Studies on the Steroidal Components of Domestic Plants. LV.¹⁾
The Steroidal Sapogenins of the Female Flowers and the
Seeds of *Dioscorea tokoro* MAKINO

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Steroidal sapogenins were extracted from the seeds and the female flowers of *D. tokoro* MAKINO. The sapogenins isolated as crystals from the female flowers were diosgenin, yonogenin, tokorogenin and a new sapogenin, igagenin, and that from the seeds was tokorogenin. Marked differences between the seeds and the female flowers were found in regards to the sorts and the amounts of sapogenins.

When one of the authors (A.A.) isolated yonogenin and tokorogenin from the male flowers of *D. tokoro* MAKINO³⁾ (Japanese name "onidokoro"), he attempted to investigate the sapogenins of the female flowers of this plant, but could not collect a sufficient amount of material because the female flowers are readily fertilized and develop to fruits. He also investigated the sapogenins contained in the seeds⁴⁾ of this plant. However, he could only estimate the existence of diosgenin, yonogenin and tokorogenin by paper chromatographic analysis, because the available material was limited. According to his studies, the male flowers contained the same sapogenins as those found in the other aerial parts, while the amounts of sapogenins contained in the flowers were greater than those of the other parts. It also appeared that the seeds differed from the other aerial parts and were rather similar to the underground parts in their steroidal constituents. The present work was carried out, because it became necessary to investigate the steroidal sapogenins contained in the female flowers and seeds of these plants in order to clarify the distribution of the sapogenins in this plant and to elucidate their variation in relation to the season and the age of the plant. The study on the variation of the sapogenins is now proceeding, and further results will be reported later.

The steroidal sapogenins were extracted and purified as reported previously.⁴⁾ The results obtained are summarized in Table I. From the female flowers, a new substance of mp 253°, $C_{27}H_{44}O_5$, $[\alpha]_D -43.6^\circ$ was isolated in addition to yonogenin, tokorogenin and diosgenin. Although the four bands characteristic of the typical steroidal sapogenins^{5,6)} were not observed in the infrared spectrum of this substance, the wave numbers of two bands (1017 and 911 cm^{-1}) were closely similar to those of isonarthogenin and isocarneagenin.⁷⁾ Furthermore, this substance was detected on a thin-layer plate as a yellow spot resembling the steroidal sapogenins after treated with cinnamic aldehyde and $SbCl_3$. It was easily acetylated with pyridine and acetic anhydride at room temperature and afforded a triacetate, mp 191—192°, $C_{33}H_{50}O_8$, $[\alpha]_D -15.1^\circ$. No free hydroxyl band was assigned in its infrared spectrum and a band at 910 cm^{-1} was similar to those of the acetates of isonarthogenin and isocarneagenin. From these results this substance was assumed to be a new steroidal sapo-

1) Part LIV: K. Takeda, G. Lukacz, and F. Yasuda, *J. Chem. Soc.*, 1968, 1041.

2) Location: a) *Fukushimaku, Osaka*; b) *Aburahi, Shiga*.

3) A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **13**, 68 (1963).

4) A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **11**, 93 (1961).

5) M.E. Wall, M.L. McClennan, C.R. Eddy, and M.E. Klump, *Anal. Chem.*, **24**, 1337 (1952).

6) R.N. Jones, *J. Am. Chem. Soc.*, **75**, 158 (1953).

7) K. Takeda, H. Minato, A. Shimaoka, and Y. Matsui, *J. Chem. Soc.*, 1963, 4815.

genin possessing three primary or secondary hydroxyl groups one of which exists at C₂₇, and was named igagenin.

TABLE I. Steroidal Sapogenins isolated from the Seeds and the Female Flowers of *D. tokoro* MAKINO

Material	Dry weight		Steroidal sapogenins isolated				
			Dios	Yono	Tokoro	Koga	Iga
Seeds	205	free sapogenins	—	0.005	+	+	—
		aglycones of saponins	0.002	+	0.060	+	—
Female flowers	236	free sapogenins	+	4.200	0.021	+	0.007
		aglycones of saponins	0.014	1.787	0.028	+	0.011

Figures denote grams. +, detected by thin-layer chromatography; —, not detected

Since Marker, *et al.*⁸⁾ discussed a correlation between the structures of the sapogenins and the physiological stages of the plant, the problem concerning the variation of the sapogenins was reported by several authors. Woodbury, *et al.*⁹⁾ investigated the sapogenins of *Yucca brevifolia* ENGELM. and reported that smilagenin is found only in the wood tissue, the concentration of the sapogenins is low in the leaves and highest in the reproductive parts and the predominant sapogenins are hecogenin in the capsules and tigogenin in the seeds. The flowers are the reproductive organs of the plants and the other parts are the vegetative organs and the seeds are considered to be the new-plants rather than parts of their mother plants. In *D. tokoro*, a marked change in the constitution of the steroidal sapogenins was found among these parts. The sapogenins found in the female flowers are very similar to those in the male flowers except igagenin. The concentration of yonogenin is much higher in these parts than in the other parts,⁴⁾ although considerable amounts are contained as free sapogenin in the flowers as well as the other aerial parts.⁴⁾ Igagenin is only found in the female flowers. In contrast to the report by Woodbury, *et al.*, when the female flowers are fertilized and develop seeds, the concentration of the sapogenins diminishes markedly. It is particularly conspicuous in yonogenin which is the predominant sapogenin of the female flowers but contained only in trace amounts in the seeds.

Experimental¹⁰⁾

Thin-Layer Chromatography (TLC)—Kiesel gel G (Merck) plate, 0.25 mm 25 × 25 cm. Solvents (I), for aglycones, CHCl₃–acetone–AcOH (14:6:1); (II), for aglycone acetates, CH₂Cl₂–acetone (20:1). Color reagent, 1% cinnamic aldehyde in EtOH and 25 g of SbCl₃ in 5 ml of nitrobenzene. *R_f* values of the standard steroidal sapogenins: with solvent (I); diosgenin 0.78, yonogenin 0.51, tokorogenin 0.30, kogagenin 0.16; with solvent (II); yonogenin diacetate 0.67, tokorogenin triacetate 0.30, kogagenin triacetate 0.16.

Materials—The seeds were collected from the plants cultivated in Aburahi Farm in October, 1965, air dried and preserved in a desiccator until April, 1967. The female spikes were collected in and around Aburahi Farm in July, 1967 and air-dried.

Isolation of the Steroidal Sapogenins from the Seeds—1) The Free Sapogenins: 205 g of seeds were immersed and ground in 800 ml of benzene, then refluxed for 4 hr. The residue was extracted further three times with benzene under reflux. After removal of the solvent, 0.585 g of dark greenish tar were obtained. This was chromatographed on 50 g of Al₂O₃ (Merck, activity 1). The *R_f* values of the steroidal sapogenins which were detected by TLC and not isolated were as follows: 0.30, 0.16 and 0.07 in CHCl₃–MeOH (90:10) fraction (yellowish green tar, 83 mg). 68 mg of the greenish tar obtained from CHCl₃–MeOH (98:2–50:50)

8) R.E. Marker, R.B. Wagner, P.R. Ulshafer, E.L. Wittbecker, D.P.J. Goldsmith, and C.H. Ruof, *J. Am. Chem. Soc.*, **69**, 2167 (1947).

9) A.M. Woodbury, M.E. Wall, and J.J. Willaman, *Economic Botany*, **15**, 79 (1961).

10) All melting points were uncorrected. Infrared (IR) spectra were recorded with a Nippon Bunko double-beam spectrophotometer model DS 201 B.

fractions were acetylated with pyridine and Ac_2O under reflux to yield 44 mg of a greenish tar. This was rechromatographed on 3 g of alumina to yield 5 mg of white crystals mp 180—184°. The IR spectrum of this substance was identical to that of yonogenin acetate.

2) Sapogenins contained as the Aglycones of the Saponins: After extracted with benzene, the residue was again extracted four times with 800 ml of MeOH for 4 hr under reflux. The MeOH extract was hydrolyzed with 200 ml of 5% HCl-MeOH for 5 hr under reflux, then poured into water and extracted with ether to yield 1.61 g of a brownish tar. This was chromatographed on 80 g of Al_2O_3 . 181 mg of the yellowish green substance obtained from the benzene- CHCl_3 fraction were crystallized from MeOH to yield 25 mg of white needles, mp 115—130°. This was found to be a mixture of β -sitosterol and stigmasterol by gas chromatography. The mother liquor was evaporated and the residue was acetylated with pyridine and Ac_2O to yield 81 mg of a brown tar. This was rechromatographed on 4 g of Al_2O_3 to give 2 mg of white needles, mp 178—193° were isolated. The IR spectrum of this substance was identical with that of diosgenin acetate. 143 mg of the yellowish tar obtained from the CHCl_3 -MeOH (90:10)-MeOH fractions were crystallized from MeOH to yield 20 mg of white needles, mp 263—265°. *Anal.* Calcd. for $\text{C}_{27}\text{H}_{40}\text{O}_5$: C, 72.28; H, 9.89. Found: C, 72.21; H, 9.90. This was identified as tokorogenin by mixed melting point and IR spectra. 88 mg of the greenish tar recovered from the mother liquor were acetylated with pyridine and Ac_2O to yield 81 mg of yellowish tar. This was rechromatographed on 4 g of Al_2O_3 to yield 40 mg of white needles of tokorogenin triacetate mp 252—258° after recrystallization from MeOH. *Anal.* Calcd. for $\text{C}_{33}\text{H}_{50}\text{O}_8$: C, 68.96; H, 8.77. Found: C, 68.77; H, 9.05. The *Rf* values of the steroidal sapogenins which were detected only by TLC, were as follows: 0.58, 0.51 and 0.41 in benzene- CHCl_3 (50:50)- CHCl_3 -MeOH (98:2) fractions (yellowish green tar 57 mg).

Isolation of the Sapogenins from the Female Flowers—1) The Free Sapogenins: 236 g of the female flowers were extracted five times with 2 liter of benzene for 4 hr under reflux. Dark greenish jelly (11.1 g) was obtained after removal of the solvent which was chromatographed on 500 g of Al_2O_3 . The green jelly (7.07 g) obtained from CHCl_3 - CHCl_3 -MeOH (95:5) fractions was crystallized from MeOH to yield 4.2 g of white needles, mp 238—240°. *Anal.* Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_4$: C, 74.95; H, 10.25. Found: C, 74.66; H, 10.13. This was identified as yonogenin by mixed melting point and IR spectra. 573 mg of a green tar obtained from CHCl_3 -MeOH (90:10—50:50) fractions were subjected to preparative thin-layer chromatography to yield 50 mg of white powder (1) and 18 mg of white needles (2). (1) was recrystallized from MeOH to yield 21 mg of tokorogenin mp 267—268°. (2) was recrystallized from acetone to yield 7 mg of white needles (*Rf* 0.26, igagenin), mp 248—249°. The spots of steroidal sapogenins were also detected in benzene- CHCl_3 (30:70)- CHCl_3 fractions (*Rf* 0.88, 0.85, 0.78, yellowish brown tar, 673 mg).

2) The Sapogenins contained as Saponins: After extraction with benzene, the residue was extracted four times with 2 liter of MeOH for 4 hr under reflux. 55 g of a brown tar obtained from the MeOH extract were hydrolyzed with 360 ml of 5% HCl-MeOH for 5 hr under reflux, poured into water and extracted with ether to yield 9 g of a dark greenish substance. This was chromatographed on 50 g of Al_2O_3 . The yellowish green jelly (3.638 g) obtained from CHCl_3 -MeOH (98:2—95:5) fractions was crystallized from MeOH to yield 1.787 g of white needles of yonogenin, mp 238—240°. Yellowish greentar (1.07 g) obtained from the CHCl_3 -MeOH (95:5)-MeOH-MeOH-pyridine (95:5) fractions was subjected to preparative thin-layer chromatography to yield 28 mg of white needles of tokorogenin and 56 mg of white crystals (*Rf* 0.26). The latter was recrystallized from acetone to yield 11 mg of white needles of igagenin, mp 248—249°. The melting point was raised to 253° after further recrystallization, $[\alpha]_D^{25} -43.6^\circ$ ($c=0.220$, MeOH). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_5$: C, 72.28; H, 9.89. Found: C, 72.13; H, 9.90. IR, cm^{-1} (Nujol): (OH), 3256; (E, F ring), 1017, 974, 962, 911.

Acetylation of Igagenin—12 mg of igagenin were dissolved in 1 ml of pyridine and 0.5 ml of Ac_2O and allowed to stand overnight at room temperature, then poured into water and extracted with CHCl_3 to yield 16 mg of the acetate. From this 9 mg of white plates were obtained after recrystallization from MeOH, mp 191—192°, $[\alpha]_D^{25} -15.1^\circ$ ($c=0.351$, CHCl_3). *Anal.* Calcd. for $\text{C}_{33}\text{H}_{50}\text{O}_8$: C, 68.96; H, 8.77. Found: C, 69.11; H, 8.77. IR cm^{-1} (CHCl_3): (acetate) 1735, 1260; (E, F ring) 975, 963, 921, 910, 900.