Gas Chromatography in Tobacco Research [1]

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Introduction

Natural products occur ordinarily as complex mixtures. When such mixtures are pyrolyzed, their products will be even more complex. Cigarette smoke is typical of such products. Although it is probably composed of hundreds or thousands of compounds, most of the components present will have one property in common; they all exist, at least momentarily, in the gas phase. This property suggests that vapor pressure methods may be used to advantage in their separation. Even with the most refined distillation technique, however, sample size is sharply limited somewhere in the semimicro range. This limitation precluded the use of these techniques in our problem of separating the constituents present in the gas phase of cigarette smoke. This led to an early and favorable evaluation of the initial paper on gas chromatography by Martin and James (1952) and to the application of their procedures to our work.

The present paper describes the techniques which were developed and tried on a 17-component synthetic mixture whose heterogeneous composition was suggested by literature reports on cigarette smoke (Osborne *et al*, 1954, 1956). Both adsorption and partition gas chromatographic techniques were used in the early stages of this work. Adsorption was limited in that it would separate only relatively non-polar compounds efficiently, holding back all others. Gas-liquid-partition chromatography was a much more versatile tool, although some of the initial trials were disappointing. Repeated chromatography of primary fractions with selective and different stationary liquids led to the isolation of pure compounds.

Each fraction that was separated on the chromatographic columns was trapped and was analyzed mass spectrometrically by methods previously used and reported from this laboratory (Seligman *et al*, 1955). The present work again clearly demonstrates that isolated fractions should be characterized unequivocally by a secondary detector.

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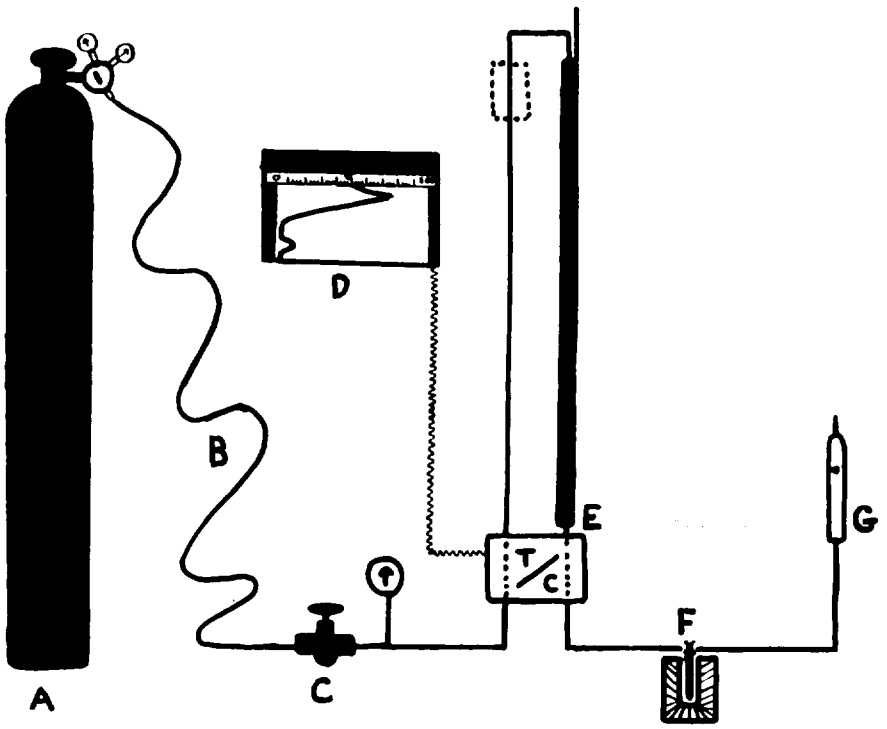

Figure 1. Schematic Diagram of Apparatus.

A. Helium tank.
B. Drying tube.
C. Secondary pressure regulator and pressure gauge.
D. Recording potentiometer.
E. Chromatographic column and thermal conductivity cell.
F. Fraction collection trap.
G. Flowmeter.

Apparatus

Thermal conductivity cell (Model RCT): Gow-Mac Instrument Co., Madison, N. J.

Pressure regulator: a) primary, (Automatic Regulator No. 8): The Matheson Co., East Rutherford, N. J. b) secondary, (Rego 2403U): The Bastian-Blessing Co., Chicago, Ill.

Electric vibrator (Vibra-Tool): Burgess Vibrocrafters, Inc., Lake

Zurich, Ill.

Rotameter (Size 1-15-6): Brooks Rotameter Co., Lansdale, Pa.

Recording potentiometer (12 point, 3 mv. fullscale deflection): Bristol Co., Waterbury, Conn.

Mass spectrometer (Model 21-103B): Consolidated Engineering Corp., Pasadena, Calif.

Chromatographic column: borosilicate glass, 5 mm. ID, 4 foot length.

Coolant containers: Homart Styrofoam toilet tank floats (Sears, Roebuck and Co.) are hollowed out sufficiently to hold the shaft of a collection trap surrounded by 30 ml. of liquid nitrogen.

Fraction collection traps: These are of all-glass construction. Two arms of a three-way stopcock of 2 mm. bore are connected to size 12-2 ball and socket joints, 9.5 cm. from the ball and 5.5 cm. from the socket. The third arm is connected to a 5 mm. I.D. tube of 9.5 cm. length to form a T. This tube is sealed inside another tube of 15 mm. I.D. The inner tube extends to within 10 mm. of the bottom of the outer tube. A side arm of 2 mm. bore is attached to the outer tube 7.5 cm. from the bottom. This arm connects to one side of a two-way stopcock of 2 mm. bore. This stopcock, in turn, is connected to the shaft of the ball joint 3.5 cm. from the three-way stopcock and 4.5 cm. from the ball. The total height of the trap is 15 cm.; the volume, 10 cubic centimeters.

Reagents

Celite 545: Johns-Manville Co., New York, N. Y.

Tricresyl phosphate (Kronitex AA): Ohio-Apex Division, Food Machinery and Chemical Corp., Nitro, W. Va.

Silica (Commercial Grade, sized to 20-60 mesh): Davison Chemical Corp., Baltimore, Md.

2-Phenoxyethanol: Eastman Organic Chemicals, Rochester, N. Y.

Hyvac oil: Central Scientific Co., Chicago, Ill.

Butyl butoxyethyl phthalate (Santicizer B-16): Monsanto Chemical Co., St. Louis, Mo.

Helium: Air Reduction Co., Inc., New York, N. Y.

Molecular sieve 4A: Linde Air Products Co., Div. of Union Carbide & Carbon, New York, N. Y.

Liquid nitrogen: Air Reduction Co., Inc., New York, N. Y.

Spectro-vac stopcock grease (Type III): Dr. Robert R. Austin, Pasadena, Calif.

Synthetic Mixture

Butane (extra pure), 1,3-butadiene (instrument grade), 1-butene (C.P.), propane (instrument grade), carbonyl sulfide, and methyl chloride: The Matheson Co., East Rutherford, N. Y.

Methane and ethane: City of Richmond natural gas.

Acetaldehyde, acetonitrile, and propionaldehyde (Eastman White Label): Eastman Organic Chemicals, Rochester, N. Y.

Furan: Matheson Coleman & Bell, East Rutherford, N. J.

Isoprene (pure grade-99 mole percent): Phillips Petroleum Co., Bartlesville, Okla.

Methanol (reagent grade): Baker and Adamson Products, General Chemical Division, Allied Chemical and Dye Corp., New York, N. Y.

Acetone (spectro grade): Eastman Organic Chemicals, Rochester, N. Y.

Ammonium hydroxide ('Baker Analyzed'): J. T. Baker Chemical Co., Phillipsburg, N. J.

Experimental Procedure

Preparation of Chromatographic Columns. The Celite was sized, washed and dried by the method of James and Martin (1952). The partitioning phases were stirred with the Celite to give a 30% mixture (w/w) of the liquid on the inert support. Columns were made from glass tubing (5 mm. I.D., 120 cm. length) with glass wool plugs inserted at both ends to retain the packing. The columns were filled with 10.5 g. of the Celite mixture and were packed by using an electric vibrator. The silica columns contained 16.9 g. of Davison silica sized to 20-60 mesh. Before use, the columns were swept with helium until the detector cells balanced, as indicated on the recorder.

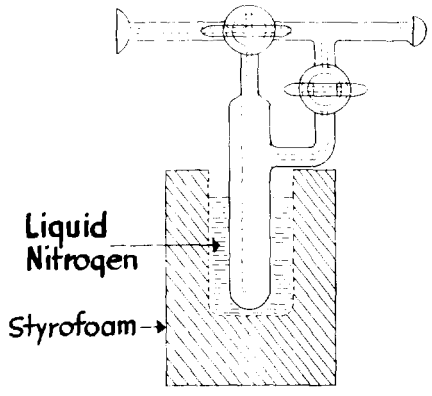

Figure 2. Fraction collection trap.

Preparation of the Synthetic Mixture and Introduction onto the Column

Gases. A 10 ml. burette was inverted and connected to a separatory funnel by a rubber hose. The separatory funnel was mounted on a ring stand so it could be raised or lowered. The hose and burette were partially filled with mercury. The tapered end of the burette was connected to an evacuated trap (figure 2) which, in turn, was connected to one arm of a 3-way stopcock. The other two arms of the stopcock were connected, respectively, to a vacuum pump and to a lecture bottle containing one of the sample gases.

Air was expelled from the burette by raising the mercury to the burette stopcock. This was accomplished by raising the separatory funnel and closing the stopcock. The remainder of the system was evacuated by the pump which was connected to the 3-way stopcock. The sample gas was released from the lecture bottle and

Table 1. 17-Component Synthetic Mixture.

Compound	B.P.°C.	Molecular Weight
Methane	—161.5	16
Ethane	— 88.3	30
Carbonyl Sulfide	— 48.0	60
Propane	— 42.2	44
Ammonia	— 33.3	17
Methyl Chloride	— 24.2	50.5
n-Butylene	— 5.0	56
1,3-Butadiene	— 3.0	54
n-Butane	— 0.6	58
Acetaldehyde	21.0	44
Furan	32.0	68
Isoprene	34.0	68
Propionaldehyde	49.0	58
Acetone	56.5	58
Methanol	64.7	32
Acetonitrile	82.0	41
Water	100.0	18

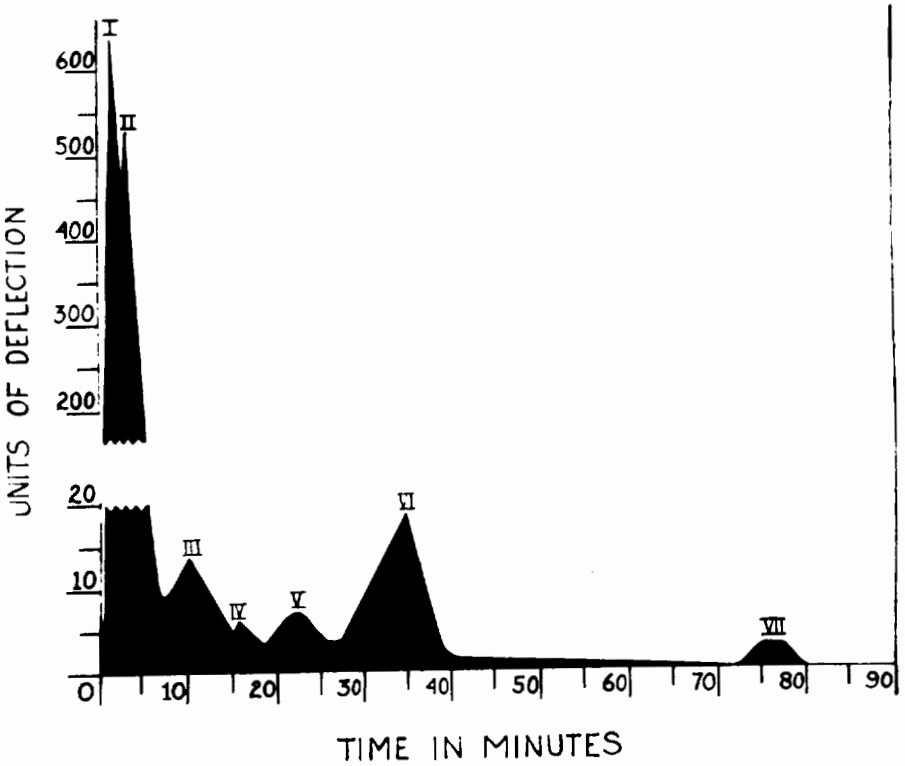

Figure 3. Chromatogram of the synthetic mixture on Celite-TCP at 25°C. and 20 ml./min. helium flow. The number of theoretical plates was 280.

allowed to fill the evacuated space across the top of the trap as far as the burette stopcock. The 3-way stopcock was then closed, and the burette stopcock was opened carefully to collect one cubic centimeter of gas. The burette stopcock was closed and the excess of sample gas was pumped off through the 3-way stopcock. The evacuated trap was placed in liquid nitrogen, one of its stopcocks was opened, and the sample gas was passed from the burette into the trap. The trap was then closed and the procedure was repeated with each of the other sample gases.

Six gases, viz., methyl chloride, propane, carbonyl sulfide, butane, 1, 3-butadiene, and 1-butene, were obtained from lecture bottles. Methane and ethane were collected from a sample of natural gas. Ammonia and water vapor were obtained by freezing ammonium hydroxide in a container placed in liquid nitrogen. By warming the container to room temperature, these vapors were transferred to the gas burette.

The 10 gases in this trap (designated Gas Trap) were maintained at liquid nitrogen temperature until the trap was connected to the chromatographic column.

Liquids. A mixture of equal volumes of acetaldehyde, acetone, acetonitrile, furan, isoprene, methanol, and propionaldehyde was prepared. Seven microliters of this mixture were transferred to a glass wool plug placed at the entrance of a U-shaped trap made of capillary tubing. This trap (designated Liquid Trap) was equipped with two 3-way vacuum stopcocks so helium could flow through or by-pass the sample chamber. The vapors from the liquid sample were swept by a flow of helium into the evacuated trap, which was cooled in liquid nitrogen. With the sample still frozen, the helium was pumped off by using a vacuum pump. The liquids were then allowed to vaporize before they were introduced onto the column.

Sample Introduction. The two traps containing the two portions of the synthetic mixture were connected in series to the chromatographic column by means of the glass joints. The Gas Trap was kept cold while helium, which by-passed both traps, swept air from the chromatographic system. The Liquid Trap containing the volatilized liquids was opened directly onto the column. The coolant was then removed from the Gas Trap and the gases were allowed to diffuse through the Liquid Trap and onto the column. Both traps were swept with helium for one minute, and then were by-passed for the duration of the chromatographic development.

Chromatographic Development. The apparatus used for the gas chromatographic procedure is shown schematically in figure 1.

Helium was withdrawn from a cylinder through a pressure regulator, was dried by being passed through a three-foot copper coil containing Linde molecular sieve No. 4A, and was delivered via a sensitive diaphragm regulator to the reference chamber of the thermal conductivity cell which was the detector. The gas

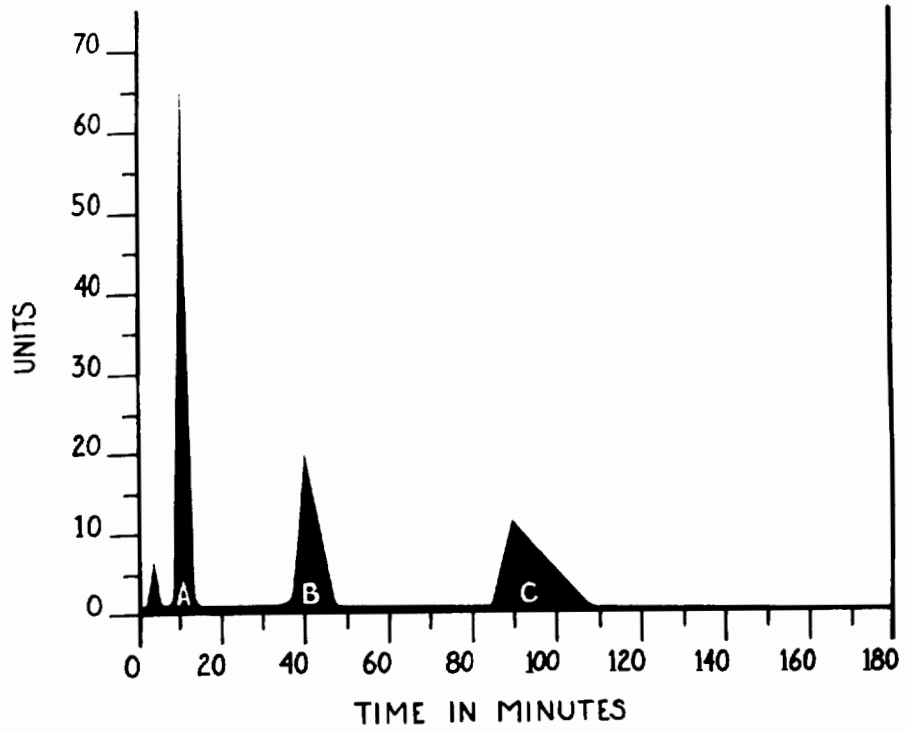

Figure 4. Chromatogram of fraction I on silica at 25° C. and 40 ml./min. helium flow.
A. Carbonyl sulfide plus propane.
B. n-Butane.
C. 1-Butene.

was next passed through a glass column which was packed with an adsorbent, or with a stationary liquid supported on Celite. Upon emerging from the column, the gas stream passed through the sample chamber of the detector and was vented through a rotameter. (Neither the columns nor the detector chamber were thermostatted.)

A recording potentiometer traced the chromatographic pattern from the signal given by the detector. This signal represented the difference in composition between the gases in the reference and in the sample chambers of the thermal conductivity cell (operated at 138 ma).

Fraction Collection. Prior to use the fraction collection traps (figure 2) were cleaned, the stopcocks were well greased with vacuum stopcock grease, and the assembled traps were evacuated while warm. They were then cooled in liquid nitrogen and filled with helium. This procedure prevented back-diffusion of air into these traps when the stopcocks were first opened to collect a sample. (These precautions were necessary to prevent contamination of the sample by moisture and carbon dioxide because relatively large amounts of these contaminants contribute large partial pressures to the total pressure of the sample. When these are present, the mole percents of small amounts of unknown vapors are very low, and their peak heights in the mass spectrometric patterns are of insufficient magnitude for identification.)

The traps were constructed with a by-pass so several could be used in series. The trap farthest from the column's exit was used first so it could be disconnected and its contents analyzed while other fractions were being collected.

All the traps were kept immersed in liquid nitrogen to insure complete condensation of the column's effluent vapors. Containers readily fabricated from Styrofoam were very convenient for holding this coolant and were much less expensive than Dewar flasks.

Mass Spectrometry. The ball and socket joints on the fraction collection traps permitted easy connection to the mass spectrometer's gas inlet system. Precision ground vacuum stopcocks were used in these traps to prevent air contamination during the introduction of the sample into this evacuated system. The trap from the chromatographic system contained both helium and sample; the helium was removed prior to analysis by evacuation at liquid nitrogen temperature. Both stopcocks on the trap were then closed, the liquid nitrogen was removed, and the trap was warmed to room temperature before the sample was introduced into the mass spectrometer. The sample was analyzed in the usual manner and the unknown chromatographic fraction was identified from its mass

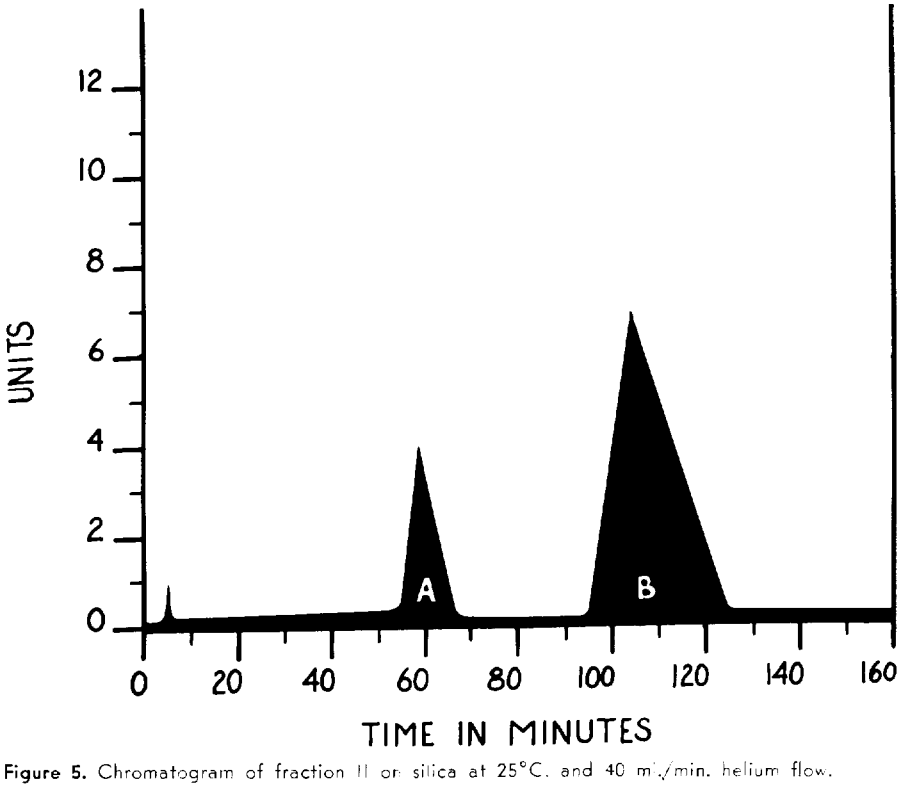

Figure 5. Chromatogram of fraction II on silica at 25°C. and 40 ml./min. helium flow.

A. Methyl chloride.
B. 1-Butene.

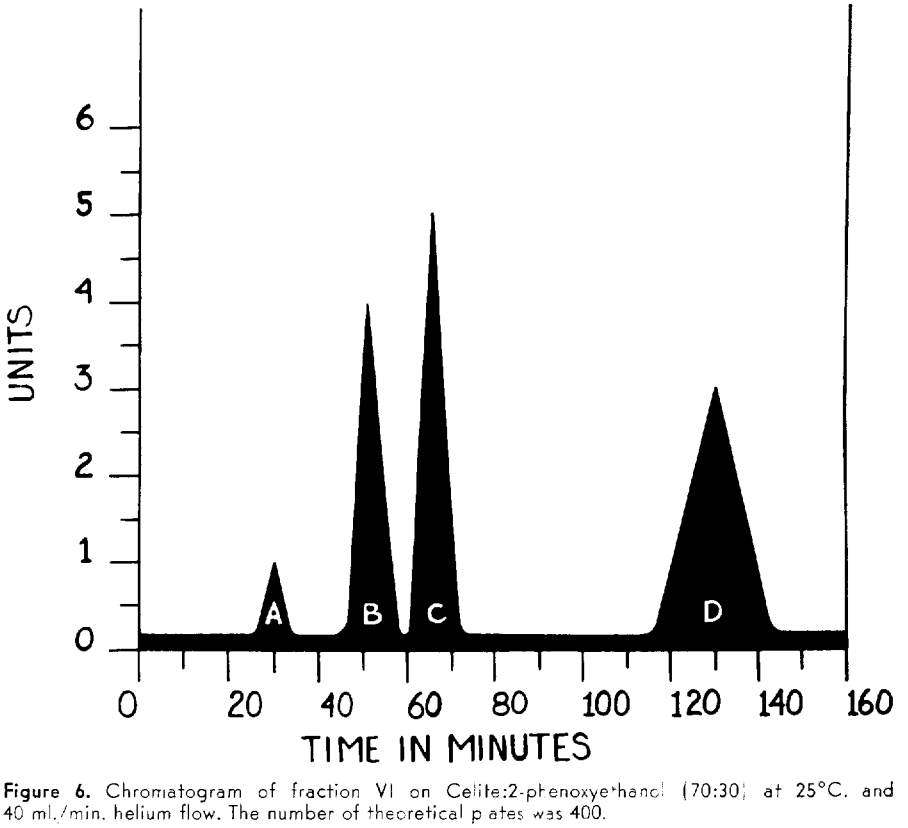

Figure 6. Chromatogram of fraction VI on Celite:2-phenoxyethanol (70:30) at 25°C. and 40 ml./min. helium flow. The number of theoretical plates was 400.

A. Propionaldehyde.
B. Acetone.
C. Methanol.
D. Water.

I	II	III	IV	V	VI	VII
NH_3*	CH_3Cl	$CH_3\text{-}CH{=}O$	$CH_2{=}C(CH_3)\text{-}CH{=}CH_2$		$CH_3\text{-}C(=O)\text{-}CH_3$	CH_3CN
CH_4*	C_4H_8				$C_2H_5CH{=}O$	
C_2H_6*	$CH_2CHCHCH_2$				CH_3OH	
C_3H_8					HOH	
C_4H_{10}						
C_4H_8*						
COS						

*Trace Quantities

Figure 7. Mass spectrometric analysis of the original fractions.

spectrum.

If the spectrum of the sample were too complex for positive identification, the vapors were returned to the trap by submerging the trap in liquid nitrogen. This sample was then rechromatographed on a new column which was packed with a different immobile phase.

Separation of the Synthetic Mixture

The compounds that were present in the original synthetic mixture are listed in table 1. This mixture was partitioned at 25°C. between tricresyl phosphate supported on Celite and helium which flowed at 20 ml./min. Seven primary peaks were recorded, as shown in figure 3. The first fraction, indicated by peak I, was trapped and further purified by adsorption chromatography on silica. The chromatogram was developed at room temperature with helium flowing at 40 ml./min. Four secondary fractions resulted (figure 4). The first of these, emerging with the air, contained methane and ethane. The second, emerging in 11 minutes, contained carbonyl sulfide and propane. (These pairs of compounds were not further separated since it was a simple matter to identify them by mass spectrometry.) The third secondary fraction, emerging at 36 minutes, contained n-butane; the fourth, emerging at 92 minutes, contained 1-butene.

Primary fraction II, emerging from the initial column at 5 minutes, was rechromatographed, again using tricresyl phosphate as the immobile liquid. This produced two rather broad peaks. The first peak represented methyl chloride and ammonia, and those compounds that trailed from fraction I (carbonyl sulfide, propane, butane, and 1-butene). These compounds were further separated at room temperature on a column filled with silica. This duplicated the separation just described for primary fraction I, with an added fraction identified as methyl chloride. Ammonia was held tightly and was not recovered from the adsorption column.

The constituents from primary fraction II (figure 3) which were represented by the second broad peak were trapped during the three to nine minute period. This fraction was rechromatographed on silica at room temperature; it yielded two single component peaks (figure 5). Methyl chloride emerged in 62 minutes, 1-butene in 108 minutes, 1,3-Butadiene was not desorbed from the silica under these conditions.

Primary peaks III, IV, and V represented simple binary mixtures. The principal component of the 11 minute fraction was acetaldehyde; that of the 18 minute fraction, isoprene; that of the 21 minute fraction, furan. Fraction III and fraction IV were contaminated with 30% of each other. Further separation of the two constituents in these fractions was accomplished on a column filled with Hyvac oil coated on Celite. Acetaldehyde emerged in three minutes and isoprene in 18 minutes. Improved separation of furan from isoprene was obtained with butyl butoxyethyl phthalate as the immobile liquid; isoprene emerged in 13 minutes, and furan in 26 minutes.

The fraction represented by primary peak VI was rechromatographed at room temperature on a column filled with a Celite—2-phenoxyethanol mixture. Four pure compounds were isolated: propionaldehyde at 32 minutes; acetone at 52 minutes; methanol at 66 minutes; and water at 135 minutes (figure 6).

The seventh and last fraction collected during the initial chromatographic development contained essentially pure acetonitrile.

Discussion

Figure 7 lists the compounds which were present in the seven primary fractions, as identified by mass spectrometric analysis. The synthetic mixture consisting of 17 compounds was completely broken down; each recovered compound was obtained in pure form. Exceptions were the methane-ethane mixture and the carbonyl sulfide-propane mixture. These pairs might have been resolved by using longer columns of either silica or tricresyl phosphate or by operating at lower temperatures.

A secondary detector is essential to supplement gas chromatography when one is working with very complex mixtures whose composition is completely unknown. The mass spectrometer is excellent for this purpose. Its value is well illustrated by the carbonyl sulfide-propane peak in figure 4. This pair remained unresolved even after passage through a partition-type column and then through an adsorption-type column. The final elution curve for this pair was so extremely sharp and symmetrical that there was no indication of the presence of a mixture. Only mass spectrometric analysis proved that this fraction was not unicomponent.

The utility of the mass spectrometric analysis was demonstrated further in the identification of the methyl chloride fraction (figure 5). Without this analysis, that fraction would certainly have been identified as a known hydrocarbon having a similar retention volume.

These examples should serve to caution those who may wish to utilize gas chromatography as a qualitative tool. They should beware of placing too great reliance upon retention times alone as identifying criteria. They should not accept the occurrence of single sharp peaks as assurance of the separation of a single compound from a complex mixture. Repeated chromatography with varied liquid phases will greatly reduce the possibility of these errors. Mass spectrometry is ideally suited as a guide for avoiding these pitfalls.

Summary

A synthetic mixture of 17 components was prepared to simulate the heterogeneous mixture that might be produced by the pyrolysis of a na-

tural product such as tobacco.

Each of the constituents was separated by gas chromatography through the use of the adsorption and partition techniques. Mass spectroscopy insured unequivocal identification after the constituents had been isolated by repeated chromatography through various stationary phases.

Acknowledgment

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Literature Cited

James, A. T., and Martin, A. J. P., "Gas-Liquid Partition Chromatography: The Separation and Microestimation of Volatile Fatty Acids from Formic Acid to Dodecanoic Acid", *Biochem. J.*, **50**: 679-690 (1952).

Osborne, J. S., and Hobbs, M. E., "Some Constituents of the Gas Phase of Cigarette Tobacco Smoke", presented at the Eighth Annual Tobacco Chemists' Conference, Richmond, Virginia, November 12, 1954.

Osborne, J. S., Adamek, S., and Hobbs, M. E., "Some Components of Gas Phase of Cigarette Smoke", *Anal. Chem.*, **28**: 211-215 (1956).

Seligman, R. B., Resnik, F. E., O'Keeffe, A. E., Holmes, J. C., Morrell, F. A., and Murrill, D. P., "New Techniques of Smoke Analysis: I. Vapor Phase Chromatography", presented at the Ninth Annual Tobacco Chemists' Conference, Raleigh, North Carolina, October 6, 1955.