Isolation and Purification of the Saponins of Glottidium vesicarium

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The range of *Glottidium vesicarium* (bladderpod, coffeebean weed or castlebean) has been described (Featherly, 1945) as ". . . . common in the southern tier of counties with losses . . . reported as far north as Cleveland and Creek counties". The mature beans (toxic to cattle, rabbits and chickens) were listed as the poisonous part of the plant.

Later work (Foote and Gramling, 1940; Nuessle and Lauter, 1958) on the poisonous principle of this plant revealed saponins to be present, but these compounds have been neither chemically characterized nor shown to be the toxic constituents of this plant. A thorough review of the literature in this area by Kingsbury (1964) shows three species of closely related legumes to be toxic to livestock. These plants are *Sesbania punicea, S. drummondii,* and *S. vesicaria.* The latter appears in Small's Manual of the Southeastern Flora to be the same as *Glottidium vesicarium.* The range of these plants is reported to be from Florida through Texas along the Gulf coastal plain extending inland several hundred miles.

Experimental Procedure

Two procedures were used in the isolation of crude saponins (Morris and Hussey, 1965; Shaver, Camp and Dollahite, 1964). Both involve extraction of the finely ground plant material with 70% ethanol, combination and concentration of these extracts, filtration and then acidification. This last step was accomplished in the first procedure by the direct addition of hydrochloric acid and in the second with a hydrochloric acid activated I.R.C.-50 ion exchange column. At this point the extracts, treated with hydrochloric acid directly, yield a precipitate of the saponin. In the second procedure the acidified extract from the I.R.C.-50 column was decolorized with activated carbon, further concentrated and the saponins extracted into butanol. As the butanol extract was concentrated on a rotary evaporator the saponins were precipitated from solution as a thick syrup. Absolute ethanol was used to remove water until a white, crystalline precipitate was formed. The precipitated saponins were further washed separately with the solvent series, absolute ethanol, acetone, and ether, then dried under vacuum in a P_2O_5-charged desiccator.

Various characterization tests and chromatographic techniques which seemed appropriate were utilized in the partial characterization of the products (Clark, 1964). The presence of a saponin in various isolates and chromatographic fractions was determined by the ability of these fractions to hemolyze washed red blood cells.

Results

The isolation of saponin compounds from *G. vesicarium* by the hydrochloric acid precipitation procedure gave a gelatinous white product which was water-insoluble and would hemolyze red blood cells. This procedure, used by many workers, did not yield a water-soluble saponin from the mature beans so it was abandoned.

A white crystalline solid and a light tan glass were isolated as products from mature beans by the ion exchange column technique. Both products were water-soluble and contained saponins. Approximately 2 grams of the crystalline saponin isolate were obtained from 1.2 kilograms of dried, finely ground beans. A comparison of the saponin activity of isolates from different plant tissues is shown in Table I.

TABLE I. DESCRIPTION AND SAPONIN ACTIVITY OF ISOLATES FROM TISSUES OF *Glottidium vesicarium*.

Plant Tissue	Description of Isolate	Red Blood Cell Hemolysis		Isolate Yield (%)
		Saponin Conc. (%)	Time	
Green Tops	Brown precipitate	—	30 min.	Less than 0.1
Roots	White solid	0.6	2 min.	Less than 0.1
Mature Beans	White solid	0.6	16.5 min.	0.15
Mature Beans	Tan glass	0.6	7 min.	0.10
	Commercial saponin**	0.6*	overnight	—

*Usual saponin concentration for rapid red blood cell hemolysis is 1%.

**Matheson Coleman and Bell, Inc., Norwood, Ohio, Reagent Grade, Saponin, plant source unknown.

Various chromatographic techniques were applied to the isolates along with other saponin check samples. Thin layer chromatography, two types of silicic acid column chromatography, Dowex 1 - 8 formate form and Sephadex column chromatography were not effective in the separation of components of these isolates. Paper chromatography with three solvent systems was tried and purity checks were obtained. The results for one solvent system are shown in Table II.

Certain other characteristics of the crude saponin isolate from *G. vesicarium* mature beans were determined. The mixture is very hygroscopic, does not have a sharp melting point, is soluble in water, insoluble in absolute ethanol, methanol and all organic solvents tested, nontoxic to rats and gives the color tests shown in Table III.

A large scale paper chromatographic separation of the saponin isolates was attempted with some success. Further characterization of these compounds must await the development of a more efficient purification procedure.

Discussion and Conclusions

A partial characterization of the saponins present in *Glottidium vesicarium* indicates the compounds are more active than commercial saponin as red blood cell hemolytic agents. The purified isolates show positive carbohydrate and sterol tests, precipitate with cholesterol in a manner similar to digitonin and are free of phenolic contaminants. A complete characterization must await a large scale purification process. Root tissue of this plant appears to be a rich source of a very active saponin. The saponins are not toxic to rats at the usual toxic dose levels for these compounds.

Table II. Separation of Components of Saponin Isolates by Paper Chromatography.*

Material	Number of Spots	R_f (range)	Saponin Activity
Glottidium vesicarium (mature bean) White solid	4	0.00	+
		0.18-0.19	−
		0.32-0.35	−
		0.55-0.56	+
Tan glass	3	0.00	+
		0.51-0.57	+
		0.68-0.70	−
		0.81-0.83	+
Commercial saponin	4	0.05	−
		0.15-0.26	+
		0.53-0.61	+
		1.00	(?)

*Detection spray: periodate-benzidine; 3 mm Whatman No. 1 paper; solvent system; pyridine, butanol, water, 3:2:1.

TABLE III. CHARACTERIZATION TESTS ON SAPONIN ISOLATES.

Isolate	Salkowski*	Liebermann-Burchard*	Molisch**	$FeCl_3$†	Precipitation with Cholesterol††
G. vesicarium (Bean) white solid	+	+	+	–	+
Tan glass	+	+	+	–	+
Root saponin	+	+			+
Commercial isolate	+	+	+	–	
Digitonin					+

*Test for sterol nucleus

**Carbohydrate color test

†Test for phenolic impurities (pigments)

††Test for digitonin-type structure

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