

# Tobacco Chemistry. XIV. Sampling, Concentration, and Examination of Tobacco Headspace Vapors<sup>1</sup>

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A charcoal trapping system for tobacco headspace vapors has been evaluated. Adsorption on charcoal of a test mixture containing compounds of different polarities and subsequent extraction with propane and dimethyl ether followed by distillation showed that artifact formation under these conditions was absent. The recoveries of individual compounds varied somewhat but were typically 50%. Distillation losses were also estimated and found to be of the order of 10-15% for the compounds used.

Application of these procedures to the headspace volatiles of both fermented and nonfermented Virginia tobacco yielded extracts of complex nature, the gas chromatographic patterns of which were significantly different. Identification of some 30 constituents, mainly in the extract from the fermented tobacco, was accomplished by mass spectrometry in combination with capillary gas chromatography. The method is applicable for studies of tobacco fermentation.

The headspace vapors over tobacco are of considerable interest, since they may reflect the state of maturity of tobacco and should represent an important part of the aroma of tobacco smoke. It was therefore considered desirable to study these vapors and hence initially to obtain a method suitable for evaluation of the individual constituents. Earlier studies of the volatile constituents of tobacco have been extensive and have been critically reviewed by Stedman in 1968. The tobacco headspace has previously been investigated (Swain *et al.*, 1966) and its complexity has been well demonstrated by Grob and Grob in 1970. Many of the significant aroma constituents occur only in very low concentrations (Kimland *et al.*, 1972) and therefore a sampling method which could provide larger quantities of material would be required for examination of tobacco headspace vapors. Accordingly, direct headspace analyses of volatile organic compounds, already an important technique for obtaining information on the quality of food products (Teranishi *et al.*, 1971), was considered inadequate for the present problem. This is because certain significant aroma compounds will evidently be below the accessible detection range if some concentration step is not included in the procedure, and formation or disappearance of compounds important for the aroma may not be observable when the headspace vapors of tobaccos of different maturities are compared.

An attractive sampling method appeared to be adsorption on active charcoal. Charcoal has successfully been used for small-scale collection of tobacco volatiles (Grob and Grob, 1970) as well as for the collection of the flavor of food products, *e.g.*, apples (Paillard, 1965; Strackenbrock, 1961), onions (Carson and Wong, 1961), peas (Ralls *et al.*, 1965), and apricots (Tang and Jennings, 1967). Most often, the volatiles were present together with large amounts of water or water vapor. Active charcoal shows good affinity for organic compounds and the presence of water does not seem to affect its activity (Hassler, 1963). These properties permit the collection of headspace vapors of tobacco to be carried out at room temperature and atmospheric pressure, while retaining the initial water content of the tobacco (approx-

mately 11%), thus yielding conditions resembling those in a cigarette. The constant water content will enhance the production of volatiles and the low temperature will minimize degradation of the tobacco (Jones and Weybrew, 1962).

The present work was undertaken to evaluate a charcoal-trapping system for headspace vapors of tobacco. In addition, a preliminary study of headspace volatiles of fermented and nonfermented Virginia tobacco has been carried out.

## EXPERIMENTAL

**Charcoal Purification.** Charcoal (Merck's Active Charcoal for Gas Chromatography, 20-35 mesh, 100 g) was extracted in a Soxhlet apparatus with pentane for 48 hr and diethyl ether for 48 hr. The extracted charcoal was kept under vacuum (water pump) and slowly heated to 400° over a period of 6 hr. Remaining traces of impurities were removed by reducing the pressure further ( $10^{-3}$  mm) while keeping the temperature at 400-500°. After cooling to room temperature, the charcoal was immediately transferred to glass ampoules which were sealed and stored until required.

**Transfer of the Test Mixture onto the Charcoal.** Isopropylacetate, *n*-pentan-1-ol, 2-hexen-1-al, carvone, linalool, and isovaleric acid (100 mg of each) were applied to a plug of cotton previously purified by extraction with ether. This was placed in a cylindrical glass tube (11 cm long and a diameter of 2.8 cm) which was connected in series with another glass tube of the same dimensions containing purified active charcoal (20 g). Care was taken not to contaminate the system with foreign material. Oxygen-free nitrogen was flushed through at room temperature (flow rate 200 ml/min) until quantitative transfer of the compounds was accomplished.

**Extraction and Distillation.** The active charcoal containing the transferred test mixture was extracted twice with propane (bp -42.1°, 2 × 1 l.) for 3 days at 9.5 atm and, thereafter, twice with dimethyl ether (bp -23.7°, 2 × 1 l.) for 3 days at 6.5 atm at 25°. The specially designed stainless steel extractor (Figure 1) was filled with solvent by transfer of the gas phase under pressure, which was liquified when the water cooler was switched on. The gases were of the highest commercially-available purity (dimethyl ether, 99.9%; propane, 95.2% C<sub>3</sub>, 1.1% C<sub>2</sub>, 2.1% *n*-C<sub>4</sub>, 1.6% *i*-C<sub>4</sub>). The bottom part of the extractor was then heated in thermostated water bath (25°) in order to start the extraction. After the extraction was terminated, the bottom part of the extractor was connected to a distillation flask and cooled with Dry Ice.

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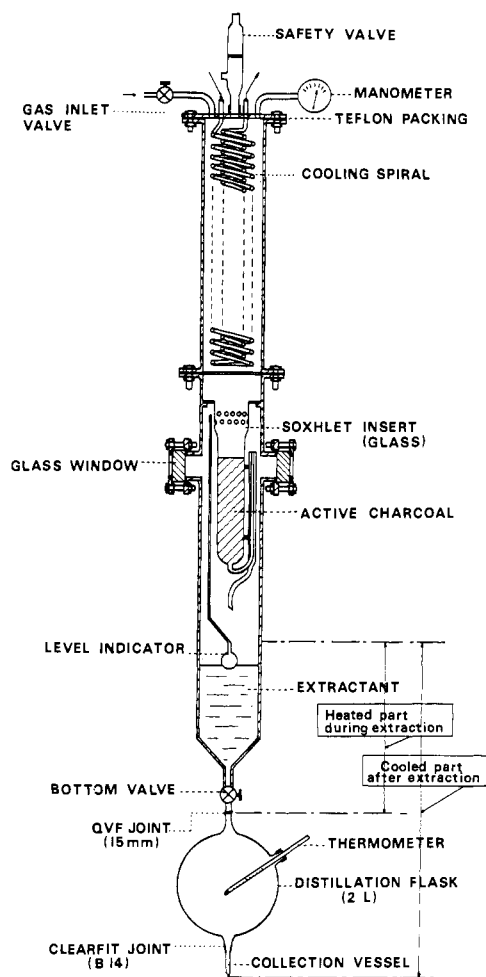


Figure 1. Stainless steel pressure extractor

When the pressure in the extractor had dropped nearly to atmospheric, some of the Dry Ice was removed to be able to open the bottom valve. The thermometer connection was loosened somewhat to prevent an overpressure in the distillation flask. After transfer of the extract to the distillation flask, some additional gas was introduced into the extractor. This allowed rinsing of the extractor, giving a more complete transfer of the extract. The distillations were carried out at ordinary pressure. A vacuum-jacketed Vigreux column (60 cm long, 1.5 cm i.d.), equipped with a cold finger distillation head cooled with  $\text{CO}_2$ /acetone, was used. The distillation flask was immersed in an acetone bath, which was kept at a few degrees above the boiling point of the solvent used for the extraction. In this way, a fairly constant distillation rate could be maintained. Most of the residual solvent was removed by increasing the bath temperature to  $0^\circ$ .

**Sampling of the Tobacco Volatiles.** Fermented and non-fermented Virginia tobacco (3 kg each) was cut to dimensions similar to those used in cigarette manufacture and loaded into a glass column (17  $\times$  100 cm, cf. Figure 5). Through this column, air (4–5 l./min) was passed. The gas stream had previously been humidified by passage through a wash bottle containing distilled water saturated with ammonium nitrate, thus holding the relative humidity at ca. 60%, a normal condition for tobacco storage, giving the tobacco a moisture content of about 11%. Before this humidified air was passed through the column it was filtered through an active charcoal filter (15  $\times$  4 cm). The volatiles of both the fermented and the nonfermented Virginia tobacco were collected at room

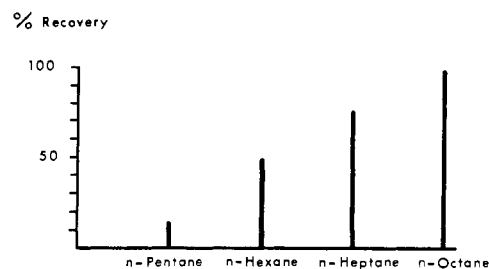


Figure 2. Recoveries of a hydrocarbon mixture in a distillation test with propane

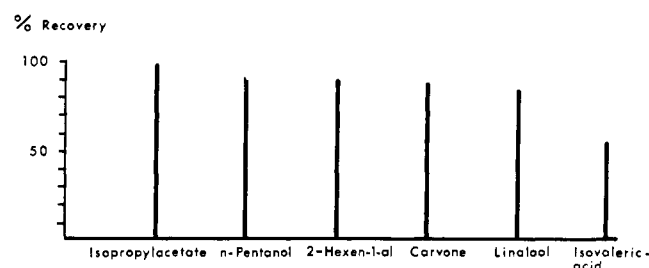


Figure 3. Recoveries of a six-component test mixture in a distillation test with propane

temperature for a period of 4 weeks on purified active charcoal (filter size 15  $\times$  4 cm, 25 g active charcoal). The charcoal was extracted for 1 week with 1 l. of propane and for 1 week with 1 l. of dimethyl ether. The extracts were worked up as described above.

**Gas Chromatography and Mass Spectrometry.** Gas chromatography was performed on a Varian 1700 instrument equipped with a capillary injector and a flame ionization detector. The capillary columns used in the present study were made by coating stainless steel tubes (50 m  $\times$  0.5 mm or 50 m  $\times$  0.25 mm, Handy and Harman grade 316-S) with a 10% solution of OV 17 or Ucon Oil HB 2000 in methylene chloride.

The gc-ms was carried out on a modified LKB 9000 instrument operated at 70 eV, in which the original gas chromatograph had been replaced by a homemade unit equipped with a capillary injector, a device for introduction of makeup gas, a splitter, and a flame ionization detector (FID). The signal from the FID could be detected a few seconds prior to that of the total ion current sensor of the mass spectrometer, due to the different traveling times after the splitter (Roeraade and Enzell, 1969). The helium separator was kept at a temperature of  $250^\circ$  and the ion source temperature was  $270^\circ$ .

## RESULTS AND DISCUSSION

The charcoal chosen for this work was selected to assure a reasonable surface area and to provide acceptable characteristics with respect to adsorption, solvent extraction of adsorbed volatiles, and pressure drop in cylindrical filters. It proved to contain certain impurities and was therefore extracted with pentane and ether for several days and subsequently heated to high temperature under vacuum to remove both the solvent and possible traces of impurities.

It has been postulated several times (e.g., Carson and Wong, 1961; Heinz and Jennings, 1966) that artifact formation may occur during adsorption or removal of sensitive organic compounds from active charcoal, but clear tests to substantiate this are lacking. In fact, investigations with low-boiling esters and alcohols (Dhont and Weurman, 1960; Paillard, 1965) have shown that such compounds do not alter after trapping on charcoal and subsequent liberation. In order to obtain

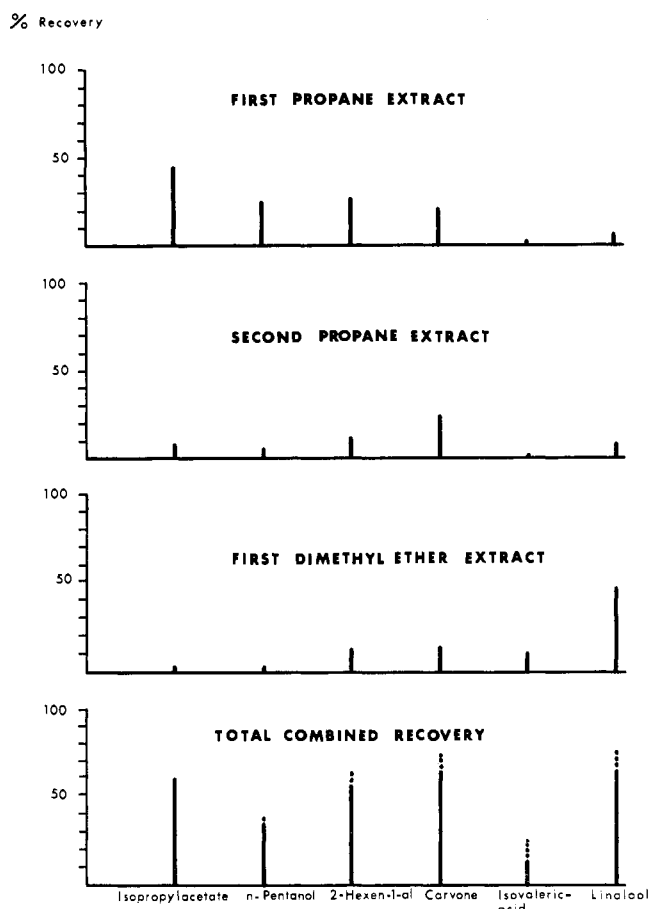


Figure 4. Recoveries of a six-component test mixture obtained after trapping on charcoal, extraction, and distillation. (The second dimethyl ether extract contained only negligible amounts and is not shown.) Full lines designate values uncorrected for distillation losses and dotted lines indicate values corrected for these losses

more data on possible artifact formation, a selected test mixture containing isopropylacetate, 1-pentanol, 2-hexen-1-al, carvone, linalool, and isovaleric acid was used. These compounds were chosen to permit evaluation of the possible occurrence of isomerization, catalytic dehydration, esterification, hydrolysis, and condensation reactions. Simultaneously, information on recoveries of compounds of different polarities could be obtained for the system used.

Removal of volatile organic compounds can be accomplished by means of vacuum desorption at elevated temperature (Paillard, 1965; Strackenbrock, 1961) or by solvent extraction (Carson and Wong, 1961; Ralls *et al.*, 1965; Tang and Jennings, 1967) and yields are usually high in both cases. Since desorption at elevated temperature is more likely to induce chemical reactions, solvent extraction seemed preferable for the present work. It has been pointed out by Hassler in 1963 that the yield obtained for compounds first adsorbed on charcoal and then removed by solvent extraction is dependent on the ratio of the weight of the charcoal relative to the weight of the trapped organic compounds. A high recovery rate can thus be obtained, when trapping is continued until the charcoal is almost saturated. Turk and van Doren (1953) found that charcoal can take up to 23% of organic compounds (apple volatiles) before this saturation point is attained. Unfortunately not all the compounds are equally strongly adsorbed on the charcoal, which makes sampling near the saturation point less attractive since certain compounds will be partly or completely displaced by others. An interesting approach has recently been reported by Grob and Grob

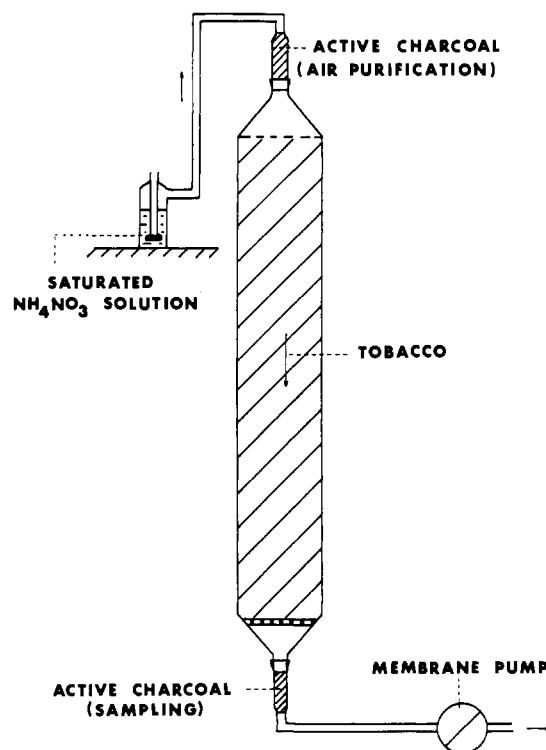


Figure 5. Collection of the tobacco headspace volatiles

(1971), where two filters are used in series. The first filter is allowed to saturate and the second collects the displaced compounds. This permits an easier estimation of the optimum amounts of charcoal required. In the present study the amount of the adsorbed organic compounds did not exceed 5% of the weight of the charcoal and the test compounds were transferred onto the charcoal, using a stream of nitrogen as the transport medium.

The yields obtained by extraction of volatile organic compounds adsorbed on charcoal are also dependent on the choice of extractant. The most complete extraction is obtained with a solvent for which the affinity of the charcoal is greater than for all the compounds to be extracted (Hassler, 1963). Although ether has commonly been used for extraction and carbon disulfide has been stated to have superior properties (Grob and Grob, 1971; Jennings and Nursten, 1967), propane and dimethyl ether were used in the present work on account of their low boiling points. The extraction was carried out in a specially designed Soxhlet apparatus at room temperature under pressure (Figure 1).

The charcoal was extracted twice with propane and thereafter twice with dimethyl ether in order to observe the influence of the solvent polarity on the extraction and also to obtain values of the extraction rates for the individual components. The solvents were removed by distillation at ordinary pressure through a Vigreux column and, although their low boiling points enhance the recoveries of the highly volatile compounds, certain losses were experienced. Such effects have been pointed out previously (Stedman and Miller, 1963; Weurman, 1969) and are particularly noticeable on concentration of very dilute solutions of volatile compounds, which can result in severe losses of volatiles, *e.g.*, Stedman and Miller have reported these losses to be more than 70% when concentrating an ether solution of methyl propionate (bp 80°, concentrate 36 mg/l.) even when rather sophisticated distillation equipment was used.

Consequently, it was of critical significance to obtain information on the recoveries after use of our low-temperature

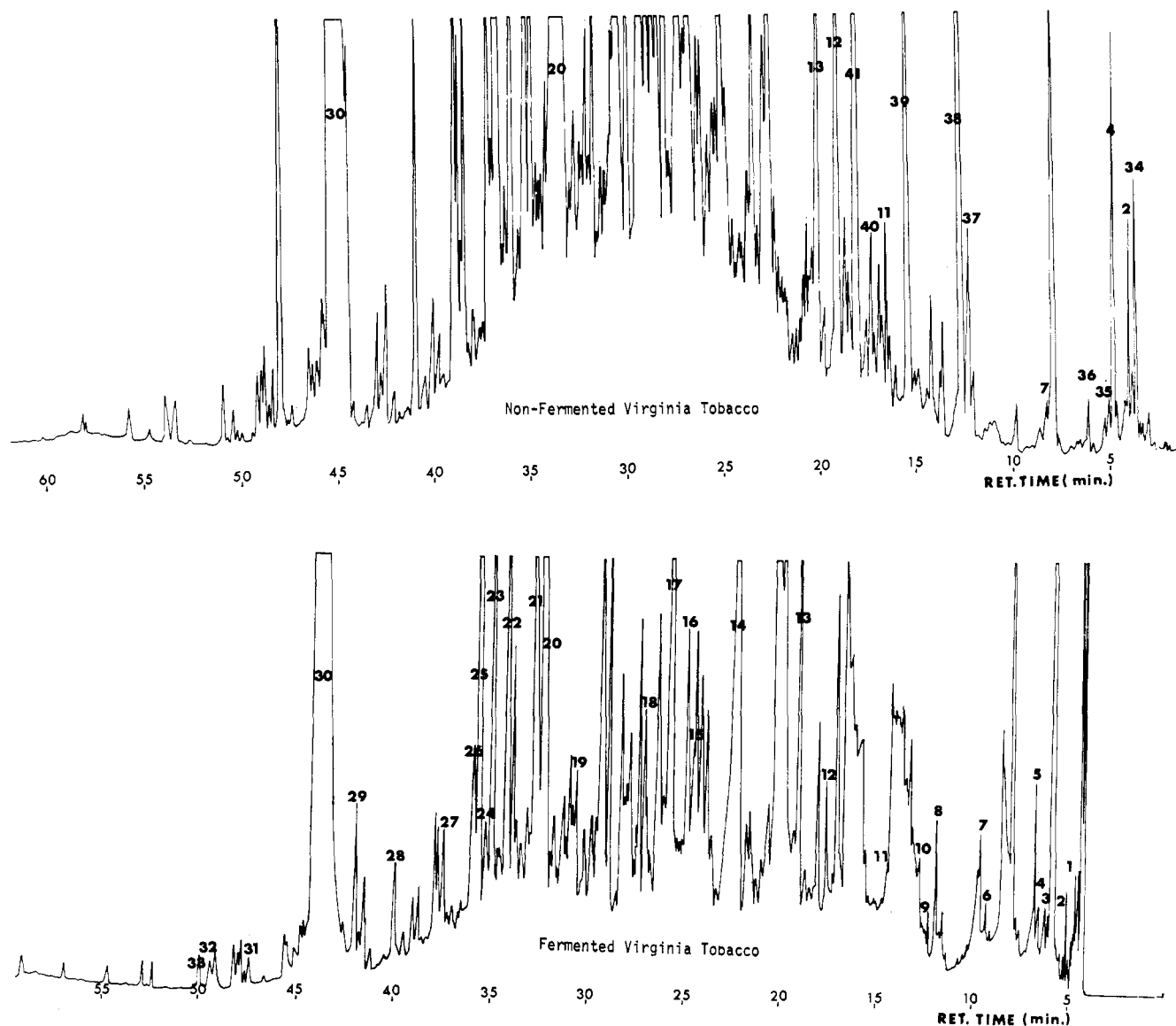


Figure 6. Gas chromatograms of the neutral fractions of the propane extracts of fermented and nonfermented Virginia tobacco headspace. Column: 50 m  $\times$  0.5 mm stainless steel capillary; stationary phase: OV 17; flow: 2 ml/min; temperature programmed from 25–250°, 4°/min

distillation system. A solution of 100 mg each of *n*-pentane (bp 36.1°), *n*-hexane (bp 69°), *n*-heptane (bp 98.4°), and *n*-octane (bp 125.6°) in 1 l. of propane was concentrated by distillation and the residue was examined by gas chromatography. The recovery rates were calculated and are presented in Figure 2, which shows that even compounds with boiling points as low as *n*-pentane will be observable and that the recovery of compounds with the same boiling points as *n*-octane (or higher) are quantitative, provided that no azeotropic effects influence the situation.

Losses of the individual compounds might also be expected during the distillation of the six-component test mixture extracted from the active charcoal. The same distillation test used for the hydrocarbons was therefore performed and the results are shown in Figure 3.

The individual and total recoveries of the six test compounds from the charcoal, corrected as well as uncorrected for distillation losses, are presented in Figure 4. The net efficiency of the overall procedure is of course shown by the uncorrected values.

Following this evaluation, the trapping, extraction, and distillation techniques described above were used in a preliminary study of fermented and nonfermented Virginia

tobaccos. It was of particular interest to note recoveries as well as differences in composition observed for the volatile concentrates obtained from the two samples. The headspace of the tobaccos was collected on charcoal, as shown in Figure 5.

After extraction of the charcoal with propane and dimethyl ether followed by distillation, extracts having extremely powerful odors were obtained. The propane extracts had a pleasant tobacco smell while the dimethyl ether extracts had a smell strongly reminiscent of short-chain fatty acids. The yields obtained were rather small, in the order of 100 mg for both tobacco types. (Later studies have led to improved yields.)

Gas chromatographic analyses of the concentrates which, before concentration were combined to give one propane and one ether extract of each tobacco type, showed, in accordance with expectation, that the extracts were extremely complex mixtures. In order to simplify the gas chromatographic patterns, the extracts were separated into acidic, basic, and neutral fractions. The propane extracts proved to contain the main part of the neutrals, while the dimethyl ether extracts contained the more polar compounds, especially acids. Capillary gas chromatograms of the neutral

**Table I. Compounds Identified by Mass Spectrometry in the Neutral Fractions of the Headspace of Fermented and Nonfermented Virginia Tobacco. (The Numbers Correspond to Those in the Gas Chromatograms of Figure 6)**

No.	Compound	Fermented tobacco	Non-fermented tobacco
1	Methylcyclopentane	X	
2	3-Methylhexane	X	X
3	Ethyl acetate	X	
4	Benzene	X	X
5	Propanol	X	
6	Dihydropyran	X	
7	Toluene	X	X
8	Dimethylbenzene	X	
9	<i>n</i> -Decane	X	
10	Furfural	X	
11	Methylethylbenzene	X	X
12	6-Methyl-5-hepten-2-one	X	X
13	Benzaldehyde	X	X
14	Benzyl alcohol	X	
15	Benzyl acetate	X	
16	2-Acetylpyrrole	X	
17	$\beta$ -Phenylethanol	X	
18	<i>n</i> -Tetradecane	X	
19	$\beta$ -Phenylethyl acetate	X	
20	Solanone	X	X
21	<i>n</i> -Pentadecane	X	
22	Damascenone	X	
23	<i>trans</i> - $\beta$ -Damascone	X	
24	Thymohydroquinone dimethyl ether	X	
25	Geranylacetone	X	
26	<i>n</i> -Hexadecane	X	
27	$\beta$ -Ionone	X	
28	Dimethyl phthalate	X	
29	Dihydroactinidinolide	X	
30	Neophytadiene	X	X
31	Eicosane	X	
32	Methyl palmitate	X	
33	Farnesyl acetone	X	
34	<i>n</i> -Hexane		X
35	2,5-Dimethylhexane		X
36	Dimethylcyclohexane		X
37	Ethylbenzene		X
38	3,7,7-Trimethylbicyclo-[3.1.1]-2-heptane		X
39	$\beta$ -Pinene		X
40	Trimethylbenzene		X
41	Limonene		X

fractions of the propane concentrates are presented in Figure 6, and considerable differences can be observed.

Mass spectrometry in combination with capillary column gas chromatography was carried out on the two neutral fractions of the propane extracts and led to the identification of the compounds listed in Table I on the basis of their mass spectra. Most of these compounds have previously been found in tobacco (Kimland *et al.*, 1972; Stedman, 1968). Although several other compounds could be identified tentatively, they are not presented here.

One reason why fewer compounds from the nonfermented tobacco could be identified is that many compounds exhibited considerable tailing during the gas chromatographic analyses, thus obscuring following peaks.

Further investigations were made on the combined acidic fractions from the fermented tobacco to provide guidelines for future work. After converting the compounds to methyl esters with diazomethane, gas chromatography-mass spectrometry revealed the presence of all the straight-chain acids between C<sub>2</sub> and C<sub>11</sub>, as well as isovaleric and benzoic acid.

The basic fractions were found to contain almost exclusively nicotine.

## CONCLUSIONS

The results obtained from the experiments with the test mixture suggest that active charcoal does not cause artifact formation. This is in accordance with previous findings (Dhont and Weurman, 1960). Moreover, the fact that many sensitive compounds previously encountered in tobacco could be shown to be present implies that the active charcoal method appears comparable to the extraction procedures used earlier to establish the presence of these tobacco constituents. Although air was used during the sampling for the sake of simplicity, the use of an inert gas, to be applied in future studies, would constitute a safeguard against oxidation.

The influence of the polarity of the solvent is difficult to evaluate on the basis of the present results. Thus, while isovaleric acid and linalool were only efficiently extracted by dimethyl ether, pentanol was readily displaced by propane. The use of the two extractants ensured that none of the compounds was completely retained on the charcoal, although the yields varied considerably.

It may be concluded from the gas chromatographic and mass spectrometric results that, when a more detailed analysis is required, a pre-separation of the extracts has to be carried out, as well as an improvement of the gas chromatographic separation. The latter is adversely affected by peak tailing of many of the polar compounds and, therefore, capillary columns with a more inert surface and more suitable stationary phases are desirable.

Although the limited separation hampered a full comparison of the two extracts, it appeared that several of the important aroma constituents do not occur in the nonfermented tobacco, or at least not to the same extent as in the fermented tobacco. This would be in accordance with the previously presented concept (Kimland *et al.*, 1972) that higher isoprenoids can degrade to components, many of which are significant for the flavor during the curing and fermentation processes.

The results obtained in this study show that charcoal is suitable for sampling of tobacco headspace vapors, and will be used in subsequent studies of the curing and fermentation processes.

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