# Chemical Similarity and Biological Activity of the Saponins Isolated from Alfalfa and Soybeans

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A number of reasons have been given to explain the growth-depressing effect of uncooked soybean and of dehydrated alfalfa leaf meal. In the former, a trypsin-inhibiting factor (*1, 2*) and the availability of amino acids (*3*) and in the latter, its high fiber content (*4*) and the presence of a saponin (*5*) have been given as reasons for the growth-depressing action. Recently (*6*) alfalfa saponins also have been shown to cause bloat in ruminants. Saponins are glycosides that can be split on acid hydrolysis into sugars and crystalline saponogenins or genins. Studies in our laboratory (*7*) indicate that alfalfa saponins and soybean soyasapogenols are similar in chemical structure and biological activity.

At least three triterpene genins were isolated from dehydrated alfalfa leaf meal. Meal of weight 7.4 kg was extracted twice with hot 95 percent ethanol, the extract was freed from solvent, and the saponins were isolated and hydrolyzed by the procedure of Wall (*8*). The resulting 900-mg mixture of genins was dissolved in benzene and passed through a 2 by 12-cm column of alumina. The column was washed with 700 ml of benzene and extruded and divided into three equal portions. The top portion (section I), upon elution with 20 percent methanol in benzene and removal of the solvent, left a solid of 500 mg that had a melting point of 247° to 248°C and a specific rotation of +59°. The eluate from the middle portion (section II) contained 11 mg of solid, mp 285° to 286°C, and the bottom portion (section III) contained 30 mg of material, mp 233° to 235°C. When the material from section I was recrystallized from methanol, its melting point was raised to 258° to 259°C and its specific rotation to +88.7°. All the alfalfa genins gave a bright red turning to a deep red color on treatment with Liebermann-Burchard reagent, suggesting a triterpene nucleus. In analysis of section I for $C_{30}H_{50}O_3$, we calculated C, 78.55; H, 10.99; we found C, 76.87; H, 10.84. The materials were sublimed under high vacuum before analysis.

Ochiai *et al.* (*9*) isolated a series of sapogenols from soybeans. Soyasapogenol B had a melting point of 259°C and a specific rotation of +92.4. The soyasapogenol B isolated by Meyer *et al.* (*10*) had a melting point of 259° to 260°C and a specific rotation of +90°. In analysis of soyasapogenol B for $C_{30}H_{50}O_3$,

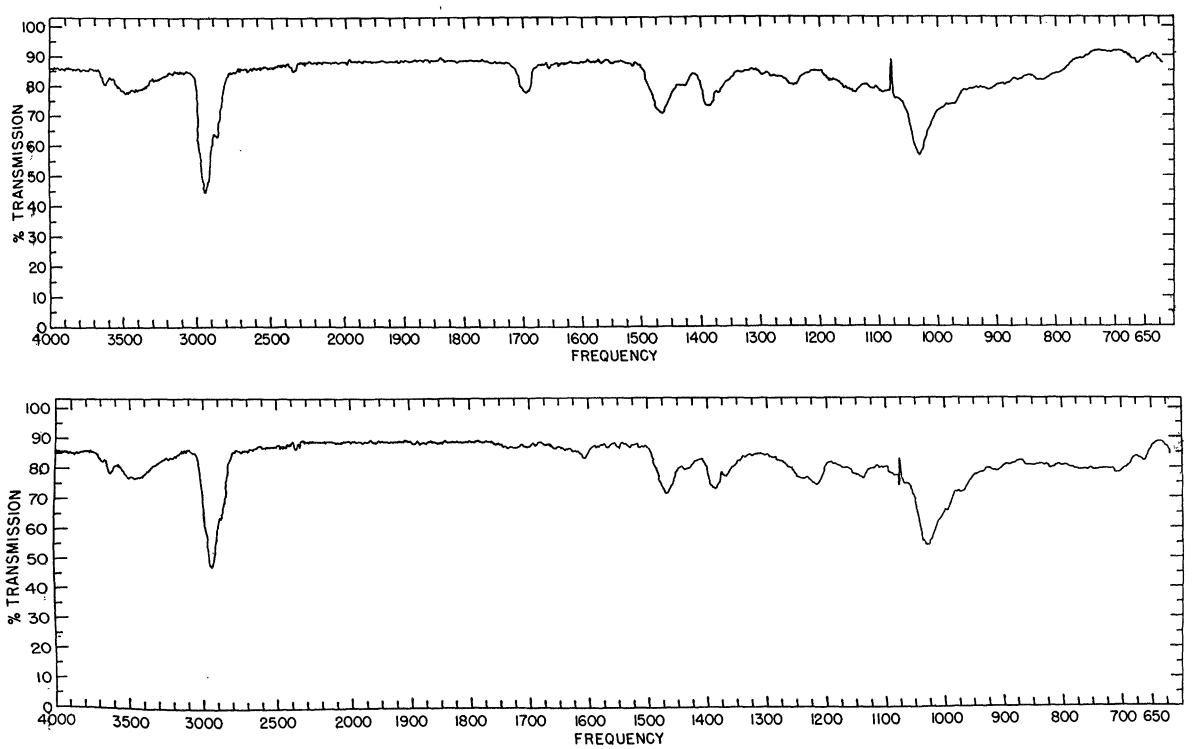


Fig. 1. The upper curve represents the infrared spectrum of the alfalfa genin, mp 258° to 259°C; the lower curve represents the spectrum of soyasapogenol B prepared in our laboratory. A Perkin-Elmer model 21 double-beam spectrophotometer with a sodium chloride prism and cell and solutions of 2.5 percent genin in chloroform were used to obtain both curves. The absorption at 1680 $cm^{-1}$ in the upper curve was probably due to a small amount of carbonyl-containing material. The amount present was too small to give a positive Lappin and Clark test (*12*).

we calculated C, 78.55; H, 10.99; we found C, 78.48; H, 10.99.

A sample of soyasapogenol B was prepared in our laboratory according to the method given by Ochiai *et al.* It had a melting point of 257° to 258°C and a mixed melting point with the alfalfa genin I of 255° to 256°C. Infrared data (*11, 12*) indicated closely related structures for the two materials (Fig. 1). It is therefore suggested that the neutral saponins of alfalfa are of the same type as the neutral saponins of soybean in that both contain a triterpene nucleus in the aglucon portion (Fig. 2).

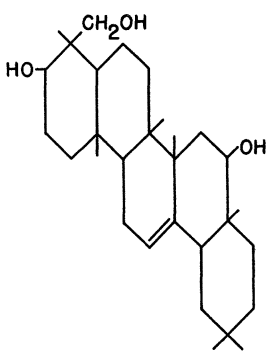


Fig. 2. The structure of soyasapogenol B, as given by Meyer *et al.* (*10*), appeared to be similar to the alfalfa genin, mp 258° to 259°C.

Both the purified alfalfa and soybean saponins inhibited the growth of chicks while their genins did not (*13*). At least part of the growth-depressing effect of uncooked soybean meal may be due, therefore, to the soyasapogenols which on cooking hydrolyze to nontoxic genins. If this cooking process is too mild, incomplete hydrolysis of the soyasapogenols could occur. Such meals might cause bloat in ruminants and would explain the occurrence of bloat in cattle fed with untoasted solvent-extracted soybean meal. On the other hand, the dehydrating conditions are too mild to hydrolyze alfalfa saponin.

### References and Notes


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## Tumor Induction in *Drosophila melanogaster* by Injection of $e^{11}tu$ Larval Fluid


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Hereditary melanotic tumors have been reported in *Drosophila melanogaster* by numerous workers (*1–9*). The expression of these pigmented growths is modified by nutrition (*2, 3, 7, 10, 11*), temperature (*12, 13*), irradiation with x-rays (*6, 14–16*), available oxygen (*15, 16*), estrogen-like compounds (*17*), and carcinogens (*18*).

A hereditary pigmented tumor arose spontaneously in our ebony stock of *Drosophila melanogaster*. This hereditary tumor is determined by a gene (*tu*) on chromosome two at approximately locus 88 (*19*). Depending upon the food available to the larvae or the temperature experienced by the larvae, the frequency of tumorous adults (homozygous) varies from 26 to 90 percent (*10, 12*). X-rays applied at the proper developmental stage also modify the penetrance of the tumor gene (*14*). Initial transplantation experiments of the pigmented growth into larvae of a nontumor strain indicated tremendous growth and subsequent appearance of multiple pigmented bodies. These findings were similar to those reported by Russell (*8*).

Both host and donor larvae of 120 hr of age (from egg laying) were chosen as test animals, because of the high postoperative survival of host animals at this age. Repeated trials were made for each test. For each trial, 50 donor larvae of the desired stock were used in the preparation of the larval extract. The larvae were washed 3 times in Waddington's insect Ringer's solution, placed in a mortar, and ground to an amorphous mash in 8 drops of insect Ringer's solution. The mixture of mashed larvae and insect Ringer's was pipetted into a centrifuge tube and centrifuged at 5400 rev/min for 15 min. The acellular supernatant fluid was then transferred into a clean, cooled tube and immediately placed in an ice bath. The acellular nature of the fluid was regularly confirmed microscopically by smears. The ice bath was employed to delay a blackening of the fluid extract that invariably occurred with the passage of time. The blackened fluid was so toxic that no animal injected with it ever survived the operation.

The 120-hr-old larval hosts were washed in three consecutive baths of insect Ringer's solution, dried, and etherized. The injection apparatus utilized in this experiment was essentially the same as that described by Ephrussi and Beadle (*20*), except that needle tips of very small diameter were used to reduce mortality. The needle was inserted at about the third or fourth segment from the posterior end of the larva. Fluid was administered until the larva was slightly distended. The host larvae were then placed on moist filter paper until they had fully recovered from the anesthetic. Only active larvae that possessed a scar