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# Composition Studies on Tobacco VIII. Total Paraffinic Hydrocarbons in Aged and Fermented Tobaccos

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# Composition Studies on Tobacco

## VIII. Total Paraffinic Hydrocarbons in Aged and Fermented Tobaccos

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### Introduction

The occurrence of saturated, long chain, solid hydrocarbons, i.e. paraffins, in tobacco leaf and smoke has been known for many years. Although Zeise (1843) and Kissling (1883) isolated paraffin-like substances from smoke and leaf, respectively, these reports were not conclusive demonstrations (Kosak, 1954). In 1901, Thorpe and Holmes presented more acceptable evidence of the occurrence of paraffinic hydrocarbons in tobacco leaf, and since that time, numerous isolations from leaf (Chibnall *et al.*, 1934; Hukusima and Oike, 1940; Palfray *et al.*, 1941; Lam, 1955; Onishi *et al.*, 1956; Wynder and Wright, 1957; Dymicky and Stedman, 1959 and from smoke (Wenusch, 1934, 1937; Schurch and Winterstein, 1935; Kosak, 1956; Wright and Wynder, 1956; Izawa *et al.*, 1957; Wynder and Wright, 1957; Clemo, 1958; Trillat and Cuzin, 1958; Van Duuren and Kosak, 1958) have been reported.

Qualitatively, tobacco paraffins of leaf and smoke are believed to consist mainly of mixtures of  $C_{25}$ - $C_{36}$  homologues,<sup>2,3</sup> the material from smoke containing 75-90 per cent of

either mixed  $C_{30}$ - $C_{32}$  components (Cuzin *et al.*, 1958) or pure hentriacontane (Wright and Wynder, 1956). Quantitatively, the levels of paraffinic hydrocarbons in the leaf are reported to vary from approximately 0.06 to 1.0 per cent (Thorpe and Holmes, 1901; Kurilo, 1930a, b; Bruckner, 1936; Shmuk, 1937; Rowland, 1957; Cuzin *et al.*, 1958; Rayburn *et al.*, 1958) and to be relatively constant (Kurilo, 1930a, b) or widely variable (Bruckner, 1936) in different tobaccos. In the case of smoke, levels of crude hentriacontane from 0.05 to 0.36 per cent, based on 100 g of tobacco burned, have been reported for various European cigarettes (Clemo, 1958).

Since interest has recently developed in the possible role of paraffins as precursors of polycyclic hydrocarbons formed during smoking (Lam, 1957a, b; Wynder, 1957; Rayburn *et al.*, 1958), valid information on levels of paraffins in various tobaccos would seem desirable. The quantitative reports cited above do not provide such information since they were not systematic surveys of a wide variety of tobaccos and, of greater importance, the data were obtained using non-specific methods.<sup>4</sup>

### Methods

Classical methods of determining paraffinic hydrocarbons in tobacco

consist of extraction with a nonpolar solvent, either low temperature crystallization of the hot ethanol solubles from the extract or acetone precipitation of the original extract, and weighing of the precipitates thus obtained. Bruckner (1936) has described in detail his version of the procedure employing crystallization from ethanol. Preliminary studies on this method (*vide infra*) showed that it is nonspecific for paraffinic hydrocarbons; therefore, the following modification was employed in the present work.

### Analytical Method for Tobacco Paraffins

Essentially, the method consists of extraction of tobacco with Skellysolve B, treatment of the extracted material with concentrated sulfuric acid, column chromatography on silicic acid of the Skellysolve<sup>5</sup> solubles from the acid mixture and cold ethanol crystallization of the petroleum ether eluate from the column.

Thirty-seven and one-half g of tobacco (50 mesh) are extracted in a Soxhlet apparatus with 200 ml Skellysolve B for 25 hours. The extract is cooled to room temperature and filtered, after which the solvent is boiled off on the steam bath. The residue is cooled to room temperature and 35 ml of concentrated sulfuric acid are added incrementally with agitation. The mixture is then heated with manual agitation on a

<sup>1</sup> Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

<sup>2</sup> Part VII of this series described the isolation of a paraffinic hydrocarbon or a mixture of such hydrocarbons having more than forty carbon atoms from flue-cured leaves (Dymicky and Stedman, *loc. cit.*).

<sup>3</sup> In an extensive study of plant paraffins, Chibnall *et al.* (1934) concluded that the evidence for the presence of only odd-numbered paraffinic hydrocarbons in plants is strong. More recently, however, Hatt (1958) has indicated that even-numbered paraffins probably occur in small amounts.

<sup>4</sup> The abstract of the presentation of Cuzin *et al.*, 1958, includes data obtained by methods which may be specific for total tobacco paraffins. However, the details of the methods were not given in this publication. Also, a wide range of tobacco types was not surveyed.

<sup>5</sup> Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

steam bath until the temperature reaches 60° C, immediately removed from the bath, and permitted to cool and remain at room temperature for 16 hours. After cooling to 0° C in an ice bath, 30 ml of cold water are added to the acid mixture in small increments so that the temperature is kept below 20°C during the addition. The mixture is then extracted with five successive 100 ml portions of Skellysolve B, and the Skellysolve layers are pooled, washed with water until neutral and dried over anhydrous magnesium sulfate. After filtering, the solvent is removed on the steam bath, and the residue is dissolved in 10 ml of redistilled petroleum ether. This solution is then chromatographed on 20 g activated<sup>6</sup> silicic acid previously wet-packed with petroleum ether in a 15 x 240 mm chromatographic column. The column is developed with petroleum ether (redistilled), and the first 200 ml of eluate are collected. The solvent is removed from this fraction on the steam bath, and to the residue are added 10 ml absolute ethanol. The mixture is heated on the steam bath for five minutes and filtered hot through a sintered glass filter, and the filtrate is placed in the freezer (-14° C) for 30 minutes. The cold suspension is filtered through a glass filter precooled to -14° C, and the solid on the filter is washed with 5 ml of ethanol similarly precooled. The precipitate is dissolved in petroleum ether by running 20 ml of boiling solvent through the filter and the solution is evaporated to dryness on the steam bath. The residue is heated in an oven at 100° C for 30 minutes, cooled to room temperature in a desiccator and then accurately weighed.

#### Development of the Method

Cured tobacco leaves contain significant amounts of uncharacterized compounds which are soluble in non-polar solvents, are relatively insoluble in cold ethanol, and can be expected to accompany paraffins in the classical methods discussed above. Infrared spectral analysis of mixtures of such compounds shows the presence of significant unsaturation, variable chain length and various oxygen-containing functions. That these substances accompany paraffins in the classical analytical method employing crystallization from ethanol, is illustrated in Figure 1, which gives the weights of fractions obtained from flue-cured Type 12 tobacco by such method, and Table 1,

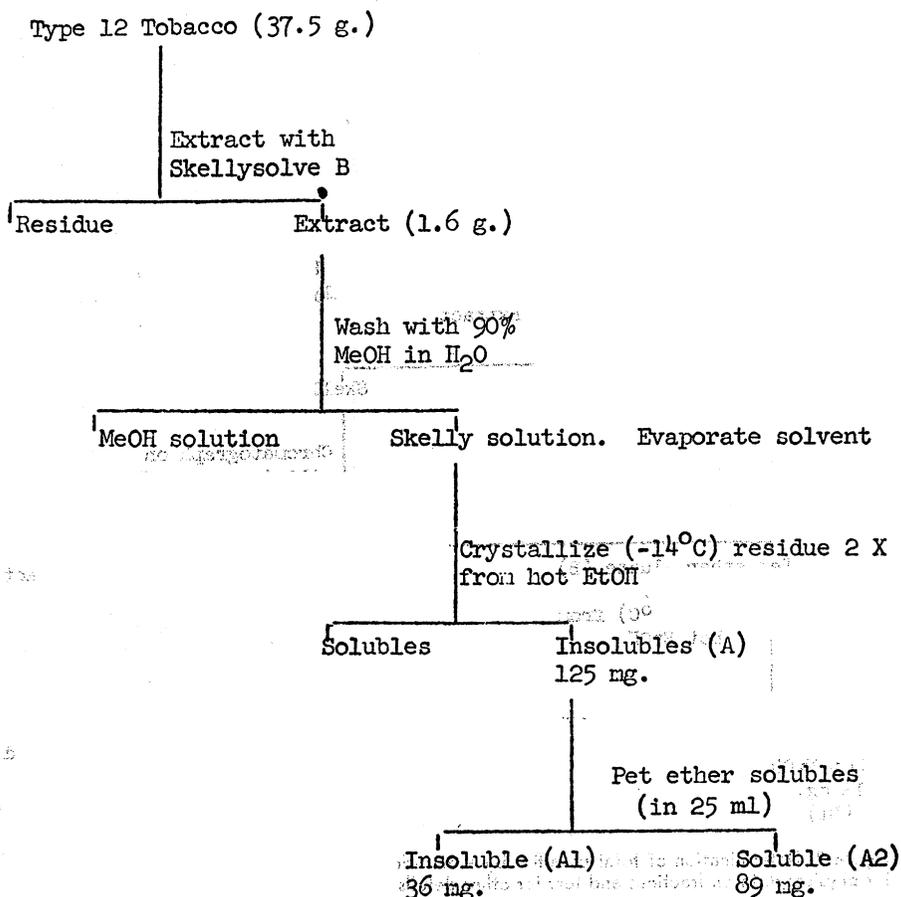


Figure 1. Determination of total paraffins by a classical procedure. See Table 1 for physical properties of fractions and text for explanation.

**Table 1. Physical characteristics of fractions obtained by two methods (see Figures 1 and 2) for the determination of total paraffins in tobacco leaf**

Frac-	Fig-	Appearance	Melting Point (°C)	Infrared spectrum	X-ray diffraction pattern
A1	1	Brown wax	87-220(d)	Unsaturation, —OH, —COOR	—
A2	1	Yellow semi-solid wax	—	—	—
B	2	Yellow wax	30-31	Long chain hydrocarbon (RH) with trace of >C=O	—
B1	2	Brown oil	—	Chain branching, —OH, >C=O	—
B2	2	White wax	63-64	Long chain, saturated RH	Paraffinic RH approx. C <sub>29</sub>
B3	2	Brown oil	—	—	—

which shows the infrared spectral characteristics of certain of these fractions. Using the classical interpretation, Fraction A would be considered total paraffins. However, approximately 30 per cent of Fraction A is a material (A1) having a wide melting range and showing unsaturation and oxygen-containing groups on infrared spectral examination. The remainder (Fraction A2) consists of a yellow, low-melting, semi-

solid mass which, by inspection, is not solely a mixture of paraffinic hydrocarbons in the range of chain length commonly found in tobacco leaves.<sup>7</sup>

In an attempt to develop a specific analytical method for tobacco paraffins, the classical procedure was

<sup>7</sup> Further work in this laboratory has shown that another classical method for separating paraffins from extracts, i.e. acetone precipitation, is also non-specific.

<sup>6</sup> By heating at 150° C for four hours.

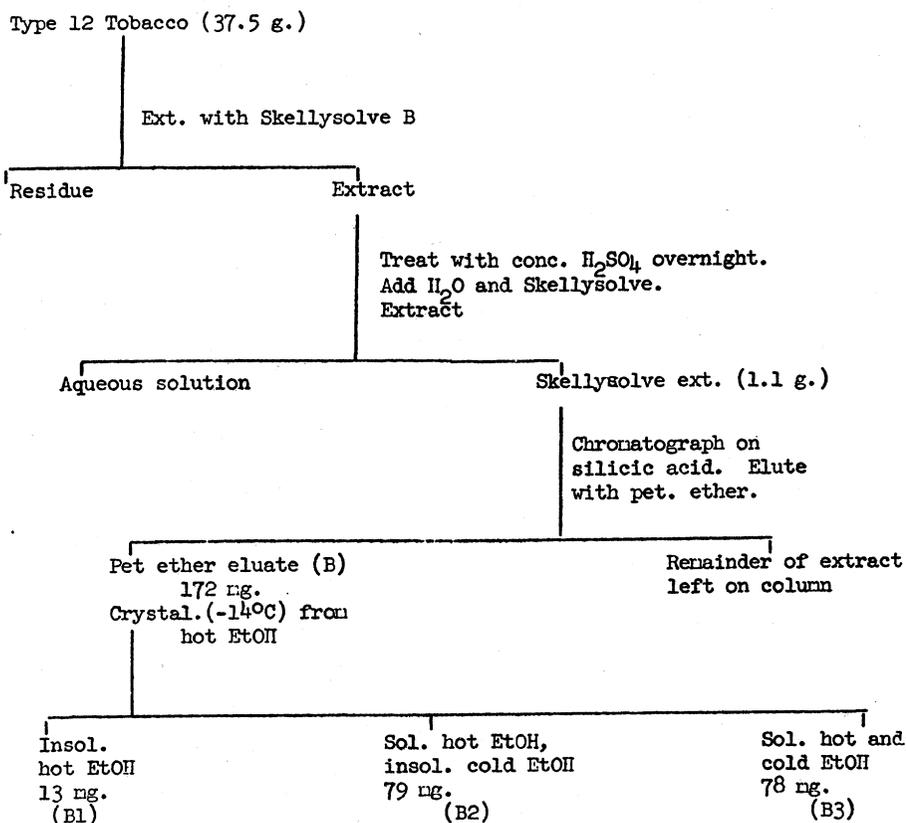


Figure 2. Determination of total paraffins by a modification of the classical method. See Table 1 for physical data on fractions and text for other details.

Table 2. Levels of paraffinic hydrocarbons and total sterols in various aged and fermented tobacco leaves

Tobacco type	Percentage paraffins*	Percentage total sterols**
Flue-cured		
Blend of flue-cured types	0.28	0.45
Type 11, 1955 crop	0.28	0.35
Sample X	0.24	—
Burley		
Sample X	0.32	0.39
Sample Y	0.32	—
B <sub>3</sub> F grade	0.36	0.34
Maryland		
Sample T	0.34	—
1954 crop	0.43	0.38
Sample Y	0.41	—
Turkish		
Smyrna	0.36	0.26
Sample T	0.37	—
Sample X	0.37	—
Cigar types		
Filler	0.32	0.16
Binder	0.30	0.14
Fire-cured	0.24	0.20

\* Determined in present study. All values are on a moisture-free basis.

\*\* Reported in a previous study (Stedman and Kusamwskyj, 1959).

modified in a number of ways and the technique described in the preceding section was ultimately adopted. The appearance, weights, melting points, infrared spectral characteristics and X-ray diffraction pattern of various fractions obtained

with this technique are shown in Figure 2 and Table 1. Fraction B2 consists solely of paraffinic hydrocarbons without extraneous material.

Various findings of interest were obtained in the preliminary work on development of this method. A sum-

mary of these findings follows.

*Treatment with concentrated sulfuric acid.* Treatment of Skellysolve extracts with this reagent is necessary to eliminate unsaturated components. Two conditions of acid treatment were studied: the overnight exposure at room temperature adopted above and 3 hours heating on the steam bath. Neither treatment entirely destroyed carbonyl-containing components, but the latter can be subsequently removed by column chromatography and crystallization from ethanol. No significant variation was observed between the values obtained using the two acid treatments followed by column separation and crystallization. The overnight treatment was adopted since it permitted a more convenient schedule for our purposes.

*Saponification.* Hydrolysis in alkaline diethylene glycol without prior or subsequent sulfuric acid treatment removes considerable colored matter but unsaturated and carbonyl-containing substances persist. Saponification with acid treatment gives results similar to acid treatment alone when fractions are later chromatographed or recrystallized.

*Girard's reagent.* Treatment of extracts with this reagent does not contribute significantly to the elimination of non-paraffinic components regardless of other experimental variables employed.

*Column chromatography.* Chromatography on acid-washed alumina or silicic acid without prior or subsequent sulfuric acid treatment does not entirely separate the carbonyl-containing or unsaturated components from saturated hydrocarbons on elution with petroleum ether. The use of such columns in conjunction with the adopted overnight acid treatment gives reasonably clean eluted material which, on single crystallization from ethanol, yields a mixture of hydrocarbons uncontaminated by non-paraffinic substances. Chromatography without one subsequent crystallization does not give a material completely free of carbonyl-containing substances. No difference between the two adsorbents was observed. On silicic acid, 200 ml of petroleum ether is sufficient to elute all the paraffinic hydrocarbons from 37.5 g of tobacco. The next 300 ml of solvent elutes material which is relatively soluble in cold ethanol and non-paraffinic in nature.

*Crystallization.* Regardless of prior treatment, crystallization from ethanol is required as a final step in obtaining paraffinic hydrocarbons. For material eluted from columns, one crystallization from 10-25 ml

ethanol yields a white waxy product consisting entirely of paraffinic hydrocarbons. If overnight sulfuric acid treatment is used without column separation, at least three, successive recrystallizations from 25 ml of ethanol are required to give a material of acceptable melting point, but this material still shows a yellow color and slight indications of carbonyl-containing substances. Using the three hour acid treatment, two recrystallizations from 25 ml of ethanol give a yellow material of correct melting point with traces of carboxylic components. Significant losses may occur on multiple recrystallizations and are dependent, of course, on the volume of ethanol employed. With column eluates, 10 ml of ethanol can be used to give an acceptable product; material not chromatographed requires 15-25 ml ethanol. The use of 25 ml of ethanol results in approximately five per cent loss of paraffins for each crystallization of material.

**Recovery of added paraffins.** Experiments on the recovery of known amounts of tobacco paraffins added to the initial Skellysolve extracts have shown that at least 80 per cent recovery can be achieved by the adopted method. Considering the multiplicity of steps in the method this degree of recovery is considered satisfactory, at least for comparative purposes.

**Completeness of extraction.** Studies on this point have shown that greater than 96 per cent of the paraffins extractable from tobacco with Skellysolve B are removed by the adopted procedure.

**Precision.** The adopted method shows a precision of approximately  $\pm$  eight per cent.

## Results

Table 2 shows the values for total paraffinic hydrocarbons obtained on a number of aged or fermented tobacco leaf samples. The tobaccos were representative commercial types. Available descriptive information is given in the table. Each analytical value represents a single determination, and the appearance and other physical properties of the isolated hydrocarbons indicated that a valid measure of total paraffins was being obtained with all tobacco types. All inferences drawn from these data are presented with the usual reservations regarding biological variation, sample size and other factors which limit broad generalizations.

In general, the range of variability of paraffin levels in the different types was relatively small, thus con-

firmed the contention of earlier workers with respect to cigarette tobaccos (Kurilo, *loc cit*). The fire-cured and bright types gave the lowest values for total paraffins. The Maryland samples showed the highest and were most variable in range. The levels of Turkish tobacco were quite uniform and appear to be slightly more than those of burley. The cigar types were intermediate between the bright and burley samples.

It is of interest to compare the range of values for total sterols and total paraffins since most of the samples had been previously analyzed for the former (see Table 2). The range of total sterol values (0.14-0.45 per cent) was wider than that of the paraffins for all types. Also no correlation between sterol and paraffin levels of the various tobacco types was evident in the few instances where direct comparisons could be made.

## Summary

The classical method for the determination of total paraffinic hydrocarbons in tobacco in which the essential step is crystallization from hot ethanol does not give results specific for these compounds. A modification of this method is described which permits specific determination. Using this procedure, samples of aged and fermented tobaccos were analyzed. Total paraffinic levels did not vary considerably in the commercially acceptable samples of flue-cured, burley, Maryland, Turkish, fire-cured and cigar tobaccos investigated. The flue-cured and fire-cured types showed the lowest concentrations of paraffins. Although the total sterol (previously reported) and total paraffin levels of all types were in the same general range of concentration, no correlation between the levels in the various tobaccos was observed in the few cases available for direct comparison.

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## Addendum

During the preparation of this manuscript, a report appeared (Gladding and Wright, *Tobacco Science* 3, 81 (1959), describing the isolation of heptacosane and hentriacontane and giving a quantitative value (0.31 per cent) for paraffinic hydrocarbons in burley tobacco.

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