Transformations of Potato Glycoalkaloids by Rumen Microorganisms

Incubation of potato glycoalkaloids with rumen microorganisms resulted in initial hydrolysis to the alkaloid solanidine. A substantial portion of the solanidine produced was then reduced to the 5,6-dihydro analogue 5β-solanidan-3β-ol. No evidence for the subsequent involvement of esterification processes or metabolism of the nitrogen moiety was detected.

Plants of the *Solanum* genus contain trace amounts of a class of compounds called glycoalkaloids. These compounds consist of 3-hydroxysteroidal alkaloids linked glycosidically to an oligosaccharide, and they occur in all parts of the plant including the tubers. The steroidal alkaloid most common to domestic potatoes is solanidine (solanid-5-en-3β-ol), and it occurs generally in the form of its α-solanine and α-chaconine glycosides (Schreiber, 1968). Although varietal differences and genetics play an important role in the glycoalkaloid content of potatoes, factors such as exposure to light, soil types, fertilization practices, climate, tuber maturity, and mechanical or chemically induced stresses substantially alter the inherent levels.

A considerable literature exists concerning cases of potato glycoalkaloid poisoning in man and farm animals (Jadhau and Salunkhe, 1975). In recent years some concern has also been expressed regarding the potential toxicity or teratogenicity of these compounds on developing embryos (Brown and Keeler, 1978).

The increased use of cull potatoes and wastes from processing plants for animal feed and renewed interest in the potential feeding value of potato vines (Nicholson et al., 1978) prompted us to investigate the fate of potato glycoalkaloids when incubated in vitro with microorganisms of the bovine rumen.

EXPERIMENTAL SECTION

Chemicals. A mixture of α-solanine and α-chaconine glycoalkaloids was isolated from potato blossoms by removal of methanol from the 2% acetic acid–methanol extract and two recrystallizations from methanol of the solid precipitated by concentrated ammonium hydroxide. Solanidine was isolated by crystallization from 95% ethanol of the hydrolysis product (1 N ethanolic HCl at 100 °C for 1 h) from the glycoalkaloid mixture (Schreiber, 1968).

Equipment. Melting points were determined on a Kofler hot-stage microscope. Infrared (IR) spectra were determined by using a Beckman IR-20A spectrophotometer. Mass spectra (MS) were determined on a Hitachi Perkin-Elmer mass spectrometer. Optical rotations were measured in chloroform solutions at room temperature with a Kern full-circle polarimeter.

Gas–Liquid Chromatography. A Tracor 222 gas chromatograph (GLC) equipped with a Perkin-Elmer nitrogen–phosphorus detector and 1.8 m by 4 mm i.d. glass column packed with 3% OV-17 on 80–100-mesh H.P. Chromosorb W was used. The helium carrier gas flow rate was 65 mL/min. With an injection port temperature of 275 °C and a column temperature of 265 °C, the retention times for 5β-solanidan-3β-ol and solanidine were 7.1 and 8.3 min, respectively.

In Vitro Rumen Fermentation Procedure. Rumen contents were collected from a rumen fistulated cow that was maintained on timothy hay. The sample of rumen contents was taken before the morning feeding and was put into a prewarmed bottle. The bottle was completely filled to eliminate any air space. The inoculum was prepared by blending rumen contents in a Waring Blendor for 2 min under carbon dioxide, by squeezing them through four layers of cheesecloth, and then by filtering them through a 5-cm pad of glass wool (also under carbon dioxide) to remove feed particles.

Table I. Composition of Rumen in Vitro Solutions

solution A	
NH_4HCO_3	4.0 g/L
$NaHCO_3$	35.0 g/L
solution B	
Na_2HPO_4	5.7 g/L
KH_2PO_4	6.2 g/L
$MgSO_4 \cdot 7H_2O$	0.6 g/L
solution C	
$CaCl_2 \cdot 2H_2O$	13.2 g/L
$MnCl_2 \cdot 4H_2O$	10.0 g/L
$CoCl_2 \cdot 6H_2O$	1.0 g/L
$FeCl_3 \cdot 6H_2O$	8.0 g/L
solution D[a]	
cysteine hydrochloride·H_2O	0.625 g/100 mL
1 N NaOH	4.0 mL/100 mL
$Na_2S \cdot 9H_2O$	0.625 g/100 mL

[a] Prepared immediately before use by dissolving the cysteine hydrochloride in 95 mL of water and 4 mL of 1 N NaOH and then adding the Na_2S and dissolving it to give 100 mL.

To 1.0-L Erlenmeyer flasks containing 640 mg of soluble starch (DIFCO Laboratories) was added 32 mL of water, 16 mL of solution A (Table I), 16 mL of solution B, and 0.08 mL solution C. Except for controls, potato glycoalkaloids were added to give an eventual concentration of 1 mg/mL of flask contents. The flasks and contents were then saturated with and kept under carbon dioxide to a pressure of 10–20 cm of water. Saturation was judged complete when a control flask containing media and 0.5 mL of a resazurin solution (1 g of resazurin/L) remained pink. Eight milliliters of solution D was added to lower the redox potential as indicated by the media containing resazurin turning colourless.

After 30 min, 80 mL of freshly prepared rumen fluid inoculum was added. The incubation was conducted at 40 °C under a positive pressure of 10 cm of water provided by carbon dioxide. This procedure is similar to that described by Goering and Van Soest (1970).

Flasks containing no rumen fluid inoculum served as fermentation controls. Fermentations were sampled at various times by removing 10-mL aliquots of the flask contents, 0.1 mL of toluene was added, and the samples were immediately cooled in an ice bath. Samples were held at 4 °C until the analysis for glycoalkaloids was conducted.

Sample Analysis. A 5.0-mL aliquot of the rumen fermentation mixture was transferred to a separatory funnel and made alkaline with concentrated NH_4OH. The solution was then extracted with chloroform (4 × 15 mL), and the chloroform removed on a rotary evaporator. Benzene (10 mL) was added to the residue, and 10-μL aliquots were injected into the GLC for alkaloid analysis (King, 1980).

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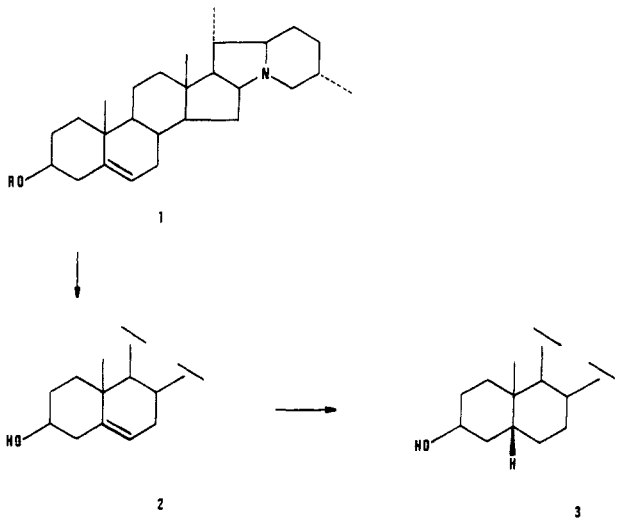

Figure 1. Reaction scheme for the transformation of potato glycoalkaloids by rumen microorganisms. R = oligosaccharides.

Alkaline hydrolysis of the rumen fermentation mixture (after chloroform extraction, etc.) was carried out by refluxing a portion (1.0 mL) for 2 h with 10% KOH in 70% ethanol (5 mL). After removal of the ethanol on a rotary evaporator, the remaining aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). Benzene (2 mL) was then added to the residue remaining after removal of the chloroform, and 10-μL aliquots were injected into the GLC for alkaloid analysis. Acid hydrolysis of the rumen fermentation mixture (after chloroform extraction) and control samples was carried out by the procedure described by King (1980).

Thin-Layer Chromatography. Solanidine and 5β-solanidan-3β-ol were separated on preparative thin-layer plates (coated with silica gel GF) by development in benzene–ethanol (10:1). The compounds were detected with a water spray and eluted from the silica gel with chloroform. They were recrystallized from 95% ethanol and subsequently determined to have the requisite melting point, optical rotation, IR, and MS data (Schreiber, 1968).

RESULTS AND DISCUSSION

GLC analysis of chloroform extracts taken from the in vitro rumen samples revealed the initial hydrolysis of the glycoalkaloid mixture (1) to solanidine (2) (Figure 1). In contrast to gastrointestinal studies involving rats (Nishie et al., 1971) where solanidine was the only product reported, we found that it was in turn converted to another compound. The conversion commenced with relatively little lag time (Figure 2). The new derivative was subsequently determined by IR and MS to be a 5,6-dihydro analogue of solanidine (2). Comparison with published melting point and optical rotation values identified it as the 5β-solanidan-3β-ol isomer (3) (Schreiber, 1968). This assignment is consistent with previous reports regarding the reduction of other Δ^5 plant sterols to their corresponding 5β-H steroid analogues by monogastric intestinal microorganisms (Rosenfeld and Hellman, 1971).

Although monogastric intestinal microorganisms exhibit the ability to esterify plant and animal sterols (Rosenfeld et al., 1967), no solanidine or 5β-solanidan-3β-ol derived esters were detected in the chloroform extracts of the fermentation mixture. Subsequent alkaline hydrolysis of the rumen fermentation mixture (after extraction of all chloroform-soluble materials) did not yield any identifiable glycoalkaloid metabolites.

As shown in Figure 2, hydrolysis of the glycoalkaloid mixture to solanidine was essentially complete within 24 h. The total available solanidines recovered averaged 83% at and beyond this period. These recoveries indicate that metabolism of the nitrogen moiety did not occur to any significant extent. The lag time shown for the production of solanidine (Figure 2) is consistent with the observations of Swain et al. (1978). Their enzymatic studies demonstrated that initial hydrolysis of the individual hexoses in the oligosaccharide portion of the glycoalkaloids was the preferred reaction sequence.

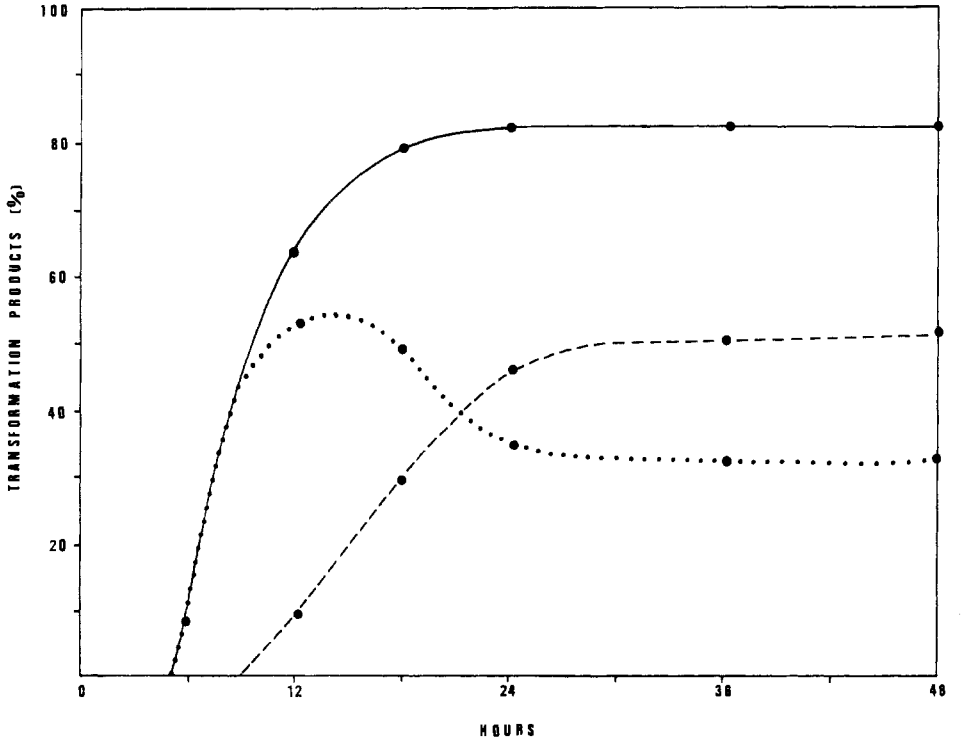

Figure 2. Rate of transformation of potato glycoalkaloids by rumen microorganisms. (···) Solanidine (2); (– – –) 5β-solanidan-3β-ol (3); (—) total solanidines (2 plus 3). Each point represents the mean of duplicate determinations.

Although two preliminary trials to determine optimum sampling times were required, the identity of the glycoalkaloid metabolites remained consistent.

This study indicates that the hydrolysis of potato glycoalkaloids by rumen microorganisms may be rapid enough so that any concern focused upon the large-scale absorption of these compounds from the gastrointestinal tract of ruminant animals is unnecessary. Attention, however, should be directed toward the fate of the relatively water insoluble alkaloid portion (solanidine) and its respective dihydro analogue (5β-solanidan-3β-ol).

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LITERATURE CITED

Brown, D.; Keeler, R. F. *J. Agric. Food Chem.* **1978**, *26*, 564.
Goering, H. L.; Van Soest, P. J. *U.S., Agric. Res. Serv., ARS* **1970**, *No. 379*.
Jadhau, S. J.; Salunkhe, D. K. *Adv. Food Res.* **1975**, *21*, 342.
King, R. R. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1226.
Nicholson, J. W. G.; Young, D. A.; McQueen, R. E.; DeJong, H.; Wood, F. A. *Can. J. Anim. Sci.* **1978**, *58*, 559.
Nishie, K.; Gumbmann, M. R.; Kely, A. C. *Toxicol. Appl. Pharmacol.* **1971**, *19*, 81.
Rosenfeld, R. S.; Hellman, L. *J. Lipid Res.* **1971**, *12*, 192.
Rosenfeld, R. S.; Paul, I.; Yamauchi, T. *Arch. Biochem. Biophys.* **1967**, *122*, 653.
Schreiber, K. In "The Alkaloids"; Manske, R. H. F., Ed.; Academic Press: New York, 1968; Vol. X, pp 1–192.
Swain, A. P.; Fitzpatrick, T. J.; Talley, E. A.; Herb, S. F.; Osman, S. F. *Phytochemistry* **1978**, *17*, 800.

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