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Histochemistry. V.[1] Soyasaponins in Soybeans (*Glycine max* MERRILL, Seeds)

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In order to clarify the distribution of soyasaponins, which are the bioactive constituents of the seeds of *Glycine max* MERRILL, in various organs of soybeans, histochemical analysis has been performed. By means of high-performance liquid chromatography of fluorescent derivatives of the saponins, it was found that soyasaponins, except soyasaponin II, are located predominantly in certain organs of soybeans (the plumule, hypocotyl, and radicle) which subsequently grow to form the adult plant.

Keywords——*Glycine max*; Leguminosae; soybean; histochemistry; seed organ; HPLC; TLC; soyasaponin; triterpene glycoside

In recent years, we have been engaging in histochemical investigations on the distribution of chemical constituents such as ginsenosides[2,3] paeonol,[1] and monoterpene glucosides[4] in various parts and tissues of medicinal plants. In the present paper, we wish to report the results of histochemical analysis of soyasaponins,[5–7] which are known to be the bioactive oligoglycosidic components of soybeans.

Soybeans are important cereals utilized as materials for edible oil and various processed foods. Furthermore, they have recently been recommended as a health food containing tocopherols (vitamin E).[8] In traditional Chinese herb formulae, soybeans have been called Glycine Semen (Daizu in Japanese and dàdòu in Chinese) and used as crude drugs for the treatment of various infectious diseases.

Results

Weight Ratio of Organs

Eleven commercially available soybeans (A—K in Table I) were divided into three

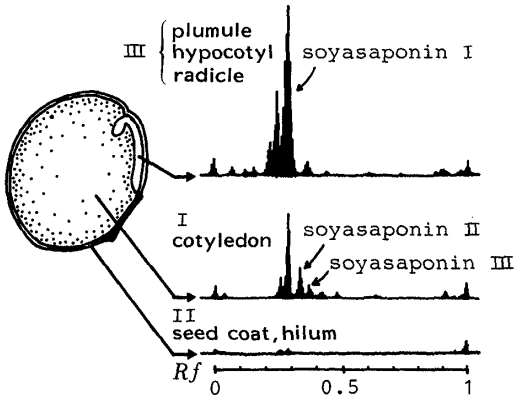

Fig. 1. TLC Profile of Soyasaponins in Organs I, II and III of Soybeans (Sample C)

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TABLE I. Weight and Weight Ratio of Organs of Various Soybeans

Materials	Whole grain weight[a] (mg)	Weight ratio (%)		
		I (cot)	II (sc, hi)	III (plm, hyp, rad)
(A) Tamanishiki daizu	383±13.0	92.5	5.4	2.2
(B) Hokkai 2-go edamame	317± 8.6	92.3	5.9	1.8
(C) Tokachi-wase edamame	300± 6.3	92.0	6.2	1.8
(D) Daizu (Hokkaido)	297± 9.7	91.8	6.2	2.2
(E) Yuzufuri-wase edamame	290±14.7	92.4	5.9	1.8
(F) Chitose-wase edamame	283± 2.6	92.1	6.3	1.6
(G) Shiratori-wase edamame	270± 7.7	92.1	6.2	1.8
(H) Wasemidori edamame	227± 7.7	92.8	6.4	1.9
(I) Dàdòu (China, Shan-tung)	207± 7.0	89.8	7.7	2.5
(J) Soybeans (South America)	180± 8.7	90.5	7.1	2.4
(K) Dàdòu (China, Peking)	176± 4.0	89.5	7.6	2.6
Average	266.4	91.62	6.45	2.05
Standard deviation	63.1	1.14	0.72	0.34
r		0.77	−0.92	−0.54

a) Mean±S.D. ($n=30$). r: correlation coefficient between whole grain weight and organ weight.

categories of organs: organ I, cotyledon (cot); organ II, seed coat (sc) and hilum (hi); and organ III, plumule (plm), hypocotyl (hyp) and radicle (rad), as shown in Fig. 1.

The whole weight and weight ratios of organs I, II and III to the whole weight were examined in each sample (A—K). Wide dispersion of whole grain weight was found, from 383±13 to 176±4 mg (average±standard deviation).

It was found that organ I, accounted for about 90% of the weight of a grain of soybean, organ II about 6%, and organ III (which subsequently grows to form the mature plant) only 2%. The regression equations for the weight ratios of organs I, II, and III to the grain weight (x) were $y(\mathrm{I})=0.046x-3.98$ ($r=0.77$), $y(\mathrm{II})=-0.086x+0.83$ ($r=-0.92$), and $y(\mathrm{III})=-0.108x+0.50$ ($r=-0.54$), respectively (r=correlation coefficient). The weight ratios were thus dominated by the cotyledon, for which the ratio increased with increase in the grain weight.

Qualitative Determination of Soyasaponins in Various Organs of Soybeans

The thin-layer chromatographic (TLC) profile of soyasaponins of sample C (Fig. 1) qualitatively shows the composition of soyasaponins in organs I, II, and III of soybeans. It was found that the concentration of soyasaponin I was highest in organ III. Soyasaponins were hardly detected in organ II.

Similar results were observed in other soybeans investigated in this study.

Quantitative Determination of Soyasaponins in Various Organs of Soybeans

The concentration of soyasaponins (soyasaponins I, II, III, A_1 and A_2) in organs I and III was determined by the high-performance liquid chromatographic (HPLC) method according to the previous report,[9] except for the column conditions.

As is apparent from Fig. 2, the average content of total soyasaponins in organ III (about 0.99%) was about six times that in organ I (about 0.16%). The content of soyasaponin I varied remarkably from organ to organ: thus, organ III (0.49—1.06%, 0.70±0.16%, coefficient of variation (C.V.)=0.23) contained about 5—10 times as much as organ I (0.062—0.104%, 0.088±0.012%, C.V.=0.14). Furthermore, the contents of soyasaponins III, A_1 and A_2 in

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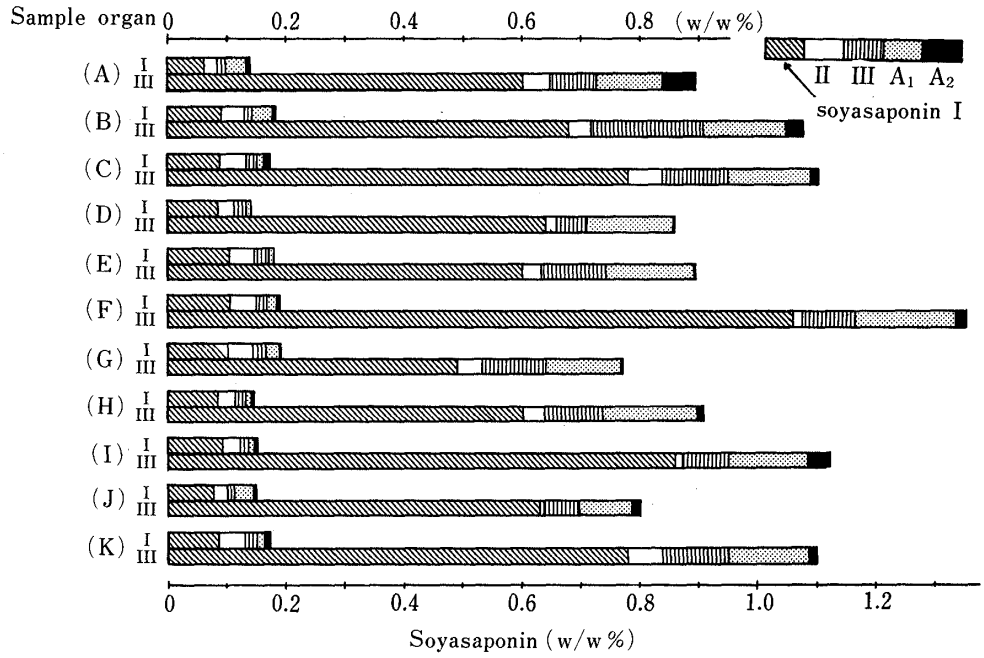

Fig. 2. Contents of Soyasaponins in Organs I and III of Soybeans

organ III were higher by 6—7 times than those in organ I. In contrast, soyasaponin II was found to be distributed almost equally in organ I ($0.035 \pm 0.009\%$, C.V. $=0.26$) and organ III ($0.032 \pm 0.019\%$, C.V. $=0.59$).

The relationship of the saponin compositions in organs I and III was:

y(soyasaponin I) $=1.06x+0.051$ ($r=0.77$ for soyasaponin II)

y(soyasaponin I) $=1.94x+0.054$ ($r=0.57$ for soyasaponin III) in organ I, and

y(soyasaponin I) $=-2.22x+0.77$ ($r=-0.28$ for soyasaponin II)

y(soyasaponin I) $=-0.17x+0.72$ ($r=-0.04$ for soyasaponin III) in organ III.

It was found that the content of soyasaponin I in organ I increased with increase in the contents of soyasaponin II and soyasaponin III.

No soyasaponins were detected in organ II.

The contents and compositions of soyasaponins of the whole grains mostly coincided with those in organ I, which accounted for about 90% of the whole grain weight. The total content of soyasaponins was found to be between 0.13% to 0.23% ($0.173 \pm 0.029\%$, C.V. $=0.17$). Interestingly, the dispersion of soyasaponin I content (0.062—0.120%, $0.097 \pm 0.016\%$, C.V. $=0.16$) was almost twice that of total soyasaponins.

The data obtained here are in accord with the reported values for different varieties of soybeans obtained by means of HPLC[9] and gas liquid chromatography.[10]

Discussion

Histochemical analysis of soybeans indicates that soyasaponins, except soyasaponin II, were not uniformly distributed in all organs of soybeans, but were contained predominantly in specific organs, *i.e.*, the plumule, hypocotyl and radicle (organ III), which subsequently grow to form the mature plant. The findings described in this report are in accord with the previous findings[2,3] that ginsenosides, ginseng-saponins, are localized in specific organs in the root of *Panax ginseng*.

The relationship between morphological characteristics and contents of soyasaponins was investigated. Soybeans (B, C, and E—I) with green-colored seed coats showed a higher

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content of total soyasaponins ($0.19 \pm 0.03\%$, $n=6$) than yellow-colored types ($0.15 \pm 0.01\%$, $n=3$); the difference was statistically significant ($t=2.637$, $p<0.05$).

The content of total soyasaponins in green-colored soybeans increased slightly with increase in the grain weight ($y=0.39x+0.08$, $r=0.64$), while that in yellow-colored soybeans was almost constant ($y=-0.062x+0.16$, $r=-0.84$).

No relationship was observed between the content of soyasaponins and morphological parameters of the soybeans, such as width/length and thickness/width, as well as the color of the hilum.

It is interesting from phyto-biochemical and physiological viewpoints that soyasaponins in soybeans are contained locally in specific organs which will grow to form the mature plant. Further work is in progress to investigate the distribution and translocation of soyasaponins in soybeans during the seed maturation and germination processes.

Experimental

Materials——Eleven commercial samples (A—K) of soybeans were examined, as listed in Table I. Four samples (D and I—K) are soybeans to be used as food materials, which the other 7 are for seeding. The seed coats of samples B, C, and E—I were green and those of the others were yellow.

The whole grain weight of 30 grains of each sample was weighed five times to calculate the means value and standard deviation of whole grain weight.

One hundred grains of each sample were peeled to obtain organ II (sc and hi), and then divided to separate organ III (plm, hyp and rad) and organ I (cot), as shown in Fig. 1. The weight ratio of each organ to the whole grain was determined.

Extraction of Chemical Constituents——Thirty grains of each sample or 100 of organs I, II and III were powdered and then treated as shown in Chart 1. Extraction was carried out to determine soyasaponins, isoflavones and tocopherols. The results for the latter two constituents will be reported in a forthcoming publication.[11]

Qualitative Profile Analysis of Soybeans——TLC examination of the saponin fraction of each soybean was carried out on a precoated silica gel plate (60 F254, Merck) developed twice with *n*-BuOH–AcOH–H_2O (4 : 1 : 5, lower layer). The plate was sprayed with H_2SO_4–EtOH (1 : 1) solution and heated to develop the color. The plate was scanned with a Shimadzu CS-910 dual-wavelength TLC scanner (zig-zag scan (reflection); 1.25×1.25 mm slit; $\lambda_S=520$ nm, $\lambda_R=700$ nm).

Quantitative Analysis of Soybeans——Soyasaponins possessing a carboxylic function in their sugar moieties were quantitatively determined by HPLC by employing a fluorescence-labelling method using 4-bromomethyl-7-methoxycoumarin (Br-Mmc: Dojindo Laboratories).

The esterification procedure with Br-Mmc was carried out under essentially the same conditions as reported.[12] The saponin fraction (Chart 1) was not subjected to the alkaline treatment used in the previous report,[9] but was treated under improved reaction conditions: a mixture of Br-Mmc (15 mg), KF (1 mg), and 18-crown-6 (5 mg) in dimethylformamide (DMF) (1 ml) in an electric oven at 90 °C for 1.5 h. After the addition of 20 ml of $CHCl_3$, the reaction mixture was filtered with a silica cartridge (SEP-PAK, Waters) and then the MeOH eluate from the cartridge was evaporated to dryness.

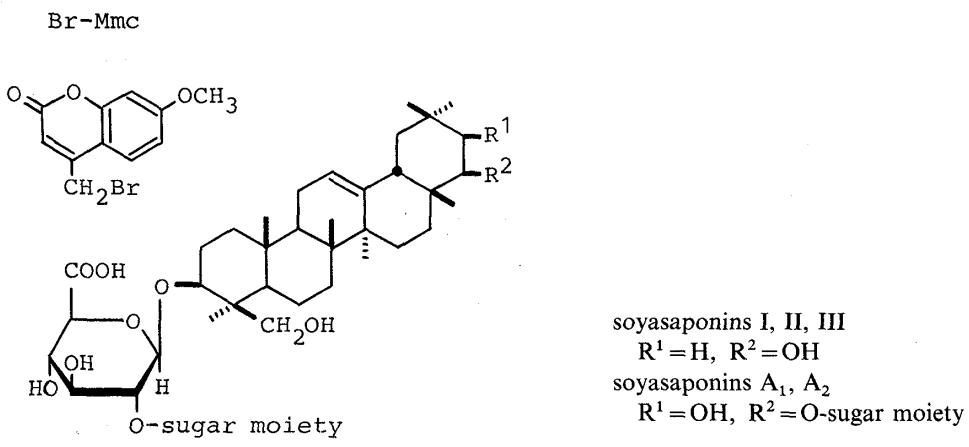

Fig. 3. Structures of Soyasaponins and Br-Mmc

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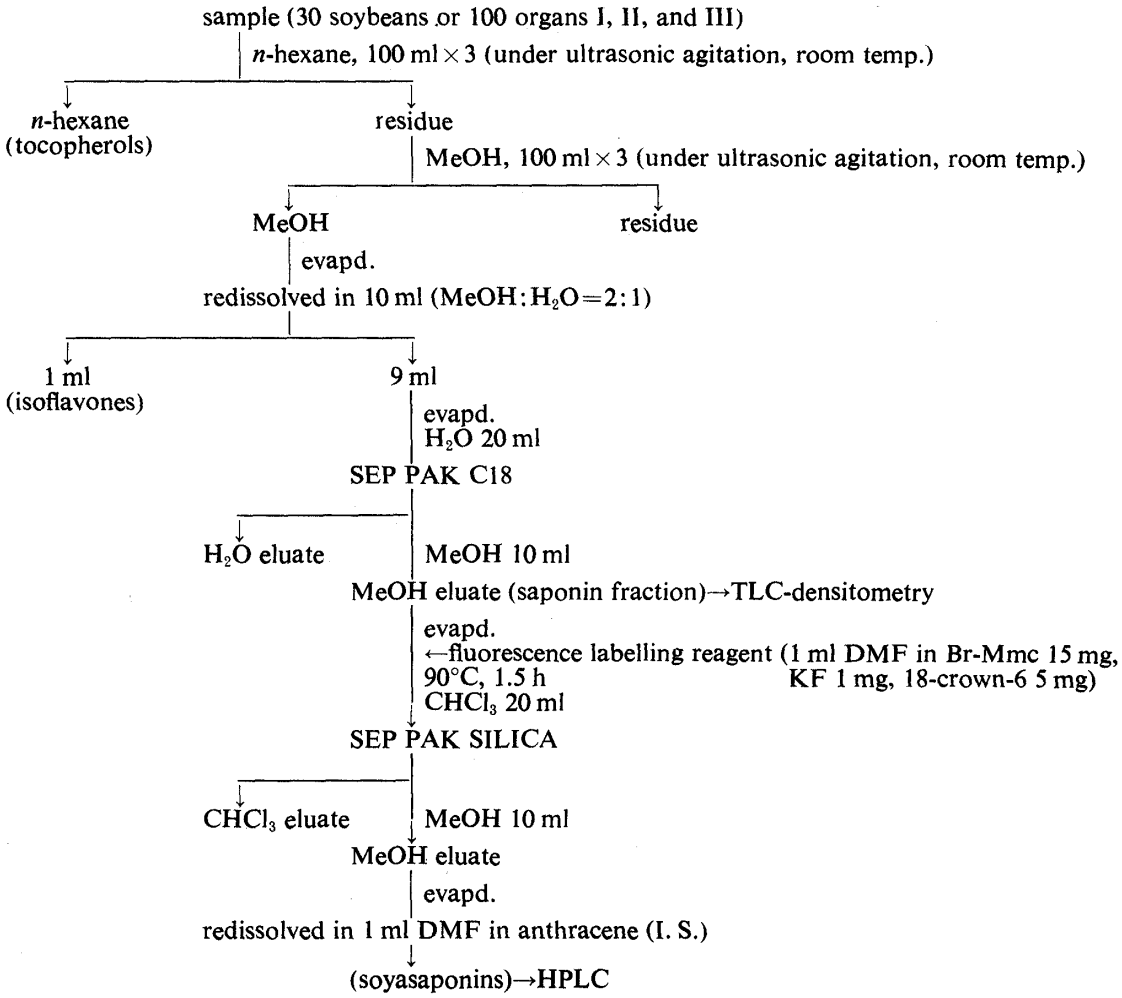

Chart 1. Extraction Procedure for Soyasaponins

The residue was taken up with 1 ml of DMF containing anthracene as an internal standard and the whole mixture was analyzed by HPLC under the conditions given below. A Shimadzu LC-3A liquid chromatograph was used with 420-E and 420-C fluorescence detectors (Waters) and a ZORBAX ODS column, 4.6 mm × 25 cm (Shimadzu); mobile phase, 30% aq. CH_3CN---(0.5%/min)---60% aq. CH_3CN---(5%/min)---100% CH_3CN; flow rate, 1 ml/min; wavelengths, $\lambda_{EX}=360$ nm, $\lambda_{EM}=400$ nm. These HPLC conditions are analogous to those described in the previous report,[9] except that the column conditions were modified to separate the peaks of soyasaponins II and III. The peaks were checked by co-chromatography with standard soyasaponins I, II, III, A_1 and A_2, and measured using a Shimadzu C-R1A computing integrator.

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