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Steryl Glycoside Formation in Seedlings of *Nicotiana tabacum* L.[1]

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PARSHALL B. BUSH[2] AND C. GRUNWALD
Department of Agronomy, University of Kentucky, Lexington, Kentucky 40506

ABSTRACT

Particulate enzyme preparations from tobacco seedlings (*Nicotiana tabacum* L.) were used in the synthesis of steryl glycoside. The data obtained by measuring cholesterol-4-^{14}C incorporation generally agree with results obtained with UDP-glucose-^{14}C. The *in vitro* reaction was linear for the first 10 minutes and had a pH optimum of 7.0 to 7.4. Addition of ATP activated while UDP-glucose inhibited slightly the reaction. In short term experiments, the percentage disappearance of endogenous and added sterol was about the same.

Intact tobacco seedlings incorporated cholesterol-4-^{14}C and sitosterol-4-^{14}C into their steryl glycosides. The acylated steryl glycosides were more rapidly labeled than the nonacylated form. After 12 hours of incubation with cholesterol-4-^{14}C, about 5% of the radioactivity was recovered as steryl glycoside and 12% as acylated steryl glycoside. Incubation for 12 hours with authentic cholesteryl-^{14}C glucoside gave only a 4% acylation, and under these conditions 21% of the radioactivity was recovered as free cholesterol. It is suggested that acylated steryl glycosides may be formed through the acylation of steryl glycosides or the transfer of an acyl-glycosyl group to sterol.

Steryl glycosides have been isolated from a variety of higher plants: groundnut (2), potato (10, 18), soybean (15, 16), pea, spinach, avocado, cauliflower (21), orange (24), barley (5, 14), mung bean (17), and tobacco (3, 4, 12, 25). The sugar moiety of the steryl glycosides has been identified as glucose and mannose (8, 24, 25). Cellular fractionation studies with tobacco leaves showed that the larger cellular organelles (500*g* and 2500*g*) have a higher percentage of steryl glycoside than the smaller organelles (12). Hou *et al.* (15, 16), Eichenberger and Newman (9), Kauss (17), Ongun and Mudd (21), and Péaud-Lenoel and Axelos (22) demonstrated that steryl glycoside biosynthesis occurs in particulate enzyme preparations, and UDP-glucose is the most active glycosyl donor. It is generally believed that the glycoside bond is formed through the meditation of nucleotide sugars (9, 15–17, 21). Hou *et al.* (16) reported that added sterol stimulated steryl glycoside formation. Kauss (17) and Ongun and Mudd (21) could not confirm this finding; however, Ongun and Mudd (21) found a dependency on added sterol if the enzyme preparation was treated with acetone. A similar observation was reported by Péaud-Lenoel and Axelos (22).

The biosynthesis of acylated glycosides in *in vitro* systems has also been examined, and it is generally believed that steryl glycosides give rise to acylated steryl glycosides (1, 6, 7, 16, 21, 22). Eichenberger and Grob (6, 7) reported that galactolipids are needed for acylation to occur; and they found that digalactosyl diglyceride was more active than monogalactosyl diglyceride. If acetone powder preparations were used, phosphatidyl ethanolamine was required for acylation to proceed (1).

In all previous experiments the biosynthesis of steryl glycosides and acylated steryl glycosides was studied by measuring the incorporation of the ^{14}C-sugar moiety. Furthermore, these investigations were performed with particulate enzyme preparations, and none compared *in vitro* with *in vivo* experiments. In the present study we examined the incorporation of ^{14}C-sterol into the steryl glycosides and acylated steryl glycosides using particulate enzyme preparations and intact seedlings.

MATERIALS AND METHODS

Chemicals. All solvents were reagent grade. Cholesterol, campesterol, stigmasterol, and sitosterol standards were purchased from Applied Science Laboratory, College Park, Pa., cholesterol-4-^{14}C from Tracer Laboratory, Waltham, Mass., and sitosterol-4-^{14}C from Amersham/Searle, Arlington Heights, Ill. Cholesteryl-^{14}C glucoside was synthesized from cholesterol-4-^{14}C according to the method of Meystre and Miescher (20) as described previously (14). Cholesterol-4-^{14}C palmitate was synthesized *via* the acid chloride by the method of Pinter *et al.* (23). The reaction products were purified by column chromatography (14) and gave only one radioactive spot by TLC.

***In Vivo* Experiments.** Tobacco (*Nicotiana tabacum* L. var. Burley 21) seeds were germinated for 6 days on Whatman No. 3 filter paper as previously described (3). The filter paper with seedlings was cut into 5-cm squares, and each square was put into a separate Petri dish. Ten milliliters of 10 mM Na-K phosphate buffer (pH 6.6) containing 0.1 mM penicillin G and 0.1 ml of ethanol containing the desired sterol were added to each Petri dish. At the end of the desired incubation period, the seedlings were washed free of excess sterol with 2 liters of distilled water. All experiments were carried out with illumination at 2.2×10^3 ergs/cm^2-sec. A portion of the seedlings was removed for dry weight determination. The remaining sample was ground for 2 min in acetone with an Omnimixer

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[2] Present address: Soil Testing and Plant Analysis Laboratory, Extension Agronomy Department, University of Georgia, Athens, Ga. 30601.

Table I. *In Vitro Cholesteryl Glycoside Synthesis*

Standard assay 6 mM Na-K phosphate buffer at pH 7.4 containing 81 μmoles of ATP, 0.4 μmole of CoA, 33 μmoles of GSH, 10 μmoles of $MgCl_2$, 0.011 μmole (34.8 μc/μmole) of cholesterol-^{14}C in 30 μl of ethanol, and 1 ml of enzyme preparation. Final volume was 10 ml, and the reaction time was 10 min.

Enzyme Preparation	Protein	Endogenous Sterol	Cholesterol-^{14}C Converted to Glycoside
	mg/assay	*μg/assay*	%
Crude fraction	3.9	46	40
Particulate fraction	3.1	41	64
Soluble fraction	2.1	5	3

and extracted in a Soxhlet apparatus for 24 hr. The free, esterified, and glycosidic sterols were isolated by column chromatography and precipitated with digitonin (14).

For cellular fractionation studies, seedlings incubated in the above cholesterol-^{14}C medium were washed free of excess radioactive sterol and ground with mortar and pestle at 3 C in 50 ml of 100 mM phosphate buffer at pH 7.5 containing 500 mM sucrose, 10 mM NaCl, and 40 mM EDTA (sodium salt). The homogenate was filtered through four layers of cheesecloth, and the cellular fractionation was by centrifugation in a refrigerated centrifuge at 2,500*g* for 30 min, 16,000*g* for 30 min, and 46,000*g* for 90 min. All pellets and the residue thus obtained were boiled in 50 ml of acetone for 30 min, and the free, esterified, and glycosidated sterols were isolated by column chromatography (14).

***In Vitro* Experiments.** With mortar and pestle, 20 g of 6-day-old tobacco seedlings were homogenized in 10 ml of 10 mM Na-K phosphate buffer (pH 7.4) containing 20 mM GSH and 1 g of soluble polyvinylpyrrolidone, mol wt 10,000. The homogenate was passed through 6 layers of cheesecloth, and the residue in the cheesecloth was reground in 10 ml of the above medium. The filtrates were combined, and the final volume was approximately 25 ml. This fraction (referred to as crude homogenate) was centrifuged at 500*g* for 10 min (discarded) and then at 20,000*g* for 30 min. The 20,000*g* pellet was resuspended in 20 ml of grinding medium with a glass homogenizer, and this suspension was used as the enzyme preparation.

The standard *in vitro* assay was carried out in 6 mM Na-K phosphate buffer at pH 7.4. The reaction mixture contained 81 μmoles of ATP, 0.4 μmole of CoA, 33 μmoles of GSH, 10 μmoles of $MgCl_2$, the desired sterol dissolved in 30 μl of ethanol, and 1 ml of the enzyme preparation. The final volume was 10 ml, and all experiments were carried out at 2.2×10^3 ergs/cm^2-sec of illumination. The protein content was determined by the method of Lowry (19). At various periods, 2-ml aliquots of the reaction mixture were removed and extracted with 15 ml of chloroform-methanol (2:1 v/v). The aqueous phase was subsequently partitioned three times against 5 ml of *n*-hexane. The chloroform and *n*-hexane fractions were pooled and evaporated to dryness under a stream of air. The residue was made up in 0.3 ml of absolute ethanol, spotted on Silica Gel G thin layer plates, and developed in chloroform-acetone (98:2 v/v). The areas containing the free and glycosidated sterols were removed, and the radioactivity in each fraction was determined by liquid scintillation.

The endogenous free sterol content was determined by adding 25 ml of acetone to a 5-ml aliquot of the enzyme preparation and heating the mixture over a steam bath for 30 min. The acetone fraction was filtered and evaporated to dryness under vacuum. The residue was dissolved in 25 ml of 75% ethanol, and the free sterols were extracted 3 times with *n*-hexane. The pooled *n*-hexane fractions were reduced to a small volume, and the free sterols were purified by column chromatography (14).

Sterol Analysis. A gas chromatograph equipped with a flame ionization detector, effluent sample splitter, and an electronic intergrator was used. The column was a 1.8-m U-shaped glass column packed with Anakrom ABS (80/90 mesh) coated with 5% OV-101 and operated at 260 C (11). Helium was the carrier gas at 60 ml/min. For sterol quantification, cholestane was used as the internal standard, and corrections were made for differences in detector response (13). The total sterol content is the summation of the individual sterol values.

RESULTS

***In Vitro* Experiments.** In a cell-free system, the 20,000*g* particulate enzyme preparation was found to be the most active in the synthesis of steryl glycoside (Table I). The soluble enzyme fraction which was ineffective in the synthesis of steryl glycoside was not further investigated. To characterize the sterol products of the particulate enzyme reaction, the sterols of the enzymatic mixture were extracted and chromatographed on Silica Gel G thin layer plates. The solvent employed was chloroform-acetone (98:2, v/v). The area corresponding to the steryl glycosides was scraped off, and the steryl glycosides were eluted with methanol. The methanol extract was reduced in volume and rechromatographed on a Silica Gel G plate in diisobutylketone-acetic acid-water (80:50:10, v/v/v). The steryl glycoside fraction showed only one component which cochromatographed with authentic cholesteryl glucoside. Acylated steryl glycosides were not formed at pH 7.4 under the standard assay conditions within a 10-min reaction time; however, a small quantity of acylated steryl glycoside was found at pH 6.4.

The synthesis of cholesteryl glycoside from cholesterol-^{14}C had a pH optimum of 7.0 to 7.4 (Fig. 1). The reaction required ATP for optimal activity and was slightly inhibited by UDP-glucose (Table II). Steryl glycoside synthesis was linear up to 50% conversion of the added radioactive cholesterol and continued until approximately 70% of the cholesterol-^{14}C was converted to its glycoside, which occurred within 40 min (Fig. 2). To test the possibility that added cholesterol was more readily

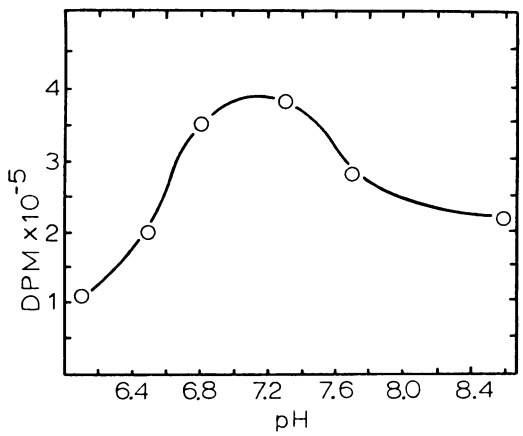

FIG. 1. Effect of pH on the *in vitro* synthesis of cholesteryl glycoside under standard assay conditions. Cholesterol, 0.011 μmole (34.8 μc/μmole); protein content, 3.4 mg; endogenous sterol content, 20.2 μg/assay. Phosphate buffer was used over the tested pH range.

available to the enzyme preparation than the endogenous free sterols, the disappearance of free sterol was determined quantitatively by gas chromatography. The standard enzyme assay with an added 10.4 μg of free cholesterol was used. After 10 and 60 min, samples were removed and analyzed for free sterols (Table III). In the first 10 min the percentage disappearance of cholesterol, consisting of endogenous and added cholesterol, was of the same magnitude as the disappearance of the endogenous campesterol, stigmasterol, and sitosterol. After 60 min slightly more cholesterol had disappeared on a percentage basis than any of the other three sterols.

***In Vivo* Experiments.** Intact 6-day-old tobacco seedlings were incubated with cholesterol-^{14}C and sitosterol-^{14}C for 24 hr, and the individual sterol fractions were isolated by column chromatography (Table IV). Of the sterols recovered from the tissue, 72% remained as free cholesterol and 67% as free sitosterol; but 27% of the cholesterol-^{14}C and 32% of the sitosterol-^{14}C were recovered in the glycoside fraction. Only about 1% of the recovered cholesterol and sitosterol was esterified during the 24-hr incubation period. The uptake of cholesterol was slightly greater than that of sitosterol, but, since the formation of steryl glycosides did not seem to be sterol-specific, cholesterol was used for most further experiments. Intact tobacco seedlings were allowed to incorporate cholesterol-^{14}C before the cellular fractions were isolated by differential centrifugation (Table V). Most of the ^{14}C-label was recovered as free sterol in all fractions. The 16,000*g* pellet contained the lowest amount of cholesteryl-^{14}C glycoside, and no sterols could be detected in the supernatant. Experiments with sitosterol-^{14}C gave similar results (unpublished data).

A time course experiment with intact tobacco seedlings incubated with cholesterol-^{14}C is shown in Figure 3. In this experiment the acylated steryl glycosides were separated from the steryl glycosides. Approximately 70% of the cholesterol-^{14}C recovered from the tissue was free cholesterol, even after 30 hr. The most unexpected observation was that the acylated steryl glycosides increased at a faster rate and obtained a higher final level of radioactivity than the steryl glycosides. Intact tobacco seedlings incubated for 12 hr with cholesteryl-^{14}C glucoside formed 4.2% acylated cholesteryl glucoside in the dark and 10.8% in the light (Table VI). However, under the same conditions the radioactivity recovered as free cholesterol was 21.2 and 22.7%, respectively. If free cholesterol-^{14}C was fed to intact plants, more cholesterol-^{14}C was incorporated into the acylated steryl glycosides than into the steryl glycosides (Table VI). Incorporation of ^{14}C into the steryl glycosides was

Table II. *Substrate Requirement for Cholesteryl Glycoside Formation*

Standard assay and cholesterol-^{14}C content as described in Table I. Protein content 2.68 mg and endogenous sterol content was 66.3 μg/assay. Reaction time was 10 min.

Reaction	Steryl Glycoside Formed	
	dpm	%
Standard assay	537,700	63.3
+ 0.88 μmole UDP-glucose	394,400	46.4
− ATP	42,500	5.0
+ 0.88 μmole UDP-glucose, −(ATP and CoA)	22,900	2.7

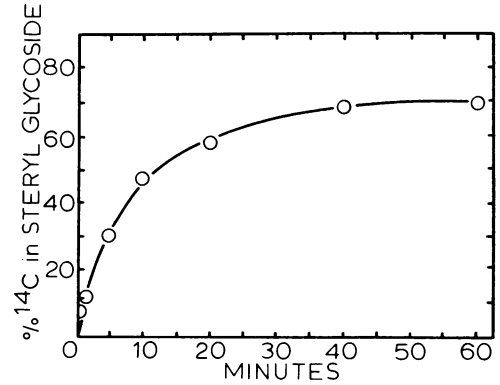

FIG. 2. Time course of *in vitro* biosynthesis of cholesteryl glycoside under standard assay conditions. Cholesterol, 0.011 μmole (34.8 μc/μmole); protein content, 3.4 mg; endogenous sterol content, 44.4 μg/assay.

Table III. *Biosynthesis of Steryl Glycosides as Determined by Disappearance of Free Sterol*

Standard assay as described in Table I. Protein content per assay was 10 mg with a final volume of 50 ml.

Reaction Time	Cholesterol		Campesterol		Stigmasterol		Sitosterol	
min	μg	%	μg	%	μg	%	μg	%
0	26.5[1]	100.0	21.3	100.0	65.6	100.0	43.1	100.0
10	9.0	34.0	7.4	34.7	21.8	33.2	13.9	32.3
60	5.4	20.4	5.5	25.8	23.2	35.4	12.5	29.0

[1] Contained 16.1 μg of endogenous cholesterol and 10.4 μg of exogenous cholesterol.

Table IV. *Incorporation of Cholesterol-^{14}C and Sitosterol-^{14}C into the Sterol Fractions of Intact Tobacco Seedlings*

Six-day-old tobacco seedlings were incubated for 24 hr in 10 ml of medium which contained 1% ethanol and 0.057 μmole of cholesterol-^{14}C (34.8 μc/μmole) or 0.025 μmole of sitosterol-^{14}C (61 μc/μmole). The steryl glycoside value is the acylated and nonacylated form.

Fraction	Sterol Content	Cholesterol		Sitosterol	
	mg/g dry wt	*dpm*	%	*dpm*	%
Free sterol	0.837	224,114	71.8	107,500	67.4
Steryl glycosides	0.058	83,834	26.8	20,020	31.6
Steryl ester	0.659	4,342	1.4	2,636	1.0
Summation	1.554	312,290	100.0	130,156	100.0

Table V. *Intracellular Distribution of Cholesterol-^{14}C in Intact Tobacco Seedlings*

Six-day-old tobacco seedlings were incubated for 12 hr in 10 ml of aqueous medium which contained 1% ethanol and 0.057 μmole of cholesterol-^{14}C (34.8 μc/μmole). The steryl glycoside value is the acylated and nonacylated form.

Fraction	Total Radio-activity	Sterol Fractions	Sterol		
			Free	Glycoside	Ester
	dpm × 10^{-2}		%	%	%
Residue	1,502	1,475	77.7	20.4	1.9
2,500*g* for 30 min	225	217	80.7	16.6	2.7
16,000*g* for 30 min	215	207	89.1	8.5	2.4
46,000*g* for 90 min	205	203	77.6	20.4	2.0
Supernatant	243	...	...	...	...

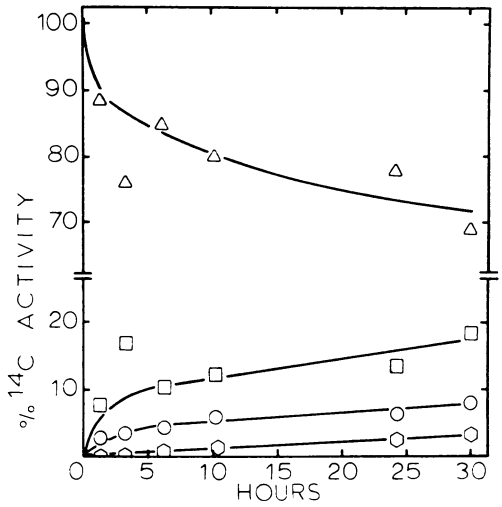

FIG. 3. Incorporation of cholesterol-4-^{14}C into the sterol fractions of 6-day old tobacco seedlings; △: free sterols; □: acylated steryl glycosides; ○: steryl glycosides; *hexagon:* steryl esters. For each time period the aqueous incubation medium was 10 ml containing 1% ethanol and 0.057 μmole of cholesterol-^{14}C (34.8 μc/μmole).

Table VI. *Interconversion of Cholesterol-^{14}C and Cholesteryl-^{14}C Glucoside in Intact Tobacco Seedlings*

Six-day-old tobacco seedlings were incubated for 12 hr in 10 ml of aqueous medium which contained 1% ethanol and 0.057 μmole of cholesterol (34.8 μc/μmole) or 0.010 μmole of cholesteryl glucoside (0.79 μc/μmole). Incubation was under fluorescent light (+) or in total darkness (−).

Sterol Supplied	Light	Sterol ^{14}C-Activity	Sterol Fraction			
			Free	Glycoside	Acylated Glycoside	Ester
		dpm	%	%	%	%
Cholesteryl glucoside	+	1,890	21.2	73.0	4.2	1.6
	−	1,760	22.7	62.6	10.8	3.9
Cholesterol	+	321,976	69.6	3.1	26.0	1.3
	−	230,124	77.3	6.4	12.8	3.5

greater in the dark, and under these conditions less radioactivity was found in the acylated steryl glycosides.

DISCUSSION

The steryl glycosides of germinating and developing tobacco seedlings constitute only a small fraction of the total sterol content (3, 4), and as seedlings develop the major steryl glycosides decrease (3). Cellular fractionation studies with seedlings incubated with cholesterol-^{14}C showed that all cellular fractions contained an appreciable amount of cholesteryl-^{14}C glycoside (Table V). Steryl glycoside biosynthesis, by measuring cholesterol-^{14}C incorporation in cell free systems, generally supports data obtained with UDP-glucose-^{14}C (6, 7, 9, 17, 21). Particulate enzyme preparations were more active in the synthesis of steryl glycoside than soluble enzyme preparations (Table I), and the reaction was linear with time up to 10 min (Fig. 2). It has been reported that steryl glycoside formation with particulate enzyme preparations from immature soybean seeds had a pH optimum of 8.0 (16), 6.3 to 6.6 from dark-grown mung bean shoots (17), and 8 to 9 with pea root preparations (21). Steryl glycoside synthesis with particulate enzyme preparations from tobacco seedlings had a pH optimum of 7.0 to 7.4 (Fig. 1).

Ongun and Mudd (21) reported that acylated steryl glycoside synthesis had a pH optimum of 6.5 to 7.0. In our *in vitro* system no acylated cholesteryl-^{14}C glycoside could be detected at pH 6.8. Probably one reason for the absence of acylated steryl glycosides in the present system was the short incubation period of 10 min. Most previous experiments were carried out at 1 to 5 hr of incubation (6, 16, 21). Kauss (17) and Péaud-Lenoel (22) reported acylated steryl glucoside formation within 10 min at pH 6.3 and 7.5, respectively. Eichenberger and Grob (6, 7) pointed out that the optimal pH for acylation depended upon the tissue, and they also found that galactolipids were required. Péaud-Lenoel and Axelos (1, 22) reported that with acetone preparations sterols were required for steryl glycoside synthesis and phosphatidyl ethanolamine for the acylation of steryl glycosides. Hou *et al.* (16) found that a chloroform-methanol extract from immature soybean seeds appreciably stimulated cell-free steryl glycoside synthesis as determined by glucose-^{14}C incorporation. They suggested that the stimulation was due to the addition of a more available form of free sterol, or that the sterol extract from soybean was a more efficient acceptor for glucose. Péaud-Lenoel and Axelos (22) did not observe a difference in steryl glycoside formation with simple sterols or plant lipid extracts. In the present investigation, with the use of gas chromatography, no difference between added and endogenous sterol disappearance could be found (Table III), and a sterol-specific reaction was not observed (Tables III, IV).

Addition of ATP stimulated steryl glycoside biosynthesis (Table II), and Hou *et al.* (16) postulated that ATP prevented phosphatase from hydrolyzing UDP-glucose. In our *in vitro* system the addition of UDP-glucose, in the presence or absence of ATP, however, decreased steryl glycoside synthesis (Table II). These results suggest that UDP-glucose protection may not be the only function of ATP.

Cholesterol incorporation studies with intact seedlings suggest that the formation of steryl glycoside and acylated steryl glycoside is rapid (Fig. 3). After 24 hr the specific radioactivity of the steryl glycoside fraction was higher than that of the free sterols (Table IV). Although the significance of this observation is not clear, it may be that the formation of steryl glycosides is associated with the plasmalemma and that externally fed sterol is more readily available than the cellular free sterol pool. Separating the steryl glycosides into acylated and nonacylated forms showed that in intact tobacco seedlings the formation of acylated steryl glycosides was favored over the formation of steryl glycosides (Fig. 3, Table VI). These results were unexpected, since experiments with particulate enzyme preparations indicated that the steryl glycosides are the precursor of the acylated steryl glycosides (1, 6, 7, 22). The present finding is supported by the only *in vivo* experiment reported in the literature (9). In the radioautogram published by Eichenberger and Newman (Fig. 1 in Ref. 9) it is evident that in lettuce leaf discs incubated with UDP-galactose-^{14}C a large portion of the radioactivity was in the acylated steryl fraction; as a matter of fact, this fraction seemed to have as much or more radioactivity than the steryl glycoside fraction. A similar observation has been made with excised barley roots, where it was found that, even though the acylated steryl glycosides constituted only 15% of the total steryl glycoside fraction, more radioactivity from fed cholesterol-4-^{14}C and sitosterol-4-^{14}C was associated with the acylated steryl glycosides than with the nonacylated steryl glycosides (Grunwald, unpublished results). These results suggest that not all of the acylated steryl glycosides may be formed through the acylation of steryl glyco-

sides. This suggestion is partly supported by feeding authentic cholesterol-^{14}C glucoside to intact seedlings (Table VI). In the light only 4% of the cholesteryl glucoside was acylated while 21% was recovered as the free sterol. Acylation of cholesteryl glucoside was higher in the dark (11%), but still more (23%) of the ^{14}C label was recovered in the free sterol fraction. These data suggest that acylation of steryl glycosides is not a rapid reaction in the *in vivo* system. Furthermore, under the same condition free cholesterol-^{14}C was incorporated to a greater extent into the acylated steryl glycosides than into the steryl glycosides (Table VI). The present results appear to be in conflict with published *in vitro* reports (1, 6, 7, 22) which suggest that steryl glycosides are the precursors to acylated steryl glycosides. A number of explanations can be proposed, but it must be remembered that all published *in vitro* experiments which were carried out with radioactive steryl glycoside had the label in the sugar moiety. In these experiments acylation of steryl glycoside was measured, but the formation of free sterol was not determined (1, 6, 7, 22). Our results with the label in the sterol moiety suggest that, in the *in vivo* system at least, acylated steryl glycosides may possibly be formed through the transfer of an acyl-glycosyl unit to the sterol moiety. This pathway of acylated steryl glycoside biosynthesis would not exclude the established pathway, the acylation of steryl glycosides. The theoretical possibility that acylated steryl glycosides could be formed *via* two pathways has been discussed by Hou *et al.* (16) and Eichenberger and Grob (6). The latter workers pointed out that acylated glucose has been isolated; however, the isolation of ^{14}C-labeled acylated glucose has never been reported. The biosynthesis of acylated steryl glycosides requires further study, especially in the *in vitro* system.

LITERATURE CITED

1. Axelos, M. and M. C. Péaud-Lenoel. 1971. Identification de l'activité phosphatidyléthanolamine:sterylglucoside transacylase extraite du Blé. Comptes Rendus 273, Series D: 1434–1437.
2. Aylward, F. and B. W. Nichols. 1958. Isolation of β-sitosterol-D-glucoside from groundnut phospholipids. Nature 181: 1064.
3. Bush, P. B. and C. Grunwald. 1972. Sterol changes during germination of *Nicotiana tabacum* seeds. Plant Physiol. 50: 69–72.
4. Bush, P. B. and C. Grunwald. 1973. Mevalonic acid incorporation into the sterols of six-day-old tobacco seedlings. Plant Physiol. 51: 110–114.
5. Bush, P. B., C. Grunwald, and D. L. Davis. 1971. Changes in sterol composition during greening of etiolated barley shoots. Plant Physiol. 47: 745–749.
6. Eichenberger, W. and E. C. Grob. 1969. Zur enzymatischen Bildung von Acyl-Steringlycosid in Pflanzen. Chimia 23: 368–369.
7. Eichenberger, W. and E. C. Grob. 1970. Enzymatische Acylierung von Sterin-^{14}C-Glucosid durch ein lösliches Enzym aus Karotten. Chimia 24: 394–396.
8. Eichenberger, W. and W. Menke. 1966. Sterole in Blättern and Chloroplasten. Z. Naturforsch. 21b: 859–867.
9. Eichenberger, W. and D. W. Newman. 1968. Hexose transfer from UDP-hexose in the formation of sterol glycoside and esterified sterol glycoside in leaves. Biochem. Biophys. Res. Commun. 32: 336–374.
10. Galliard, T. 1968. Aspects of lipid metabolism in higher plants. Phytochemistry 7: 1907–1914.
11. Grunwald, C. 1969. Analysis of free phytosterols by gas chromatography using liquid phase OV-101. J. Chromatogr. 44: 173–176.
12. Grunwald, C. 1969. Sterol distribution in intracellular organelles isolated from tobacco leaves. Plant Physiol. 45: 663–666.
13. Grunwald, C. 1970. Quantitative analysis of free phytosterols by gas chromatography using stationary phase OV-101. Anal. Biochem. 34: 16–23.
14. Grunwald, C. 1971. Effects of free sterols, steryl ester, and steryl glycoside on membrane permeability. Plant Physiol. 48: 653–655.
15. Hou, C. T., Y. Umemura, M. Nakamura, and S. Funahashi. 1967. Enzymic synthesis of sterol glycoside by a particulate preparation from immature soybean seeds. J. Biochem. 62: 389–391.
16. Hou, C. T., Y. Umemura, M. Nakamura, and S. Funahashi. 1968. Enzymic synthesis of sterol glucoside by a particulate preparation from immature soybean seeds. J. Biochem. 63: 351–360.
17. Kauss, H. 1968. Enzymatische Glucosylierung von pflanzlichen Sterinen. Z. Naturforsch. 23b: 1522–1526.
18. Lepage, M. 1964. Isolation and characterization of an esterified form of steryl glycoside. J. Lipid Res. 5: 587–592.
19. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
20. Meystre, C. and K. Miescher. 1944. Über Steroid. Zur Darstellung von Saccharidderivaten der Steroide. Helv. Chim. Acta 20: 231–236.
21. Ongun, A. and J. B. Mudd. 1970. The biosynthesis of steryl glucosides in plants. Plant Physiol. 45: 255–262.
22. Péaud-Lenoel, M. C. and M. Axelos. 1971. Uridine diphosphate glucose: stérol transglucosylase. Purification et activité dé préparations particulaires et solubles extraites du Blé. Comptes Rendus 273, Series D:1057–1060.
23. Pinter, K. G., J. G. Hamilton, and J. E. Muldrey. 1964. A method for rapid microsynthesis of radioactive cholesterol esters. J. Lipid Res. 5: 273–274.
24. Swift, L. J. 1952. Isolation of β-sitosteryl-glucoside from the juice of Florida Valencia oranges. (*Citrus sinensis*). J. Amer. Chem. Soc. 74: 1099–1100.
25. Wright, H. E., Jr., W. W. Burton, and R. C. Berry. 1962. Identification of stigmasterol-D-glucoside in aged burley tobacco. J. Org. Chem. 27: 918–920.