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SAPONINS: CHARACTERIZATION AND SOME PROPERTIES FROM *PISUM ARVENSE* SEED

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ABSTRACT

Saponins from *Pisum arvense* L. seed were fractionated according to their solubility in the presence of excess calcium oxide. The insoluble saponin fraction showed seven spots to paper chromatography besides having a very bitter taste and a high surface activity. In this preparation soysapogenols B, C, and E plus an unidentified sapogenin were present as saponin aglycones while arabinose, glucuronic acid, and xylose were idenfied as saponin carbohydrate moieties. Even though the soluble saponin fraction showed four spots to paper chromatography, it exhibited only slight bitterness or surface activity, and it contained no identifiable sapogenins or monosaccharides. Thus most, if not all, *Pisum arvense* saponins appear to be precipitable with excess calcium oxide. Neither saponin preparation showed significant *in vitro* hemolysis. Since the insoluble saponin fraction contained the known triterpenoid soybean sapogenins, *Pisum arvense* saponins can be classified as triterpenoid.

INTRODUCTION

Saponins are minor constituents of many plants and are composed of one or more sugar units attached in glycosidic linkage to aglycones, termed sapogenins, which resemble steroids or triterpenoids (Basu and Rastogi, 1967). These bitter-tasting glycosides have been found and studied in a number of edible members of the *Leguminosae* family (Applebaum *et al.*, 1969), most notably *Glycine max* (soybean) Birk, *et al.*, 1963; Wolf and Thomas 1970, 1971). Without exception all edible legume sapogenins that have been chemically characterized belong to the triterpenoid group, the most ubiquitous being those isolated and characterized from the soybean and named soysapogenol A, B, C, D, and E. respectively. (Meyer *et al.*, 1950; Ochia *et al*, 1937; Smith *et al.*, 1958; Wilner *et al.*, 1964). Several antinutritional and antibiological properties attributed to forage legume saponins (Shany, 1970) are not found in edible legume saponin preparations (Applebaum, 1969); however, most legume saponin preparations cause *in vitro* lysis of red blood cells. We are engaged in ascertaining heretofore unstudied legume chemical constituents which may be of physiological and/or medicinal significance and now report on the study of the saponins from *Pisum arvense* L. seed, a legume commonly used for human as well as livestock consumption (Uphof, 1968).

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EXERIMENTAL METHODS

Saponin Preparations. Pisum arvense L. (Field Pea, Crowder variety) seed saponin preparations were obtained according to Applebaum *et al.* (1969) with the exception that the initial 80% ethanol saponin extract was concentrated by flash evaporation in order to minimize foaming. The calcium oxide precipitable saponin fraction was labeled *P. arvense* saponin preparation I (PASP I) while that saponin fraction soluble in the presence of excess calcium oxide was labeled *P. arvense* saponin preparation II (PASP II). For comparative purposes soybean saponins were isolated by a method similar to that of Birk *et al.*, (1963).

Analysis of Saponin Preparations. All saponin and sapogenin paper chromatograms were carried out on Whatman 3MM chromatography paper and visualized with a saturated solution of antimony trichloride in chloroform (Coulson, 1958). *Pisum* saponin preparations were subjected to ascending paper chromatography in n-butanol—ethanol—water (6:2:3, v/v/v) (Birk *et al.*, 1963). Hydrolysis of *Pisum* and soybean saponin preparations in 1 N sulfuric acid (in dioxane—water 1:3) followed by ether extraction yielded saponin aglycones (Gestetner, *et al.*, 1966). *Pisum* saponin carbohydrate moieties were obtained by barium carbonate neutralization of the PASP I and PASP II acid hydrolysates. Sapogenins were analyzed by circular paper chromatography in n-hexane—chloroform—glacial acetic acid (100:10:2.5, v/v/v) (Gestetner, 1964), and standard chromatographic procedures (Macek, 1960; Trevelyan *et al.*, 1950) were used to identify the *Pisum* saponin monosaccharides using glucose, galactose, glucuronic acid, glucuronic acid lactone, rhamnose, fucose, 2-deoxyribose, xylose, and aribinose as markers.

Pisum Saponin Preparation Characteristics. Kofler's method (1927) as modified by O'Dell *et al.* (1959) was used to determine *in vitro* hemolytic activities. Foam characteristics were assayed as reported elsewhere (O'Dell *et al.*, 1959). Relative bitterness was ranked by eight persons each placing at 1 hr intervals 1 drop of a 15 mg/ml aqueous solution of either PASP I or PASP II to the back of his tongue.

RESULTS AND DISCUSSION

Previous work (Barker, 1970) had indicated the presence of saponins in *P. arvense* seed; however, repetition of this isolation procedure resulted in low yields of a product which was not entirely saponin since silicia gel thin-layer chromatography in benzene— ethyl acetate (3:1, v/v) showed the product to contain at least three relatively nonpolar components with Rf values greater than 0.7. To obtain an adequate amount of *P. arvense* saponins, a previously reported method for the isolation of legume seed saponins was used (Applebaum, 1969).

Paper chromatography of the *Pisum* saponin preparations is shown in Figure 1. PASP I shows at least seven fractions while PASP II shows at least four, each of which is presumed to be saponin or a mixture of saponins even though the visualization reagent is not saponin specific.

Relative bitterness (Dieckert and Morris, 1958) and surface activity (O'Dell, 1959) tests have been used as criteria for saponin detection and estimation, respec-

tively. These tests are based on the fact that the magnitude of bitterness and foam stability (in contrast to height of foam column formed) is dependent upon the total amount of saponin present. The *Pisum* saponin preparations had similar foam formations (Table 1), but since PASP I had a much greater foam stability and was decidedly more bitter than PASP II, the former preparation probably contains more total saponin than the latter. The greater apparent percent yield of PASP II over PASP I (Table 1) is not entirely indicative of saponin content since PASP II could also contain 80% ethanol solubles which extracted along with the saponins.

Table 1. Some properties of *Pisum arvense* saponin preparations.

PROPERTY	PASP I	PASP II
Yield(%)‡	1.30	3.50
Foam formation(mm)	28	24
Foam stability(min)	240	10
Ranked bitterness	very bitter	slightly bitter
Hemolysis(%)	negligible	negligible
Aglycones*	soysapogenols B, C, E; X_1**	X_2**, X_3**
Monosaccharides	arabinose, xylose, glucuronic acid	unidentified

‡Based on lipid-free meal.

*Analyzed by circular paper chromatography(10).

**X_1, X_2, and X_3 are unidentified aglycones which stained brown to visualization reagent and had R_f values of 0.97, 0.97, and 0.63, respectively.

Presumably the high *in vitro* hemolytic activity of saponins arises from interactions of the sapogenin entity of the saponin molecule with the erythrocyte membrane (Segal, *et al.*, 1970). Saponins from soybean (Birk, *et al.*, 1963) and alfalfa (Shany *et al.*, 1970) which were prepared similarly to these from *Pisum* show strong *in vitro* hemolytic activity. Even though soysapogenol A appears to be the aglycone primarily responsible for the high hemolyzing power of soybean saponins (Gestetner *et al.*, 1963) the presence of this or any other soybean sapogenin does not necessarily mean that hemolyzing power will be enhanced since clover (Walter *et al.*, 1955) and alfalfa (Gestetner, *et al.*, 1971) saponin fractions which contain only various soysapogenols (including soysapogenol A) show no hemolysis. In *Pisum* saponin preparations PASP I contained three of the soysapogenols (Table 1). Neither it nor PASP II showed significant hemolysis, however.

Other aglycones of the *Pisum* saponins remain unidentified (Table 1). The one remaining in PASP I had a similar migration and color reaction to a PASP II sapogenin while another PASP II aglycone was similar to none in the chromatogram.

After hydrolysis of PASP I and PASP II and extraction of the sapogenins, the sugar residues of each saponin preparation were examined by paper chromatography (Table 1). PASP I contained xylose, arabinose, and glucuronic acid, these being in the group of sugars most commonly involved in glycosidation(1). PASP II showed no monosaccharide chromatographically identical to any chosen standard. Even though paper chromatography indicates that saponins are present in PASP II (Figure 1), its relatively low bitterness, foam stability, and lack of containing sugars commonly found in saponins raises questions as to whether saponins are indeed present in this preparation or not. Thus it appears that most, if not all, *Pisum* saponins are probably precipitable with excess calcium oxide.

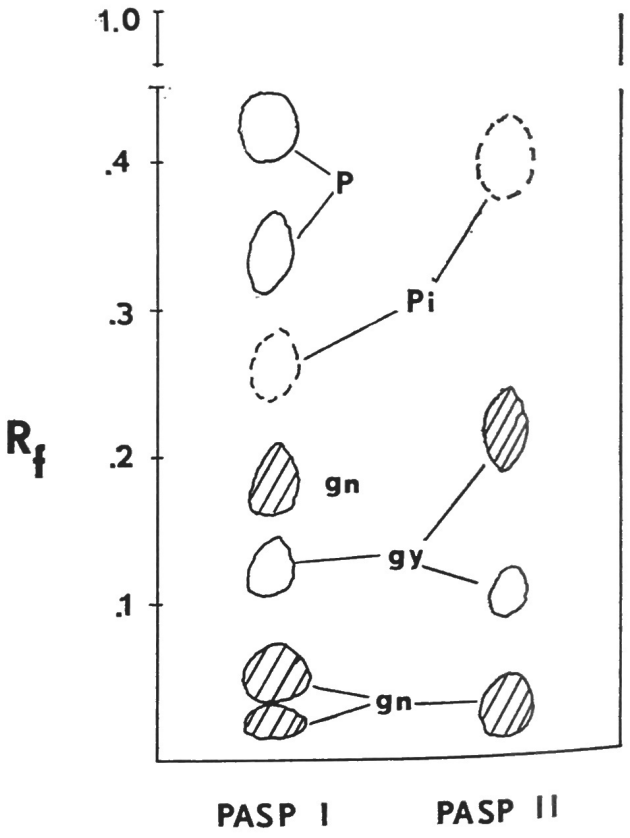

Figure 1. Whatman 3MM paper chromatographic pattern of *Pisum arvense* saponin preparations developed in n-butanol—ethanol—water (6:2:3, v/v/v) using ascending techniques (Birk *et al.*, 1963). Antimony trichloride saturated chloroform visualization reagent yielded colors designated as: (Green(gn), Gray(gy), Pink(pi), and Purple(p).