Molluscicidal Activity of Natural Products. The Effect of Solanum Glycosidic Alkaloids on *Lymnaea cubensis* Snails[1, 2]

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ABSTRACT

The progressive increase of bovine fascioliasis in Puerto Rico demands the control of the population of the intermediate snail host. This report deals with preliminary results of the effect of extracts of *Solanum mammosum* fruits on *Lymnaea cubensis* snails. The methanolic crude extract shows 95% molluscicidal activity at 25 p/m. One component of the active extract was identified as solasod-5-en-3β-ol (solasodine).

INTRODUCTION

Molluscicidal research has made considerable advances, especially in the control of bilharziasis. For *Fasciola hepatica*, however, there is still need for molluscicides which can be obtained from local plant material. A progressive increase in the percentage of cattle infected with this parasite in Puerto Rico has been reported in recent slaughterhouse studies (4). A 1978 survey of dairy cattle on farms throughout the island revealed that the increase in bovine fascioliasis is higher than previously reported (5). In connection with our study on biologically active compounds from plants we examined the fruits of the plant *Solanum mammosum* (berenjena de cucaracha), which was previously observed to have molluscicidal properties (1, 6). The present paper reports the evaluation of the toxicity of water and methanol extracts of *Solanum mammosum* fruits on the local snail vector *Lymnaea cubensis* and the chemical analysis of the strong molluscicidal methanolic extract.

MATERIALS AND METHODS

Solanum mammosum fruits were collected in Dorado and Caguas, Puerto Rico, during March, April, and July. *Lymnaea cubensis* snails used in this study were cultured under standard laboratory conditions in

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plastic boxes provided with mud from the original snail habitat under continuous flow of dechlorinated and charcoal-sand filtered tap water.

PREPARATION OF CRUDE EXTRACTS

The ripe fruits were dried at 50° C and ground in a blender; 375 g of this material was extracted wih methanol:water (9:1). After filtration through glass wool and filter paper, vacuum evaporation of the solvent gave 23.3 g (6.2%) of a brown residue. For the water extract, 20 g of the dried and ground ripe fruits were stirred at room temperature for 24 hours with 500 ml of distilled water. After filtration, the residue was dried and weighed, and the solution was made up to a desired concentration for analysis.

SEPARATION OF ACTIVE FRACTIONS

Thin layer chromatography of the crude methanolic extract on silica gel plates with chloroform:methanol (5:1) revealed the presence of nine spots. Addition of ammonia to a hot solution of the methanolic crude extract in 3% acetic acid produced a light brown precipitate which was filtered, washed with cold water and dried with air. Column chromatography of this precipitate on silica gel with chloroform:methanol (3:1) afforded a solid that showed only two spots on silica gel plates, chloroform:methanol (5:1). This mixture (300 mg) was heated with 5% hydrochloric acid for 3 hours. The reaction mixture was basified with ammonia and extracted with chloroform. The organic phase was washed with water, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. Column chromatography of the resulting residue on Al_2O_3 with ether and recrystallisation from methanol yielded 98.1 mg (70%) of crystalline plates, m.p. 197–199° C. This product was identified by comparison of the IR and MS spectra as the steroidal solanum alkaloid solasod-5-en-3β-ol (solasodine).

TOXICITY TESTS

Bioassays were carried out with 30-day-old *Lymnaea cubensis* snails of uniform sizes (average length: 4 mm). Each test was made with two replicates of 10 snails placed in petri dishes with dechlorinated and filtered water solutions of known extract concentration. The test included a 24-hour exposure and a 24-hour recovery period. After this time, the snails were observed under the microscope. Control runs were carried out with dechlorinated and filtered tap water.

RESULTS AND DISCUSSIONS

The toxicity tests showed that the water, and especially the methanol extract of *Solanum mammosum* fruits, had molluscicidal properties. The water extract killed 25% of the population of the snails *Lymnaea cubensis*

at the concentration of 100 p/m (table 1). The molluscicidal activity is strongly increased by extraction with methanol. Water solution of the methanolic crude extract showed 95% mortality at 25 p/m. Therefore, special attention was given to the chemical composition of the methanol extract. As described in the separation of fractions, column chromatography of the crude methanol extract afforded a fraction containing a mixture of two components. Water solution of this mixture produced 100% mortality at 10 p/m (table 1). From these results, it is evident that in this mixture two strong molluscicidal products from *Solanum mammosum* were obtained. Isolation and further activity studies of each of these products is now in progress. Nevertheless, hydrolysis of the mixture afforded only one alkaloid which was identified as solasodine. These results suggest that the strong molluscicidal products are the steroid glycoalkaloids solasonine and solamargine. Both glycosidic alkaloids contain solasodine and differ only in the sugar moiety (2, 3, 8). Earlier studies

TABLE 1.—*Molluscicidal effect of active extracts obtained from* Solanum mammosum *fruits on* Lymnaea cubensis *snails expressed as mortality percentage*

Active principle	Concentration (p/m)						
	100	75	50	25	10	5	1
Water extract	25	17	10	5	0	0	0
Methanol extract	100	100	100	95	35	10	0
Mixture of glycosidic alkaloids	100	100	100	100	100	30	10

also report the presence of solasonine and solamargine in *Solanum mammosum* fruits (7, 9). Solasodine itself showed no toxicity in water. This can be explained on the basis of the low water solubility of the alkaloid. Extraction with other less polar solvents has yielded residues with no significant toxicity.

RESUMEN

El incremento en la incidencia de la *Fasciola hepatica* en el ganado de Puerto Rico hace necesario controlar el caracol transmisor del parásito. Este estudio determinó el efecto de los frutos de la planta *Solanum mammosum* (berenjena de cucaracha) sobre caracoles *Lymnaea cubensis*. El extracto metanólico bruto demostró una actividad moluscicida de 95% a la concentración de 25% en solución acuosa. Una fracción cromatográfica del crudo metanólico aumentó su potencia moluscicida a 100% en una concentración de 10 ppm. Se identificó un producto del extracto metanólico activo como solasod-5-en-3β-ol (solasodina).

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