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Synthesis of 6-O-Palmitoyl- β -D-Glucosyl β -Sitosterol*

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Acylated steryl glucosides have been isolated from soybean oil foots in a crystalline state and shown to be widely distributed in oil seeds and other plant tissues. The sterols were characterized as steryl 6-acyl-D-glucoside on the basis of their chromatographic behavior, infrared spectra and periodate oxidation as well as analytical data of their hydrolyzates.^{1,2)} In this report synthesis of 6-O-palmitoyl- β -D-glucosyl β -sitosterol is presented to obtain further confirmation for the assigned structure. β -Sitosterol and palmitic acid were the sterol and fatty acid, respectively, chosen for this purpose; they have been shown to be the major components in soybean steryl 6-acyl-D-glucoside. At first, β -sitosterol was converted to its glucoside by Koenigs-Knorr reaction where the sterol was treated with acetobromoglucose in ethereal solution in the presence of silver oxide as catalyst. β -Sitosteryl- β -D-glucoside thus obtained possessed properties identical with those of the mixed steryl glucosides from soybeans. The synthetic β -sitosteryl- β -D-glucoside was esterified with palmitoyl chloride in dry pyridine to give 6-O-palmitoyl-

β -D-glucosyl β -sitosterol. The latter showed the similar properties with those of the steryl 6-acyl-D-glucosides from soybeans, a preparation heterogeneous in sterol and fatty acid composition.

EXPERIMENTAL AND RESULTS

Materials

β -Sitosterol. Commercial preparation by Tokyo Chemical Industry Co., Ltd.

α -Acetobromoglucose. Prepared by the method of Bárczai-Martos and Korösy.³⁾ Recrystallization from ether gave practically pure acetobromoglucose, m.p. 86°C, which was ready for use for later processes. Yield: 71%.

Palmitoyl chloride. Prepared in this laboratory. b. p._{1mm} 152~154°C.

Synthesis of β -sitosteryl- β -D-glucoside.⁴⁾ Eighteen g of β -sitosterol and 18 g of acetobromoglucose were dissolved in dry ether and shaken for 8 hours at room temperature with 28 g of freshly precipitated dry silver oxide and 30 g of anhydrous sodium sulfate. The mixture was then added with a small amount of dilute nitric acid to coagulate the silver oxide, and filtered. The ethereal filtrate was washed twice with water, dried over anhydrous sodium sulfate and evaporated in vacuo. The residue was recrystallized from ethanol to remove a small amount of soluble by-product formed from the acetobromoglucose. The product

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thus obtained consisted of a mixture of tetraacetyl β -sitosteryl- β -D-glucoside and original β -sitosterol. It was dissolved in hot ethanol and the solution was digested for a few minutes with an excess of aqueous potassium hydroxide to eliminate the acetyl group from the tetraacetyl glucoside. After cooling, the free sterol and glucoside were collected. Water and ether were added to the substances and the fluffy layer produced at the interface was collected. When the crude glucoside thus obtained was purified by precipitation from pyridine-95% ethanol mixture, 680 mg of β -sitosteryl- β -D-glucoside was obtained. m.p. 270°C (decomp.), $[\alpha]_D^{20} = -40.0^{\circ}\text{C}$ (c 1, pyridine), *Anal.* Found: C, 72.16; H, 10.22. Calcd. for $\text{C}_{35}\text{H}_{60}\text{O}_6$: C, 72.87; H, 10.48%.

Synthesis of 6-O-palmitoyl- β -D-glucosyl β -sitosterol

β -Sitosteryl β -D-glucoside was esterified with palmitoyl chloride according to the method of Asselineau for esterification of methylglucoside.⁵⁾ It gave a mixture of mono-, di- and tetrapalmitate, which was separated into the individual compound by alumina column chromatography.

To 826 mg of β -sitosteryl β -D-glucoside dissolved in dry pyridine, 400 mg of palmitoyl chloride in dry benzene was added dropwise,

FIG. 1. Thin-layer Chromatogram of the Crude Synthetic Products.

Synthetic products were spotted on the plate coated with Silica Gel G and developed with chloroform-methanol-acetic acid-water (90:8:1:1). A, authentic sterols from soybeans; 1, steryl glucosides 2, acylated steryl glucosides

B, synthetic products

TABLE I. PROPERTIES OF SYNTHETIC AND SOYBEAN STERYL GLUCOSIDES, ACYLATED AND NON-ACYLATED

	Soybean glucosides*			Synthetic glucosides	
	Steryl glucoside	Acyl steryl glucoside	Steryl glucoside, deacylated**	β -Sitosteryl β -D-glucoside	6-O-Palmitoyl β -D-glucosyl β -sitosterol
R_F values on TLC***	0.1	0.3	0.1	0.1	0.3
m.p.	$273 \sim 276^{\circ}$ (decomp.)	$130 \sim 150^{\circ}$	—	270° (decomp.)	$158 \sim 159^{\circ}$
$[\alpha]_D^{20}$ (pyridine)	-46.5°	-25.5°	-44.2°	-40.0°	-32.7°
Analysis: Found	C, 71.05% H, 9.94%	C, 75.21% H, 10.86%	C, 71.93% H, 10.08%	C, 72.16% H, 10.22%	C, 74.60% H, 11.06%
Calculated				C, 72.87% H, 10.48%	C, 75.11% H, 11.12%

* Glucosides isolated from soybean are heterogeneous in composition, carrying β -sitosterol, stigmasterol and campesterol as steryl moieties and palmitic, stearic, oleic, linoleic and linolenic acids as the main acyl moieties of acylated glucoside.

** Steryl glucosides prepared by deacylation of acylated steryl glucosides.

*** Thin-layer chromatography was effected on Silica Gel G. Developing solvent: chloroform-methanol-acetic acid-water (90:8:1:1).

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and the mixture was kept standing for 3 hours at room temperature. After acidification with diluted sulfuric acid the reaction mixture was extracted with chloroform. The chloroform layer was washed twice with water and evaporated in vacuo. A small portion of the residue was dissolved in a small volume of pyridine and subjected to thin-layer chromatography. As shown in Fig. 1, the main product was monopalmitate (Fig. 1, spot 2); the other spots corresponded to those of di- and tetra-palmitate (Fig. 1, spots 3 and 4).

The monopalmitate was isolated from the main portion of the residue by Florisil chromatography; yield, 250 mg. Recrystallization from acetone gave 145 mg of pure monopalmitoyl-glucosyl β -sitosterol. By periodate oxidation, it was confirmed that the palmitoyl residue was linked to C-6 of the glucose moiety. Properties of the synthetic glucosides, acylated and non-acylated, are listed in Table I for comparison with those of natural preparations from soybeans. Infrared spectra of the synthetic 6-O-Palmitoyl- β -D-glucosyl β -sitosterol and natural mixed acylated steryl glucosides are shown in Fig. 2.

FIG. 2. Infrared Spectra (potassium bromide disks) of 6-O-Palmitoyl β -D-Glucosyl β -Sitosterol and a Natural Mixture of Acylated Steryl Glucosides from Soybeans.

A, Synthetic 6-O-palmitoyl- β -D-glucosyl β -sitosterol

B, Acylated steryl D-glucoside isolated from soybeans

DISCUSSION

In the previous report¹¹ we have described the characterization of acylated and non-acylated steryl glucosides isolated from soybeans. However, their anomeric configuration remained to be determined, because our attempts to hydrolyze these glucosides with a β -glucosidase preparation had been unsuccessful. Lepage also described his unsuccessful attempts to hydrolyze the acylated glucosides with β -glucosidase, maltase and amylase,⁶¹ though mention had been made of an assignment of β -configuration to the steryl glucosides from the root of *Levisticum officinali* L. by Nielsen and Kofod without detailed description of the enzymic hydrolysis.⁷¹

In the present investigation β -sitosteryl glucoside was synthesized by the Koenigs-Knorr reaction involving treatment of α -acetobromoglucose with β -sitosterol in ethereal solution in the presence of silver oxide. The reaction is known to take place with inversion of the anomeric carbon.⁸¹ Thus, the β -anomeric structure may be assigned to the β -sitosteryl D-glucoside, and 6-O-palmitoyl-D-glucosyl β -sitosterol as well. As shown in Table I, optical rotations of the steryl glucosides isolated from soybeans as well as those obtained by deacylation of the natural acylated steryl glucosides are in agreement with those of the synthetic glucosides. Therefore, β -glucosidic linkage may be assigned to the free and acylated steryl glucosides isolated from soy-

TABLE II. SPECIFIC ROTATION CALCULATED ACCORDING TO "HUDSON'S RULES OF ISOROTATION" OF STERYL GLUCOSIDES

	β -Sitosteryl α -D-glucoside	β -Sitosteryl β -D-glucoside	6-O-Palmitoyl- β -D-glucosyl β -sitosterol
Calculated	+26.4°	-38.1°	-27.0°
Observed		-40.0°	-32.7°

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beans. When the optical rotations of β -sitosteryl D-glucoside, free and acylated, are calculated according to "Hudson's Rules of Isorotation", the negative rotation results for the β -configuration of these glucosides (Table II). The negative specific rotation, $[\alpha]_D^{20}$, -40.0°C and -32.7°C of the free and acylated synthetic β -sitosteryl glucoside, respectively, will give further support to the assignment of

the β -configuration to these glucosides.

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