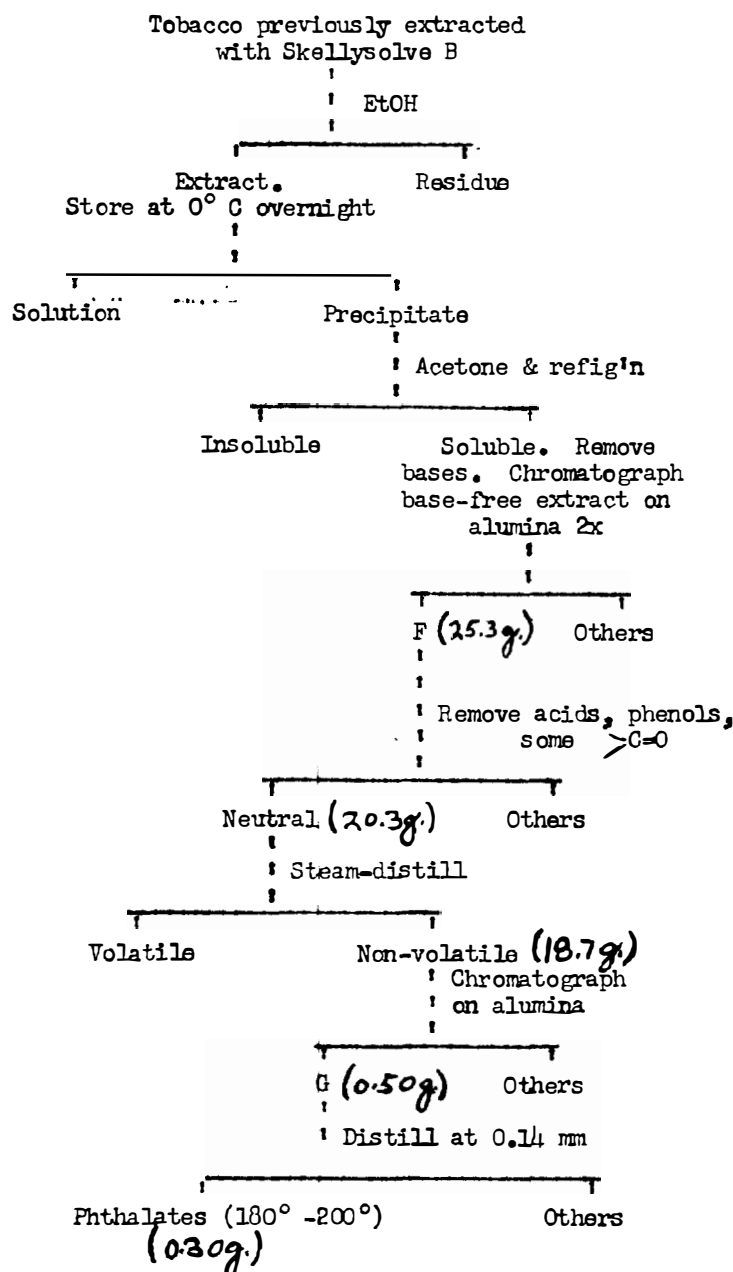


Composition Studies on Tobacco

VI. Phthalates from Flue-cured Leaves

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Introduction

This report concerns the isolation of a mixture of esters of phthalic acid from unaged, flue-cured tobacco. Although Frankenburg (1946) has stated that terephthalic acid occurs in tobacco and Quinn and Hobbs (1958) have identified phthalic acid in cigarette smoke, the presence of esters of phthalic acid in leaves has not been previously shown, as far as we are aware.

Methods and Results

The esters were isolated by a lengthy fractionation which is given in simplified form in **Figure 1**. Details of this scheme are as follows:

Thirty-seven kg of cured, unaged Type 12 tobacco (mixed U. S. grades) were successively extracted with Skellysolve B² and ethanol as described in Parts I and II (Dymicky and Stedman, 1958; Grossman and Stedman, 1958). The ethanolic extract (320 l) was concentrated to 16 l and stored overnight at 0° C. The waxy precipitate which formed during storage was filtered off, and this solid material served as the source of the phthalates.

The solid was dissolved in 500 ml acetone, the solution was stored at 0° C for 15 hours and the precipitate which appeared was filtered off. The filtrate was evaporated *in vacuo*, the residue was dissolved in diethyl ether and the ethereal solution was washed with aqueous 10 percent hydrochloric acid solution followed by water. After drying over magnesium

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

² Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

Figure 1. Isolation of phthalates from flue-cured leaves.

sulfate, the ethereal solution was evaporated to dryness, and the residue was dissolved in petroleum ether. This solution was then chromatographed on acid-washed alumina (3 lbs). and the column was washed with petroleum ether (8 liters), 15 percent ethyl acetate in petroleum ether (6 liters) and methanol (6 liters). The fraction eluted with 15 percent ethyl acetate in petroleum ether was evaporated to dryness, and the residue dissolved in 100 ml petroleum ether. This solution was again chromatographed on acid-washed alumina (400 g), and material eluted with petroleum ether (250 ml) was collected (*F* in Figure 1).

F was evaporated to a viscous, dark residue which was dissolved in diethyl ether and successively extracted with 5 percent sodium bicarbonate, 5 percent sodium hydroxide and 40 percent sodium bisulfite, all in aqueous solutions. The ethereal layer containing neutral substances was then evaporated to dryness, and the residue was steam-distilled. The non-volatile residue was chromatographed on 200 g acid-washed alumina and eluted with a succession of solvents: petroleum ether (1300 ml); petroleum ether successively containing 1 percent chloroform (200 ml), 10 percent chloroform (100 ml) or 50 percent chloroform (100 ml); and other solvents including benzene, benzene with diethyl ether, diethyl ether, ethanol and water. The eluates (*G*) obtained with petroleum ether, alone and in mixtures, contained the phthalates; these eluates were evaporated *in vacuo* to give an oily residue.

This residue was distilled at 0.14 mm pressure, and a colorless oil, b r 180°-200° C, was collected. The infrared spectrum of this fraction showed sharp bands suggestive of high purity and structural features indicative of an ortho substituted aromatic ester. Comparison with spectra of known substances showed the oil to be almost identical with dibutyl phthalate.²

The oil was saponified in 5 percent methanolic potassium hydroxide by refluxing for two hours. On cooling the saponification mixture a crystalline solid separated and was removed by filtration. This solid showed a m p > 300° C and was water-soluble. The neutral equivalent of this salt (to phenolphthalein) was 231 (m w dipotassium phthalate, 242). The solution used in the determination of neutral equivalent was acidified to pH 1.0 with hydrochloric acid and then

Table 1. Gas chromatographic separation of the alcoholic components of the phthalates from tobacco*

Sample	Column temp (°C)	Carrier gas rate (ml He/min)	Retention times (minutes)
Authentic			
n-Propanol	123	70	2.2
Isopropanol	123	70	<1.0
n-Butanol	123	70	4.4
n-Hexanol	118	60	23.0
n-Heptanol	120	62	27.5
n-Octanol	120	62	45.2
n-Heptanol	177	70	6.4
3-Heptanol	177	70	3.9
n-Heptanol	177	40	10.8
3-Heptanol	177	40	6.9
Menthol	177	40	10.6
Unknown			
Peak #1	123	70	2.2
#2	123	70	4.1
#3	123	70	19.1
#3	120	62	28.3
#3	177	70	5.4
#3	177	40	8.3

* Aerograph Model A-100 instrument, 5 ft. column dinonyl phthalate, 150 ma filament current and other conditions as indicated. Authentic alcohols injected in trace amounts (<10ul) to simulate peak size obtained with unknown peaks. "Unknown" was ethereal concentrate described in text, 40-50 ul being injected in all instances.

extracted several times with diethyl ether. On evaporation of the diethyl ether a white solid was obtained which, on mixed melting point determination with authentic phthalic acid, gave no depression.

The alcoholic components of the original ester were then determined. To the filtrate of the above saponification mixture was added sufficient water to give two layers with diethyl ether, and the aqueous layer was exhaustively extracted with this solvent. The ether extract was concentrated to a small volume and examined by vapor-phase chromatography, using the conditions shown in Table 1.

Three components were found in the ether concentrate, showing the original material to be a mixture. Two of the three were identified as n-propanol and n-butanol (Table 1). The third component was of longer chain length and apparently possessed a branched structure. On the basis of chromatographic behavior, the unknown component may be an aliphatic alcohol with perhaps six or more carbon atoms in the longest chain or a saturated ring compound of indeterminate structure. The quantity of the unknown alcohol in the mixture was slightly greater than the amounts of propanol and butanol.

To confirm the chromatographic findings, a portion of the ether concentrate was further evaporated under a stream of nitrogen until the

ether was removed, and the infrared spectrum of the residue was determined. The findings indicated that the sample contained only alcohols and that the average chain length was in the range of approximately four to six carbon atoms.

It should be noted that the presence of methyl or ethyl phthalate could not be detected by the above method since neither methanol nor ethanol could be separated from diethyl ether in the chromatographic apparatus under the conditions used.

Discussion

Reports on the occurrence of phthalates in plants or plant products are occasionally encountered in the literature. Among the plant substances from which phthalic acid or its esters have been isolated are raspberries (Schinz and Seidel, 1957), grapes (Haagen-Smit *et al*, 1949), the Kewda plant (Dhingra *et al* 1954), the poppy (Schmid and Karrer, 1945) and stored orange juice (Huskins, 1952). Aebi *et al* (1953) have also shown the presence of free pythalic acid and esters thereof in the tubercle bacillus. The reports on the occurrence of terephthalic and phthalic acids in tobacco and its smoke were cited above.

Since certain alkyl phthalates are employed commercially as plasticizers and lubricants, the possibility that the phthalates isolated from tobacco were derived from such extra-

² We are indebted to Dr. H. Susi of this laboratory for a detailed study of these spectra and for suggesting the identity of the oil.

neous sources has been investigated. Among these sources are plastic tubing, stopcock grease and solvents, especially those used in chromatographic separations.⁴ In no instance were phthalates obtained from such sources. The chance that the phthalates were derived from substances other than tobacco is remote.

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Summary

A mixture of esters of phthalic acid was isolated by extensive fractionation of unaged, cured Type 12 tobacco. The identity of the acidic component of the esters was established by mixed melting point determination with authentic material. The alcoholic components of the esters were shown to be n-propanol, n-

butanol and an unidentified alcohol by vapor phase chromatographic procedures.

Possible extraneous sources of phthalates in the isolation procedure were also investigated. The possibility that the phthalates were derived from such sources appears remote.

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⁴ We are indebted to Dr. R. L. Rowland, R. J. Reynolds Tobacco Company, for the suggestion that thiophene-free benzene may contain dibutyl phthalate.

