

# Composition Studies on Tobacco IX. Campesterol from Flue-cured Leaves

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# Composition Studies on Tobacco IX.

## Campesterol from Flue-cured Leaves

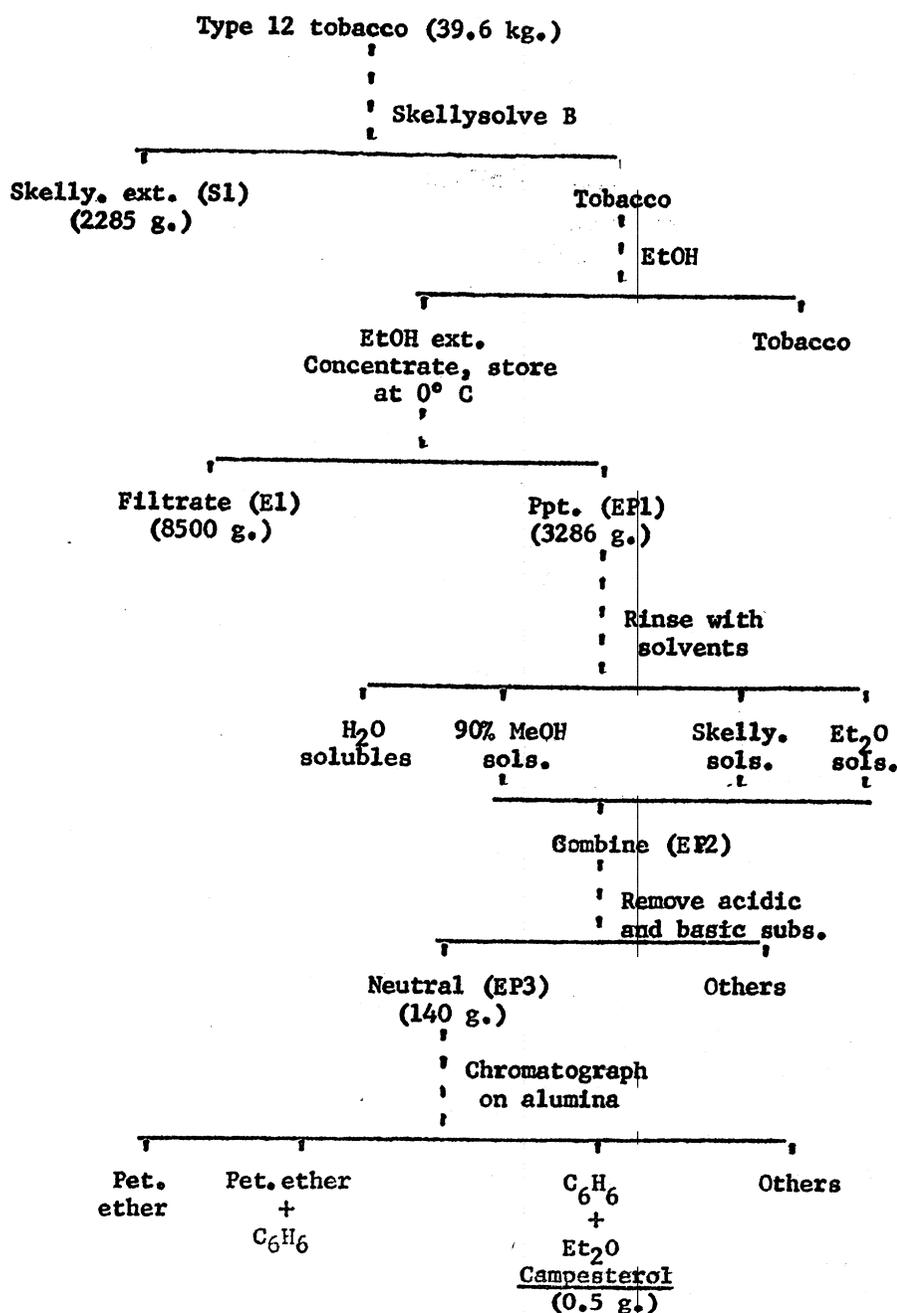


Figure 1. Large scale extraction of tobacco leaves and isolation of fraction containing campesterol.

### Introduction

Past reports in this series have described the isolation of  $\beta$ -sitosteryl monoglucoside, stigmasterol,  $\gamma$ -sitosterol, ergosterol, and a partially characterized steroidal glycoside from unaged flue-cured tobacco leaves (Dymicky and Stedman, 1958; 1959a,b; Grossman and Stedman, 1958). The present report concerns the isolation of another phytosterol, campesterol, from the same source.

### Methods and Results

Campesterol was isolated from a fraction obtained in a large scale extraction of unaged, flue-cured Type 12 tobacco leaves. Since this extraction will be referred to in future publications, a detailed description of the procedure is given below and in Figure 1.

Thirty-nine and six-tenths kg of ground leaf webs<sup>2</sup> were extracted with 400 l. of Skellysolve B<sup>3</sup> in a 1000 l. stainless steel kettle. In this procedure, the tobacco was placed on a screen in the base of the tank and the solvent was poured on the tobacco and stirred. A pump below the tank drew the solvent through the tobacco, circulated the extract upward through a heated section of pipe, and directed the warm (60° C) extract into the top of the tank where the solution was discharged through a nozzle and onto the tobacco. The extraction was performed by circulation and agitation in this manner for 4 hours, and the tobacco was soaked in the extract overnight. The extract was then drawn off, 320 l. of fresh solvent were added and the

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

<sup>2</sup>Webs of mixed grades of Type 12 tobacco pulverized so that all particles passed a 10 mesh screen and most of them passed a 20 mesh screen.

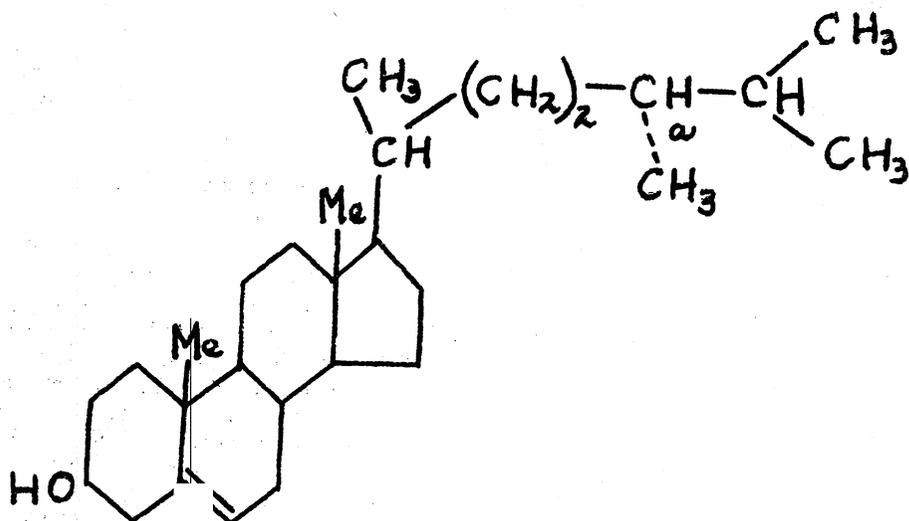
<sup>3</sup>Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

cycling repeated for 1.5 hours. After drawing off this solution, a third extraction with fresh solvent was performed for one hour using 290 l. of Skellysolve B followed by soaking overnight. All extracts were combined (S1, Figure 1).

The tobacco was then extracted with two successive 440 l. portion of absolute ethanol for 4.5 and 9.5 hours, respectively, in the same manner as above. The ethanolic extract was drawn off and the tobacco was then soaked in another 430 l. of ethanol overnight. This extract was drawn off and the overnight soak repeated with 340 l. of absolute ethanol. All ethanolic extracts were pooled and concentrated to 20 l. During the concentration, precipitation occurred and the solids were filtered off. The filtrate was stored at 0° C for two days and the resulting precipitate was filtered off and pooled with the above solids (EP1).

EP1 was successively washed with 8 l. of water, 2 l. of 90 per cent methanol, 2 l. of Skellysolve B and 2 l. of diethyl ether. Water removed most of the solids. Methanol and Skellysolve dissolved only negligible amounts of material. Ether extracted all of the remaining material and the ethereal solution was combined with the methanol and Skellysolve extracts, which were evaporated to a thick, brown, viscous residue (EP2). The residue was dissolved in 2 l. of diethyl ether, and the ethereal solution was dried over magnesium sulfate and successively extracted with 2 l. portions of 10 per cent sulfuric acid and five per cent potassium hydroxide solutions. The ethereal layer containing neutral substances was then dried and evaporated to 300 ml (EP3) which was then chromatographed on an 8 x 10 cm column containing 4.5 kg of acid-washed alumina. Elution was performed with the following series of solvents: petroleum ether, 5.5 l.; petroleum ether-benzene, 1:1, 2 l.; benzene, 4.5 l.; benzene-diethyl ether, 1:1, 3 l.; diethyl ether, 4 l.; methanol, 6 l.; chloroform, 4 l.; and acetone 8 l. Fractions of five hundred ml. each were collected for each eluting solvent. On evaporation of the solvent from the first two fractions eluted with 1:1 benzene-diethyl ether, brown, viscous residues were obtained from each fraction. The residues were combined (1.5 g) and dissolved in 5 ml. ether.

After two days storage at room temperature, a crystalline precipitate was observed which was filtered off. This solid showed m.p. 140°-150° C and gave a positive Liebermann-Burchard reaction. Two recrystalliza-



Campesterol

tions from acetone gave 0.5 g of a white solid, m.p. 156°-157° C,  $[\alpha]^{25D} -34.3$ ,<sup>4</sup> lit., campesterol: m.p. 157°-158° C,  $[\alpha]^{23} -33$ . (Fernholz and MacPhillamy, 1941)). The infrared spectrum of the solid showed the characteristics of a phytosterol in the "fingerprint" region (800-1100 cm.<sup>-1</sup>).

The sterol was acetylated in pyridine-acetic anhydride by heating the mixture at 110°-115° C for 2.5 hours. The solvents were removed under nitrogen and the residue crystallized twice from ethanol, giving white crystals, m.p. 139°-140° C.  $[\alpha]^{28D} -38.5$  (lit., campesteryl acetate: m.p. 139°-140° C,  $[\alpha]^{24D} -37.0$  (Schuette and Link, 1954)).

Benzylation of the sterol was performed in a pyridine-benzoyl chloride mixture heated at 100° C for 1.5 hours. The solvents were removed under nitrogen and the residue was crystallized twice from a 1:1.5 benzene-ethanol mixture. The benzoate showed m.p. 158°-159° C and  $[\alpha]^{28D} -9.4$  (lit., campesteryl benzoate: m.p. 158°-160° C,  $[\alpha]^{28D} -9.1$  (Fernholz and MacPhillamy, 1941)). The benzoate was saponified in five per cent potassium hydroxide in methanol under reflux for 1.5 hours, and the saponification mixture was evaporated under nitrogen during which water was added dropwise. The precipitated sterol was filtered, and the filtrate was neutralized with 0.1 N hydrochloric acid giving an equivalent of 495 (calcd. mol. wt., campesteryl benzoate: 504).

The sterol was hydrogenated for six hours in glacial acetic acid at 6

atmospheres using platinous oxide as the catalyst. The stanol obtained from this reaction was recrystallized twice from acetone and showed m.p. 145.5°-146.5° C,  $[\alpha]^{28D} +31.6$  (lit., campestanol: m.p. 146°-147° C,  $[\alpha]^{24D} +31$  (Fernholz and MacPhillamy, 1941)).

On admixture with an authentic sample of campesterol provided by Dr. O. Wintersteiner, Squibb Institute for Medical Research, the isolated sterol showed no depression of melting point.

#### Discussion

Campesterol is a comparatively rare phytosterol, but the structure has been fully characterized (Fernholz and Ruigh, 1941). The sterol occurs in rapeseed, soybean, wheat germ and rye germ oils (Fernholz and MacPhillamy, 1941; Nomura, 1949; Matagrin, 1950; Schuette and Link, 1954). Campesterol has also been isolated as an intermediate in the hydrogenation of chalinasterol (Bergmann *et al*, 1951). Apparently, the free sterol is a minor steroidal component of tobacco since the isolated material represents less than one per cent of the weight of total sterols present therein.

Since fractions obtained in the above large scale extraction will be referred to in the future, some comment should be made on the completeness of extraction attained. As shown in Figure 1, Skellysolve B extracted 2285 g of solids equivalent to 5.8 per cent of the tobacco weight (moist basis). Small scale extraction of this tobacco (Soxhlet, 24 hours) gave 7.5 per cent solids. Thus the large scale procedure removed approximately three-quarters of the substances extracted by the Soxhlet. This appears

<sup>4</sup>All rotations, including literature citations, were determined using CHCl<sub>3</sub> as the solvent and c equivalent to 1.04-2.20.

to be quite satisfactory, considering the scale of the operation. Similar values for the ethanolic extraction of tobacco (previously extracted with Skellysolve B) were 29.8 (large scale) and 22.9 (Soxhlet) per cent. This difference is greater than that of the Skellysolve extraction; thus, the larger amounts of ethanolic extractives of the large scale operation are not entirely attributable to the removal of substances not previously extracted with Skellysolve B. Regardless of the reason for the difference, it is apparent that the large scale extraction was quite thorough and the extracts can be used for comparative quantitative estimations of constituents.

#### Summary

Mixed grades of Type 12 tobacco leaves were successively extracted with Skellysolve B and ethanol. On concentration of the ethanolic extract and storage of this concentrate at 0° C, precipitates were obtained from which campesterol was ultimately isolated. Free campesterol is a minor component of the steroidal complement of flue-cured leaves.

#### Acknowledgments

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#### Literature Cited

- Bergmann, W., R. J. Feeney and A. N. Swift, "Marine Products. XXXI. Palysterol and Other Lipide Components of Sea Anemones", *J. Org. Chem.* **16**: 1337-44 (1951).
- Dymicky, M. and R. L. Stedman, "Composition Studies on Tobacco. I.  $\beta$ -Sitosteryl D-Glucoside from Flue-Cured Tobacco Leaves", *Tobacco Science* **2**: 99-101 (1958).
- Dymicky, M. and R. L. Stedman, "Composition Studies on Tobacco. IV. Ergosterol, Gamma-Sitosterol and a Partially Characterized Steroidal Glycoside from Flue-Cured Leaves", *Tobacco Science* **3**: 4-8 (1959a).
- Dymicky, M. and R. L. Stedman, "Composition Studies on Tobacco. VII. Isoeugenol, Hydrocarbons and Probable Bound Stigmasterol from Flue-Cured Leaves", *Tobacco Science* **3**: 60-1 (1959b).
- Fernholz, E. and H. B. MacPhillamy, "Isolation of a New Phytosterol: Campesterol", *J. Am. Chem. Soc.* **63**: 1155-6 (1941).
- Fernholz, E. and W. L. Ruigh, "Constitution of Campesterol", *J. Am. Chem. Soc.* **63**: 1157-9 (1941).
- Grossman, J. D. and R. L. Stedman, "Composition Studies on Tobacco. II. Isolation and Identification of Stigmasterol from Flue-Cured Leaves", *Tobacco Science* **2**: 115-6 (1958).
- Matagrín, A., "The Phytosterols of Soybean and the Synthesis of Hormones and Vitamins of Steroid Composition", *Inds. agr. et aliment. (Paris)* **67**: 221-9 (1950).
- Nomura, D., "Sterols in Crude Phosphatides (Oil Foots) of the Soybean", *J. Chem. Soc. Japan* **3**: 196-7 (1949) (cited by Bergmann, "The Plant Sterols", *Ann. Rev. Plant Physiol.* **4**: 383-426 (1953)).
- Schuetz, H. A. and W. E. Link, "Isolation of Campesterol and  $\Delta^7$  Stigmasterol from Rye Germ Oil", *J. Am. Chem. Soc.* **76**: 4192 (1954).