A SAPONIN FROM THE SOY BEAN*

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INTRODUCTION

In a study of the carbohydrate <=> fat metabolism of germinating and maturing soy bean seeds an attempt was made to demonstrate the presence of hydroxy acids. A paper by Muramatsu (1) came to our attention, which described a procedure for the isolation of a hydroxy acid from soy bean meal. By this procedure a product was obtained with properties similar to those reported by Muramatsu. As the original procedure is quite long and tedious, a modified method was devised whereby larger yields of the same material could be more easily obtained.

Subsequent investigation proved that the product is in reality not a simple hydroxy acid, but an insoluble acid saponin.

A search of the literature revealed that recently four other investigators have obtained saponins from the soy bean by various methods. Sumiki (2) reports the isolation of a crystalline saponin and in a later paper (3) the preparation of its sodium salt. Walz (4) claims to have identified three different saponins in soy beans, which he designates as C, C1, and C2. C and C2 are water-soluble saponins, while C1 is insoluble. More recently, Okano and Ohara (5) report two kinds of saponins occurring in soy bean meal, one of which is crystalline and the other amorphous.

In general, the literature dealing with saponins contains many inconsistent and discordant results which have their origins largely in the great difficulty of obtaining pure, unchanging samples of these compounds.

*The work described in this paper is based upon part of a dissertation submitted by E. D. Walter in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the Ohio State University.
Saponin from Soy Bean

Isolation and Purification

The improved method of isolation and purification was as follows: 4 kilos of finely ground soy bean meal were refluxed for 1 day with 5 liters of 80 per cent ethyl alcohol in a specially constructed copper boiler. The boiler was placed 6 inches above a steam hot-plate which kept the temperature of the mixture at 50-70°. The alcohol extract was poured off, the residue was pressed out with a fruit press, and the combined liquid extracts filtered. The alcohol was then distilled off, the heavy liquid remaining was placed in an electrodialyzer, and about 200 cc. of water were added. After being dialyzed at 100 volts for 24 hours, the supernatant liquid was siphoned off; and the precipitate was filtered out and dried in an evaporating dish on the steam bath. The dried residue was placed in extraction thimbles and extracted with ether in a Pickel extractor for 24 hours. The residue was then dissolved in 70 per cent ethyl alcohol; and after being clarified with Darco, the solution was concentrated to about half its volume. On standing, crystals separated. The crystals taken directly from the mother liquor appeared as rosettes under the microscope. On repeated recrystallization and drying in a desiccator over concentrated H₂SO₄, they assumed the form of very thin plates which melted with decomposition at 220-225°. In the descriptions which follow this substance will be referred to as Compound I.

Identification and Properties

Compound I—It was observed that when a few drops of concentrated H₂SO₄ were added to a small quantity of Compound I a bright red color appeared, which on standing changed to a deep violet or purple. This is a general, though not specific, color reaction of many saponins.

Although only slightly soluble in water and ether, Compound I dissolves readily in methyl or ethyl alcohol and in acetone. It also forms a soluble salt when treated with potassium or sodium hydroxide solution (Fig. 1). Such solutions foam strongly when shaken.

The neutral equivalent value of Compound I is 769, and the averages of eight combustion analyses were C 59.24 and H 8.52 per cent. Muramatsu (1) reported a neutral equivalent value (prob-
able molecular weight) of 768 for his product, C 56.03, H 8.86, and a melting point of 224°.

Acetylation gave a product of much lower melting point than the original material. It was probably an acetyl derivative of one of the sugars resulting from a partial hydrolysis.

At first our attempts to hydrolyze this substance were unsuccessful. However, following the procedure of Miyamichi and Onishi (6), a new product which resisted any further hydrolysis (sapogenin) and sugars were obtained. The hydrolysis and isolation of the sapogenin were effected as follows: 1 gm. of Compound I was dissolved in 30 cc. of methyl alcohol and 3 cc. of concentrated H₂SO₄ were carefully added with shaking. The mixture was refluxed on the steam bath for 100 hours. After cooling, beautiful long blades crystallized out (Compound II, Fig. 2). These crystals were separated by filtration with suction, washed with water to remove adhering acid, and recrystallized several times from 90 per cent methyl alcohol. The melting point after drying in a desiccator over concentrated H₂SO₄ was 198–200°. The crystals were insoluble in water and in petroleum ether, but soluble in ether and ethyl alcohol. About half the alcohol was evaporated from the original filtrate and water added to precipitate an additional quantity of
Compound II which remained dissolved in the mother liquor of the reaction mixture. The precipitate was filtered off and the acid washed out with water. It was recrystallized several times from 90 per cent methyl alcohol. The melting point was 198–200°. These crystals were combined with the first fraction, making the total yield 0.4 gm.

The identity of Compound I as a saponin was confirmed by hemolysis tests with sheep blood, toxicity to goldfish, and toxicity to water snails (7).

**Analysis and Properties of Sapogenin (Compound II)**—Duplicate combustion analyses of Compound II gave the following results: C 77.74, 77.93; H 11.13, 11.12.

A few mg. of Compound II, when heated over a small flame in an evaporating dish covered with a watch-glass, gave a resinous odor and formed a sticky deposit on the watch-glass. This material still gave a reddish color with concentrated H₂SO₄, as did the original material. This behavior is similar to that of certain compounds known to contain a terpene grouping.

A test of some of Compound II with bromine dissolved in chloroform showed it to be unsaturated. Two determinations of the molecular weight by the Rast method each gave a value of 453. Duplicate titrations with alcoholic potassium hydroxide gave neutral equivalent values of 430 and 455. Optically, the crystals are biaxial, positive. Blades show characteristic crosswise cleavage. Elongation is positive and extinction parallel. α across the broad blades is 1.543 to 1.545; β lengthwise, 1.55; and γ perpendicular to the blades, measured on fragments turned on edge, is 1.565 to 1.57. The values for γ may not be exact because the crystals are slightly soluble in the ordinary oils and rapid estimates must be made.

Between crossed Nicols, first and second order interference colors are observed. Since all crystals show parallel extinction, except those with an optic axis emerging, the system is apparently orthorhombic.

The specific rotation for Compound II dissolved in chloroform at 25.2° is +73.7°.

**Identification of Sugars Hydrolyzed from Saponin**—The mucic acid test was positive, the identity of the mucic acid crystals being confirmed by melting point and optical properties. This is interpreted to mean that galactose is one of the sugars produced by the
hydrolysis of the saponin. Less reliable qualitative tests (Bial's reaction, shape of osazone crystals, and the ammonium molybdate test (8)) indicated rhamnose in addition to galactose.

**DISCUSSION**

Since the solubility, melting point, and probable molecular weight of Compound I are the same as those of the hydroxy acid reported by Muramatsu (1), it seems probable, in the light of the additional chemical tests, that the compound in question is in reality an insoluble acid saponin (9) which is quite resistant to hydrolysis.

**TABLE I**

*Comparison of Saponins and Their Sodium Salts As Made by Different Investigators*

<table>
<thead>
<tr>
<th>Saponin</th>
<th>M.p. °C.</th>
<th>Crystal habit</th>
<th>M.p. of Na salt °C.</th>
<th>Crystal habit of Na salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound I</td>
<td>220-225</td>
<td>Thin plates</td>
<td>260</td>
<td>Thin hexagons</td>
</tr>
<tr>
<td>Sumiki</td>
<td>222-224</td>
<td>&quot;Squama&quot;</td>
<td>259-260</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Walz</td>
<td>272</td>
<td>Scales</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>225</td>
<td>Radial druses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>280</td>
<td>Hexagonal plates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okano and Ohara</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystalline</td>
<td>225-227</td>
<td>&quot;Squama&quot;</td>
<td>259</td>
<td>Thin hexagons</td>
</tr>
<tr>
<td>Amorphous</td>
<td>216-218</td>
<td>Powder</td>
<td>260</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

Judging from the melting points, the saponin reported in this paper, the one reported by Sumiki, the C₁ of Walz, and the crystalline saponin of Okano and Ohara (all mentioned in the introductory part of this paper) are identical. This may be more clearly seen in Table I.

Since, as Kofler (10) points out, saponins readily retain inorganic materials, sterols, lecithins, and plant pigments which can be removed only with great difficulty, it is rather remarkable that the physical properties reported by these different investigators agree as well as they do. It is also apparent from Table I that the reported sodium salts are all probably the same compound.
SUMMARY

1. An improved method is described for the preparation of soy bean saponin.
2. Soy bean saponin on long continued hydrolysis yields a sapogenin which probably contains a terpene grouping, galactose, and possibly rhamnose.
3. Some physical and chemical properties of both the saponin and the sapogenin are reported. These include the optical properties of crystals of the sapogenin.
4. Attention is called to the probable identity of several recently reported saponin preparations from the soy bean.
5. Evidence is presented that Muramatsu’s hydroxy acid from the soy bean was probably a difficultly hydrolyzable, insoluble, acid saponin.

BIBLIOGRAPHY

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