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Composition Studies on Tobacco I. β -Sitosteryl D-Glucoside from Flue-cured Tobacco Leaves

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Introduction

Within the past few years, interest in the chemical composition of tobacco and its smoke has intensified.

In contrast to earlier studies which were limited, in many cases, to the detection of classes of chemical compounds (Frankenburg, 1946) the

newer investigations have emphasized precise isolation and characterization of components. The phytosterols are an example of this trend. Although the occurrence of such compounds in tobacco leaves and smoke has been known for many years (Mosca, 1913; Kobel and Neuberger, 1935; Schurch and Winterstein, 1935; Shmuk, 1937), only two structures have been identified. Khanolkar et al. (1955) have isolated a glucoside of γ -sitosterol from processed chewing tobacco, and Kosak et al. (1957) have found stigmasterol in cigarette smoke. In no instance has the identification of a steroid from the leaves of flue-cured tobacco been reported. The present publication concerns the isolation of such a compound, β -sitosteryl D-glucoside, from flue-cured tobacco.

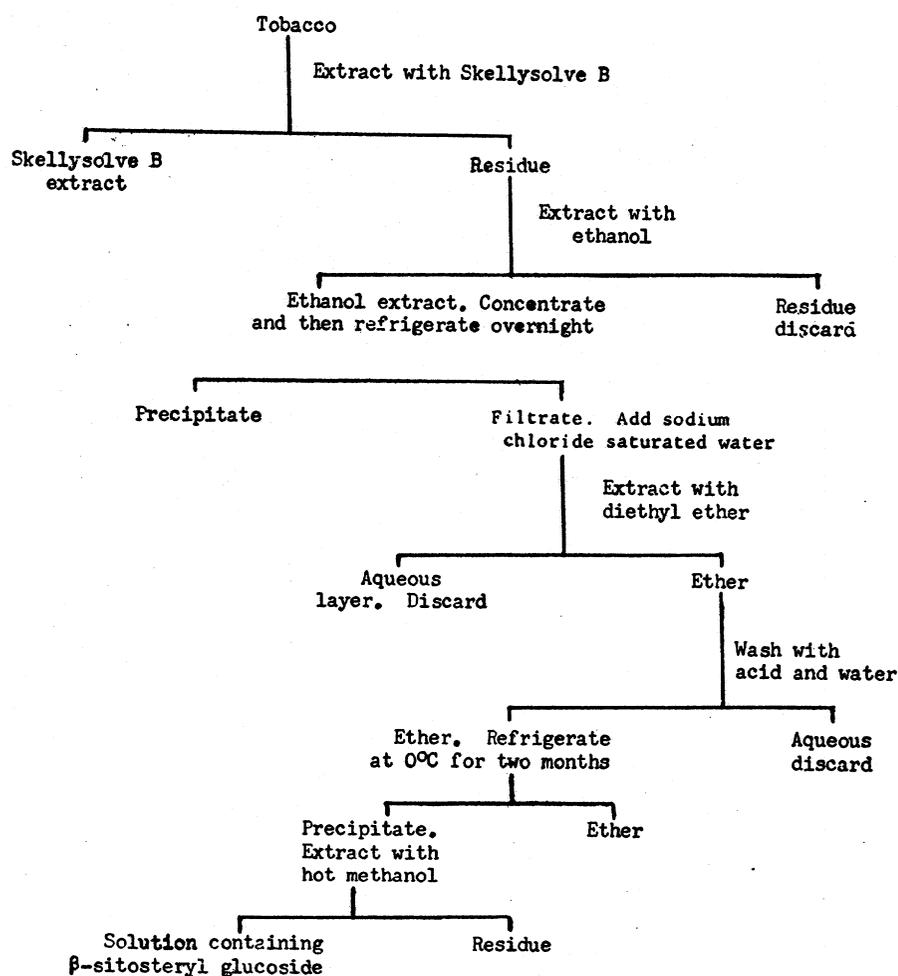


Figure 1. Fractionation scheme for the isolation of β -sitosteryl D-glucoside from flue-cured tobacco leaves.

Experimental and Results

The essential elements of the fractionation procedure are outlined in figure 1. Details of the separation are as follows: 37 kg of mixed grades of U. S. Type 12 flue-cured (but unaged) tobacco were successively extracted under reflux with 320 l. Skellysolve B and the same volume of 95 per cent ethanol. The ethanolic extract, which contained the sitosteryl glucoside, was evaporated in vacuo to 16 l., stored at 0°C overnight and the waxy precipi-

¹ Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Table 1. Reported melting points (°C.) (IMP and specific optical rotations (SOR) of β -sitosteryl D-glucoside, β -sitosterol and derivatives.

Compound	Present Study MP	Study SOR*	Swift MP	(1952) SOR	Jantzen and Gohdes (1934)		Stoll and Jucker (1955)	
					MP	SOR	MP	SOR
Glucoside	287-289**	...	298**	-40.1°	..	**	..	**
Acetylated glucoside	164.5-165.0	-20.7	171	-33.7°	166.3-166.7	-23.0°
Sterol	138-139	-37.0	137-138	-38.2°	138.4-139.0	..	135-137; 139-140	-34°
Acetylated sterol	121-122	-39.4	125-126	-40.3°	120-121	..

*Solvents for SOR determinations: present study, acetone; Swift, pyridine for acetylated glucosides and chloroform for sterol and its acetate; Stoll and Jucker, chloroform. Temperatures for SOR determinations were 20°C (sterol and acetate) and 23°C (acetylated glucoside) in the present study; temperatures for literature values were 20°-25°C. The Fisher-John melting point apparatus was used through this work; use of any commercial product does not constitute endorsement by the U. S. Department of Agriculture.

**See Discussion.

tate filtered off. To four liters of the alcoholic filtrate containing the glucoside was added sufficient sodium chloride saturated water to give two layers and permit partitioning with 12 l. diethyl ether which extracted the glycoside. The ether fraction was evaporated to 3 l. and successively washed with 20 per cent sulfuric acid solution and water. One-half of the ether extract was then refrigerated at 0°C for two months at which time a crude, greenish-white precipitate containing the glycoside formed.

By extraction of the crude precipitate with boiling methanol and several recrystallizations from the same solvent, 154 mg of β -sitosteryl glucoside was obtained. The purified glucoside softened at 282°C and melted with decomposition at 287°-289°C. The compound gave an emerald color in the Liebermann-Burchard test, and the infrared spectrum showed the presence of a low degree of unsaturation, extensive hydroxylation and the absence of other oxygenated functional groups. X-ray diffraction analysis indicated the absence of polymeric substance and of straight chains of more than approximately four carbon atoms.

The glycoside was then acetylated by the method of Swift (1952). The melting point and specific rotation of the resulting acetate are given in table 1 in comparative form since some discrepancies in physical constants appear in the literature. The acetylated derivative was saponified and, assuming four acetyl groups, a molecular weight of 784 was obtained (calcd. molecular weight, β -sitosteryl D-glucoside tetraacetate, 745). The regenerated compound was then hydrolyzed by prolonged treatment with weak acid (Swift, 1952). The aglycone was precipitated from the mixture by the addition of water and recrystallized from acetone. The

product gave the physical constants listed in table 1 and an infrared spectrum identical with authentic β -sitosterol, including the distinctive absorption bands at 959, 974, 1009, 1025 and 1053 cm^{-1} . Acetylation of the sterol formed a derivative having the physical properties listed in table 1. A mixed melting point of this compound with authentic β -sitosteryl acetate gave no depression. The sugar obtained from the hydrolysis mixture was converted to an osazone, m.p. 206°-207.5°C. lit., D-glucosazone, D-mannosazone, and D-fructosazone, m.p. 205°C. (Shriner and Fuson, 1940)). An aqueous solution

of the sugar gave $[\alpha]_D^{20} + 48.2$

(lit., hydrated glucose $[\alpha]_D^{20} + 47.7$

(Shriner and Fuson, 1940)).

The above data indicated that the compound was a monoglucoside of β -sitosterol.

Discussion

The literature contains many references to isolated natural products which yield "sitosterol" and glucose on hydrolysis (Bergman, 1953; Stoll and Jucker, 1955). Many of these studies were conducted prior to knowledge of the heterogeneity of the sitosterol group. The reports of Jantzen and Gohdes (1934) on soya oil, of Swift (1952) on oranges and of Stoll and Jucker (1955), who reviewed phytosterolins in general, appeared sufficiently reliable for comparative purposes and were thus employed here.

Discrepancies are observed in table 1 for the melting points and optical rotations of the free and acetylated glucosides. The melting point of the free glucoside is an un-

reliable criterion for identification since an erratic behavior is noted. Jantzen and Gohdes found that their compound browned on heating to 250°-260°C. and decomposed with gas formation at approximately 290°C., although the latter temperature varied with conditions. Swift reported a similar variation and expressed his data in terms of the time required (20-30 sec.) for melting when the sample was plunged into a bath preheated to 298°. In the present study, softening was noted at 282° prior to reaching the reported range at which decomposition occurred.

The optical rotation for the acetylated glucoside checked well with the value of Jantzen and Gohdes but was at variance with Swift's data. Some if not all of this variation may be due to a difference in solvents: Swift employed pyridine, Jantzen and Gohdes did not state their solvent, and the present study used acetone. Fieser and Fieser (1949) have discussed the influence of solvent on optical rotation measurements of steroids and indicated that more polar solvent sometimes give a more positive reading.

Concurrent work by another member of this laboratory has shown the presence of at least one other sterol, stigmasterol, in the leaves of flue-cured tobacco.² Thus, there are strong indications³ that flue-cured tobacco may contribute at least part of the steroids reported in the smoke of American blended cigarettes (Kosak *et al.*, 1957). Without the current study, the possibility existed that the steroids arose from other constituents of the cigarettes, such as burley or Turkish tobaccos or natural oils added as flavoring

² Isolated by Dr. J. D. Grossman and to be reported at a later date.

³ An indication rather than proof is claimed since the tobacco used here was not "aged."

agents.

Sitosterol has been shown to possess certain physiological properties, one of which is the ability to increase the rate of elimination of the carcinogenic agent, 3,4-benzopyrene, in mice (Weil-Malherbe, 1947). This property is of particular interest when related to reports of the isolation of this polynuclear hydrocarbon in the tar obtained from cigarette smoke (Cardon *et al.*, 1956; Kosak *et al.*, 1956; Strong *et al.*, 1957).

Acknowledgments

The assistance of Drs. J. D. Grossman and C. R. Eddy and Mr. D. A. Lutz is gratefully acknowledged.

Summary

The initial attempt to identify the steroidal composition of American flue-cured tobacco is described. β -Sitosteryl D-glucoside was isolated and characterized. Difficulties in characterizing sitosterol glycosides and the correlation between the current report and other published studies on related subjects are discussed.

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