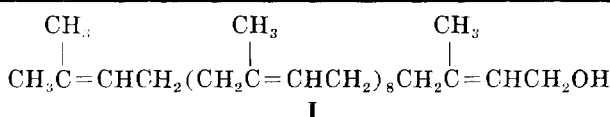


# Flue-cured Tobacco. IV. Isolation of Solanesyl Esters<sup>1</sup>

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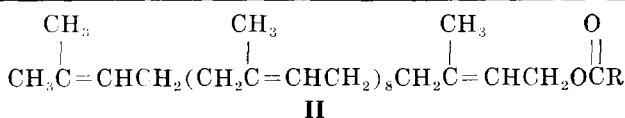
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We have previously reported the isolation of solanesol (I) from flue-cured tobacco. In the course of investigating the extractable materials in flue-cured tobacco, we have isolated three ester fractions which we have identified as mixtures of solanesyl esters (II). Our study has shown that these fractions were solanesyl esters of which the following are



cured tobacco. In the course of investigating the extractable materials in flue-cured tobacco, we have isolated three ester fractions which we have identified as mixtures of solanesyl esters (II). Our study has shown that these fractions were solanesyl esters of which the following are

solanesyl oleate. The solanesyl esters were isolated by chromatographic separation of a hexane extract of aged flue-cured tobacco. Three ester fractions, A, B and C were obtained by chromatography using silicic acid. The esters were subsequently crystallized from



present in largest amounts: solanesyl acetone at  $-27^\circ$ . Each of these ester

fractions showed infrared absorption essentially identical with that of authentic solanesyl palmitate.

Saponification of each of these fractions gave a good yield of solanesol, identified by melting point and by the infrared absorption both of the liquid and solid states. It is interesting to note that solanesol crystallizes in two forms which gave significantly different infrared spectra. Form I was usually formed by solidification of solanesol at room temperature. Form II resulted from chilling liquid solanesol to  $-65^\circ$  and was formed occasionally by solidification at room temperature. In several cases, the infrared absorption appeared to be a mixture of forms I and II. The infrared spectrum of form I showed absorption bands at  $3.0 \mu$  (m),  $3.5 \mu$  (s),  $6.0 \mu$  (m),  $7.0 \mu$  (s),  $7.3 \mu$  (s),  $8.0 \mu$  (w),  $8.3 \mu$  (m),  $8.7 \mu$  (m),  $9.2 \mu$  (m),  $9.5 \mu$  (m),  $10.1 \mu$  (s),  $11.3 \mu$  (s),  $11.7 \mu$  (s),  $12.5 \mu$  (s),  $13.7 \mu$  (w), where s=strong, m=moderate, and w=weak. The infrared spectrum of form II showed absorption bands at  $3.0 \mu$  (m),  $3.5 \mu$  (s),  $6.0 \mu$  (m),  $7.0 \mu$  (s),  $7.3 \mu$  (s),  $7.8 \mu$  (w),  $7.9 \mu$  (w),  $8.3 \mu$  (w),  $9.1 \mu$  (w),  $10.0 \mu$  (m),  $10.4 \mu$  (w),  $11.4 \mu$  (s),  $11.9 \mu$  (w),  $12.5 \mu$  (s), and  $13.3 \mu$  (m).

The solanesol obtained by hydrolysis of solanesyl ester C was converted to the p-phenylazobenzoate ester identical in melting point and

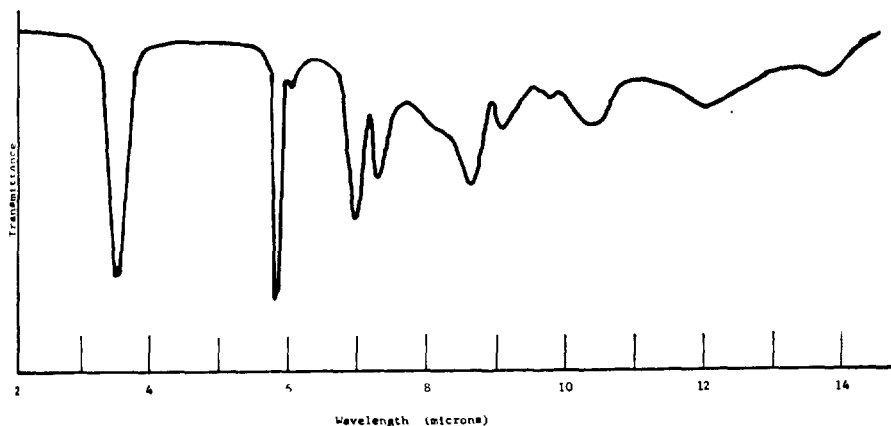


Figure 1. Infrared spectrum of synthetic solanesyl palmitate.

<sup>1</sup> Presented at the Tobacco Chemists' Conference at Durham, N. C., October 23, 1958.

infrared absorption with the p-phenylazobenzoate ester prepared from authentic solanesol.

The acid fractions from the saponification of solanesyl ester fractions A, B and C were converted to the methyl esters by reaction with diazomethane. By comparison with the known methyl esters in vapor phase chromatography, the methyl esters were shown to be primarily methyl palmitate, methyl myristate, methyl linolenate, methyl linoleate and methyl oleate. The presence of small amounts of methyl caprylate and methyl caprate was indicated as was the presence of the methyl esters of several unknown acids.

In the vapor phase chromatography of the methyl esters, the separation of methyl oleate and methyl stearate is the most difficult. Methyl oleate and methyl stearate were eluted so closely together that it is difficult to decide from the point of elution which of these two esters was present in our methyl ester mixture. The presence of methyl oleate rather than methyl stearate was indicated by the fact that the peak due to methyl oleate, which was of significant size in the runs of freshly prepared methyl ester mixture, had almost completely disappeared from runs made on the methyl ester mixture which had stood in acetone for six weeks. This is considered to be a result of oxidation and polymerization of the unsaturated oleate ester.

The estimation of the relative amounts of the methyl esters detected by vapor-phase chromatography was accomplished by integration of the curves. These values are given in Table 1. One apparent discrepancy, the quantity of esters of unsaturated acids in solanesyl ester fraction A compared to solanesyl ester fraction B is explained as follows: The chromatographic separation using silicic acid was accomplished in two steps, the first giving separation into the source of solanesyl ester fraction A and the source of solanesyl ester fractions B and C. The second separation using silicic acid chromatography gave a further and more complete separation with the solanesyl esters of saturated acids predominating in B and the solanesyl linolenate predominating in C.

A loss of the esters of unsaturated acids during the chromatographic separation using Florisil indicates that the amount of the solanesyl esters of unsaturated acids in tobacco is greater than the values which we are noting. Accordingly, the content of solanesyl esters in flue-cured

**Table 1. Saponification of Solanesyl Ester Fractions A, B, and C**

Ester Fraction	Alcohol Yield	M.P.	Yield	Acids	
				Approximate Composition as Estimated By Vapor-Phase Chromatography of the Methyl Esters <sup>1</sup>	
A, 0.29 g.	0.14 g.	36.5-38.5°	0.071 g.	Palmitic	38%
				Linoleic	31%
				Linolenic	15%
				Myristic	7%
				Oleic	6%
B, 0.25 g.	0.16 g.	37-39°	0.066 g.	Palmitic	65%
				Oleic	13%
				Myristic	7%
				Linolenic	5%
				Linoleic	4%
C, 0.17 g.	0.10 g.	38.5-40.5° <sup>2</sup>	0.046 g.	Linolenic	84%
				Linoleic	6%
				Unidentified acid	4%
				Unidentified acid	3%

<sup>1</sup> In all three fractions, small amounts of caprylic and capric acids and an unidentified acid were noted.

<sup>2</sup> The p-phenylazobenzoate ester, after chromatography from silicic acid and crystallization from hexane, amounted to 112 mg. (88%), m.p. 53-60°. The infrared absorption was identical with that of solanesol p-phenylazobenzoate prepared from solanesol which had been isolated from tobacco.

tobacco is undoubtedly higher than what we are reporting.

An approximation of the content of the solanesyl esters in flue-cured tobacco is given in Table 2.

The solanesyl esters which we have found in flue-cured tobacco in definite amounts (palmitate, linolenate, linoleate, oleate, myristate) are in agreement with the report of Shirokaia, who noted the acids derived from mahorka resins to be palmitic, linolenic, linoleic and oleic acids.

### Experimental

*I. Isolation of Solanesyl Esters.* Aged flue-cured tobacco (20 kg. dry weight) was extracted with 130 l. hexane at 60° for 2.5 hours. The extract was concentrated under reduced pressure. The residue was chromatographed on silicic acid. The material eluted from silicic acid by hexane-benzene mixtures was then chromatographed on alumina (Merck). The material eluted from alumina by benzene was subsequently chromatographed on silicic acid, whereby separation was effected into three main fractions. Subsequent purification was accomplished by crystallization from acetone and chromatography using Florisil, giving the following fractions:

1. Aliphatic ester fraction X, m.p. 78-80°, 0.0016 per cent of the dry weight of the tobacco, was

eluted from silicic acid by 4:1 hexane-benzene and crystallized from acetone at room temperature. X was separated from Y (described below) by the insolubility of X in ether at room temperature.

2. Ester fraction Y, m.p. 62-63°, 0.008 percent of the dry weight of the tobacco, was eluted from silicic acid by 4:1 hexane-benzene and crystallized from acetone at room temperature. Y was separated from X by the solubility of Y in ether at room temperature. Partially completed studies suggest that Y is

**Table 2. Content of Solanesyl Esters in Aged Flue-Cured Tobacco**

Esters	Percent of Dry Weight
Solanesyl Myristate	0.0015
Solanesyl Palmitate	0.011
Solanesyl Oleate	0.001
Solanesyl Linoleate	0.004
Solanesyl Linolenate	0.008
Minor esters	
Solanesyl Caprylate	0.0003
Solanesyl Caprate	0.0003
Solanesyl esters of unidentified acids	0.001

a mixture of esters.

3. Solanesyl ester fraction A, m.p. 31-33°, 0.012 percent of the dry weight of tobacco leaf, was eluted from silicic acid by 4:1 hexane-benzene and was separated from X and Y by the solubility of A in acetone at room temperature. A was crystallized from acetone at -27° and was eluted from Florisil by 4:1 hexane-benzene.  $n_D^{27}=1.4980$ .
4. Solanesyl ester fraction B, 0.010 percent of the dry weight of tobacco, was eluted from silicic acid by 1:1 hexane-benzene and was crystallized from acetone at -27°. After elution from Florisil by 4:1 hexane-benzene, B was again crystallized from acetone at -27°.  $n_D^{27}=1.5023$ .
5. Solanesyl ester fraction C, 0.008 percent of the dry weight of tobacco, was eluted from silicic acid by 1:1 hexane-benzene after the elution of B. C was crystallized from acetone at -27° and was then chromatographed using Florisil from which it was eluted by 4:1 hexane-benzene.  $n_D^{27}=1.5023$ .

## II. Saponification of Solanesyl Ester Fractions A, B and C. Saponi-

fication of the ester fractions (0.17—0.3 g.) was accomplished by refluxing for two hours with a mixture of 5g. of potassium hydroxide, 35 ml. of ethanol and 35 ml. of benzene. The mixture was concentrated under reduced pressure. The residue was acidified with 2 N hydrochloric acid. Extraction with 100 ml. of ether and concentration of the ethereal extract gave a residue from which the alcohol and acids were separated by chromatography using Florisil. The alcohol was eluted from Florisil by benzene and the acids were eluted as salts by methanol. The residues from concentration of the methanol eluates were acidified with 2 N hydrochloric acid. Extraction with ether and concentration of the ethereal extracts gave the acids which showed infrared absorption similar to that of palmitic acid. The acids were esterified with excess diazomethane in ether. The methyl esters were studied by vapor-phase chromatography using a Perkin-Elmer 154B Vapor Fractometer and the procedure of Lipsky and Landowne.

*III. Solanesyl Palmitate.* A mixture of 1 g. of solanesol, 50 ml. of benzene, 1 ml. of pyridine and palmityl chloride prepared from 1.5 g. of palmitic acid and 10 ml. of thionyl

chloride was heated under reflux for 2 hours after which time the mixture was washed with two 50-ml. portions of water. The residue from concentration of the benzene layer was chromatographed on Florisil, giving 0.76 g. of solanesyl palmitate eluted by benzene. Crystallization from acetone gave 0.7 g. of white solid, m.p. 47-48°, with an infrared absorption identical with that of solanesyl ester fractions A, B and C. Anal. Calcd. for  $C_{66}H_{112}O_2$ : C, 84.55; H, 12.04. Found: C, 84.48; H, 12.00.

## Acknowledgments

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