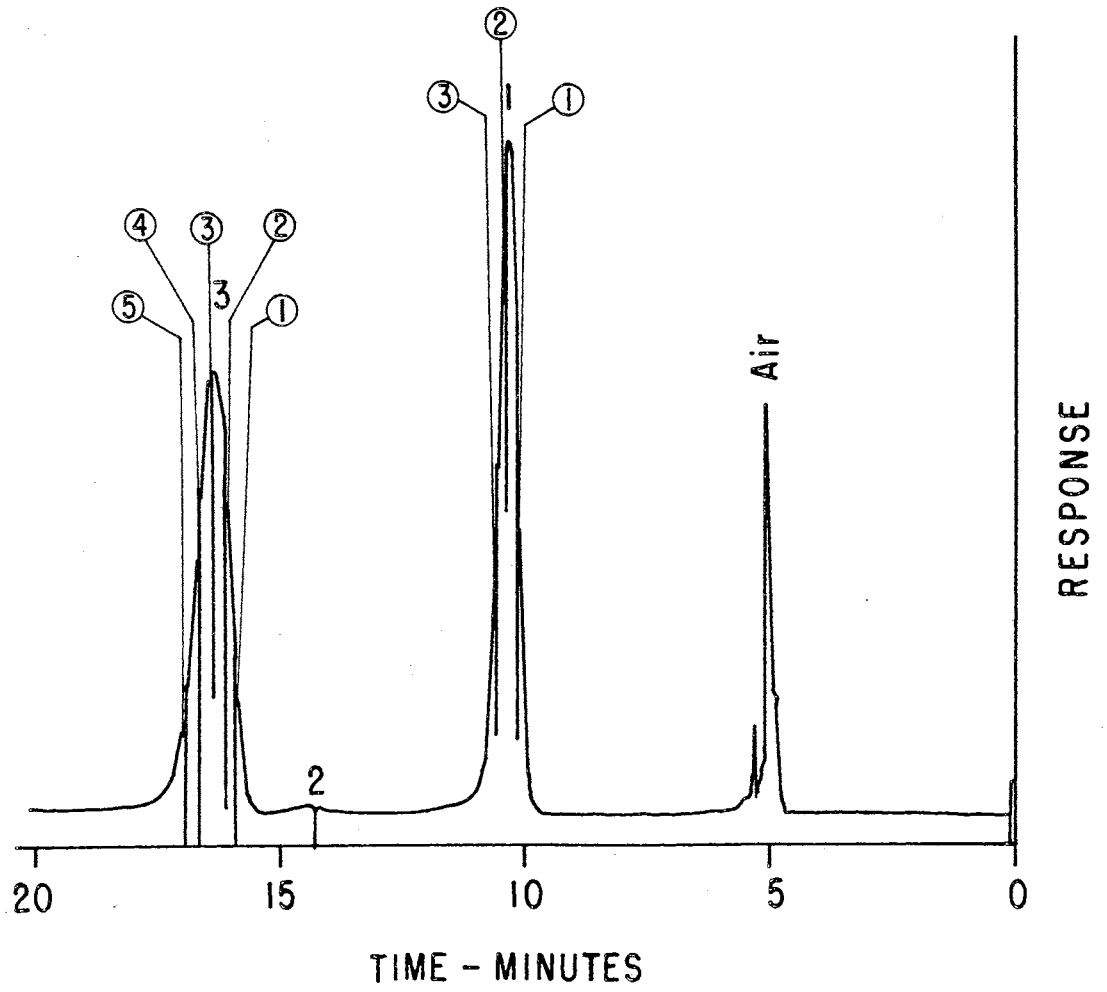

Figure 2. Gas Chromatogram from the GC-MS Instrument of 250 μl of Vapors from Regenerate of 9 mg of 2,4-DNPH Derivative

1 and 3 were clearly the major monocarbonyl compounds present. Complete spectra of these two major components taken at the apex of the peaks is shown in Table I; intensities of major peaks in successive spectra taken of each component as it eluted from the gas chromatograph are also included. Spectra of these components were obtained at points indicated by discontinuities on the curves marked ①, ②, ③, etc. in Figure 2. Spectra of standard compounds are included for reference.

Successive spectra showed that component 1 was homogeneous and the spectrum at the apex showed that it was isobutyraldehyde. Conversely, successive spectra revealed that component 3 was a mixture consisting mostly of 2-methylbutanal with some 3-methylbutanal in the trailing edge of the peak. This was noted by sharp increases in relative intensities of m/e values of 29, 41, 44, and 58 in spectra 4 and 5 of component 3 while other fragment intensities were nearly the same as in the first three spectra. In comparing the reference standard spectra for 2-methyl- and 3-methylbutanal, shown in Table I, the major differences in intensities of fragments, relative to a m/e of 57, were noted at m/e values of 29, 44, and 58. Thus, the increase of relative amounts of these fragments in spectra 4 and 5, particularly at m/e of 44, was probably due to the presence of some 3-methylbutanal.

The standards shown in Table I, with the exception of 2-methylbutanal, were obtained by analyzing commercially

TABLE I

MASS SPECTRA OF COMPONENTS FROM GLC SEPARATION OF 250-μl OF VAPORS FROM REGENERATE OF 9-MG. 2,4-DNPH DERIVATIVES

	Complete Spectra of Components and Standards					Significant Ions of Successive Spectra							
						Spectra of Component 1			Spectra of Component 3				
m/e	1	Isobutyr-aldehyde	3	2-me-butanal	3-me-butanal	1	2	3	1	2	3	4	5
15	1.5	4.4	1.5	1.5	5.4								
26	3.3	6.0	3.9	3.9	3.0								
27	39.6	48.5	29.2	26.9	35.5	39.6	38.8	39.5	23.4	29.2	31.5	37.3	33.9
28	11.3	19.5	13.0	8.9	1.1								
29	25.8	22.7	91.2	88.2	36.2	25.8	24.2	24.6	86.5	91.2	88.8	97.1	95.5
30			3.6	3.2	1.1								
31	1.0	1.8		1.5	1.5								
37	2.1	2.6											
38	3.3	5.5		1.9	4.4								
39	18.9	20.5	18.9	17.0	28.5	18.9	19.8	19.2					
40	2.5	4.1	3.3	2.2	5.8								
41	64.3	65.0	87.2	81.0	79.0	64.3	64.0	67.5	81.5	87.2	89.8	107.0	125.0
42	8.3	9.4	5.7	4.3	16.8								
43	100.0	100.0	15.7	10.0	73.5	100.0	100.0	100.0	15.9	15.7	20.5	36.5	
44	4.6	8.0	7.5	1.3	100.0				4.5	7.5	17.0	42.2	67.5
45	0.5	2.2	1.8	3.2	15.5								
50			1.6	1.5	2.4								
51			1.8	1.6	2.3								
53	1.0	1.4	3.9	3.0	5.2								
55	1.5	2.4	8.9	8.5	5.4								
56			8.3	8.8	3.0								
57	2.9	4.9	100.0	100.0	23.5	2.9	2.9	2.6	100.0	100.0	100.0	100.0	100.0
58	1.0	4.6	72.5	65.0	51.8				71.5	72.5	75.5	85.4	94.0
59			3.3	2.6	3.5								
61					1.6								
67					1.6								
68					2.0								
69					2.2								
70					1.4								
71	1.0	1.2	6.4	4.5	21.2								
72	52.8	42.6			1.9	52.8	49.2	48.8					
73	2.6	5.8			4.4								
74				4.3									
85					2.5								
86			11.1	15.3	6.7				11.0	11.1	10.8	12.2	13.4
87			0.85	3.1	0.77								

available compounds on the GC-MS instrument. The standard spectrum of 2-methylbutanal was from Dow Uncertified Spectra (36).

Later 40 mg of the 2,4-DNPH derivatives were regenerated and 5 ml of the vapors introduced onto the column attached to the GC-MS instrument in order to obtain good spectra of component 2 (Figure 2) and any other minor monocarbonyls present. The column temperature, initially at 50°C, was raised abruptly at several points during the chromatogram as shown in Figure 3. The chromatogram obtained (Figure 3) shows the number of components separated, marked by arabic numbers and the number of spectra taken on each peak (discontinuities). Again each discontinuity on the curves, except for one point where an attenuation was made, marks the points at which successive spectra were taken.

Components 2 and 4 of Figure 3 were found to be the same as 1 and 3 of Figure 2. Spectra of components 1, 3, 7, 9, and 10 compared well with reference standards (Table II). Agreement was considered sufficient for identification in all cases with the possible exception of component 9. In this case the disagreement of intensities of the low mass fragment ions with that of the reference standard (Dow Uncertified Spectrum) was explained by column bleed. Intense fragment ions in the background caused considerable trouble in interpretation when the amount of component being analyzed was small (note small size of

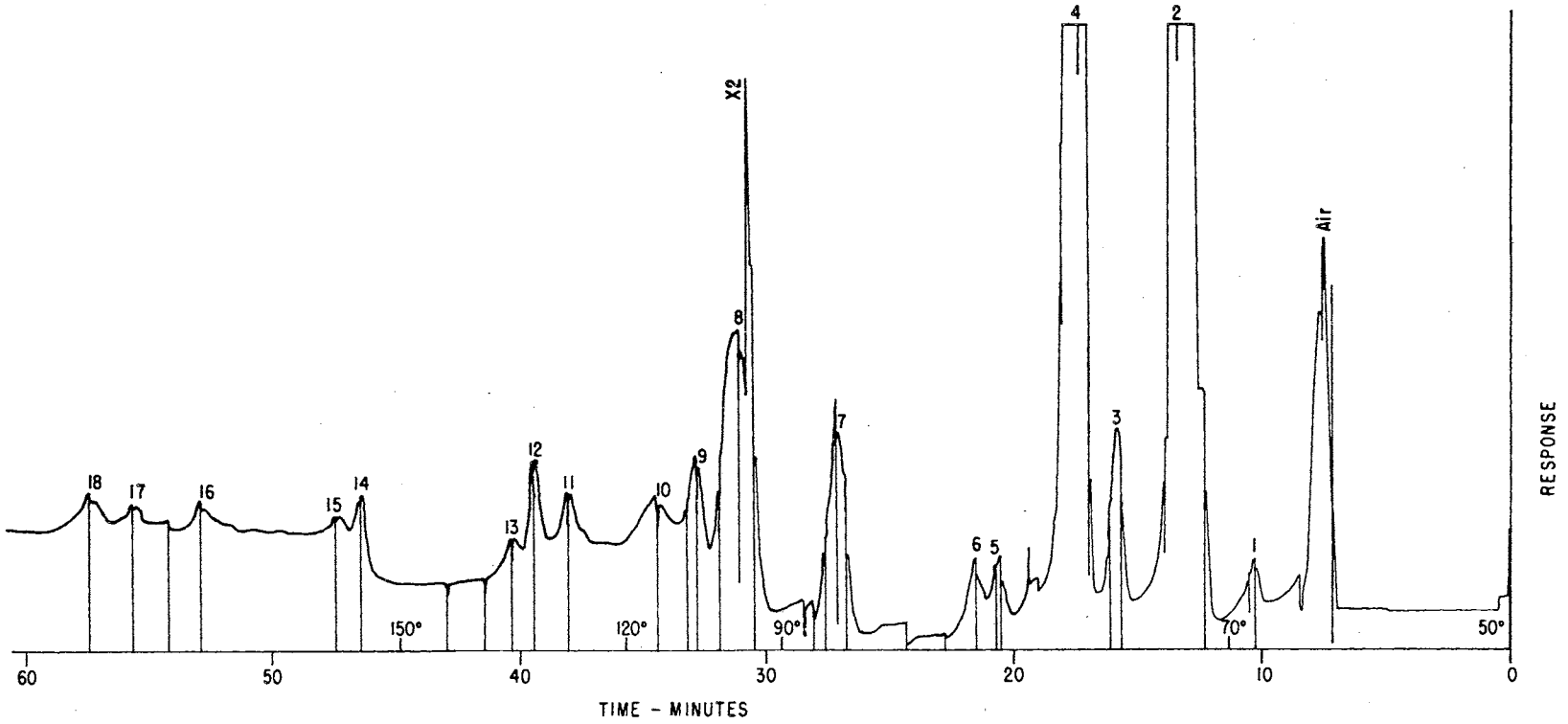

Figure 3. Gas Chromatogram from the GC-MS Instrument of 5 ml of Vapors from Regenerate of 40 mg of 2,4-DNPH Derivatives

TABLE II

MASS SPECTRA OF COMPONENTS FROM GLC SEPARATION OF 5-ML. OF VAPORS FROM REGENERATE OF 40-MG. OF 2,4-DNPH DERIVATIVES

	RELATIVE INTENSITIES OF COMPONENTS AND STANDARD COMPOUNDS									
m/e	1	Acet-aldehyde	3	Acetate	7	Toluene	9	Tigaldehyde	10	N,N-dimethyl-formamide
15	22.5	32.2	4.5	9.4		1.2	5.2	1.3	13.2	14.0
25	3.3	3.6								
26	5.9	6.2	1.5	2.7		1.5	4.9	6.9		
27	3.2	4.0	8.7	10.6		5.0	31.4	42.9	3.7	3.1
28			4.2	4.1			37.5	14.1	24.8	19.3
29	100.0	100.0	17.5	20.1			44.1	62.5	10.0	9.2
30							6.6	1.5	16.7	19.8
31							1.7	1.3		
37						1.5		3.0		
38						4.5	5.2	4.8		
39			6.0	1.2	9.4	18.8	30.3	10.9	2.5	1.6
40						2.3	5.7	4.8	4.4	4.8
41	6.2	7.3	3.5	3.4	4.8	2.2	61.3	9.8	7.9	5.4
42	14.5	16.3	7.5	7.2			15.8	2.3	35.5	39.3
43	47.5	43.7	100.0	100.0	3.4		15.6	4.1	9.8	8.3
44	88.0	83.3	6.0	6.5			4.0	5.9	70.0	87.2
45	2.7	4.6	17.5	19.1	3.0	4.4	1.0	1.6		
45.5						2.1				
46						3.4				
49								1.9		
50					3.8	6.0	5.1	7.7		
51					7.2	10.3	8.4	8.1		
52						1.2	1.9	2.6		
53						1.2	16.0	18.7		
54							4.8	4.7		
55							90.0	101.5		
56							17.1	9.8	2.4	2.0
57				1.1			10.2	2.0		
58				1.4					8.2	7.2
60			0.2	1.1						
61			16.5	16.7						
62						1.6				
63					2.4	4.1				
64					7.2	9.6				
65					12.8	14.1				
66						1.7	1.9	1.3		
67							2.1	1.1		
69							4.1	3.3		
70			17.5	9.5						
71				1.6						
72									8.8	8.0
73			5.7	9.2					100.0	100.0
74						1.2			5.1	4.5
75							6.3			
77				1.3						
83							14.2	9.8		
84							100.0	100.0		
85							0.8	5.7		
88				4.7						
89			0.2	0.4	4.2	4.5				
90					6.8	9.0				
91					100.0	100.0				
92					88.0	79.3				
93					6.2	5.6				

component 9 in Figure 3). Reference spectra for acetaldehyde, ethyl acetate, and N, N-dimethylformamide were obtained on the GC-MS instrument. A number of API spectra for toluene (serial nos. 176, 305, 418, and 251) were consulted and agreement was sufficient to preclude the necessity of obtaining toluene spectra using the combination instrument. The tigaldehyde (2-methyl-2-butenal) spectrum was taken from uncertified spectra (36) because the authentic compound was not available. It became apparent from the size of peaks in Figure 3 that all monocarbonyls except isobutyraldehyde and 2-methylbutanal and possibly 3-methylbutanal were minor constituents of the condensates. It was not at all obvious just how such compounds as ethyl acetate, toluene and dimethylformamide could appear in a regeneration of carbonyl compounds. The most likely explanation was that these compounds were occluded in or absorbed to the 2,4-DNPH derivatives such that they were not freed during the washing process.

This explanation seems feasible since ethyl acetate has been reported as a volatile constituent from roasted peanuts by Pattee et al. (16). Other possibilities are that such compounds arose during the regeneration process or may have been impurities in the 2,4-DNPH reagent or α-ketoglutaric acid used in the regeneration; these seem less likely, however. Some of the minor constituents may be of major importance in the flavor; for example, phenylacetaldehyde will probably prove to be of considerable

importance to the sweet "bouquet" of roasted peanut aroma.

Table III contains a listing of the remaining components in Figure 3 for which tentative identification was obtained from the mass spectra. Probable identities were not discernable for the latter five components (14-18 in Figure 3) due to relatively high background.

Benzaldehyde and phenylacetaldehyde were not sufficiently volatile to produce peaks large enough to obtain good mass spectra when regenerated carbonyls were chromatographed. Therefore methylene chloride extracts of the condensate were chromatographed on the GC-MS instrument. Table IV shows mass spectra obtained for components 18 and 21 of Figure 1, together with reference standards obtained from commercially available chemicals.

Even though the contribution of these monocarbonyl compounds to flavor has not been objectively determined, their odors and abundance suggest they must exert a very important contributory effect. For instance, when phenylacetaldehyde was removed from the total condensate by gas chromatography, and the remaining components recombined, the "sweet" background or bouquet of roasted peanut aroma was lost. When the low molecular weight aldehydes were removed the "harsh" aroma usually associated with warm freshly roasted peanuts was lost.

In both instances, considerable differences in intensity and type of aroma resulted. Since gas chromatography

TABLE III

TENTATIVE IDENTITIES OF MINOR COMPONENTS SHOWN IN FIGURE 2 BASED ON MASS SPECTRAL DATA

Component	Identity
5	3-methylfuran
6	pentanal
8	thiocyclohexane
11	3-methylthiocylohexane
12	4-methylthiocylohexane
13	heptanal
14-18	unknown

TABLE IV

MASS SPECTRA OF COMPOUNDS CORRESPONDING TO COMPONENTS 18 AND 21 IN FIGURE 1 AND REFERENCE STANDARDS

	RELATIVE INTENSITIES			
m/e	Benzaldehyde Standard	Component 18	Phenylacetaldehyde Standard	Component 21
29	3.8	11.1		
37	4.0	3.8		
38			1.2	1.9
39	9.0	15.2	7.3	10.3
41			2.1	5.4
49	4.1	5.7		
50	23.4	28.7	2.5	3.4
51	44.8	42.2	4.5	6.6
52	15.8	12.0		
53	2.1	6.1		
61	2.0	3.1		
62	2.5	2.3	2.4	2.5
63	3.9	3.2	5.8	6.0
64			1.3	2.2
73	5.1			
74	13.1	25.3		
75	6.7	15.6		
76	7.5	7.5		
77	100.0	100.0		
78	26.8	19.0		
89			3.6	4.7
90			2.8	2.8
91			100.0	100.0
92			23.8	24.0
93			1.8	3.2
105	96.5	109.9		
106	96.8	109.9		
107	12.7	12.7		
120			26.5	27.0
121			2.6	4.2

was used to remove these, it is possible that small amounts of other components were also lost which could have an effect on the aroma. However, compounds having typical "roasted" and "nutty" aromas were still present and the "roasted" aromas have been attributed to certain pyrazine compounds and their mixtures (Chapter 4).

The probable source of these monocarbonyl volatile constituents was by Strecker degradation of the corresponding free amino acids in the peanut. Strecker degradation involves oxidative deamination and decarboxylation of amino acids to give aldehydes with one less carbon atom. Dicarbonyl compounds, which are produced from reducing sugars under browning conditions, are needed to effect the degradation.

It has been reported that low-boiling volatiles common in food aroma may each be produced separately and in about the same quantity as from cooked foods from boiling dilute (0.01 M) solutions of a mixture of an individual amino acid with a sugar (2). Efforts to correlate quantities of amino acids and the Strecker degradation aldehydes in black teas (37) showed no absolute correlation between the two. Thus it appeared that the relationship could not be as simple as a direct comparison of the quantity of amino acid and the aldehyde product. Casey et al. (2) reported that variation in the quantity of volatiles produced depended on the oxidant involved, activation energy of each breakdown reaction, and conditions such as pH, ionic strength, and temperature.

Apparently, isobutyraldehyde, 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde arise as a result of Strecker degradation of valine, isoleucine, leucine and phenylalanine, respectively, and that the concentration of these aldehydes is probably not absolutely correlated with the corresponding amino acid concentrations.

Before exact roles of each of the monocarbonyls in the overall roasted peanut aroma can be determined, it will be necessary to quantitate each one, to determine threshold values, to subject all possible combinations of components to a sensory evaluation panel, and to determine the effect of subthreshold amounts of one component on the odor quality of another.

CHAPTER IV

IDENTIFICATION OF PYRAZINES AND A PYRROLE IN THE VOLATILES OF ROASTED PEANUTS

Apparatus

The apparatus used in roasting and homogenizing the peanuts, and the vacuum system used in degassing are described in Chapter 3. The gas chromatographs and mass spectrometers employed are also described in Chapter 3.

Nuclear magnetic resonance (NMR) spectra were determined on a Varian Model A-60 spectrometer.

Ultraviolet spectra were determined on a Cary Model 14 recording spectrophotometer.

Reagents

SE-52. Wilkins Instrument and Research, Inc., Box 313, Walnut Creek, California.

Carbon tetrachloride. Reagent grade, Baker Chemical Co., Phillipsburg, New Jersey.

Tetramethylsilane. Peninsular Chemical Research Inc., Gainesville, Florida.

Methylpyrazine and 2,5-dimethylpyrazine. Wyandotte Chemical Corp., Wyandotte, Michigan.

Pyrazine and N-methylpyrrole. K and K Laboratories, Plainview, New Jersey.

Cyclohexane. Practical grade, Eastman Organic Chemicals, Rochester, New York.

Glycerol, Gas-Chrom Q, Carbowax 2-M, Apiezon L, and Methylene Chloride as described in Chapter 3.

Procedures

The procedures used for preparation of the condensate, extraction of flavor components from condensate and gas chromatographic and mass spectral analysis were outlined in Chapter 3 with the following exceptions:

Two-hundred microliter quantities of the methylene chloride extracts were separated on a 3/8 inch by 12 foot aluminum column packed with 15% W./W. Carbowax 20-M on Gas-Chrom Q, prepared according to procedures of Sawaradeker and Slonecker (38). The column had been cured at 240°C for about 48 hours. The column was temperature programmed from 125° to 200°C at 5.6°/minute on an F and M model 500 gas chromatograph employing a four-filament hot-wire detector. A typical chromatogram is shown in Figure 4.

Samples were collected for NMR spectra in the manner described by Brame (39). NMR spectra were determined in 50 μl of CCl_4 containing about 1% tetramethylsilane and all chemical shift data were relative to tetramethylsilane which was taken as 0.0δ. All spectra were determined with the Varian model A-60 spectrometer operating at a spectrum

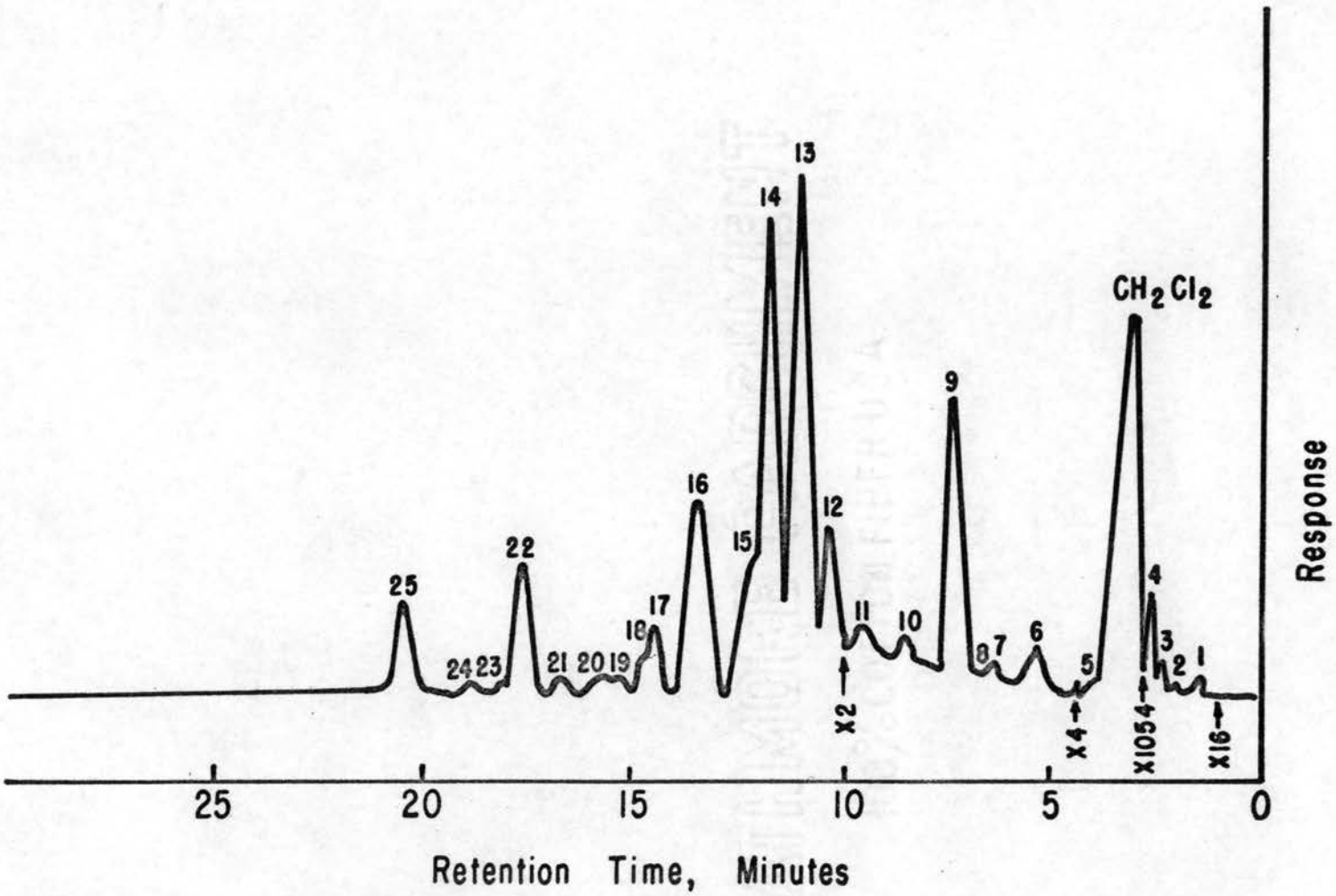

Figure 4. Typical Preparative Gas Chromatogram of Volatile Mixture (Condensate) Obtained from Roasted Peanuts

amplitude of 80, sweep width of 500 c.p.s., and other adjustments set to give optimum signal-to-noise ratio at this sensitivity. This high sensitivity was necessary for the small samples collected which amounted to less than 1 μl total volume in most cases, although approximately 1 μl quantities of N-methylpyrrole and 2,5-dimethylpyrazine were obtained. Spectra obtained in this manner were sufficient to identify protons by comparison of chemical shifts or to approximate their number from total response but the resolution was insufficient to evaluate coupling constants; thus, isomeric forms were not determinable from the data.

Ultraviolet spectra were determined on the compounds in cyclohexane by simply scanning the solutions on the Cary model 14 recording spectrophotometer in a 1 ml quartz cell with a 1 cm light path.

Results and Discussion

The first evidence for the presence of pyrrole and pyrazine compounds was obtained from mass spectra of vapors from the total condensates before separation by gas chromatography. Unexpected peaks were noted above mass 100 at even numbered peaks. These peaks were first thought to be due to oxygenated compounds (such compounds have intense molecular ion peaks at even mass numbers). The peaks above mass 100 were intensified in the purified samples while fragmentation peaks did not show the characteristic peaks of oxygenated compounds. The cracking patterns (consisting

of fragment ions) were more indicative of nitrogen compounds than oxygenated compounds and the presence of two nitrogen atoms per molecule would then account for the even numbered molecular ion peaks.

Four pounds of roasted peanuts were degassed in two-pound batches, as previously described, the condensates extracted and the extracts combined, reduced in volume, and preparative gas chromatographic separations made. Components 6 through 18, and 22 and 25 (Figure 4) were collected in sufficient quantities to perform mass spectral analyses. These spectra corresponded closely to spectra found upon examination of reference mass spectral data. In particular peak 9 corresponded to N-methylpyrrole, peak 12 to methylpyrazine, and peak 14 to 2,5-dimethylpyrazine. Also peaks 16 and 17 appeared to be pyrazine drivatives with parent masses of 122 and 136, respectively. Identifications reported here concern primarily components 9, 12, 14, 16, and 17.

Retention times were determined for authentic N-methylpyrrole and some commercially available pyrazine compounds on two different columns and it was noted that compounds having the same retentions as N-methylpyrrole, methylpyrazine and 2,5-dimethylpyrazine were present on both columns. Thus, in order to obtain sufficient material for instrumental analysis to establish the identities of the components, 16 pounds of roasted peanuts were processed as

previously described and preparative gas chromatographic separations were made (Figure 4).

Figure 5 shows the ultraviolet (UV) spectra for the isolated components and for some commercially available standards. The fact that peak 16 consisted of two components was not realized at first since it eluted as a symmetrical peak from the preparative (3/8 inch) column. However, on an analytical column (1/4 inch x 24 foot, 5% Carbowax 20-M) a second component was clearly discernable and the two peaks were approximately equal in area. Consequently, the peak was collected as approximately two equal fractions and these were purified further by rechromatography until further processing would have resulted in prohibitive losses. The component corresponding to N-methylpyrrole was not included in this figure since good UV determination was not obtained. Presumably this was due to the fact that UV maxima for pyrrole occur at shorter wavelengths (210 and 240 mμ) than for pyrazines and the extinction at the longer wavelength is very low (40).

The spectra of methylpyrazine and 2,5-dimethylpyrazine matched very well those of the isolated compounds and left little doubt that the latter were indeed substituted pyrazines. Comparison of the standard spectra of pyrazine to those of mono- and di- substituted pyrazine showed progressive bathochromic shifts of the major band ($\pi \rightarrow \pi^*$ or benzenoid transition) and loss in fine structure to both the major and minor bands ($n \rightarrow \pi^*$ transition) as the degree

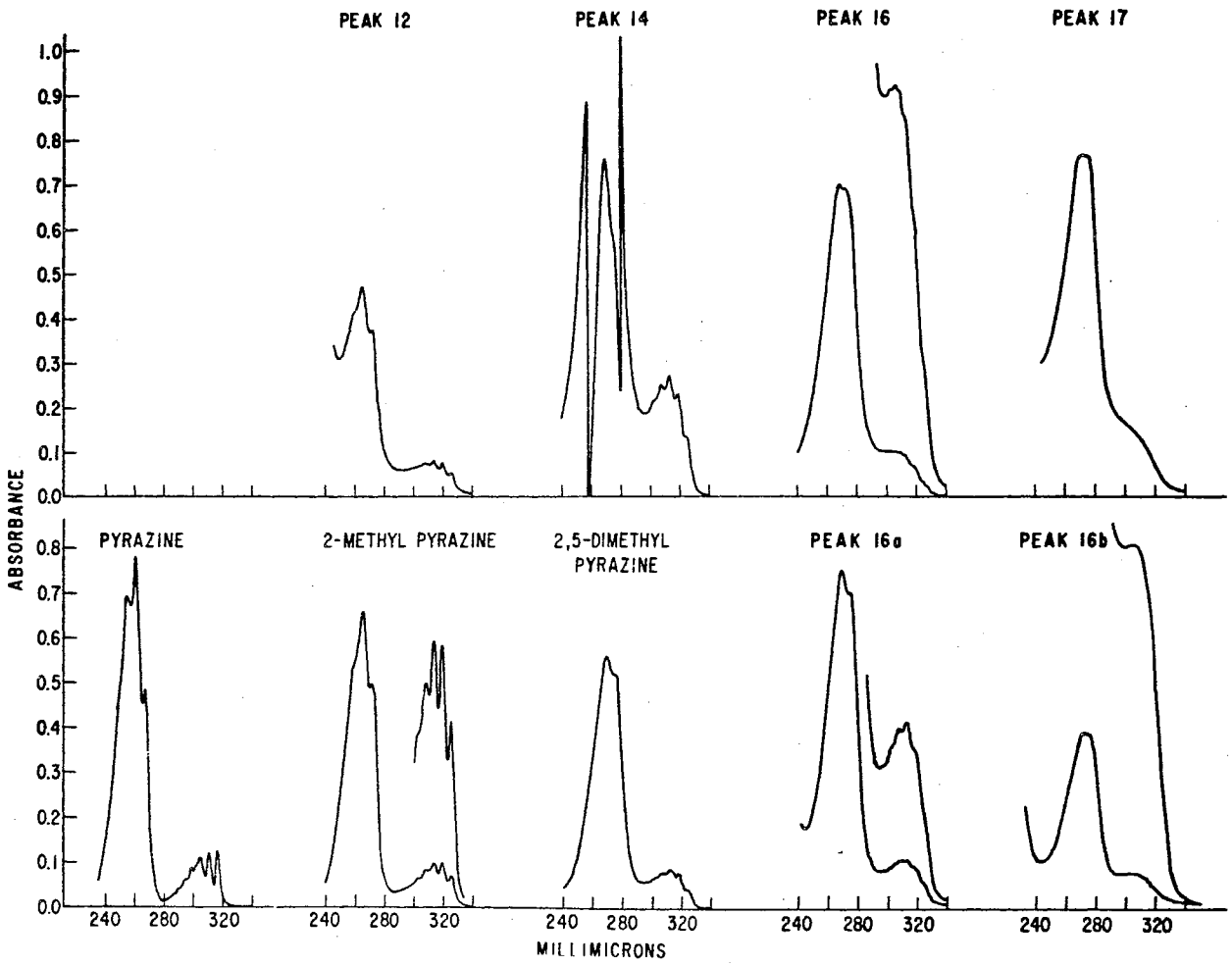

Figure 5. Ultraviolet Spectra of Peak 12, 14, 16a, 16b, and 17 Along with Some Authentic Pyrazines

of alkyl substitution increased. This was also the trend of the spectra of the samples who parent masses of 94, 108, 122, and 136 indicated a series of compounds formed by adding CH_2 to each consecutive component.

Thus mass spectral, retention time and UV spectral data of components 9, 12, and 14 left little doubt that they were N-methylpyrrole, methylpyrazine, and 2,5-dimethylpyrazine. Also the mass- and UV spectral data of components 16 (16a and 16b) and 17 showed these components to be alkylated pyrazines of molecular weights 122 and 136, respectively. With compounds of higher molecular weight, the possibility of geometric isomers arose, which was apparently the case with components 16a and 16b. 16a and 16b could be any two of the following isomers: propyl-, isopropyl-, 2,3-, 2,5-, and 2,6-methylethyl-, and trimethylpyrazine. Component 17 had, of course, a greater number of possible geometric isomers. Nuclear magnetic reasonance (NMR) spectra on these compounds provided a method to determine which geometrical isomers were present and additional evidence to support the proposed identities of component 9, 12, and 14.

Figure 6 shows the NMR spectrum for component 9. Chemical shifts relative to tetramethylsilane (TMS) were those expected for N-methylpyrrole and correspondend to those for the authentic compound. Absorption at 6.15- and 6.65 δ corresponded to four ring protons, each peak being equivalent to two protons. The latter peak presumably

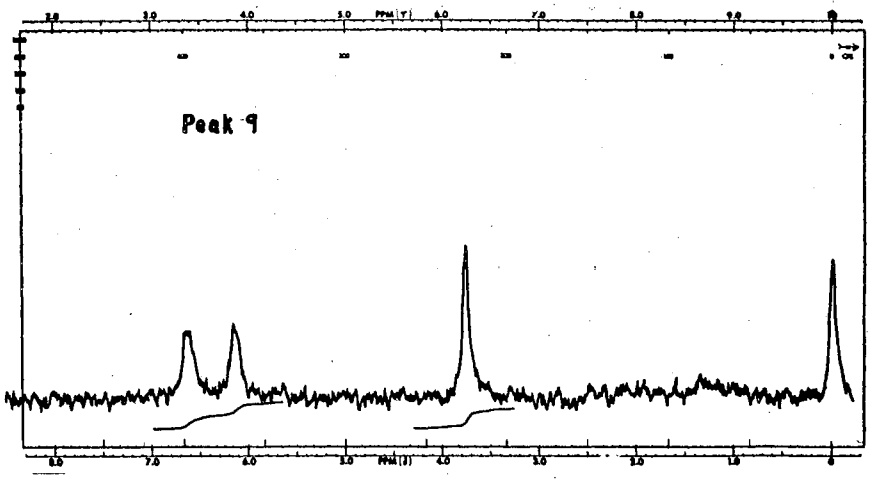

Figure 6. NMR Spectrum of Peak 9

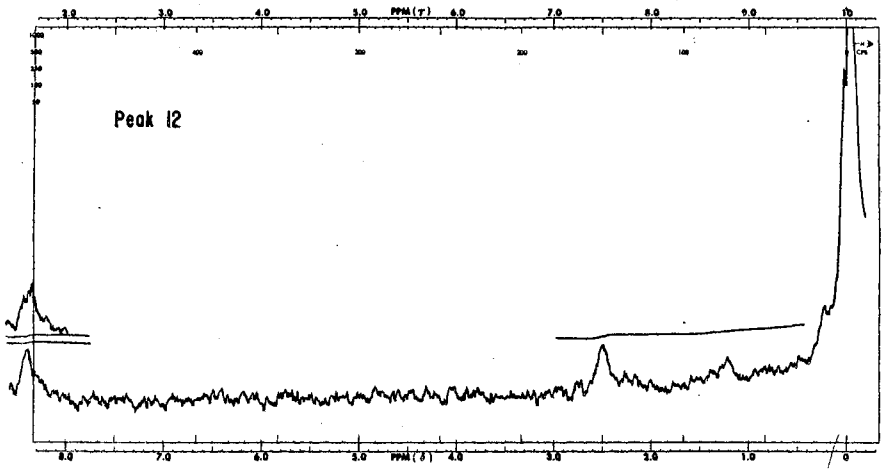

Figure 7. NMR Spectrum of Peak 12

corresponded to the two protons nearest the heterocyclic nitrogen. Absorption at about 3.75 δ corresponding to the three N-methyl protons was equivalent to 9 units of area whereas the ring protons accounted for 11 units of area or 5.5 units each. Theoretically the ratio of areas should have been 1:1:1.5 (moving upfield); whereas an experimental ratio of 1:1:1.6 was observed. Splitting of the two downfield peaks was expected, but resolution was not sufficient. The data from this spectrum combined with the mass spectrum was considered sufficient to establish the identity of component 9 as N-methylpyrrole.

Figure 7 shows the best NMR spectrum obtained of component 12. This NMR spectrum, if considered alone, would be poor experimental evidence for component 12, but when considered along with the fact that component 12 was a pyrazine compound with molecular weight 94 (known from UV- and mass spectral data) left no doubt that this compound was methylpyrazine. The chemical shifts at 2.5 δ and 8.45 δ were the same as those for authentic methylpyrazine and the areas were approximately the same, corresponding to the three equivalent ring protons and three equivalent ring methyl protons.

The data in Figure 8 shows chemical shifts obtained from component 14. The sweep width was 1000 cycles per second (c.p.s.) in this case so that all chemical shifts were twice the values read from the figure. Thus, the small and large peaks represented absorption at 8.4 δ and 2.5 δ

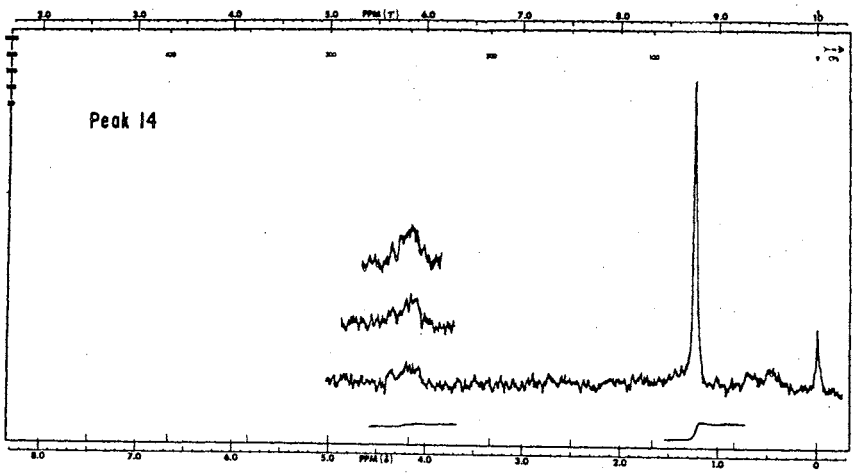

Figure 8. NMR Spectrum of Peak 14

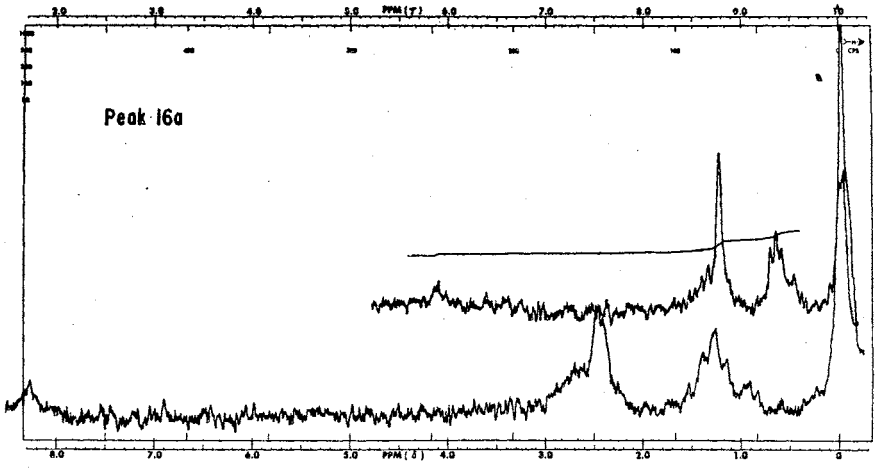

Figure 9. NMR Spectrum of Peak 16a

respectively. Relative areas were in a 1:3 ratio corresponding to those expected for 2,5-dimethylpryazine and agreed well with the spectrum of an authentic sample. As with component 12, the NMR data corroborated UV- and mass spectral data, identifying component 14 as 2,5-dimethylpyrazine.

NMR spectra for components 16a and 17 (Figures 9 and 10) were not sufficiently resolved to permit assignment of the particular isomeric form of the molecules. To accomplish this, splitting would have had to be clear enough to calculate coupling constants. Nevertheless, these spectra were sufficient to establish that 16a was a methylethylpyrazine and that 17 was a dimethylethylpyrazine.

Component 16a was deduced to be methylethylpyrazine from the information given previously and from its NMR spectrum (Figure 9) using the following rational. Lack of sufficient absorption due to methylprotons separated from the ring by two methylene groups, which should have been at about 0.95 δ, eliminated the possibility that 16a was a propyl derivative. The possibility of its being a trimethyl derivative was discounted because of the presence of considerable absorption of methylene protons on carbon attached to the ring (2.7 δ) and also because component 16b was found to be trimethylpyrazine. An ethyl radical was indicated by the presence of methyl protons appearing as a triplet (1.25 δ), indicating an adjacent methylene group. Integral areas for the various absorptions were in a ratio

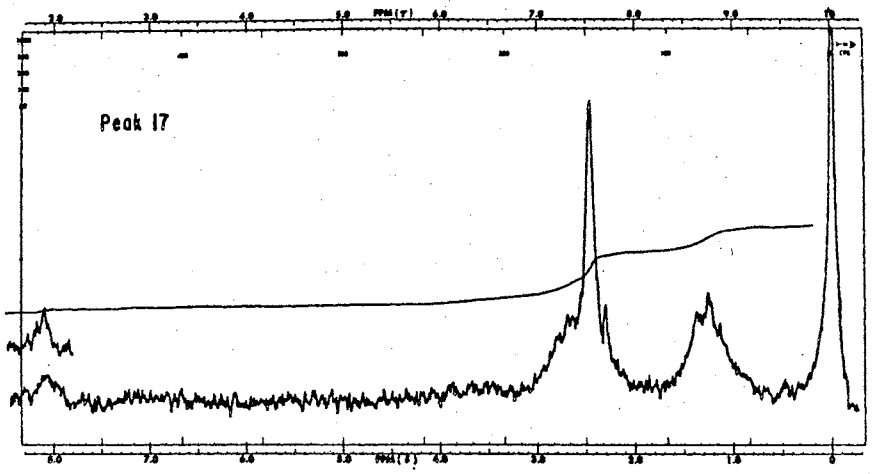

Figure 10. NMR Spectrum of Peak 17

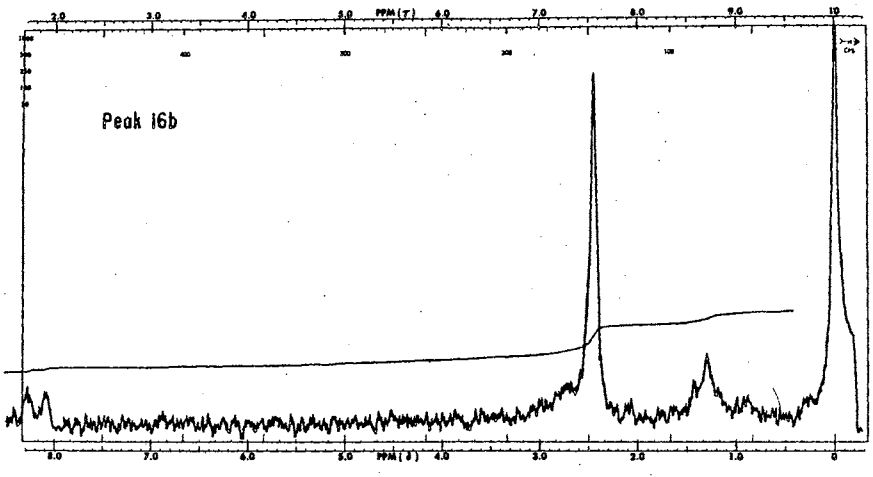

Figure 11. NMR Spectrum of Peak 16b

of 2:2:4:3 for the ring protons (8.25 δ), methylene protons (2.7 δ), ring methyl protons (2.5 δ), and alkyl methyl protons (1.25 δ) respectively, whereas the theoretical ratio is 2:2:3:3. The discrepancy in absorption due to ring methyl protons was probably due to some contamination from peak 16b (trimethylpyrazine) which had an intense absorption at 2.5 δ. A sweep width of 1000 c.p.s. was included in this spectrum also to aid in the difficult task of integration of absorption superimposed on a noisy baseline.

Of the three possible isomers for methylethylpyrazine, one isomer could be established or eliminated as the possible structure on the basis of splitting of absorption due to the two ring protons. If the unknown were 2-methyl-3-ethylpyrazine, the ring protons on adjacent carbon atoms would appear as a doublet. However, if it were 2-methyl-5-ethyl- or 2-methyl-6-ethylpyrazine the protons would be on opposite sides of the ring (not adjacent) and splitting would not occur. Insufficient compound was present to determine this with the model A-60 spectrometer. High resolution instrumentation or a time averaging device such as that used by Lundin et al. (41) was needed to obtain this information. Nevertheless, a prediction of the identity of this isomer was made from mass spectral data.

Figure 11 shows the NMR spectrum of component 16b whose most probable structure, from consideration of the mass spectra, was assigned as trimethylpyrazine. Chemical shifts for the two types of protons present in

trimethylpyrazine (8.1 δ and 2.45 δ) occurred in approximately a 1:9 ratio as expected. Presence of considerable contamination from component 16a was seen in the small amount of absorption at 1.25 δ, 2.7 δ, and the presence of the additional peak for ring protons at 8.3 δ which corresponds to that for component 16a.

By the same reasoning used with component 16a, component 17 was judged to be a dimethylethylpyrazine on the basis of the NMR spectra (Figure 10). Indications of methyl protons corresponding to those present in butyl-, methylpropyl-, or methylisopropylpyrazine were lacking in the spectrum. However, absorptions indicative of ethylpyrazine protons were present. Methylene protons on carbons adjacent to pyrazine ring (2.7 δ) and methyl protons separated from the pyrazine ring by only one methylene group (1.25 δ) were evident. Standard compounds were not available to determine these chemical shifts but NMR spectra of alkylated derivatives were obtained from G.C. Bassler and R.M. Silverstein (42) and agreement was observed in chemical shifts between the borrowed and unknown spectra. For example, with 2,3-dimethyl-5-ethylpyrazine, absorption due to methylene protons appeared as a quartet centered at 2.7 δ split by the three adjacent methyl protons. Theoretical integral values for a dimethylethylpyrazine were in a ratio of 1:2:6:3 for the ring proton, two methylene protons, six methyl protons on carbons attached to the ring, and three methyl protons attached to the terminal carbon of the ethyl

radical, respectively. Figure 10, the spectrum of component 17, showed a corresponding ratio of 1:2.1:6.4:3.2 which is in good agreement with theoretical values considering the difficulty in measuring the area of the smaller peak.

Mass spectra of the isolated components and standards obtained from various sources are included in Table V. Standard spectra of N-methylpyrrole, methylpyrazine, and 2,5-dimethylpyrazine were obtained by analyzing commercially available chemicals of high purity on the CEC-103C mass spectrometer. However, unpublished spectra, obtained from G. C. Bassler and R. M. Silverstein (42), of trimethylpyrazine and isomers of methylethyl- and dimethylethylpyrazines are included in the tables since these compounds were not commercially available.

Spectra of components 9, 12, 14, and 16b and the standard spectra were in good agreement. Only two spectra of the three possible positional isomers for methylethylpyrazine were available. The spectrum which agreed most closely with the unknown is included in the table (2-methyl-5-ethylpyrazine). The same was true for dimethylethylpyrazine where three possible positional isomers existed; the 2,3-dimethyl-5-ethylpyrazine isomer was eliminated as a possibility because the 100% peak was at m/e = 108. A 70 ev mass spectrum for 2,6-dimethyl-3-ethylpyrazine was not available but that of the corresponding 2,5-dimethyl-3-ethylpyrazine was available and was included in the table

TABLE V

MASS SPECTRAL DATA OF NITROGEN CONTAINING COMPOUNDS IN CONDENSATES ISOLATED FROM ROASTED PEANUTS AS COMPARED TO STANDARD COMPOUNDS

m/e	N-Methylpyrrole		Methylpyrazine		2,5-Dimethylpyrazine		2-Methyl-5-ethyl-pyrazine		Trimethylpyrazine		2,5-Dimethyl-3-ethylpyrazine	
	Std.	Peak 9	Std.	Peak 12	Std.	Peak 14	Std.	Peak 16a	Std.	Peak 16b	Std.	Peak 17
26	10.1	11.3	35.3	36.4	7.8	10.8	8.	14.2	5.	8.1	6.	20.4
27	16.6	23.0	10.8	21.0	10.0	16.4	20.	44.5	15.	20.8	27.	38.2
37	7.9	8.5	7.6	10.5	4.8	4.0	4.	7.7			—	4.5
38	13.0	13.6	17.9	15.8	12.6	13.2	11.	18.3	6.	7.9	8.	10.4
39	41.0	44.9	34.0	36.6	43.5	47.0	51.	79.1	26.	35.4	47.	57.8
40	10.8	11.0	28.7	35.0	30.0	32.5						
40.5	5.6	6.3										
41	7.5	13.2	10.8	20.2	5.1	14.0	7.	11.9	5.	9.9	14.	25.0
42	35.0	37.3	16.4	19.7	100.0	100.0	17.	39.1	100.	100.0	59.	41.1
49	3.0	4.0										
50	9.9	10.4										
51	11.0	11.8	5.7	10.5	3.7	4.4	7.	11.9	5.	6.5	5.	13.1
52	8.2	8.7	10.2	8.9	6.5	7.5	11.	15.1	7.	8.6	9.	13.2
53	33.2	35.7	19.3	18.3			11.	18.6	8.	9.3	12.	11.1
54	15.2	16.0					13.	21.2	10.	11.8	7.	12.9
55	19.0	22.5									—	
56							25.	33.3			29.	27.3
64												
65											3.	4.3
66	4.3	4.5	4.3	4.0			5.	11.5			5.	5.3
67			56.3	48.8			3.	10.2			8.	8.7
68			3.0	3.7								
80	82.1	80.6			11.1	11.7	4.	6.3			5.	6.4
81	100.0	100.0							13.	14.5	3.	5.6
82	5.7	6.7										
93							4.	5.0				
94			100	100			15.	19.1				
95			6.3	6.5			3.	5.7				
107							5.	5.1			11.	10.0
108					59.1	55.8					23.	24.9
109					4.4	4.5					4.	5.0
120							8.	5.1				
121							100.	100.0	—	12.8	9.	15.5
122							72.	76.4	52.	54.1		
123							7.8	7.5	4.	4.6		
134											9.	6.1
135											100.	100.0
136											90.	97.6
137											8.	8.3

with component 17, since it supported the conclusion that this component was a dimethylethyl derivative.

Some disagreement between these spectra at lower m/e ratios was present and expected since there was some column bleed material in every sample collected. This contamination contributed primarily to fragment masses below 50 and caused little difficulty at higher m/e ratios.

Ionizations less than 3% of the 100% peak were not included in the spectral data. Also, some fragments above 3% were included for the collected samples which were not included in the borrowed spectra, a line was drawn in the corresponding position in the standard where no data was available. The borrowed spectra had no digits to the right of the decimal, and are presented as such.

The mass spectrum for component 16b agreed well with that of trimethylpyrazine and when considered along with UV- and NMR data, there was no doubt of the identification.

Trace amounts of other pyrazine compounds were indicated by mass spectra of later components on the gas chromatograph.

The pyrazine derivatives encountered were considered to have a "roasted" aroma in the concentrations at which they emerged from the preparative columns. Flavor responses, reported by Deck and Chang (19), of 2,5-dimethylpyrazine at concentrations of 10 ppm in oil are "earthy" and "raw potatoe."

Component 16 possessed an extremely potent and pleasing "nutty" aroma reminiscent of roasted peanuts, before separation into two components. Whether the pleasant aroma was due to the mixture, the trimethyl derivative, or the methyethyl derivative remained unknown, but the pyrazine derivatives appeared to be among the "character impact" compounds of typical roasted peanut using the term as defined by Jennings and Sevenants (3) and more recently reported by Rodin et al. (43).

A review by Hodge (44) reported pyrazines and pyrroles as having been detected in and actually isolated from sugar-amine browning reactions in model systems. The sugar-amine systems contained mixtures of sugar and amino acids. Pyrazines have been isolated from potato chips (19) and coffee (18), both pyrolytically processed food products as are peanuts.

Four facts support the view that these compounds are not artifacts: (1) Repeated rechromatography did not result in breakdown of purified components in the gas chromatograph as would have been evidenced by disappearance of the compound and appearance of new peaks. Also no changes in the components were observed when all glass gas chromatographic systems and on-column injections were used. (2) Mass spectrometry of vapors of the total condensate before separation revealed the same series of $[M]^+$ and $[M-1]^+$ ions that were found after separation. (3) The occurrence and amounts of these components were surprisingly consistent

in condensates obtained over a period of a year. (4) The isolated components had odors which were similar to the original condensate and to typical roasted peanut aroma.

CHAPTER V

SUMMARY

The purpose of the study reported in this thesis was to isolate and identify the volatile constituents of roasted peanuts. Isolation was accomplished by vacuum degassing of a homogenate of roasted peanut cotyledons, glycerol, and water. The aqueous condensate was reacted with 2,4-dinitrophenylhydrazine reagent to obtain derivatives of the carbonyl components. These were then regenerated and analyzed on the combination GC-MS instrument. Of the 18 components regenerated the following were identified: acetaldehyde, isobutyraldehyde, 2-methylbutanal, 3-methylbutanal, 2-methylbutenal, benzaldehyde, phenylacetaldehyde, ethyl acetate, N,N-dimethylformamide and toluene.

The aqueous condensate from a 16 pound batch of roasted peanut cotyledons was extracted with methylene chloride and the extract reduced to about 1 ml on a rotatory evaporator. This was separated by preparative gas chromatography and the components collected in small cold traps as they passed out the exit port. Ultraviolet-, nuclear magnetic resonance-, and mass spectral data were obtained on these components. N-methylpyrrole, methylpyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine were identified. A

single isomer of each methylethylpyrazine and a dimethylethylpyrazine was characterized but sufficient sample was not available to permit determination of the particular isomers present. Higher molecular weight pyrazine derivatives were suspected from interpretation of the mass spectra of components beyond component 17.

The carbonyl components probably occupy a contributory role to the aroma of roasted peanuts while the pyrazine derivatives may be among the character-impact compounds.

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