[CONTRIBUTION FROM THE INSTITUTE OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY-BELLEVUE MEDICAL CENTER]

Isolation and Identification of Some Components of Cigarette Smoke Condensate[1]

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Alkali extraction of a fraction from cigarette smoke condensate gave a mixture of long chain acids and phenols. Chromatographic separation of the methyl esters and ethers gave a mixture of the esters of normal long chain monocarboxylic acids. Mass spectral and vapor phase chromatographic analysis showed that the mixture consists mainly of the C_{13}–C_{18} acids, with palmitic acid predominating. In addition trace amounts of the higher acids up to C_{27} are present. Oleic acid and linolenic acids were isolated and identified by comparison with authentic samples. Other products from the acid fraction were more highly unsaturated fatty acids and their oxidation products and phenols. The neutral fraction contained long chain hydrocarbons and saturated and unsaturated long chain ketones.

In an earlier report from this laboratory[3] the preparation and fractionation of cigarette smoke condensate was described. Long term tests for carcinogenicity on mice[4] focused attention on the two most active fractions designated[3] K and M. In the present report some findings of a chemical investigation of fraction K are described. This fraction contains a large number and variety of compounds and can best be described as cigarette smoke condensate from which volatiles, bases, acetone-insolubles, and some of the aliphatic hydrocarbons have been removed. Fraction K constitutes 4.9% of the whole tar.

Preliminary experiments on fraction K showed that a satisfactory separation of the components could not be obtained by either high vacuum distillation or by chromatography. The material could be readily separated into a petroleum ether soluble and a petroleum ether insoluble fraction by repeated extraction with petroleum ether (b. p. 30–60°) at room temperature.

Both these fractions showed in their infrared absorption spectra pronounced bands in the C—H stretching, carbonyl, and carbon-carbon double bond regions. The petroleum ether soluble fraction was treated with aqueous alkali and separated into acidic, basic, and neutral components by extraction under appropriate conditions.

The ether soluble part of the crude mixture of acids and phenols obtained in the alkali treatment was chromatographed on acid-washed alumina. The ether eluate on evaporation left a light yellow wax which from its infrared absorption spectrum consisted of a mixture of *normal* long chain saturated and unsaturated monocarboxylic acids in the range C_{14} to C_{18}. No further separation could be affected by chromatography. The yellow wax was treated with diazomethane and after rechromatography on acid-washed alumina gave a number of distinct fractions:

(*a*) a colorless methyl ester m.p. 33–34°, the infrared spectrum of which is superimpossible on that of methyl palmitate. The analysis of the ester agreed with that of methyl palmitate and hydrolysis gave an acid m.p. 53–55°. This material was subjected to mass spectrographic analysis,[5] Table I. Acids with both even and odd numbers of carbon atoms were present and 94% of the mixture consisted of acids in the C_{13}–C_{18} range. Corroborative evidence for the mass spectral results was obtained from a vapor phase chromatographic analysis[6] conducted on the same sample.

TABLE I

MASS SPECTRAL ANALYSIS OF ACID MIXTURE AS METHYL ESTERS

Number of Carbon Atoms in Acid	Percent Composition
12	1
13	5
14	5
15	3
16	66
17	4
18	11
19	1
20	1

Shiroskaya[7] and Shmuk[8] have reported the presence of palmitic acid in tobacco leaf. It is probable that the material which they had in hand was a mixture similar to that obtained in the present investigation. Recently[9] the nature of the steam

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[3] A. I. Kosak, J. S. Swinehart, and D. Taber, *J. Nat. Cancer Inst.*, **17,** 375 (1956).

[4] W. E. Smith, N. Nelson, L. Orris, and A. I. Kosak, unpublished.

[5] By Drs. A. H. Boultbee and G. P. Hinds of Shell Oil Co., Houston, Tex.

[6] By Dr. K. E. Wilkens and staff of Wilkens Instrument and Research, Inc., Berkeley, Calif.

[7] V. N. Shiroskaya, *Sborn. Rabot Khim. Tabak Bull.*, **125,** 151 (1935).

[8] A. Shmuk, *Vsesoyuz Inst. Tabach i Makhoroch, Prom.*, **133,** 3 (1937).

[9] D. E. Buyske, P. Walder, and M. E. Hobbs, *Anal. Chem.*, **29,** 105 (1957).

volatile fatty acids up to C_{12} in cigarette smoke condensate was described.

(*b*) a liquid methyl ester, n_D^{45} 1.4560. The infrared absorption spectra of the methyl ester and the free oily acid were identical with those of authentic samples of methyl oleate and oleic acid, respectively. The acid gave a crystalline *p*-phenylphenacyl ester, m.p. 54–55° which gave no depression on admixture with an authentic sample of *p*-phenylphenacyl oleate. The refractive index for oleic acid is 1.4522 at 45°.[10] The higher value found may be due to the presence of traces of more highly unsaturated acids of higher refractive indices. The acid could be hydrogenated with platinum as catalyst. One mole of hydrogen was absorbed and stearic acid, m.p. 68°, undepressed on admixture with an authentic sample, obtained.

(*c*) a yellow oil, n_D^{45} 1.4730, which was hydrolyzed and brominated to yield 9,10,12,13,15,16-hexabromostearic acid, m.p. 180–181°. The melting point was not depressed on admixture with an authentic sample and the infrared absorption spectra were identical thus identifying the unsaturated acid as linolenic acid.

Since in the fractionation of whole tar by which fraction K is obtained basic components are removed by acid extraction,[3] the bases obtained in the alkali treatment of fraction K must have arisen by hydrolysis of structures such as amides which are hydrolyzed by alkali. The mixture of acids obtained from the alkali treatment (22.9% of fraction K) could have been present in K as such or as derivatives such as esters, amides, and anhydrides. Exhaustive extraction of fraction K with sodium bicarbonate gave a 14% yield of acids, *i.e.*, more than two-thirds of the acids and phenols in fraction K are present as free carboxylic acids. No indications were obtained from infrared absorption spectra for the presence of anhydrides so that the balance of the acids are probably present as esters and amides. The nature of the phenols in fraction K was not studied.

(*d*) a yellow oil, n_D^{45} 1.5112 which differed in its infrared absorption spectrum from the methyl esters described above in that it showed, in addition to aliphatic C—H and acid C=O, the presence of hydroxyl group (3.0μ), and carbon-carbon double bond absorption (6.2μ). This suggests the presence of more highly unsaturated long chain fatty acids and their auto-oxidation products. The infrared absorption spectra of such acids and their autoxidation products have been studied[11–13] and show a close similarity to the materials obtained in this study.

In addition to the materials listed above a number of other products were obtained as mixtures of various compound types. A mixture of phenols was obtained but not separated further. A mixture of long chain ketones, m.p. 65–75° gave an infrared absorption spectrum virtually identical with that of palmitone. This product gave a negative iodoform test for a methyl ketone. The available evidence suggests that this mixture consisted of long chain saturated ketones (symmetrical or with the carbonyl group near the center of the chain), with chain lengths approximating that of palmitone, $C_{31}H_{62}O$. Schürch and Winterstein[14] reported the isolation of a material which they tentatively identified as dipalmityl ketone. In addition a product which from its infrared spectrum appears to be a mixture of unsaturated long chain ketones was obtained. This material showed in the infrared absorption spectrum bands at 5.95 μ (conjugated carbonyl) and at 6.20 μ (C=C). A saturated, long chain aliphatic hydrocarbon was obtained from fraction K. This material was identical in infrared absorption spectrum and melting point with the product previously isolated in this laboratory[3] from a similar fraction of cigarette smoke condensate. This hydrocarbon was identified as a mixture of hentriacontane and tritriacontane[3] and is present in much lower concentration in the fraction described in this study.

EXPERIMENTAL[15]

Preparation of fraction K. The preparation and fractionation of cigarette smoke condensate from five popular brands of American cigarettes has been described in an earlier paper.[3] Fraction K represents a mixture of water-insoluble acidic and neutral components of high boiling points.

The acids and phenols from fraction K. Fraction K, a viscous dark brown liquid, 34.5 g., was stirred with petroleum ether (b.p. 30–60°) and the extract decanted off. This process was repeated until only minute amounts of material could be extracted at a time. The solvent was removed from the extract at 25°/20 mm. and gave 23.2 g. of a dark brown viscous liquid. The petroleum ether insoluble residue, 11.2 g., was a viscous brown resin. The petroleum ether soluble fraction was dissolved in 100 ml. ether and stirred with 30 ml. of 10% aqueous sodium hydroxide for one hour under nitrogen. The alkali extract was removed and the alkali extraction repeated three times by which time only negligible quantities of material were extracted by the alkali. The combined aqueous alkaline extracts were acidified with dilute aqueous hydrochloric acid (1:1) and extracted with ether (10 × 100 ml.). The ether extract was washed with water and evaporated to dryness under nitrogen. The product was finally dried at 1.0 mm. over phosphorus pentoxide; a dark brown resin, 7.34, g., was obtained. The ether solution left after removal of the acids was extracted with aqueous hydrochloric acid (1:1; 6 × 50 ml.). Removal of the ether gave 12.1 g. of a neutral fraction. The acidic aqueous solution was made alkaline with aqueous sodium hydroxide, extracted with ether and the solvent distilled off to give 0.30 g. of a

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(10) A. W. Ralston, *Fatty Acids and Their Derivatives*, J. Wiley and Sons, Inc., New York, 1948.

(11) O. S. Privett, W. O. Lundberg, N. A. Khan, W. E. Tolberg, and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **30**, 61 (1953).

(12) L. R. Dugan, B. W. Beadle, and A. S. Henick, *J. Am. Oil Chem. Soc.*, **26**, 681 (1949).

(13) C. S. Privett, C. Nickell, and W. O. Lundberg, *J. Am. Oil Chem. Soc.*, **32**, 505 (1955).

(14) O. Schürch and A. Winterstein, *Z. Krebsforsch.*, **42**, 76 (1935).

(15) All melting points are corrected.

brown oil with a strong amine odor. The amine gave a positive isocyanide test for a primary amine.

Chromatography of the acids and phenols. The crude mixture described in the previous experiment was separated into a petroleum ether soluble and a petroleum ether insoluble fraction. The petroleum ether soluble fraction, 6.6 g., was chromatographed on 200 g. of acid-washed alumina. No material could be eluted with eluents less polar than ether; the latter gave 4.5 g. of a yellow wax, m.p. 35–40°. The rest of the material was eluted with ethanol to give 0.6 g. of an orange colored resin. The wax from the ether eluate was soluble in aqueous alkali, ether, petroleum ether, chloroform, and ethanol, and insoluble in water. This product gave a *p*-phenylphenacyl ester, m.p. 87–88°. The infrared absorption spectrum of this was very similar in all respects to that of long chain *normal* monocarboxylic acids in the range C_{14}–C_{18}.

Chromatography of the methyl esters. The wax from the ether eluate described in the previous experiment was dissolved in ether and converted to methyl esters and ethers by treatment with an excess of diazomethane in ether at 0°. After standing overnight and removal of solvent, the residue was chromatographed on acid-washed alumina. Elution with a petroleum ether (b.p. 30–60°)-ether mixture (9.5:0.5) gave 1.8 g. of a colorless liquid, n_D^{24} 1.4521, neut. equiv. 285. Elution with ether gave 0.80 g. of a yellow oil and elution with ethanol gave 0.10 g. of a brown resin.

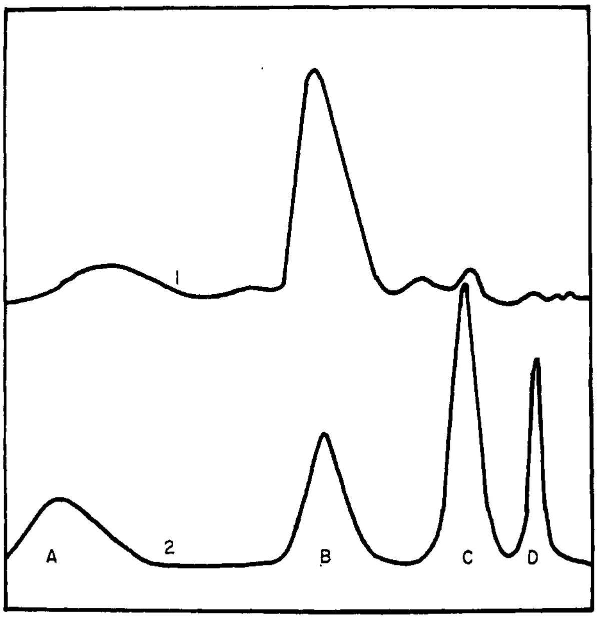

Fig. 1. Vapor phase chromatographic separation of long chain saturated acids (as methyl esters): 1. unknown mixture; 2. mixture of methyl esters of (a) stearic, (b) palmitic, (c) myristic, and (d) lauric acids. Silicone column five feet long, 225°, flow rate 100 ml./min.; chart speed 16 in./hr.

Rechromatography of the oil from the petroleum ether-ether eluate on alumina gave the following components:

(*a*) *Long chain monocarboxylic acids.* The petroleum ether eluate gave a colorless solid which was distilled in vacuum, n_D^{45} 1.4300 (reported:[10] methyl myristate, n_D^{45} 1.4281; methyl palmitate n_D^{45} 1.4317; methyl stearate n_D^{45} 1.4346), m.p. 33–34°.

Anal. Calcd. for $C_{17}H_{34}O_2$: C, 75.6: H, 12.6. Found C, 75.2; H, 12.5.

The results of the mass spectral analysis of this material is given in Table I. The various peaks obtained in a vapor phase chromatographic analysis corroborates the findings of the mass spectral analysis and are shown in Fig. 1. Hydrolysis of the mixture of methyl esters gave an acid, m.p. 53–55° (reported: myristic acid, 53–54°; palmitic acid, 62°; stearic acid, 69–70°).

(*b*) *Oleic acid.* A second fraction with the same eluent gave 0.20 g. of a colorless oil, n_D^{45} 1.4560. Hydrolysis of this ester gave a liquid acid which with *p*-phenylphenacyl bromide gave a crystalline ester, m.p. 50–53°. The product was purified by chromatography on acid-washed alumina with petroleum ether (b.p. 30–60°) as eluent followed by recrystallization from ethanol; m.p. 54–55°, undepressed on admixture with an authentic sample of *p*-phenylphenacyl oleate, m.p. 55–57°. The infrared absorption spectra of the free acid and the methyl ester were identical in every respect with that of oleic acid and methyl oleate, respectively. On hydrogenation of the free acid in ethanol with Adams catalyst one mole of hydrogen was absorbed. The product was recrystallized from ethanol-water to give a white solid, m.p. 67–68°, undepressed on admixture with an authentic sample of stearic acid, m.p. 69–70°.

(*c*) *Linolenic acid.* With petroleum ether (b.p. 30–60°)-ether (1:1) as eluent there was obtained 0.56 g. of a yellow oil, n_D^{45} 1.4730. The ester was hydrolyzed and the free acid isolated. The yellow oily acid was dissolved in 5 ml. of ether, cooled to 0°, and bromine added dropwise to the stirred solution until the yellow color persisted. On standing in ice yellow crystals separated. After standing at 0° for 8 hr. the mother liquor was decanted, the crystals washed with ether and recrystallized from dioxane; m.p. 180–181°. The melting point was undepressed on admixture with authentic 9,10,12,13,15,16-hexabromostearic acid and their infrared absorption spectra were identical. No other crystalline bromo acids could be obtained from the oily residue from the mother liquor. Chromatography on acid-washed alumina gave traces of the same hexabromostearic acid but no other crystalline compounds.

Infrared absorption spectra. All spectra were obtained in chloroform solutions with a Baird instrument equipped with sodium chloride optics.

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