Paper Chromatography of Sugar Beet Saponin Compounds

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Sugar beet saponin has been described as a glucuronic acid glycoside of a triterpene, oleanolic acid (2)[2]. Many workers have studied this and related compounds (1) (2) (3). The authors felt it advisable to study oleanolic acid, its glycoside, and their salts using paper chromatography. Knowledge thus gained should be useful in further studies of beets, beet products, and other plant materials containing similar substances. An attempt was made to utilize the chromatographic characterizations thus obtained to determine the nature of saponins as they occur in beets. Certain difficulties were encountered, perhaps due to the presence of colloids or other substances capable of forming more or less firmly bound additional products.

In the laboratory many experimental beets are analyzed for various constituents by paper chromatography. An isopropanol solvent has been developed by the laboratory which, with descending paper chromatography, is suitable for the separation of sugars, such as raffinose, galactinol, inositol, and for separation of at least nine of the amino acids commonly found in sugar beets. The above juice compounds align themselves in identical positions on paper chromatograms with the respective pure chemicals thereof. Such is not the case with purified saponin and saponin of beet juice. The pure saponin moves with the solvent front while the beet juice saponin has moved only half the distance. Smolenski (3) in 1935 found magnesium saponin present in diffusion juice. Thus saponin, possibly present in the form of salts, might account for its unusual chromatographic properties.

Methods

One and two dimensional chromatographs were made on Whatman No. 4 paper using the following solvents: a. benzene; b. isopropanol; c. methanol-water; and d. water. The spots to be chromatographed contained either a few micrograms of the relatively pure salts of 2.5 milligrams of total dry substance when sucrose was present, and 100 micrograms of egg albumin when used.

Solvents

Isopropanol Solvent consists of:
- 70 parts isopropyl alcohol
- 10 parts N-Butanol
- 25 parts benzene
- 25 parts water

Methanol Solvent consists of:
- 95 parts 95% methanol
- 5 parts distilled water

Water

Benzene

The solvents traveled down the paper chromatographs.

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[2] Numbers in parentheses refer to literature cited.

Color Reagents

Antimony Pentachloride:
5 ml. Antimony pentachloride
95 ml. chloroform
0.5 gm. antimony trichloride

Alpha Naphthol:
1 gm. alpha Naphthol
100 ml. solox
15 ml. phosphoric acid

8-Hydroxy quinoline (oxine)
0.5 gm. 8-hydroxy quinoline
100 ml. N-Butanol

The antimony pentachloride reagent reacts with saponin to form a pink color. The dried chromatograms were dipped in antimony pentachloride and placed in an oven for a few seconds at 90° C.

Chromatographs, after being dipped in antimony pentachloride, were then treated with alpha Naphthol reagent heated to 90° C. to bring out the purple color of sucrose when present.

The chromatographs were dipped in oxine reagent, allowed to dry at room temperature, exposed to ammonia fumes, then examined under ultraviolet light. Magnesium, calcium and barium each produce a bright fluorescence.

Table 1.—Rf Values of Purified Saponin Compounds.

Compounds	Rf Values in Indicated Solvents			
	Isopropanol	Water	Methanol-Water	Benene
Mg saponin	0.0	0.0	0.0	0.0
	0.3 - 0.6*	0.6 - 0.8*	0.8 - 1.0	
Ca saponin	0.0	0.0	0.0	0.0
Ba saponin	0.0	0.0	0.0	0.0
Na saponin	0.0	0.0	0.0	0.0
	0.3 - 0.6*	0.7 - 0.9*	0.3 - 0.4*	0.0
			0.9 - 1.0	
K saponin	0.0	0.0	0.0	0.0
		0.6 - 0.7*	0.2 - 0.3	
			0.7 - 1.0	
NH_4 saponin	0.3 - 0.8*	0.0	0.0	0.0
	1.0		0.6 - 1.0	
beet juice saponin	0.4 - 0.6*	0.0	0.0	0.0
		0.6 -0.8*	0.0 - 0.3	
			0.4 - 0.7*	
saponin	1.0	0.0	1.0	0.0
oleanolic acid	1.0	0.0	0.0	1.0

*Strong pink

The saponin was purified according to the method of Walker and Owens (4). The salts of saponin were prepared by mixing purified saponin and the respective oxide or hydroxide of the metal in water, filtering and using the filtrates for the various chromatographic tests.

Results and Discussion

The term Rf as used here is the value of the ratio of the distance traveled by the compound to the distance traveled by the solvent. Instead of giving the average Rf for a spot, the upper and lower limits are listed in the tables.

Table 1 reports the results obtained with purified saponin salts only.

In benzene, none of the compounds showed any mobility except oleanolic acid. Calcium and barium saponin had 0.0 Rf in all the solvents. The magnesium saponin preparation produced a pink spot at 0.0 Rf in all four solvents; stronger pink spots occurred at locations which matched the beet juice saponin when using isopropanol or water, but not with methanol. In each case magnesium, barium, or calcium was found associated with the pink spots produced by Mg saponin, Ba saponin, or Ca saponin.

Table 2.—Rf Values of Saponin Compounds When Beet Juice Was Present.

Compounds	Rf Values in Indicated Solvents		
	Isopropanol	Water	Methanol-Water
Mg saponin	0.0	0.0	0.0
	0.4 - 0.6*	0.6 - 0.8*	0.0 - 0.3
			0.5 - 0.9*
Ca saponin	0.0*	0.0*	0.0*
	0.4 - 0.6	0.6 - 0.8	02. - 0.4
			0.6 - 0.8
Ba saponin	0.0*	0.0	0.0*
	0.4 - 0.6		0.0 - 0.2
			0.5 - 0.8
Na saponin	0.0	0.0	0.0
	0.2 - 0.4*	0.6 - 0.9*	0.1 - 0.4
			0.5 - 0.8*
K saponin	0.0	0.0	0.0
		0.5 - 0.6*	0.0 - 0.2
		0.6 - 0.8*	0.5 - 0.9*
NH_4 saponin	0.3 - 0.8*	0.0	0.0
	1.0		streak
beet juice	0.4 - 0.6	0.0	0.0
		0.6 - 0.8*	0.0 - 0.3
			0.4 - 0.7*
saponin	0.4 - 0.6*	0.0	0.0
	1.0	0.6 - 0.8*	0.0 - 0.3
			0.4 - 0.7*
			1.0*

* Strong pink

An extensive series of tests using all solvents indicated that incorporation of sucrose in the salt solutions did not alter the Rfs of the various salts.

Study of Table 2 reveals that the Rfs of the pink spots resulting from the mixtures of sodium saponin and beet juice or magnesium saponin and beet juice were similar to those of beet juice alone in all three solvents. The calcium saponin and barium saponin fixed some of the mobile beet juice saponin at 0.0 Rf, probably due to an excess of calcium and barium ions present.

Table 3.—Rf Values of Saponin Compounds When Sucrose and Egg Albumin Were Present.

Compounds	Rf Values in Indicated Solvents		
	Isopropanol	Water	Methanol-Water
Na saponin	0.0	0.0	0.0
	0.5 - 0.6*	0.5 - 0.8*	0.3 - 0.5*
	1.0		
K saponin	0.0	0.0	0.0*
	0.5 - 0.6*	0.4 - 0.7*	0.0 - 0.2
	1.0		
NH_4 saponin	0.5 - 0.6*	0.0	0.0
	0.8 - 1.0	0.3 - 0.7*	0.2 - 0.5
Mg saponin	0.0	0.0	0.0
	0.4 - 0.6*	0.6 - 0.8*	0.4 - 0.6*
saponin	1.0	0.0	1.0

* Strong pink

Table 3 shows the effect of the presence of sucrose and albumin. They seemed to affect the Rf values of the saponin salts, in that sodium and magnesium saponin moved to positions similar to the major part of the saponin of beet juice, but the presence of sugar and albumin did not affect purified saponin. Using isopropanol solvent or water solvent, potassium and ammonium saponin acted similarly to beet juice saponin, but not with the methanol solvent.

Beet juice was chromatographed. The pink spots resulting from treatment with antimony pentachloride were cut out, ashed, and analyzed for sodium, potassium, calcium, and magnesium by flame spectrophotometer. Five ml. of 10 percent dry substance beet juice was spotted along a four-inch line of Eaton-Dickman No. 301 paper and irrigated in two directions. This was done in water then methanol; methanol then isopropanol; and in water then isopropanol. The colors were developed with antimony pentachloride and the pink spots cut out and ashed. Blanks were also run. Similar areas of the blank sheets were treated in the same manner. With the water then methanol irrigation, potassium was found present in the greatest amount, some sodium, very little calcium, and no magnesium. The same pattern persisted for methanol then isopropanol, however, with water then isopropanol, all were present.

Chromatographic characteristics of saponin of beet juice by two dimensional chromatography were determined in several solvent systems as follows:

a. Water then Isopropanol

In the first direction in water, two saponin spots were formed, one at the 0.0 Rf and the other (major portion) at 0.5 Rf. In the second direction with isopropanol, both spots had an Rf of 0.4.

b. Isopropanol then Water

In the first direction, only one spot appeared and that at an Rf of 0.5. In the second direction in water, two saponin spots were formed one at 0.0. Rf and the other at 0.7.

c. Water then Methanol

In water two spots were found at 0.0 Rf and at 0.6 Rf. In the other direction in methanol, three saponin spots were formed, one at 0.0. Rf, another moved out from the 0.0 Rf in the first direction to an Rf of 0.7, and the third at 0.7.

The sucrose was always nearby the major part of the beet juice saponin. In isopropanol, saponin located itself immediately below the sucrose, while in water or methanol solvents, the two usually occupied the same position.

Summary

1. The Rf values of the saponin salts were determined.

2. Magnesium and sodium saponin produced pink spots which appear to be similar in Rf to part of the beet juice saponin. Sucrose and albumin together seemed to affect the chromatographic values of the salts.

3. Potassium, sodium, calcium and magnesium were found in pink spots of beet juice saponin by flame spectrophotometer.

4. Beet juice saponin was separated into several areas by two dimensional chromatography.

References

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