

# Composition Studies on Tobacco VII. Isoeugenol, Hydrocarbons and Probable Bound Stigmasterol from Flue-cured Leaves<sup>1</sup>

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## Introduction

Isoeugenol occurs less frequently in plants than its isomer, eugenol (Fujita, 1950). Although eugenol has been previously reported in tobacco flowers (Sabetay, 1941a, 1941b, 1942) and, more recently, in leaves (Onishi and Yamamoto, 1955), conclusive demonstration of the presence of isoeugenol in the tobacco plant is apparently required. According to

Onishi and Yamamoto (1955), the reports of Pyatnitskii (1929, 1931) on the occurrence of eugenol and isoeugenol in leaves need further clarification, although Frankenburg (1946) has cited the reports without comment.

A portion of the present communication describes a conclusive demonstration of the presence of isoeugenol in flue-cured leaves. The remainder of the report concerns the isolation of certain paraffinic hydrocarbons, one of which has not been previously described in the literature, and pre-

sents some evidence for the occurrence of bound stigmasterol in flue-cured leaves.

## Methods and Results

*Isoeugenol*—Figure 1 gives a simplified diagram of the scheme of fractionation. Detail of the method are as follows. Seven hundred fifty of cured, unaged Type 12 tobacco (50 mesh) were exhaustively extracted under reflux with seven successive 1.5 liter portions of 95 per cent ethanol. Each extract was then filtered, reduced *in vacuo* to a volume of 300 ml and stored at room temperature for two weeks. During storage, a precipitate appeared in each of the seven extracts. These extracts were filtered independently, and the seven precipitates designated P1 to P7 according to the original order of extraction. Purification of certain of these precipitates gave the substances described below.

The seven filtrates were pooled, and the resulting solution evaporated to 1000 ml *in vacuo*. The concentrate was then saponified by adding 80 g NaOH, 200 ml water and refluxing for six hours. The saponification mixture was extracted with ten successive one liter portions of diethyl ether and the ethereal solutions were combined, dried and evaporated to a viscous residue. This residue was distilled at 100° C/0.5 mm and a distillate collected. The latter was then

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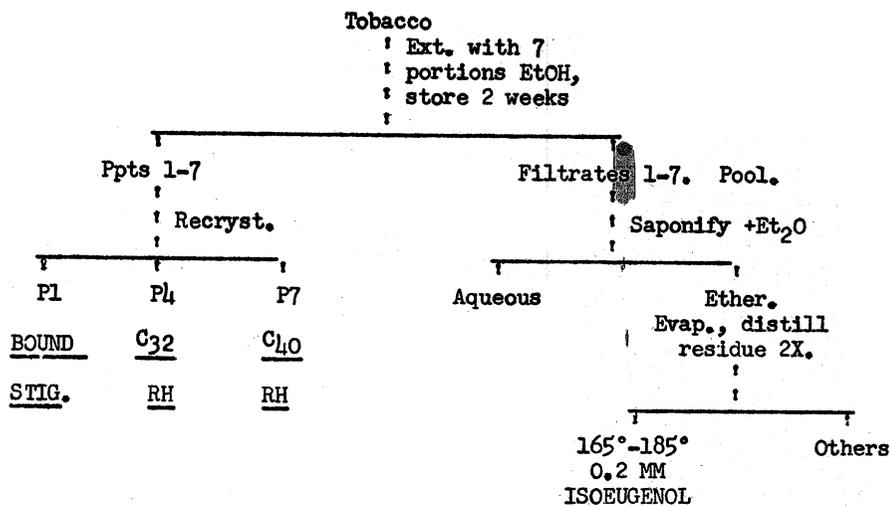


Figure 1. Isolation of isoeugenol, hydrocarbons (RH) and probable bound stigmasterol from flue-cured leaves. See text for limitations on indicated identities.

distilled at 0.2 mm and the first fraction, boiling up to 185° C, was selected for further study. The infrared spectrum of this fraction contained bands characteristic of a phenolic compound with *trans* unsaturation in addition to other unidentified bands.

The fraction was reacted with 3,5-dinitrobenzoyl chloride, and a 3,5-dinitrobenzoate was recovered. After several recrystallizations from ethanol, the 3,5-dinitrobenzoate showed m p 164-166° C, and gave no depression of melting point on admixture with the corresponding derivative of authentic isoeugenol.

**Hydrocarbons**—Precipitate P4 was recrystallized several times from ethanol and gave m p 70°-71° C. The x-ray diffraction pattern and infrared spectrum of the purified material indicated the presence of a long-chain, unbranched, saturated hydrocarbon having approximately 30 carbon atoms. The melting point matched that of n-dotriacontane. It was concluded that P4 was either a pure sample of this hydrocarbon or, more probably, a mixture of homologues of longer chain length.

Precipitate P7 was recrystallized several times from n-hexane and showed a sharp melting point, recorded at 83° C.<sup>2</sup> The infrared spectrum and x-ray diffraction pattern showed the same essential features as P4 except that a longer chain length was indicated. Although reported melting points for paraffinic compounds of chain length greater than forty carbon atoms are perhaps unreliable (Egloff, 1939), a comparison of melting point would show the hydrocarbon to be n-hentetracontane, n-dotetracontane, n-tritetracontane, or, more probably, a mixture of higher homologues.

**Probable Bound Stigmasterol**—Precipitate P1 had m p 295°-300° C (d) in the crude state. A single recrystallization from ethanol gave a solid fraction, m p 295°-297° C (d), which was filtered off, and a filtrate which contained significant amounts of unknown material on evaporation

of the ethanol. The solid fraction, m p 295°-297° C, gave an infrared spectrum suggestive of a steroidal glycoside and showed  $[\alpha]_D^{20} -43.2$  (chloroform). On hydrolysis under mild acid conditions (Dymicky and Stedman, 1958), an aglycone, m p 169°-172° C, was recovered, which, on mixed melting point determination with authentic stigmasterol, gave no depression. The nature of the non-steroidal substance(s) in the hydrolysis mixture was not determined.

#### Discussion

It should be noted that isoeugenol<sup>3</sup> was extracted from the saponification mixture by ether before acidification of the mixture. Of course, this is contrary to expectations and no explanation for this behavior is apparent.

Although isoeugenol can be formed by isomerization of eugenol, it is highly improbable that this occurred in the isolation procedure since the isomerization requires more drastic conditions than those employed here.

Paraffinic hydrocarbons in the approximate C<sub>27</sub>-C<sub>33</sub> range are frequently isolated from tobacco and such isolations cannot be considered a novelty. However, as far as we are aware, hydrocarbons of a chain length exceeding forty carbon atoms have not been previously reported in tobacco.

The steroidal substance isolated above appears to be a glycoside of stigmasterol. The degree of hydroxylation in the infrared spectrum, the optical rotation and the melting point would indicate that the non-steroidal portion of the molecule is a sugar. However, all of this is speculation since a definite claim of the isolation of a pure bound form of stigmasterol cannot be made on the basis of the above evidence. As noted in Part II (Grossman and Stedman, 1958), proof of the isolation of a stigmasterol glycoside should be rigid.

#### Summary

The partial fractionation of an alcoholic extract of unaged Type 12

tobacco leaves is described. From this work, isoeugenol (free or esterified), a probably bound form of stigmasterol and two fractions containing paraffinic hydrocarbons were isolated. Studies on one of the hydrocarbon fractions showed the presence of a pure compound or a mixture of homologues having chain lengths greater than forty carbon atoms.

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<sup>2</sup> The Fisher-Johns melting point apparatus was used in all determinations. Use of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

<sup>3</sup> It is recognized that isoeugenol may have occurred in esterified form in the leaves.