The Incorporation of Mevalonic Acid-2-C^{14} into the Triterpene Saponin of Xanthocephalum

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Characterization of the toxic constitutent of perennial broomweed as a triterpene saponin by Shaver, Camp and Dollahite (1964) suggested that the compound may be formed by a known terpene biosynthetic path. If it could be established that the plant incorporates the terpene biosynthetic intermediate, mevalonic acid, the existence of this biosynthetic route would be partially established. Two methods of administration of the suspected precursor were utilized in this incorporation study on perennial broomweed. The presence or absence of a saponin from three other related plant species, annual broomweed *(Amphiachyris dracunculoides)*, sneezeweed *(Helenium tenuifolium)* and bitterweed *(Actinea odorata)* was determined.

Contamination of the isolated saponin from the above-ground portion of the plant by tannin-like and flavanoid compounds has hindered structural studies. An isolate of perennial-broomweed root-tissue saponin has been prepared and when chromatographed on silicic acid a relatively pure saponin was obtained.

Procedure

In the incorporation study, plant material was obtained from an area with a known history of broomweed toxicity. Mevalonic acid-2-^{14}C lactone was administered to the plant material in two ways; first, through the stem of the upper portion of the plant and second, as a substrate in a tissue homogenate. In the first study the entire above-ground portion of the plant was inserted into a solution of labeled mevalonic acid containing 0.1% glucose. The plans were permitted to metabolize for 36 hours, then they were homogenized with 95% ethanol and carried through the saponin isolation procedure. For the second procedure, homogenates of the plant tissues were prepared according to the procedure of Heinstein, Tove and Smith (1962), which involves simple grinding with pH 7 potassium phosphate buffer which also contains glucose. After incubation of the tissue homogenates with the substrate for 36 hours, the homogenates were carried through the saponin isolation procedure.

The isolation of the saponin followed the procedure described by Dollahite, Shaver and Camp (1962), modified for application to small amounts of plant tissue. The yield, purity and presence of saponin were determined with the following tests. The hemolysis of saline washed and suspended red blood cells was used as an index of saponin concentration. Clarity of the hemolyzed cell preparation indicated purity as a protein precipitate was observed when the isolates contained tannins. An aqueous solution of ferric chloride gave a blue-black color and precipitate with impure isolates while the formaldehyde sulfuric acid reagent was used as a qualitative test for saponins.

The carbon-14 assay of the products was effected by the combustion procedure of Buyske, *et al.* (1961). The carbon dioxide from the combustion was absorbed into Hyamine 10x and an aliquot of this solution was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer.

The abortifacient properties of the annual broomweed isolate was tested by I. P. injection of a pregnant rat.

Results and Discussion

Table I shows yield and characterization tests on the saponin isolates from four plant species. Although a light brown hygroscopic powder with a positive foam test was obtained from annual broomweed and sneezeweed, there was no saponin activity as shown by red blood cell hemolysis. The annual broomweed isolate did not cause abortion in rats. There was no saponin isolated from the top portion of bitterweed.

The percent recovery of administered ^{14}C is shown in Table II. The very low incorporation of mevalonic acid into the isolate from the first sample of perennial broomweed is as expected since no saponin was present in this isolate. This plant material was taken from an area with a

TABLE I. *Yield and Characterization Tests on Saponin Isolates from Various Plant Species*

Plant or Source	Grams Compound	% Yield	Red Blood Cell Hemolysis	$FeCl_3$ Test	Formaldehyde + H_2SO_4
Annual Broomweed	5.45	0.11	None	Positive	Negative
Sneezeweed	0.53	0.29	None	Positive	Negative
Bitterweed	0.00	—	—	——	——
Perennial Broomweed	1.21	0.11	Complete Slight Precipitate	Slight Positive	Positive
Commercial Saponin	—	—	Complete	Negative	Positive
Cholesterol	—	—	—	Negative	Positive

TABLE II. *Incorporation of Mevalonate-2-^{14}C Into the Saponin From Broomweed*

Plant	Tissue	Grams Compound Isolated	% Yield	^{14}C Administered	^{14}C Recovered	% Recovery
Annual Broomweed	Plant Top	0.220	0.20	2 μc	0.085 mμc	0.004
Perennial Broomweed	Plant Top	0.090	0.03	15 μc	3.15 mμc	0.021
Perennial Broomweed	Top Homogenate	9.470	0.88	30 μc*	12.28 mμc	0.041
Perennial Broomweed	Leaf Homogenate	1.200	0.48	10 μc*	4.80 mμc	0.048
Perennial Broomweed	Root Homogenate	0.120	1.60	10 μc*	0.35 mμc	0.004

* Compound administered was Mevalonic-2-^{14}C lactone.

soil type which produces a toxin-free broomweed. Although the saponin isolates from the tissue homogenates show a slightly higher recovery of administered ^{14}C, it may be seen that mevalonic lactone is a poor precursor of the saponin in this system.

Table III shows the characterization tests on the saponin isolates from the incorporation study. Saponin activity was found in the three perennial

TABLE III. *Characterization Tests on Saponin Isolates from Incorporation Study*

Plant	Tissue	Red Blood Cell Hemolysis	$FeCl_3$ Test	Foam Test
Annual Broomweed	Plant Top	Negative	Dark blue + ppt.	+
Perennial Broomweed	Plant Top	Negative	Dark-blue + ppt.	+
Perennial Broomweed	Top Homogenate	Positive 50% in 10 min.	Dark-blue Green	+
Perennial Broomweed	Leaf Homogenate	Positive 25% in 10 min.	Dark-blue green	+
Perennial Broomweed	Root Homogenate	Complete 10 min.	Negative No color	+ +
Perennial Broomweed	Reference from Shaver	Complete	Light blue green	+ + +

broomweed homengate isolates but those from the top portion of the plant and from the leaf were contaminated with tannin and flavanoid compounds: The root tissue yielded a relatively pure saponin isolate.

Silicic acid column chromatography of the three isolates from the tissue homogenates showed that the radioactivity remains with the saponin. Since the structure of this compound is not known at this time, a degradation study could not be done.

SUMMARY

Annual broomweed, sneezeweed and bitterweed contain no toxic saponin. A relatively pure saponin can be isloated from the root tissue of perennial broomwood.

Tissue homengates of the top portion of the plant, leaf and root tissues of perennial broomweed show a low rate of incorporation of mevalonic acid-2-^{14}C lactone into the toxic saponin in this plant. Silicic acid column chromatography has been useful in the purification of saponin isolates.

LITERATURE CITED

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