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SHORT COMMUNICATION

GLUCOSYLATION OF SOLASODINE BY EXTRACTS FROM *SOLANUM LACINIATUM*

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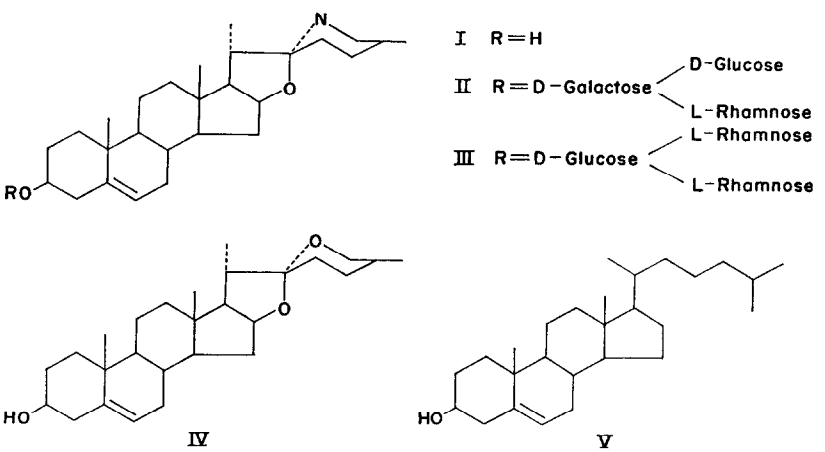
Abstract—The C-27 nitrogenous steroid solasodine has been shown to be an intermediate in the synthesis of the glycoalkaloids solamargine and solasonine by *Solanum laciniatum*. Administration of solasodine-^{14}C to excised plant stems led to the isolation of labelled glycoalkaloids and an enzyme preparation catalyzed the conversion of solasodine to the 3β-glucoside with UDP-glucose acting as donor in the reaction.

INTRODUCTION

THE LATTER stages in the synthesis of the steroidal alkaloids solasonine (II) and solamargine (III) are unknown. These compounds are found widespread in *Solanum* species, particularly in members of the subgenera Solanum[1] and Archaesolanum.[2] Their common aglycone, solasodine (I), is closely related to the spirostanol diosgenin (IV), and diosgenin and similar C-27 steroidal sapogenins are frequently found in *Solanum* species containing steroidal alkaloids.[1]



Both these classes of compounds have been shown to be derived from cholesterol (V) and it is likely that common intermediates are involved in their synthesis. Administration of acetate-^{14}C or mevalonic acid-2-^{14}C to *Solanum laciniatum* led to the isolation of solasodine with a labelling pattern identical with that observed with cholesterol.[3] Cholesterol-

[1] K. SCHREIBER, in *The Alkaloids* (edited by R. H. F. MANSKE), Vol. X, Academic Press, New York (1968).
[2] D. C. LEWIS and D. R. LILJEGREN, *Phytochem.* **9**, 2193 (1970).
[3] A. R. GUSEVA and V. A. PASESHNICHENKO, *Biokhimiya* **27**, 853 (1962).

4-^{14}C has been shown to be incorporated into solanidine,[4] tomatidine,[5, 6] and into spirostanols such as diosgenin.[7] The pathway to diosgenin is known to involve intermediates hydroxylated at the terminal and 22-position of the cholesterol side-chain.[8]

In contrast, no information is available regarding the sequence from cholesterol to the steroidal alkaloids, or the origin of the nitrogen atom, or on the stage of glycosylation of the 3β-hydroxyl group. This paper reports the results of an investigation into the glucosylation of solasodine, which is probably one of the last steps in the synthesis of the steroidal glycoalkaloids.

RESULTS AND DISCUSSION

Sodium acetate-2-^{14}C was fed to one *Solanum laciniatum* plant to obtain radioactive solasodine for further feeding experiments and enzyme studies. The solasonine and solamargine isolated were hydrolyzed to solasodine of specific activity 0·33 μc/μM.

A portion of this solasodine was administered to an excised stem from *S. laciniatum*. The specific activities of the isolated glycoalkaloids are shown in Table 1. Both solasonine and solamargine showed similar incorporations, indicating similar rates of synthesis and breakdown within the plant. This result can be compared to the 1% conversion of diosgenin (IV) to dioscin in *Dioscorea composita*,[8] a sequence involving the addition of the same triose moiety to the aglycone as is involved in the synthesis of solamargine from solasodine. It also indicates that solasodine is a likely intermediate in the biosynthesis of the glycoalkaloids; that is, that synthesis of the steroidal moiety is completed before glycosylation to the end products found in the plant. Further evidence to support this hypothesis was sought at the enzymic level.

TABLE 1. SYNTHESIS OF SOLASONINE AND SOLAMARGINE FOLLOWING ADMINISTRATION OF SOLASODINE-^{14}C (2·0 μc)

Glycoside isolated	Specific activity	% age yield
Solasonine	0 011 μc/μM	2·5
Solamargine	0·016 μc/μM	2 2

Several pathways for this glycosylation are possible. Sugar molecules could either be added sequentially to solasodine or in the form of a disaccharide or complete trisaccharide unit. Of these possibilities, the former seemed most likely. Barber has shown[9] that quercetin is formed from rutin by the addition of glucose followed by rhamnose in two discrete steps. A similar addition of monosaccharides is involved in the synthesis of anthocyanins.[10] Also, a glycosidase isolated from *Solanum nigrum* which is responsible for the breakdown of solasonine and solamargine removes the sugars in a stepwise manner.[11] Therefore, evidence was sought for the presence of an enzyme that would catalyze the first step in the synthesis of solamargine—the addition of glucose to solasodine.

[4] R. TSCHESCHE and H. HULPKE, *Z. Naturforsch.* **22b,** 791 (1967).
[5] R. TSCHESCHE and H. HULPKE, *Z. Naturforsch.* **21b,** 893 (1966).
[6] E. HEFTMANN, E. R. LIEBER, and R. D. BENNETT, *Phytochem.* **6,** 225 (1967).
[7] E. HEFTMANN, *Lloydia* **30,** 209 (1967).
[8] R. D. BENNETT, E. HEFTMANN and R. A. JOLY, *Phytochem.* **9,** 349 (1970).
[9] G. A. BARBER, *Biochem.* **1,** 463 (1962).
[10] J. B. HARBORNE, *Phytochem.* **2,** 85 (1963).
[11] D. R. LILJEGREN, unpublished results.

TABLE 2. CONVERSION OF SOLASODINE TO SOLASODINE-3β-GLUCOSIDE AFTER 4 hr INCUBATION AT 30°. COMPOSITION OF ASSAY MIXTURES DESCRIBED IN TEXT

Labelled precursor	Dis/min in 3β-glucoside	% conversion
Solasodine-^{14}C ($3{\cdot}6 \times 10^4$ dis/min)	$1{\cdot}36 \times 10^3$	3·8
UDP-glucose-^{14}C ($2{\cdot}2 \times 10^5$ dis/min)	$6{\cdot}4 \times 10^2$	0·3

A crude enzyme preparation was made from *S. laciniatum* leaves and incubated with either solasodine-^{14}C and excess UDP-glucose or UDP-glucose-^{14}C and excess solasodine. ATP was added in an attempt to minimize possible hydrolysis of the sugar nucleotide.[9] Synthesis of the glucoside occurred in both cases (Table 2), demonstrating the presence of a glucosyltransferase and the role of UDP-glucose as donor. The lower conversion in the experiment using UDP-glucose-^{14}C is explained by incomplete protection against hydrolysis of the small amount of sugar nucleotide present. Further experiments following the formation of the glycoside with time are represented in Fig. 1. The decrease in the amount present in the mixtures after 2 hr is probably due to the presence of a glycosidase in the crude extracts. The isolation of such an enzyme from *Solanum tuberosum* has been described.[12]

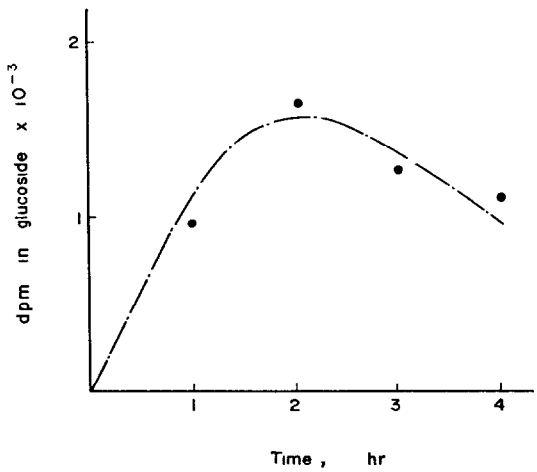


FIG. 1.

The above results further support the theory that glycosylation is the last step in the synthesis of solasonine and solamargine. Similar sequences have been demonstrated for the formation of glycosides of other secondary plant metabolites such as the cyanogenic glycosides[13] and flavonoids.[9, 14] Whether or not one enzyme of wide specificity catalyzes the addition of the different sugars found in solasonine and solamargine remains to be demonstrated using a purified enzyme system. Such a study is being made.

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[13] K. HAHLBROCK and E. E. CONN, *J. Biol. Chem.* **245**, 917 (1970).
[14] C. D. MILES and C. W. HAGEN, *Plant. Physiol.* **43**, 1347 (1968).

EXPERIMENTAL

Source of materials. Solanum laciniatum plants were grown in soil in a glasshouse from seed collected near Adelaide, South Australia. Sodium acetate-2-^{14}C (15·9 mc/mM) and UDP-glucose-^{14}C (204 mc/mM) were obtained from the Radiochemical Center, Amersham. Polyclar AT was purified as described by Loomis and Battaile.[15]

Isolation of solasodine-^{14}C. A solution of sodium acetate-2-^{14}C (500 μc) was fed to one *S. laciniatum* plant by the wick-feeding method.[16] After a growing period of 6 days the above-ground portion was harvested (wet wt. 16 g), frozen with liquid N_2, and ground to a fine powder in a mortar. The powder was extracted with boiling methanol (60 ml) and the residue after filtration washed with the same solvent. The methanol solution was evaporated to dryness, the residue dissolved in 5% HOAc (20 ml) and extracted with ether (3 × 25 ml). The aqueous phase was heated to boiling, basified with NH_3, and stored at 0° overnight. The gelatinous precipitate was collected by centrifugation, washed with dilute NH_3, and dissolved in 95% ethanol. Total alkaloid concentration (13·8 mg) was determined[2] using aliquots of this solution.

TLC analysis of the mixture (Si gel G; solvent: water-sat. *n*-BuOH–$NHEt_2$–MeOH, 85:10:3, system 1) showed 41% of the incorporated activity associated with solamargine (R_f 0·4), 36% with solasonine (R_f 0 28), and 17% with alkaloids of lower R_f (0·15–0·23) believed to be tetrasaccharides of solasodine [2]

The alkaloid solution was taken to dryness and heated under reflux for 3 hr with 5% HCl in methanol (2 ml). After treatment with NH_3 to liberate the free base, the solasodine was separated from a small amount of solaso-3,5-diene by chromatography on alumina (activity 2, 1 × 5 cm column) Elution with benzene–$CHCl_3$ (1:1) gave solasodine (5·4 mg) m.p. and mixed m.p. 200–202° (lit. m.p.[17] 203–205°). The sample was shown to be radiochemically pure by TLC chromatography (Si gel G; solvent cyclohexane–EtoAc, 1:1, system 2), where all activity was associated with the solasodine area (R_f 0·25). The specific activity was 0 33 μc/μM (0 86% incorporation from acetate).

Glycoalkaloid synthesis from Solasodine-^{14}C. Solasodine-^{14}C (2·5 mg, 2·0 μc) was dissolved in a few drops of 95% ethanol, diluted with H_2O, and the end of an excised stem of *S. laciniatum* immersed in the solution. Water was added as uptake proceeded to maintain the volume. After 24 hr, the stem (9 g) was ground with liquid N_2 and the alkaloid mixture (10 4 mg) isolated as described above. Resolution of the mixture on an alumina column using H_2O-saturated *n*-BuOH yielded solamargine (3·8 mg) and solasonine (2 3 mg) with specific activities shown in Table 1.

Enzyme extraction. Leaves from actively growing shoots of *S. laciniatum* were ground to a fine powder with liquid N_2 in a mortar. Portions of the powder were then added to an equal weight of Polyclar AT premixed at 0° for 1 hr with 0·2 M K_3PO_4, pH 7 0 (1 · 3, w/v). The mixture was ground further, strained through several layers of muslin, and centrifuged for 10 min at 3000 *g*. The supernatant was adjusted to 5% saturation $(NH_4)_2SO_4$ and the precipitate containing colored material removed by centrifugation. $(NH_4)_2SO_4$ was added to the supernatant to 60% saturation, the precipitate collected by centrifugation at 10,000 *g*, and redissolved in 0 05 M potassium phosphate, pH 7 0. After overnight dialysis against the same solution, the enzyme preparation was used without further purification.

Enzyme assays. (a) *Using UDP-glucose-^{14}C.* A mixture containing ATP (1 μM), UDP-glucose-^{14}C (0 5mμM), solasodine (50 mμM), and potassium phosphate (200 μmoles, pH 7·0) in 1·0 ml was added to 2·0 ml of the above enzyme preparation and incubated at 30°. The reaction was stopped by boiling in H_2O-bath for 10 min, the mixture basified with NH_3, and the precipitate collected by centrifugation. The residue was washed with water and extracted first with hot light petroleum and then with 95% EtOH Aliquots of the ethanol solution were spotted alongside reference compounds on silica gel plates, developed with solvent system 2, and following drying, redeveloped with system 1. Reference areas were stained using a 25% solution of Sb Cl_3 in $CHCl_3$, followed by heating at 110° Radioactivity was determined after scraping the silica gel into vials containing 5 ml of scintillation fluid.

(b) *Using solasodine-^{14}C.* Solasodine-^{14}C (50 mμmoles), ATP (10 μmoles), UDP-glucose (5 μmoles), and K_3PO_4 (200 μmoles, pH 7 0) in 1·0 ml were added to 2·0 ml of enzyme Incubation and extraction of products were as described above.

Determination of radioactivity. Aliquots of solutions in 95% EtOH or methanol were added to 10 ml of a toluene scintillation fluid containing 2,5-diphenyloxazole (PPO, 7 g), and 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP, 0·3 g) per liter. Radioactivity was measured using a Packard Tricarb liquid scintillation spectrometer model 3375.

Acknowledgement—Thanks are due to Mr. D. E. Symon for growth of *S. laciniatum* plants.

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