bioflavonoids. Some of the early reports by Rusznyák and Szent-Györgyi (*24*) attempted to assign vitamin activity to the crystalline phenolic fraction from lemon juice. More recently, Martin and Szent-Györgyi (*18*) reviewed the physiological activity of the bioflavonoids. Hendrickson and Kesterson (*6*) reviewed the literature on hesperidin which also included many reports of its therapeutic activity. Some authorities (*15*) still are skeptical, however, due to the lack of well controlled clinical studies. Nevertheless, on the basis of these claims a market for citrus bioflavonoids flourishes. The authors' procedure could be very useful in assaying bioflavonoid preparations.

An earlier paper from this laboratory (*28*) established a high correlation between the citric acid content of lemon juice and the total phenolics. The measure of total phenolics in the juice was in terms of absorbance at a standard dilution. This absorbance could not be attributed to any single compound owing to the complexity of the phenolic fraction. A plot of the total phenolics, determined by paper chromatography *vs.* absorbance at both the 275 mμ peak or inflection point and the 320 to 330 mμ peak in Figure 2, showed the two methods agreed rather closely. The slope of the line for the short wavelength peak had a smaller variance than the slope for the long wavelength peak, probably because of the predominance of the eriocitrin λ_{max} at 284 mμ. Least-squares lines of the data intercept the positive absorbance axis when the phenolics are zero. The extra absorbance is probably by nonphenolic, UV-absorbing compounds.

Because of the high correlation between the total phenolics and the citric acid content in lemon juice (*28*), the total phenolics have been quite useful commercially in determining juice authenticity. However, since the phenolics were estimated in terms of absorbance, one might be tempted to add various UV-absorbing compounds to extend the juice. By these chromatographic methods, the addition of anything other than the natural mixture could be detected.

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PEANUT COMPOUNDS

Flavor Components of Roasted Peanuts. Some Low Molecular Weight Pyrazines and a Pyrrole

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TYPICAL roasted peanut flavor is one of the most desirable and universally enjoyed flavors. However, very little is known about the compounds which constitute this unique flavor. Pickett and Holley (*9*), and more recently Young and Holley (*15*), have published information which gives some insight into the nature of some of the volatiles produced during roasting and the precursors of peanut flavor. Pattee, Beasley, and Singleton (*8*) recently reported the identities of compounds obtained from off-flavored peanuts, but there is yet to appear a definitive paper on structures of compounds which contribute to roasted peanut flavor.

This paper describes the identification of a number of nitrogenous organic compounds isolated from roasted Spanish peanuts and their possible contribution to typical roasted flavor.

Experimental

Reagents. Water, distilled and deionized.

Methylene chloride, n_D^{20}, 1.4238 Aldrich Chemical Co., Milwaukee 10, Wis., redistilled at 40.0° C.

Mixtures of volatile components have been isolated from roasted Spanish peanuts using high vacuum and low temperature trapping techniques. Components of the condensates have been separated using preparative gas chromatography and a number of the components identified by physical methods. Nuclear magnetic resonance, ultraviolet, and mass spectrometry were used to identify five pyrazines and a pyrrole: methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, methylethylpyrazine, dimethylethylpyrazine, and *N*-methylpyrrole. The possible role of pyrazines in typical roasted peanut flavor, as well as their possible origin from flavor precursors, is discussed.

Glycerol, USP, Procter and Gamble Co., Cincinnati 1, Ohio.

Gas-Chrom Q, 100- to 120-mesh, Applied Science Laboratories, Inc., P. O. Box 140, State College, Pa.

Carbowax 20M and SE-52, Wilkins Instrument and Research, Inc., Box 313, Walnut Creek, Calif.

Carbon tetrachloride, reagent grade, Baker Chemical Co., Phillipsburg, N. J.

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

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

Pyrazine and *N*-methylpyrrole, K and K Laboratories, Plainview, N. J.

Cyclohexane, practical grade, Eastman Organic Chemicals, Rochester, N. Y.

Preparation of Condensates from Roasted Peanuts. Two-pound batches of Argentine Spanish peanuts, dry roasted to a golden brown (medium roast) at 160° C. (germ and seed coats removed after roasting), were homogenized in glycerol and water as follows: To 125-gram quantities of cotyledons were added 25 ml. of water and 225 ml. of glycerol. The mixtures were homogenized a few minutes on a Serval Omnimixer fitted with a quart jar attachment. The combined homogenates were placed in a 12-liter round-bottomed flask attached to a simple vacuum manifold.

The vacuum system consisted of a three-stage oil diffusion pump backed up by a Welch fore-pump. Two cold traps, one a large cold finger and one a smaller U-tube trap, were attached to the manifold leading to the 12-liter flask. Another small cold trap was situated between the diffusion pump and the other two traps to protect against diffusion of silicone oil into the manifold. All traps were cooled in liquid nitrogen.

The 12-liter flask containing the homogenates was degassed under positive vacuum at room temperature until foaming ceased. Then the temperature of the slurry in the flask was raised slowly by means of a heating mantle until it was about 60° C. At this time the degassing was discontinued. To remove the volatiles from a 2-pound batch required about 12 hours, although aroma was never completely removed. Most of the condensate, consisting of water and entrained volatile flavor components, was collected from the large cold finger trap by thawing and allowing the aqueous suspension to run into a collecting vessel. The small U-trap collected only small amounts of material and was emptied only occasionally. Condensates from repeated extractions were treated in the manner described in the next section.

Extraction of Flavor Components. The aqueous suspensions were extracted three times with redistilled methylene chloride, with approximately 0.1 volume being used for each extraction. As determined by gas chromatography of the aqueous phase, this treatment extracted all but traces of organic components. Combined methylene chloride extracts were evaporated to about 1-ml. volume on a rotary evaporator just prior to separation by gas chromatography. No attempt was made to dry the extract for fear of loss of flavor components by adsorption or decomposition on the drying agent.

Gas Chromatographic Separation of Methylene Chloride Extracts. Two-hundred-microliter quantities of the methylene chloride extracts were separated on a $^3/_8$-inch × 12-foot aluminum column packed with 15% (w./w.) Carbowax 20M on Gas-Chrom Q which was prepared according to procedures of Sawardeker and Sloneker (*12*). The column was cured at 240° C. for about

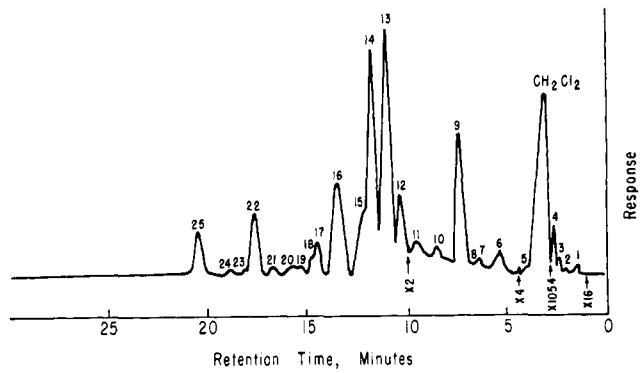


Figure 1. Typical preparative gas chromatogram of volatile mixture (condensate) from roasted peanuts

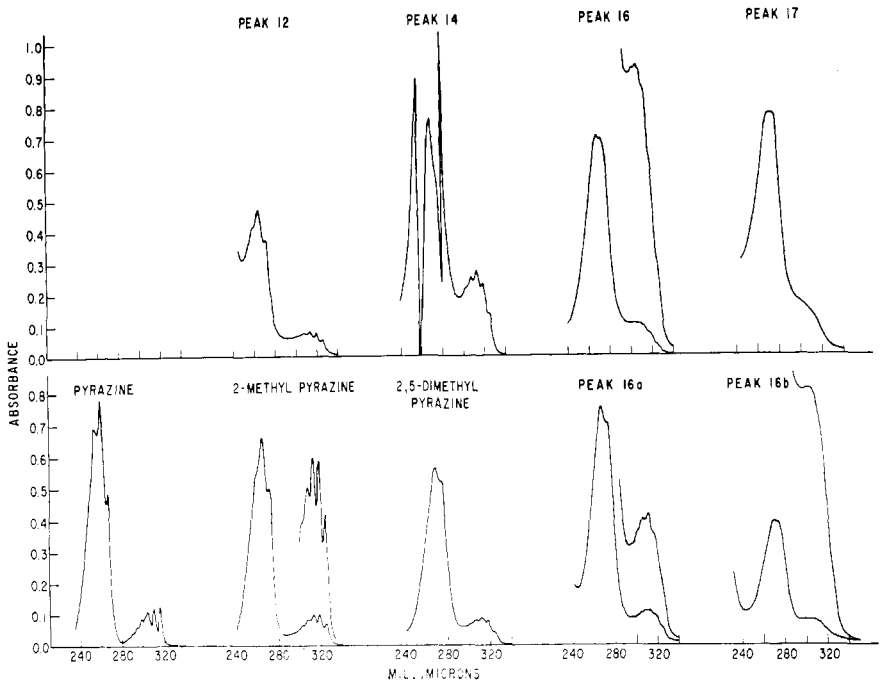


Figure 2. Ultraviolet spectra of peaks 12, 14, 16a, 16b, and 17 with some authentic pyrazines

48 hours. The column was temperature programmed from 125° to 200° C. at 5.6° per minute, and a flow rate of 140 ml. per minute was used. Separation was performed on an F and M Model 500 gas chromatograph employing a four-filament hot-wire detector, and a typical chromatogram is shown in Figure 1.

Individual components were collected in several different types of traps, depending upon the identification procedures to be used. For collection of samples for mass spectral analyses, simple U-tubes (6-mm. o.d.) constructed of borosilicate glass and containing a small plug of steel wool, according to the suggestion of Teranishi *et al.* (*14*) to prevent aerosol formation, were used. The inlet of each trap was reduced to 3.8-mm. o.d. so that it could be inserted into the exit of the gas chromatograph through a silicone rubber septum and attached to the 300° C. glass inlet on the mass spectrometer. Cryogenic trapping was accomplished with dry ice–ethanol slush baths.

Samples for NMR spectra were collected in the manner described by Brame (*3*).

For ultraviolet analyses, the collected samples were washed out of the U-tube traps with cyclohexane in which the spectra were determined.

Collected samples were purified from small amounts of surrounding peaks by rechromatography on the same column, or rechromatographed on an SE-52 column which had good selectivity for impurities and poorer selectivity for the nitrogenous compounds. The column was a $^1/_4$-inch × 6-foot glass column packed with 5% (w./w.) SE-52 on Gas-Chrom Q, and was operated at 70° C. and a helium flow rate of 35 ml. per minute.

Methods of Identification. Mass spectral data were obtained by inserting the U-tube into the heated inlet valve of a Consolidated Electrodynamics Corp. (CEC) Model 21-103C mass spectrometer. The union was sealed by means of an O-ring backed by a $^1/_4$-inch Swagelok nut and inverted rear ferrule. The nut was simply tightened onto corresponding threads at the terminus of the inlet valve until an air-tight seal existed. The tube was immersed in liquid nitrogen and the air pumped from above the frozen sample by opening the inlet valve to the vacuum system of the mass spectrometer. The coolant then was removed, and the tube containing the sample was allowed to warm in a stream of warm air from a heat gun (hot air blower with gun-type housing) until approximately 50 microns of pressure was obtained in the 3-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-e.v. spectrum was obtained and recorded as has been described (*2*).

To prevent samples loaded in the above manner from being diluted with appreciable amounts of carbon dioxide and water from the atmosphere, certain precautions were taken. These included closure of traps with silicone rubber plugs immediately after removing samples from the exit of the gas chromatograph, removing them from liquid nitrogen baths, and allowing them to warm somewhat before opening the trap inlet for attachment to the mass spectrometer.

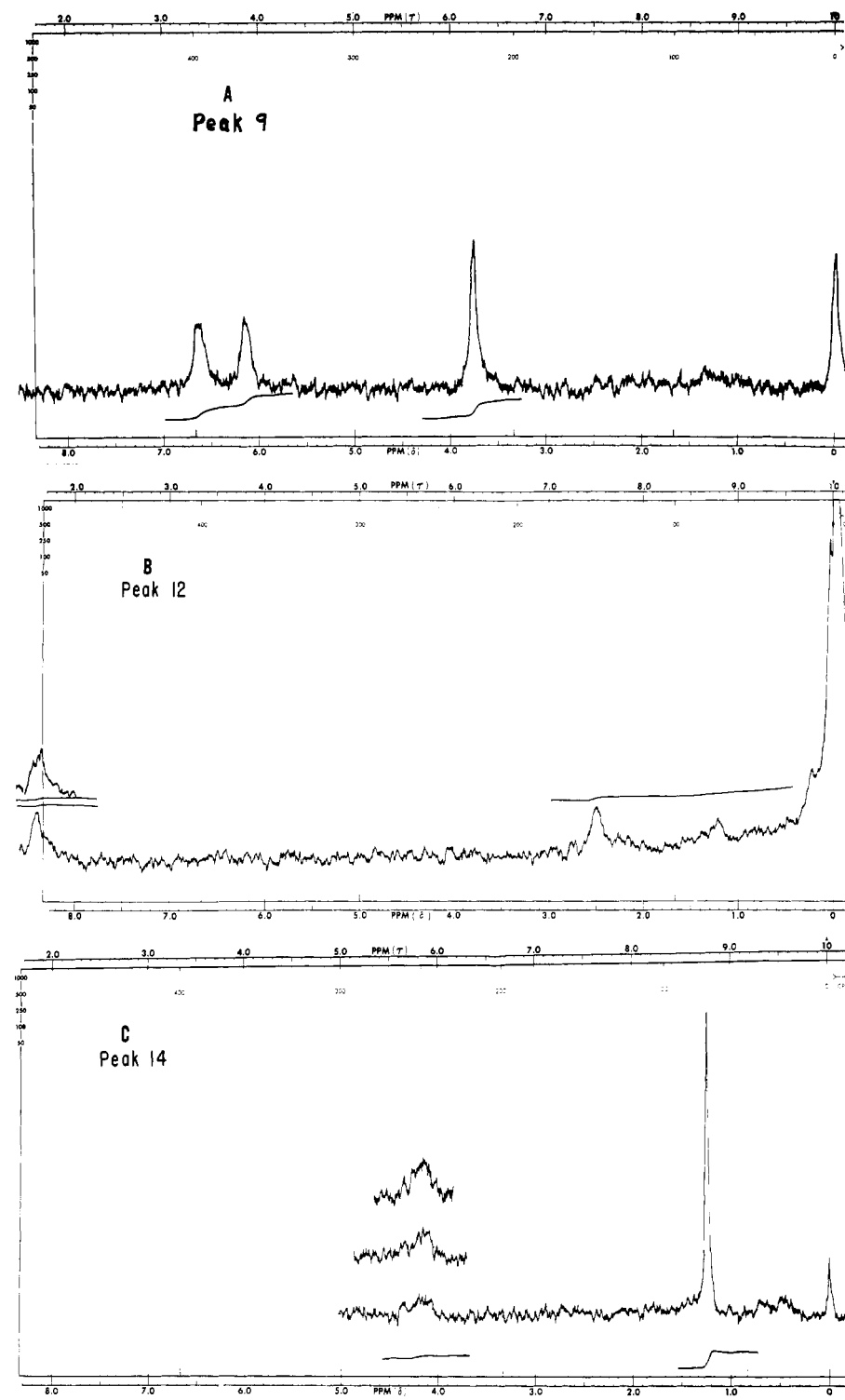
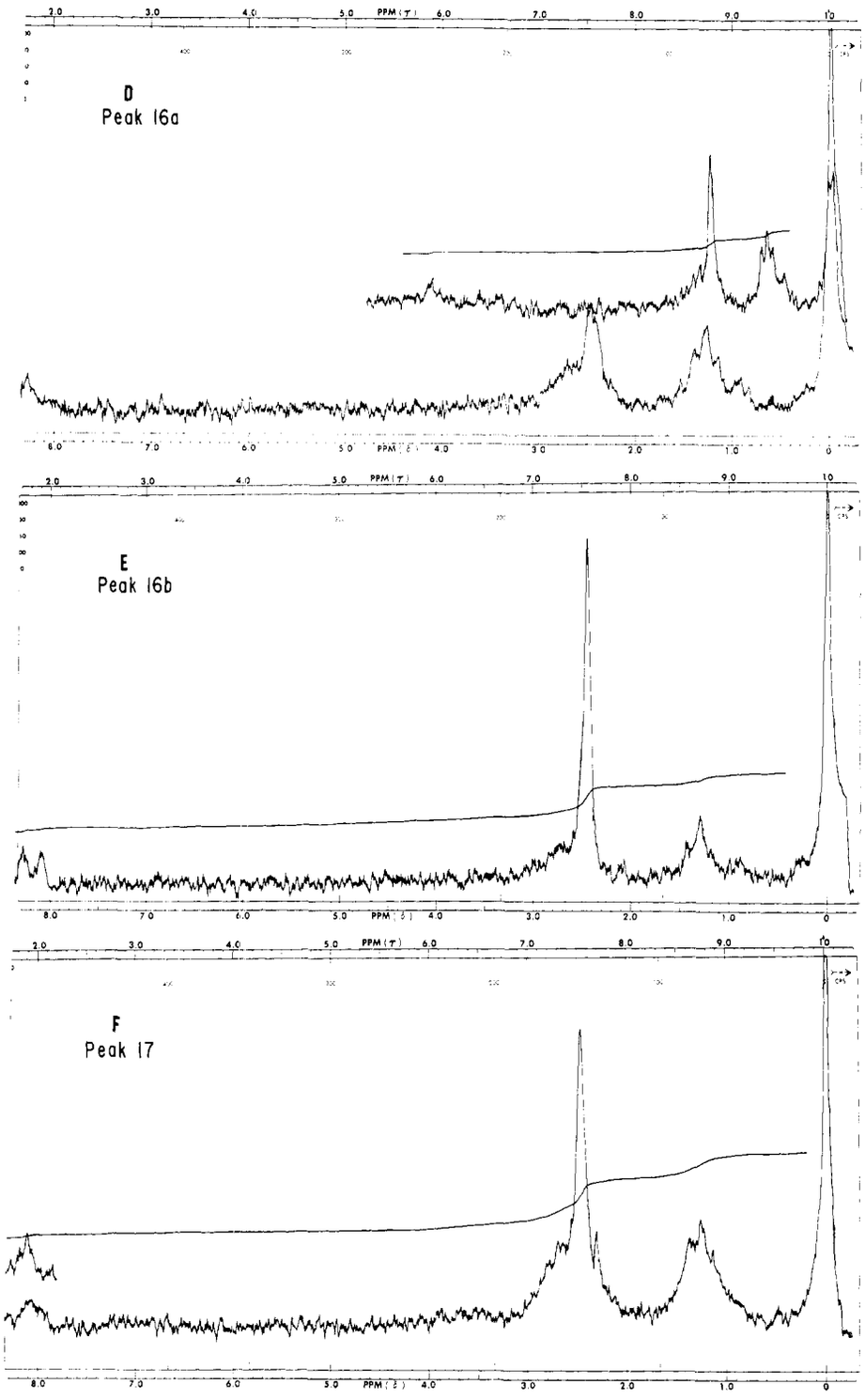


Figure 3. NMR spectra fo

A. Peak 9, 500 c.p.s. B. Peak 12, 500 c.p.s. C. Peak 14, 1000 c.p.s

Ultraviolet spectra were determined on the compounds in cyclohexane used to wash them from the traps by scanning the solutions on the Cary Model 14 recording spectrophotometer in a 1-ml. quartz cell having a 1-cm. light path.

Nuclear magnetic resonance (NMR) spectra were determined in 50 μl. of CCl_4 containing about 1% tetramethylsilane, and all chemical shift data were relative to tetramethylsilane equal to 0.0. All spectra were determined with the Varian Model A-60 spectrometer operating at a spectrum amplitude of 80, sweep widths of 500 c.p.s., except that for peak 14, and the other adjustments were 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



'e pyrazines and a pyrrole

Peak 16a, 500 and 1000 c.p.s. E. Peak 16b, 500 c.p.s. F. Peak 17, 500 c.p.s.

volume in most cases with the exception that approximately 1-μl. quantities of *N*-methylpyrrole and 2,5-dimethylpyrazine were obtained. Spectra obtained in this manner were sufficient to identify protons by virtue of chemical shifts or to approximate their number from total response but of insufficient resolution for evaluation of coupling constants; thus, isomeric forms were not determinable from the data.

Results and Discussion

The first implication of 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 at even-numbered peaks above mass 100. At first these peaks were thought to be due to oxygenated compounds—such compounds have intense 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. Intense peaks at one mass unit below the even-numbered peaks suggested that these compounds could be pyrazines, and the presence of two nitrogen atoms per molecule then would account for the even-numbered molecular ion peaks. Also, cracking patterns —consisting of fragment ions—were more indicative of nitrogen compounds than oxygenated compounds. Examination of reference mass spectral data showed these spectra corresponded closely to the typical fragmentation of pyrrole and pyrazine compounds.

Retention times were determined for authentic *N*-methylpyrrole and some commercially available pyrazine compounds on two different columns, and compounds having the same retentions as *N*-methylpyrrole, methylpyrazine, and 2,5-dimethylpyrazine on both columns were present. Thus, 16 pounds of roasted peanuts were extracted in 2-pound batches as previously described, the condensates extracted, the extracts combined, and reduced in volume, and preparative gas chromatographic separations made. Components 6 through 18, 22, and 25 (Figure 1) were collected in sufficient quantities to perform spectral analyses. Identifications reported here concern components 9, 12, 14, 16, and 17.

Figure 2 shows the ultraviolet spectra for the isolated components and 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—$^1/_4$-inch × 24-foot 5% Carbowax 20M on Gas-Chrom Q—column, a second component was clearly discernible, and the two were approximately equal in area. Consequently, the peak was collected as approximately two equal fractions, and these were purified by rechromatography until further processing would have resulted in prohibitive losses. The component corresponding to *N*-methylpyrrole was not included in this figure, since a good determination was not obtained. Presumably, this was due to the fact that ultraviolet maxima for pyrrole occur at shorter wavelengths (210 and 240 mμ) than for pyrazines, and the extinction at the longer wavelength is very low (*13*).

The spectra of methylpyrazine and 2,5-dimethylpyrazine matched very well those of the isolated compounds and left little doubt that they were substituted pyrazines. By comparing the spectra of the isolated components with those of pyrazine in the figure, 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), were noted as the degree of alkyl substitution increased. Thus, the three isolated

components, for which no standard compounds were available, were thought to be alkyl-substituted pyrazines of a higher degree of substitution than dimethylpyrazine.

Figure 3*A* shows the NMR spectrum for component 9. Chemical shifts from tetramethylsilane (TMS) were those expected for *N*-methylpyrrole and corresponded to those for the authentic compound. Absorption at 6.15 and 6.65δ corresponded to four ring protons, each peak being equivalent to two protons. The latter peak presumably corresponded to the two protons nearest the heterocyclic nitrogen. Absorption at about 3.75δ, corresponding to three *N*-methyl protons, was equivalent to 9 units of area, whereas the ring protons accounted for 11 units of area of 5.5 units each. Theoretically, the ratio of areas obtained moving upfield (toward TMS) should have been 1:1:1.5, but a ratio of 1:1:1.6 was obtained experimentally. Resolution was not sufficient to show splitting of the two downfield peaks. Consideration of this spectrum, in conjunction with the mass spectrum, left no doubt that component 9 was *N*-methylpyrrole.

Figure 3*B* shows the best NMR spectrum obtained of component 12. The chemical shifts at 2.5 and 8.4δ were the same as those for authentic methylpyrazine, and the areas were approximately equivalent corresponding to three equivalent ring protons and three equivalent ring-methyl protons. This NMR spectrum, if considered alone, would be poor experimental evidence, but when it was considered in conjunction with the ultraviolet and mass spectral data, there was no doubt about the identity of component 12. Slight absorption at 1.25δ was found to be due to material bleeding from the column during preparative chromatography.

Analyses of data in Figure 3*C*, in a similar manner, showed chemical shifts and relative areas corresponding to those expected for 2,5-dimethylpyrazine and agreed well with the spectrum of an authentic sample. 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 peak and large peaks represented absorption at 8.4 and 2.5δ, respectively. Relative areas were in a 1:3 ratio corresponding to those expected for 2,5-dimethylpyrazine and agreed well with the spectrum of an authentic sample. As with component 12, the NMR data corroborated ultraviolet and mass spectral data, so that component 14 was identified as 2,5-dimethylpyrazine.

NMR spectra for components 16a and 17, Figure 3, *D* and *F*, as in the other NMR spectra, were insufficiently resolved to permit assignment of the particular isomeric form of the molecule. To accomplish this, splitting would have had to be clear enough to calculate coupling constants. Nevertheless, these spectra established the fact that component 16a was methylethylpyrazine and 17 was a dimethylethylpyrazine. The reasons for these conclusions were as follows: The possibility of component 16a being a propyl derivative was eliminated because of the lack of sufficient absorption due to methyl protons separated from the ring by two methylene groups. These would have appeared at about 0.95δ. The possibility of its being a trimethyl derivative was discounted because considerable absorption due to methylene protons on carbon attached to the ring (2.7δ) was present, methyl protons attached to the methylene group appeared as a triplet (1.25δ) as was predicted for an ethyl radical, and component 16b was found to be trimethylpyrazine. Comparison of the spectrum to NMR spectra for methylethylpyrazines obtained from Bassler and Silverstein (*1*) showed good agreement between positions of absorption.

Integral areas for the various absorptions showed a ratio of 2:2:4:3 for the ring protons (8.25δ), methylene protons (2.7δ), ring-methyl protons (2.5δ), and alkyl methyl protons (1.25δ), respectively, although from theoretical values for a methylethylpyrazine a ratio of 2:2:3:3 was predicted. The discrepancy in degree of absorption at 2.5δ from ring-methyl protons was probably due to some contamination from the component of peak 16b (trimethylpyrazine). A sweep width of 1000 c.p.s. was included in this spectrum, also, to aid in the difficult task of integration of absorptions superimposed on noisy base lines.

Absolute assignment of the particular positional isomer of methylethylpyrazine was not possible from the experimental data. Of the three possible isomers, 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 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, but if high resolution instrumentation, or a time-averaging device such as that used by Lundin *et al.* (*7*), were available this information could have been obtained. Nevertheless, a tentative identification of this particular isomer was made from the mass spectra data.

By the same reasoning used with component 16a, component 17 was judged to be a dimethylethylpyrazine on the basis of the NMR spectra. Indication of methyl protons corresponding to those present in butyl- or methylpropylpyrazine was lacking in the spectrum. Similarly, absorptions for methylene protons which would be present in an ethyl derivative (methylene protons on carbon adjacent to pyrazine ring), and methyl protons separated from the pyrazine ring by only one methylene group, were present at 2.7 and 1.25δ, respectively. Standard compounds were not available to determine these chemical shifts, but NMR spectra of alkylated derivatives were obtained from Bassler and Silverstein (*1*) and agreement was observed in chemical shifts between the unknown spectra and those for the dimethylethylpyrazines. For example, for 2,3-dimethyl-5-ethylpyrazine, absorption due to methylene protons appeared as a quartet centered at 2.7δ split by three adjacent methyl protons. Theoretical integral values for a dimethylethylpyrazine were in a ratio of 1:2:6:3, respectively, 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. Corresponding ratios for component 17 determined from this spectrum were 1:3.1:6.4:3.2, respectively, in good agreement considering the difficulty in measuring the area of the smaller peak.

Figure 3*E* shows the NMR spectrum of component 16b whose 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 and 2.45δ) occurred in approximately a 1:9 ratio, as expected. The presence of considerable contamination from component 16a was seen in the small amount of absorption at 1.25 and 2.7δ, and the presence of the additional peak for ring protons at 8.3δ corresponding to that for component 16a.

Table I includes mass spectra of the isolated components and standards obtained from various sources. Standards for *N*-methylpyrrole, methylpyrazine, and 2,5-dimethylpyrazine were obtained by analyzing commercially available chemicals of high purity on the CEC-103C mass spectrometer. However, trimethylpyrazine and isomers of methylethyl- and dimethylethylpyrazines were not commercially available, and the standard spectra in the tables for these compounds were unpublished spectra obtained from Bassler and Silverstein (*1*).

Good agreement between standard spectra and those of components 9, 12, 14, and 16b was apparent. Of the three possible positional isomers for methylethylpyrazine, spectra were available for only two, and the spectrum which agreed most closely with the unknown was included in the table (2-

Table I. Mass Spectral Data of Nitrogen-Containing Compounds in Condensates Isolated from Roasted Peanuts as Compared with Standard Compounds

	N-Methylpyrrole		*Methylpyrazine*		*2,5-Dimethylpyrazine*		*2-Methyl-5-ethyl-pyrazine*		*Trimethylpyrazine*		*2,5-Dimethyl-3-ethylpyrazine*	
m/e	*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	28	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

methyl-5-ethylpyrazine). The same was true for dimethylethylpyrazine, where three possible positional isomers existed; 2,3-dimethyl-5-ethyl pyrazine was eliminated as a possibility because the 100% peak was at m/e = 108. A 70-e.v. mass spectrum for 2,6-dimethyl-3-ethylpyrazine was not available but that of the corresponding 2,5-dimethyl-3-ethyl isomer was available and was included in the table with component 17, since it supported the conclusion that this component was a dimethylethyl derivative.

Some disagreement of these spectra at lower m/e ratios was common and expected, since some column bleed material was present in every sample collected. This extraneous material caused little difficulty at higher m/e ratios but did contribute to fragment masses below mass 50.

Ionizations which amounted to less than 3% of the 100% peak were not included in the spectral data. In addition, some magnitudes above 3% were obtained in collected samples which were not included in the borrowed spectra; these were included under the unknown and a line was drawn in the corresponding position in the standard where data were not available. No digits to the right of the decimal were present in the borrowed spectra, so they were reported as such.

The mass spectrum for component 16b agreed well with that of trimethylpyrazine, and when it was evaluated along with NMR and ultraviolet data, the identification was considered positive.

Mass spectra of later components in the gas chromatograph indicated that several more pyrazine compounds were present in minute amounts.

These compounds, with the exception of *N*-methylpyrrole, were considered to have a "roasted" aroma in concentrations at which they emerged from the preparative columns. Pyrazines have been isolated from potato chips (*4*) and coffee (*10*); both processes involved a pyrolytic process, as does peanut roasting. Deck and Chang reported flavor responses of "earthy" and "raw potato" at 2,5-dimethylpyrazine concentrations of 10 p.p.m. in oil.

Component 16, before separation into two components, possessed an extremely potent and pleasing "nutty" aroma reminiscent of roasted peanuts. Whether this aroma was due to the trimethyl derivative, the methylethyl derivative, or a mixture of the two is not yet known, but pyrazine derivatives may be among the "character impact" compounds of typical roasted peanut flavor, where the term is used in the sense used by Jennings and Sevenants (*6*) and, more recently, by Rodin *et al.* (*11*).

Data showing that pyrazines and pyrroles can arise from heated mixtures of sugars and amino acids, or acetol and amino acids, have been reviewed by Hodge (*5*). Changes which occurred in sugar and amino acid contents of peanuts during roasting have led the authors to postulate that these nitrogenous compounds arose from reaction intermediates of amino acids and sugars. The data on which this postulation is based will be the subject of a later paper.

That these compounds were not artifacts was supported by four facts: (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, the same components were observed even when all-glass gas chromatographic systems and on-column injection 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) Odors of isolated components were reminiscent of the original mixture and of typical roasted peanut aroma. (4) Numerous condensates have been obtained and analyzed over a period of a year, and the occurrence and amounts of these components were surprisingly consistent.

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VINEGAR COMPOUNDS

Analysis of Vinegar by Gas-Liquid Chromatography

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Gas-liquid chromatography has been applied to the analysis of vinegars. By selecting the proper liquid phase it was possible in a single run to resolve alcohols, esters, acids, and 3-hydroxy-2-butanone. The yeast fermentation of cider yields the components of fusel oil, which in turn are partially converted to the corresponding acids. Methyl and ethyl acetates are produced during the vinegar fermentation. The vinegar fermentation of cider stock produces a large amount of 3-hydroxy-2-butanone, whereas only traces are produced by the yeast fermentation of cider.

THE SENSITIVITY of gas-liquid chromatography for detecting traces of volatile compounds has led to its extensive use for analyzing foodstuffs. A large body of information is being accumulated showing the identity of the volatile compounds in many common foods. The method is particularly well suited for the characterization of the compounds which occur in vinegar.

White or distilled vinegar is usually manufactured from ethyl alcohol denatured with ethyl acetate, formula SDA29. Such vinegars, and also those made from undenatured alcohol, contain traces of ethyl acetate and residual alcohol. Vinegars made from fermented fruit juices such as cider and wine also contain many other compounds, including higher alcohols which collectively are called fusel oil, and oxidation products of the higher alcohols. Most of these compounds, except acetic acid and water, occur in only trace amounts. The presence of so much acid and water makes it difficult to separate and identify the other components, which may impart character and flavors to specific vinegars. Compounds responsible for the aroma of apples and cider have been identified by various workers (*2, 4–8, 10–12*), but little has been done to identify the flavor components of vinegars.

A recent study of the ether-pentane extracts of wine and fruit vinegars by Suomalainen and Kangasperko (*13*) has shown the presence of seven or eight compounds in addition to the expected ethyl acetate: the alcohols found in fusel oil, their acetic acid esters, 2–3-butanedione (diacetyl), and 3-hydroxy-2-butanone (acetoin). They report that spirit vinegars contained only ethyl acetate. Maurel and Lafarge (*9*) found in fermented cider methanol and isopropyl alcohol in addition to the usual fermentation alcohols.