

# Composition Studies on Tobacco. XII. Total Hexane Extractives

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Recently, the nature and amounts of substances in flue-cured leaves extractable with n-hexane and insoluble in 90 percent methanol and water were described (Swain et al., 1961). The present report is a continuation of this work and presents corresponding data on the substances found in the 90 percent methanol fraction. As previously noted, the experimental procedures used in the isolations are not analytical methods, and the quantitative data should be considered as estimates.

Large scale extraction of cured, unaged Type 12 leaves with Skellysolve B<sup>2</sup> was performed and the extract was partially fractionated as shown in Figure 1, which gives the distribution of substances in terms of percentage of leaf weight (moisture-free basis). The substances remaining in the hexane layer after successive extraction with 90 percent methanol and water were separated using a variety of procedures, the chief of which was column chromatography by a flowing technique from petroleum ether to methanol. The nature and amounts of these substances were discussed in the above report of Swain et al, and the levels are repeated in Table 1. Approximately 23 percent of the fraction consisted of substances which could not be eluted from silicic acid by methanol; however, the bulk of the fraction was comprised of the resin-like materials which were dis-

cussed in detail in the earlier report. After removal of water-soluble material, the 90 percent methanol solubles were fractionated into basic, acidic and neutral substances. Extensive column chromatography on silicic acid of the neutral substances again resulted in a significant per-

centage of the strongly adsorbed material being encountered. Of the substances eluted from silic acid in the flowing technique, the resins were the major fraction. The remainder of the neutral substances were paraffinic and olefinic hydrocarbons, esters and sterols (Table 1). Over half of the

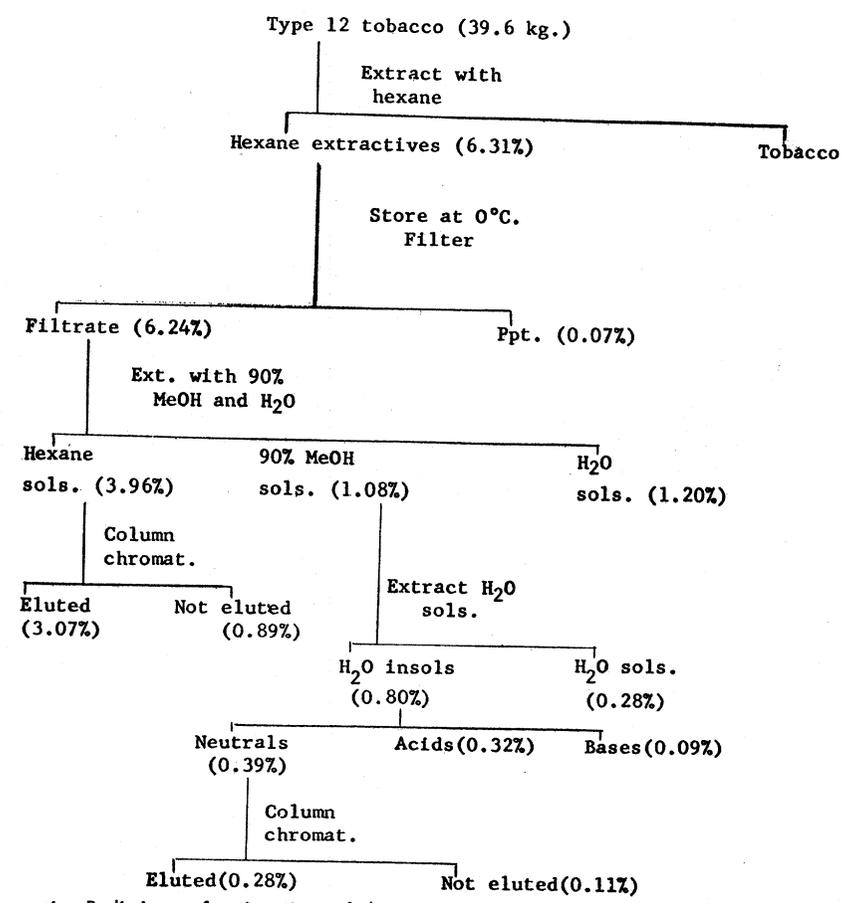


Figure 1. Preliminary fractionation of hexane extractives. Percentages are based on leaf weight (moisture-free basis.)

<sup>1</sup> Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.  
<sup>2</sup> Essentially n-hexane. Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

acidic substances were higher fatty acids. Gas chromatographic analysis showed the proportions of the major acids, palmitic and linolenic, to be quite different from those found in the hexane layer. The latter contained palmitic and linolenic in approximately similar amounts, 26 and 30 percent of the total higher fatty acids, respectively; for the 90 percent methanol solubles, comparable values were 6 and 70 percent, respectively. It is of interest to note that the total yield of higher fatty acids in all fractions was greater than 90 percent of the corresponding value obtained by a newly developed analytical method for such compounds in tobacco. Details of this method will be published in the near future.

The remaining acidic substances showed infrared spectra having strong -COOH and -OH bands but could not be fractionated. These substances are probably related to the "resin acids" reported by many workers. In general, the entire hexane extract contains a progressive series of unfractionable resins which possess the overall characteristics of high molecular weight oxidation products.

The water-soluble substances removed from the original hexane extract were of no interest and were not investigated. The occurrence of such substances in hexane extracts prepared by the large scale method used here is to be expected; the failure to precipitate these substances on 0° C storage is probably due to solubilization by other components in the concentrated extract.

## Experimental

**Extraction.** Thirty-nine and six-tenths kg of unaged, cured Type 12 leaves (mixed U. S. grades) were extracted with Skellysolve B by a procedure involving continuous percolation with warm solvent. Details of the procedure have been reported previously (Dymicky and Stedman, 1959a). The extract was concentrated and stored at 0° C, after which insoluble materials were filtered off. The filtrate was extracted with 90 percent methanol and water (Stedman et al., 1960a), and the substances remaining in the hexane layer were isolated by extensive fractionations involving adsorption and partition column chromatography, gas chromatography, distillation, precipitation and solvent partition. Essentially the methods of isolation and identification for the more well-known components were those previously reported and, except for the following citations of key references,

**Table 1. Composition of hexane extractives of cured type 12 leaves\* Percentage of leaf weight\*\***

Component	Hexane-Solubles	90% Methanol-Solubles	Total
Aliphatic paraffins	0.196	0.001	0.197
Neophytadiene	0.027	0.001	0.028
Cyclic paraffins	0.108	0	0.108
Esters***	0.162	0.041	0.203
Polyene	0.090	0.020	0.110
Phthalates	0.018	0	0.018
Solanosol	0.030	0	0.030
Sterols	0.036	0.001	0.037
Tocopherols	0.069	0	0.069
Acid salts	0.015	0	0.015
Higher fatty acids	0.195	0.172	0.367
Unidentified acidic subs.	0	0.146	0.146
Bases	0	0.089	0.089
Resins	2.12	0.226	2.346
Unidentified substances not eluted by methanol	0.888	0.105	0.993

\*Exclusive of water-soluble substances.

\*\*Values are given to the third decimal place to illustrate relative quantities with no implication of statistical significance.

\*\*\*Predominantly steryl esters of higher fatty acids (Swain et al, 1961)

will not be repeated below: isomerized neophytadiene (Stedman et al., 1960b), cyclic paraffins (Stedman et al., 1960c), steryl esters (Rodgman et al., 1959), sterols (Dymicky and Stedman, 1959b), phthalates (Stedman and Dymicky, 1959) and higher fatty acids (Stedman et al., 1960a).

**Polyene.** A labile, viscous, fluorescent oil, n<sub>D</sub> 20 1.5360, eluted from acid-washed alumina by petroleum ether-benzene, 1:1, and further purified by repeated chromatography on silicic acid. Anal Calcd for C<sub>40</sub>H<sub>66</sub>: C, 87.84; H, 12.16; mol wt 547. Found: C, 88.05; H, 12.00; mol wt (Rast), 546; microhydrogenation (platinum oxide), 6.4 double bonds per mole. Infrared spectrum: λ<sub>max</sub> 6.01, 6.07, 11.25, 12.26 μ. Ultraviolet

spectrum: λ<sub>max</sub><sup>hexane</sup> 229 mμ, log ε 3.83.

**Tocopherols.** A thick oil eluted from acid-washed alumina by benzene-ether, 1:1, and purified by chromatography on silicic acid and Florisil. The infrared spectrum resembled that of α-tocopherol, and reaction with 3,5-dinitrobenzoyl chloride yielded a corresponding 3,5-dinitrobenzoate, m. p. 72-73° C, after purification by chromatography on Florisil and crystallization from acetone. The oil was tentatively identified as a mixture of tocopherols.

**Substances Insoluble at 0° C.** Extensive attempts failed to fractionate the substances which precipitated on storage of the original hexane extract at 0° C. Although some sterols were apparently present, the large number of other components therein, each present in small amounts, made

an estimation of composition valueless.

**90 Percent Methanol Solubles.** Since this fractionation has not been previously reported details will be presented below.

**Preliminary Fractionation.** The 90 percent methanol extract (18,000 ml) was concentrated *in vacuo* to a viscous residue and diluted to 3,375 ml with ether. The ether extract was extracted four times with 5 percent aqueous potassium hydroxide (twice with 3,375 ml and twice with 1,500 ml) to remove acidic substances and all alkaline solutions were pooled (A). After washing with water, the ether layer was then washed with 12 percent hydrochloric acid in a similar manner to remove bases and all acid solutions were combined (B). The remaining ether solution containing neutrals was fractionated by methods identical to those employed above except that column chromatography was limited to separations on silicic acid.

**Acidic Substances.** The above alkaline extracts (A) were acidified to pH 1.3 with concentrated sulfuric acid, saturated with sodium chloride and extracted with ether three times (2100, 1000 and 750 ml). The ether extracts were combined and washed thoroughly with water and dried giving ether-soluble acidic substances. The acidified alkaline extract was similarly extracted with n-butanol to remove n-butanol-soluble acidic substances. The n-butanol extract was concentrated to 60 ml, 900 ml of petroleum ether were added and after overnight storage at -15° C, the insolubles (C) were filtered off.

The filtrate (3.1 g) was almost entirely water-soluble and was discarded. The insolubles (C) were separated into several water-insoluble, brown or black solids (total, 1.8 g) by solvent precipitation (mainly chloroform), which melted over various wide ranges from 142° to 243° C with decomposition, and showed ambiguous infrared spectra despite extensive attempts at purification. The above solution of ether-soluble acidic substances was reduced to a dark viscous residue and steam-distilled, giving three volatile fractions (1.2, 1.2, and 1.8 g, respectively). The infrared spectra of the three fractions showed the presence of carboxylic acids but did not resemble known acids except for the last of the three which showed some similarity to authentic nonanoic acid. The non-volatile acidic substances (97.5 g) were dissolved in methanol (1240 ml) and were twice crystallized at -15° C, giving two precipitates (15.6 and 4.8 g) which were dark, oily resins at room temperature and showed nondescript infrared spectra and gas chromatographic behavior, after methylation. The methanol solubles (77.4 g) were methylated using diazomethane and studied by the gas chromatographic and other methods discussed previously. Seventy-four percent of the fraction consisted of the common higher fatty acids with linolenic acid predominant-

ing. The remainder of the fraction was non-volatile under the gas chromatographic conditions.

**Bases.** After alkalinizing the 12 percent hydrochloric acid extracts (B), extraction with ether was performed in a manner similar to the above, and the pooled ether layers were dried and evaporated to a residue of 32.4 g. No attempt was made to fractionate the bases.

**Weights.** All weights reported above are on an "as-is" basis and do not include corrections for pilot work (+8 percent) and moisture (+10 percent).

#### Summary

As a continuation of studies on the hexane extraction of cured, unaged Type 12 leaves, the nature and amounts of substances soluble in 90 percent methanol are described. Qualitatively, the substances are generally similar to those previously reported as being present in the hexane-soluble, 90 percent methanol-insoluble fraction. However, significant quantitative differences are observed between the two fractions.

#### Literature Cited

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