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Studies on the Steroidal Components of Domestic Plants. LI.[1] Steroidal Compounds contained in the Rokkô Population of *Dioscorea tenuipes* Complex

Akira Akahori, Fumio Yasuda, and Tameto Okanishi

Shionogi Research Laboratory, Shionogi & Co., Ltd.[2]

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The steroidal components contained in the Rokkô population of the *D. tenuipes* complex were inverstigated. From its aerial parts, one triterpene, one sterol and eight steroidal sapogenins were isolated. They were taraxerol, β-sitosterol, yamogenin, yonogenin, tokorogenin, diotigenin, recently isolated,[3] three new sapogenins and an unknown steroidal compound. Two of the new sapogenins were 25L-epimers of yonogenin and tokorogenin, and named neoyonogenin and neotokorogenin, respectively. Another new sapogenin was a 25L-tetrahydroxysapogenin and named tenuipegenin. Besides these, diosgenin and stigmasterol were detected by infrared spectrum and gas chromatography, respectively. The steroidal sapogenins of this population were chiefly composed of 25L members. The coexistence of 25 D- and 25L-sapogenins in individual plants was also discussed.

A plant known as *Dioscorea tenuipes* Franch. et Savat. (Japanese name "himedokoro") is one of the most conspicuous Dioscorea in Japan and widely distributed in Honshû, Shikoku and Kyûshû. From the standpoint of plant taxonomy it has been considered to be a good species, since Savatier[4] collected it at Yokosuka, Prov. of Sagami.[5] Botanists have no doubt about the characterization of this species. Although Knuth[6] differentiated *D. tenuipes* Franch. et Savat. and *D. Maximowiczii* Uline according to the number of nerves in the leaves,[7] Prain and Burkill[8] treated the latter as a synonymy of the former. The number of nerves is a relatively invariable character in each species of Dioscorea and it is unreasonable to solely use this character to distinguish the two species. However, it was discovered (A.A)[3,9] that the variety collected in western Japan contained steroidal sapogenins abundantly in its aerial parts but the one collected in eastern Japan did not. A new trihydroxysapogenin, diotigenin, was isolated together with tokorogenin from the aerial parts of this plant during the survery of the steroidal sapogenin of Japanese Dioscorea. After further investigation, the variability of the paper chromatographic pattern of the free steroidal sapogenins was detected even among the variety distributed in western Japan.[10] Some cytological differences were also found in this plant.[10] Furthermore, the rate of germination of the seed of this

1) Part L: M. Iwasaki, *Tetrahedron*, **23**, 2145 (1967).
2) Location: *Fukushima-ku, Osaka.*
3) A. Akahori, *Phytochemistry*, **4**, 97 (1965).
4) A. Franchet and L. Savatier, *Enum. Pl. Jap.*, **11**, 523 (1879).
5) At present, Kanagawa Pref. Yokosuka is about 30 km apart from Tokyô.
6) R. Knuth, *Das Pflanzenreich*, IV-43, 178 (1924).
7) According to Knuth (*Das Pflanzenreich*, IV-43, 171 (1924)).
   I. Folia plerumque 7-nervata; lamina supra auriculas basales valde contracta, deinde longe acuminata *D. tenuipes*
   II. Folia 9-nervata
      1. Lamina ambitu ± cordata *D. enneaneura*
      2. Lamina ambitu elongato-cordata *D. Maximowiczii*
8) D. Prain and I.H. Burkill, *Kew Bull.*, 118 (1926).
9) A. Akahori, *Acta Phytotax. Geobot.*, **21**, 149 (1965).
10) unpublished data.

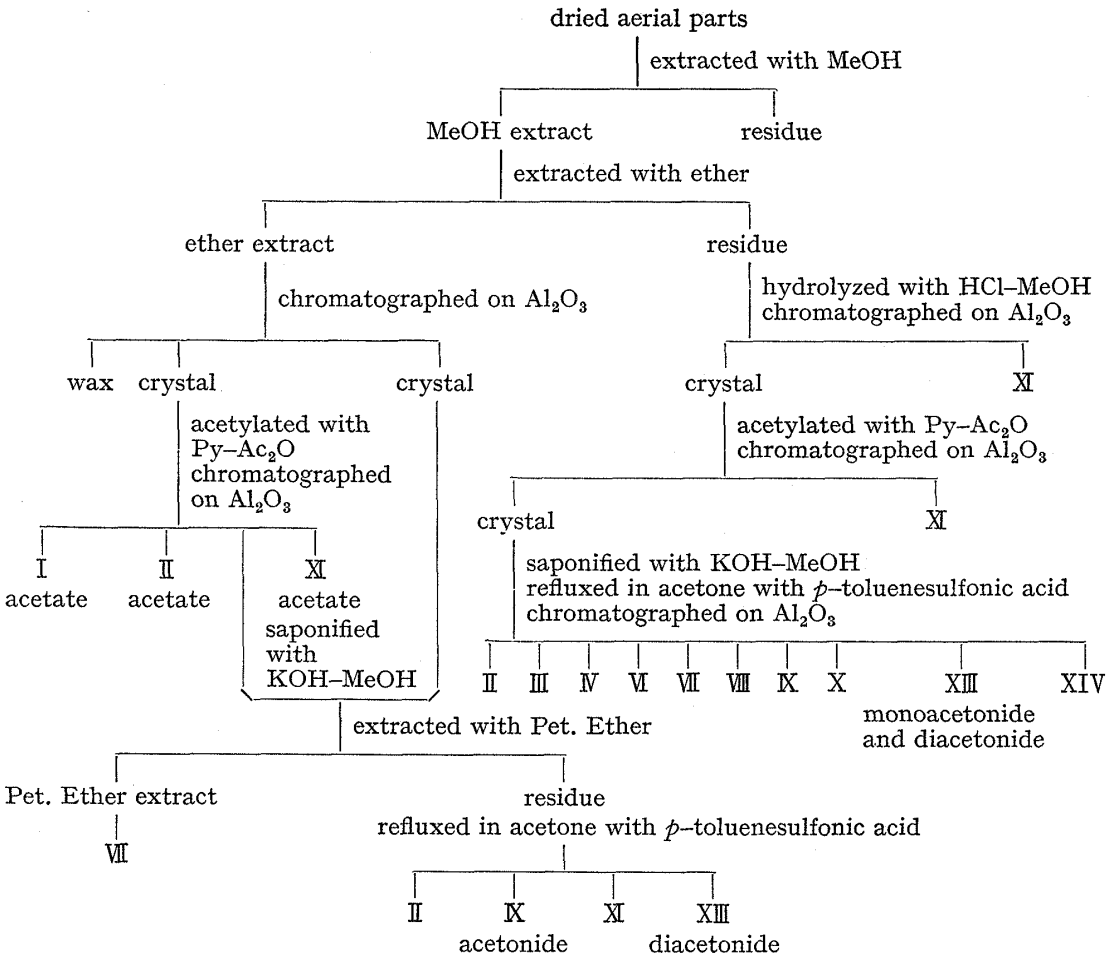

Fig. 1. Extraction and Purification of the Steroidal Components contained in the Rokkô Population of *D. tenuipes* Complex

TABLE I. Steroidal Sapogenins and Sterols contained in the Rokkô Population of *D. tenuipes* Complex

No.		as free genin	as aglycone of glycoside	color on TLC[b]
I	taraxerol	0.382[a]	—	bluish gray
II	sterols	5.344	0.983	purple
III	yamogenin + diosgenin	—	0.052	orange red
IV	sterols	—	0.181	purple
V	unknown	—	—	gray
VI	sterol	—	0.054	purple
VII	neoyonogenin + yonogenin	0.096	0.172	yellow
VIII	sterol	—	0.047	purple
IX	neotokorogenin + tokorogenin	0.917	7.180	yellow
X	sterol	—	—	purple
XI	diotigenin	7.190	32.699	yellow
XII	sterol	—	—	purple
XIII	tenuipegenin	0.818		yellow
XIV	unknown	—		yellow

a) Figures denote grams
b) After treatment with cinnamic aldehyde and antimony trichloride

plant is very low. For these reasons, this plant is assumed to be a hybrid population or a polyploid taxon. Temporarily we have named this plant a *D. tenuipes* complex. A thorough morphological and cytological investigation of this plant is now proceeding and the results obtained will be reported later.

The present work was undertaken to obtain diotigenin in order to study its structure and also to investigate the minor components of this plant. Since the amounts and varieties of sapogenins are considered to be seasonally variable in this plant as reported in other Dioscorea,[11] the authors did not know whether August would be the most suitable season for their purpose or not. However, the collection of material had to be made at its flowering period in order to collect it exclusively, because this plant is morphologically so closely similar to *D. tokoro* MAKINO that it is impossible to discriminate between these two plants when they bear neither flowers nor capsules.

The dried aerial parts were extracted with methanol. Free sapogenins were extracted with ether from the methanol extract. Saponins were then hydrolyzed with hydrochloric acid. Further purification and isolation procedures are briefly summarized in Fig. 1. The sapogenins and sterols obtained are listed in Table I and their thin-layer chromatogram is shown in Fig. 2.

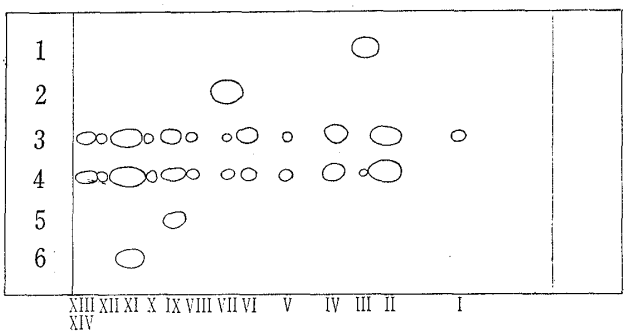

Fig. 2. Thin-Layer Chromatogram of the Crude Sapogenins

Plate: Silicagel G
Solvent: benzene: acetone: acetic acid (70:30:3)
Color Reagent: 1% cinnamic aldehyde in ethanol and 25% of $SbCl_3$ in 5 ml of nitrobenzene
1, diosgenin; 2, yonogenin; 3, free sapogenins; 4, hydrolysis product; 5, tokorogenin; 6, diotigenin.

(I) was a colorless platelet, mp 279—281°. It was identified as a neutral triterpene, taraxerol. (II) was a mixture of sterols. By gas chromatography three peaks were observed, two of which were considered to be stigmasterol and β-sitosterol by their retention times, and β-sitosterol was isolated from (II) as a colorless platelet. The infrared spectrum of (III) coincided with that of a mixture of yamogenin and diosgenin. The amounts of diosgenin contained in (III) were considered to be negligible, because the intensity of a band at 968 cm^{-1} was very weak. (VII) was a mixture of two sapogenins yonogenin and colorless needles, $C_{27}H_{44}O_4$, mp 198—199°, $[\alpha]_D^{22}$ −63.7°. The infrared spectrum of the latter showed characteristic 25L-bands. This substance was converted to its 25D-epimer after long-term refluxing with ethanol and hydrochloric acid, and yonogenin was obtained. Therefore, this substance was considered to be a 25L-isomer of yonogenin, and was named neoyonogenin. (IX) was also a mixture of 25D- and 25L-sapogenins. Tokorogenin and a new 25L-trihydroxysapogenin, $C_{27}H_{44}O_5$, mp 250°, $[\alpha]_D^{23}$ −55.1° were isolated from this mixture. The latter was demonstrated to be a 25L-isomer of tokorogenin after similar treatment to that used for neoyonogenin, and it was named neotokorogenin. (XI) was diotigenin, mp 280—281°, a principal sapogenin of this plant and isolated as a free sapogenin in addition to its saponins. The 25D-isomer of this sapogenin was not detected in this fraction. (XIII) was a 25L-tetrahydroxysapogenin, $C_{27}H_{44}O_6$, mp 299—300°, $[\alpha]_D^{22}$ −51.5° which yielded a monoacetonide, $C_{30}H_{48}O_6$, mp 255—256°, $[\alpha]_D^{22}$ −33.3° and a diacetonide, $C_{33}H_{52}O_6$, mp 210—211°, $[\alpha]_D^{22}$ −20.0°. It was readily acetylated and benzoylated with acetic anhydride and benzoyl chloride respectively in pyridine at room temperature. The acetate was assumed to be a tetracetate according to its infrared spectrum and analytical value. It was very easily soluble in any solvents and the crystallization of this acetate was not succeeded. The benzoate was obtained as a tetrabenzoate, $C_{55}H_{60}O_{10}$,

11) H.J. Cruzado, H. Delpin, and B.A. Roark, *Turrialba*, **15**, 28 (1965).

mp 167—169°. Four hydroxyl groups were assumed to be primary or secondary alcohols. This sapogenin was considered to be a new sapogenin and was named tenuipegenin. (XIV) was isolated as an acetonide, mp 168—171°. Although this substance was assumed to be a steroidal sapogenin or a saponin according to its infrared spectrum further investigation was not carried out.

Amounts of yamogenin and diosgenin were negligible as reported for the aerial parts of other Japanese Dioscorea.[3] Although yonogenin is the principal sapogenin in the aerial parts of *D. tokoro*,[12,13] neoyonogenin and yonogenin were isolated from *D. tenuipes* in only small amounts. Because spots corresponding to yonogenin are usually very weak or not detected on paper or thin-layer chromatograms of the crude sapogenins obtained from this plant, except from particular individuals, the possibility may not be entirely excluded that these sapogenins are contained only in certain individuals. Diotigenin and other four 3*α*-hydroxysapogenins were isolated as free sapogenins in addition to aglycones of saponins, but free yamogenin and diosgenin were not found. This is similar to that observed in *D. tokoro*. Another feature of the sapogenins isolated from this plant was that they were chiefly composed of 25L-sapogenins. In order to decide whether 25L- and 25D-sapogenins are both contained in the same individuals, ten fresh rhizomes were extracted separately. Diosgenin fractions obtained from these individuals were all mixtures of 25L- and 25D-sapogenins, yamogenin and diosgenin. However, in one of them a mixture of sapogenins was almost wholly composed of 25L-sapogenin. Because it is reported that 25L-sapogenins were partly converted to its 25D-isomer during acid-hydrolysis,[14] it is still impossible to exclude entirely the possibility that there are individuals containing only 25L-sapogenins, but it is certain that individuals which contain both 25L- and 25D-sapogenin exist.

Experimental[15]

Material——Aerial parts of *D. tenuipes* collected around Mt. Rokkô, Hyôgo Pref., between late July and early September were air-dried and powdered.

Extraction of Steroidal Components——5.9 kg of dried material were extracted continuously with 50 liter of MeOH. 1130 g of the dark greenish tar obtained after removal of MeOH were again extracted with ether under reflux and yielded 160 g of a dark greenish tar. The residue after extraction with ether was hydrolyzed with 750 ml of 35% HCl and 6 liter of MeOH for 5 hr under reflux. The reaction mixture was concentrated *in vacuo* and water was added. The precipitate which formed was saponified with 300 g of KOH and 6.5 liter of 90% MeOH for 2 hr under reflux. Unsaponified material was then extracted with $CHCl_3$ and 187 g of black tar were obtained. These tars were treated as shown in Fig. 1.

Taraxerol——(I) was isolated as an acetate. (I) acetate was recrystallized from $CHCl_3$–MeOH (1:1) to yield 382 mg of white platelets, mp 297—301°, $[\alpha]_D^{24}$ +9.1° (c=1.080, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.99; H, 11.26. IR cm^{-1} (Nujol): (C=C) 816, 1642; (OAc) 1253, 1727. Liebermann-Burchard test: dark red. Tetranitromethane reaction: yellow. This was identified by mixed melting point and infrared spectra with taraxerol acetate. 14 mg of (I) acetate were dissolved in 20 ml of *t*-BuOH and warmed on a water bath for 1 hr with 1 g of KOH and then water was added. From the *t*-BuOH layer 12 mg of crystals were obtained which yielded 9 mg of white platelets, mp 279—281°, $[\alpha]_D^{24}$ +1.1° (c=1.025, $CHCl_3$) after recrystallization from MeOH–$CHCl_3$ (1:1). *Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.42; H, 12.14. IR cm^{-1} (Nujol): (OH) 3475; (C=C) 817, 1643, 3054. This was identified by mixed melting point and infrared spectra with taraxerol.

(II)——Three peaks were detected in (II) by gas chromatography. Their retention times were 10.5, 13.9 and 15.8 min (standard cholesterol, 10.0; stigmasterol, 13.9; *β*-sitosterol, 15.5 min). Part of (II) was acetylated as usual and analyzed by gas chromatography. Retention times: 13.7, 19.0, 21.6 min (standard

12) A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **11**, 93 (1961).
13) A. Akahori, *Ann. Rept. Shionogi Res. Lab.*, **13**, 68 (1963).
14) M.E. Wall, S. Serota, and L.P. Witmauer, *J. Am. Chem. Soc.*, **77**, 3086 (1955).
15) All melting points were uncorrected. Infrared spectra were recorded with a Nippon Bunkô double-beam spectrophotometer model DS 201-B. Gas chromatographic data were obtained with a Barber-Colman Model 10 gas chromatograph equipped with an argon ionization detection system. The columns were glass U-tubes, 0.4×300 cm, packed with 1% SE-30 on Chromosorb-W (80—100 mesh).

cholesterol acetate, 13.7; stigmasterol acetate, 19.0; β-sitosterol acetate, 21.6 min). (II) was also subjected to preparative thin-layer chromatography on Kiesel Gel G plates with benzene–acetone–AcOH (70:30:3) and zones corresponding to β-sitosterol by their *Rf* values were eluted with $CHCl_3$–MeOH (7:3). The recovered material was recrystallized from MeOH to yield white platlets, mp 136—138°. *Anal.* Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.90; H, 12.10. This was identified by mixed melting point and infrared spectra with β-sitosterol acetate.

Yamogenin and Diosgenin——(III) was isolated from the hydrolysis product, after repeated chromatography,as 52 mg of acetate, white needles, mp 146—158°. Its infrared spectrum was almost identical with yamogenin acetate but showed a weak band at 868 cm^{-1}. This was saponified with KOH and MeOH for 2 hr under reflux and repeatedly recrystallized from MeOH to yield 7 mg of white needles, mp 179—184°. *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.45; H, 10.19. Its infrared spectrum was identical with that of yamogenin, but still showed a trace of a band at 868 cm^{-1}.

(IV)——(IV) was isolated from the hydrolysis product. By gas chromatography two peaks were detected, the retension times of which were 11.1 min and 14.5 min (standard cholesterol, 9.0 min; stigmasterol, 12.3 min; β-sitosterol, 14.1 min).

Neoyonogenin and Yonogenin——(VII) was isolated both from the ether extract and acid-hydrolysis product. Its infrared spectrum showed that (VII) was a 25L-sapogenin containing a small amount of 25D-sapogenin. This was recrystallized from MeOH and yielded 65 mg of white needles, showing 25L bands in its infrared spectrum, mp 173—180°. The melting point was raised to 198—199° after further recrystallization from MeOH, $[\alpha]_D^{22}$ −63.7° (c=0.347, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.11; H, 10.27. IR cm^{-1} ($CHCl_3$): (OH) 3410, 3560; (E, F ring) 984, 915, 895, 847 (25L, 915>895[16]). 180 mg of the crystals obtained from the mother liquor were dissolved in Ac_2O and refluxed for 30 min on an oil bath. The reaction mixture was concentrated to half volume *in vacuo* and the crystals formed were repeatedly recrystallized from MeOH to yield 7 mg of white needles, mp 180—202°. The melting point was raised to 205—208° after further recrystallization $[\alpha]_D^{24}$ −19.6° (c=1.042, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.92; H, 9.37. This was identified by mixed melting point and infrared spectra with yonogenin diacetate. 22 mg of 25L-substance was acetylated with pyridine and Ac_2O as usual and yielded 6 mg of white needles after recrystallization from MeOH, mp 184—187°. $[\alpha]_D^{22}$ −20.8° (c=0.860, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.10; H, 9.26. IR cm^{-1} ($CHCl_3$): (OAc) 1736; (E, F ring) 984, 921, 915, 895, 845 (25L 915>895).

Conversion of Neoyonogenin to Yonogenin——10 mg of neoyonogenin acetate were dissolved in 50 ml of 95% EtOH and 9 ml of 35% HCl, refluxed for 72 hr and then extracted with ether. The ether extract was acetylated with pyridine and Ac_2O. The acetate obtained was recrystallized from MeOH to yield 10 mg of white needles, mp 203—206°, $[\alpha]_D^{22}$ −21.0° (c=0.634, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.18; H, 9.47. This was identified by mixed melting point and infrared spectra with yonogenin diacetate.

Isolation of Neotokorogenin and Tokorogenin——This trihydroxysapogenin mixture was isolated as an acetonide. After repeated recrystallization from MeOH, white needles were obtained, mp 244—245°, $[\alpha]_D^{22}$ −41.7° (c=1.025, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.66; H, 10.00. IR cm^{-1} ($CHCl_3$): (OH), 3584, 3440; (E, F ring) 985, 914, 895, 848 (25L, 914>895). 4.1 g of this acetonide were dissolved in 200 ml of 80% AcOH and warmed on a water bath for 5 hr. The reaction mixture was then concentrated *in vacuo*, water was added and the mixture extracted with ether. The ether extract was saponified with 5% KOH in 90% MeOH for 2 hr and yielded 3.571 g of white crystals. This was repeatedly recrystallized from 95% EtOH to yield white platelets, mp 250°, $[\alpha]_D^{23}$ −55.1° (c=0.979, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.54; H, 9.71. IR cm^{-1} ($CHCl_3$): (OH) 3590, 3430; (E, F ring) 985, 914, 895, 848 (25L, 914>895). Part of the crystals (0.8 g) obtained from the mother liqour after isolation of 25L-sapogenin acetonide were dissolved in 80% AcOH and warmed for 5 hr. The crystals obtained from the reaction mixture were then acetylated with pyridine and Ac_2O and repeatedly recrystallized from MeOH to yield 125 mg of white needles, mp 241—248°. The melting point was raised to 251—253° after further recrystallization. *Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.96; H, 8.77. Found: C, 69.11; H, 8.88. This was identified by mixed melting point and infrared spectra with tokorogenin triacetate.

Partial Acetylation of Neotokorogenin——2.5 g of neotokorogenin was acetylated with 7 ml of pyridine and 3.5 ml of Ac_2O overnight at room temperature. The reaction mixture was then concentrated to dryness *in vacuo* and extracted with ether. The ether extract was chromatographed on neutral Al_2O_3 (Woelm, containing 3% water). A petroleum ether-benzene fraction was recrystallized from petroleum ether to yield 175 mg of white needles, mp 185—190°, $[\alpha]_D^{24}$ −20.9° (c=1.019, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.96; H, 8.77. Found: C, 68.75; H, 8.93. IR cm^{-1} ($CHCl_3$): (OAc) 1740; (E, F ring) 984, 914, 894, 847 (25L, 914>894). The $CHCl_3$ fraction was recrystallized from MeOH to yield 190 mg of white needles, mp 275—276°, $[\alpha]_D^{24}$ −36.5° (c=1.194, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 70.08; H, 9.07. IR cm^{-1} ($CHCl_3$): (OH) 3640, 3500; (OAc) 1734; (E, F ring) 984, 914, 895, 847 (25L, 914>895).

16) The intensity of the band at 915 cm^{-1} is stronger than that of the band at 892 cm^{-1}.

Neotokorogenin Acetonide Acetate——119 mg of neotokorogenin acetonide were acetylated with 2 ml of pyridine and 1 ml of Ac_2O at room temperature overnight. The acetate obtained was recrystallized from MeOH to yield 46 mg of white needles, mp 224—226°, $[\alpha]_D^{22}$ +1.5° (c=1.010, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.32; H, 9.53. IR cm^{-1} ($CHCl_3$): (OAc) 1736; (E, F ring) 984, 915, 895, 847 (25L, 915>895).

Conversion of Neotokorogenin to Tokorogenin——97 mg of neotokorogenin triacetate were dissolved in 50 ml of 95% EtOH and 9 ml of 35% HCl, and refluxed for 72 hr. The reaction mixture was concentrated *in vacuo* and extracted with ether to yield 63 mg of white crystals, mp 231—246°. This was recrystallized from MeOH to yield 7 mg of white needles, mp 265—267°. *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.00; H, 10.00. This was identified by mixed melting point and infrared spectra with tokorogenin triacetate.

Diotigenin——Diotigenin was obtained as colorless needles after recrystallization from acetone. mp 280—281°, $[\alpha]_D^{24}$ −59.8° (c=1.062, MeOH). *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.24; H, 9.98. IR cm^{-1} (Nujol): (OH) 3445, 3350; (E, F ring) 986, 926, 895, 849 (25L, 926>895).

Tenuipegenin——(XIII) was isolated as an acetonide after treatment of the sapogenin mixture with acetone and *p*-toluenesulfonic acid. The reaction mixture was chromatographed on neutral Al_2O_3 (Woelm, containing 3% H_2O). The benzene–$CHCl_3$ (4:1) fraction was recrystallized from MeOH to yield 91 mg of white platelets, mp 210—211.5°, $[\alpha]_D^{22}$ −20.0° (c=0.610, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{52}O_6$; C, 72.75; H, 9.62. Found: C, 73.06; H, 9.80. IR cm^{-1} ($CHCl_3$): (E, F ring) 985, 915, 895, 848 (25L, 915>895). The benzene–$CHCl_3$ (1:4) and $CHCl_3$ fractions were recrystallized from MeOH to yield 685 mg of white needles, mp 238—248°. The melting point was raised to 255—256° after further recrystallization from MeOH. $[\alpha]_D^{22}$ −33.3° (c=1.066, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.50; H, 9.70. IR cm^{-1} ($CHCl_3$): (OH) 3590, 3440; (E, F ring) 985, 914, 895, 848 (25L, 914>895). 12 mg of the diacetonide were dissolved in 10 ml of 70% AcOH and warmed on a water bath for 5 hr. The reaction mixture was concentrated *in vacuo*, water was added and the mixture extracted with AcOEt. The residue, after evaporation of AcOEt, was saponified in 20 ml of 90% MeOH containing 1 g of KOH for 1 hr under reflux, then extracted with AcOEt to yield 11 mg of white crystals. This was recrystallized from MeOH to give 5 mg of white needles, mp 299—300°, $[\alpha]_D^{22}$ −51.5° (c=0.899, MeOH–$CHCl_3$ 4:1). *Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.56; H, 9.77. IR cm^{-1} (Nujol): (OH) 3350 (broad); (E, F ring) 989, 924, 913, 894, 851 (25L, 924, 913>894).

Tenuipegenin Tetracetate——67 mg of tenuipegenin were acetylated with 2 ml of pyridine and 1 ml of Ac_2O overnight at room temperature. The acetylation product showed two spots after thin-layer chromatography. This mixture was then subjected to preparative thin-layer chromatography on Kiesel Gel G plates with CH_2Cl_2–acetone (20:1). Upper zones were eluted with $CHCl_3$–MeOH (7:3) and the recovered substance was dissolved in boiling 50% MeOH. After cooling in dryice-acetone 27 mg of white powders were obtained, mp 128—135°, $[\alpha]_D^{22}$ −16.5° (c=1.019, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{52}O_{10}$: C, 66.43; H, 8.28. Found: C, 66.42; H, 8.36. IR cm^{-1} ($CHCl_3$): (OAc) 1744; (E, F ring) 984, 912, 895, 847 (25L, 912>895).

Tenuipegenin Tetrabenzoate——133 mg of tenuipegenin were dissolved in 10 ml of pyridine containing 0.6 ml of benzoylchloride and allowed to stand overnight at room temperature. The reaction mixture was poured into water and extracted with $CHCl_3$. 141 mg of the recovered substance were recrystallized from MeOH to yield 64 mg of white needles, mp 167—169°, $[\alpha]_D^{23}$ +23.2° (c=0.876, $CHCl_3$). *Anal.* Calcd. for $C_{55}H_{60}O_{10}$: C, 74.97; H, 6.86. Found: C, 74.70; H, 6.93. IR cm^{-1} (CCl_4): (benzoate) 1730, 1605, 1585, 1268, 1105, 1094; (E, F ring) 985, 923, 895, 850 (25L, 923>895).

Conversion of Tenuipegenin Monoacetonide into Diacetonide——39 mg of tenuipegenin monoacetonide were dissolved in 30 ml of acetone containing 30 mg of *p*-toluenesulfonic acid and refluxed for 5 hr. The reaction mixture was concentrated *in vacuo*, water was added and the mixture extracted with $CHCl_3$ to yield 29 mg of colorless crystals. This was chromatographed on neutral Al_2O_3 (Merck, containing 3% water). The benzene fraction (4 mg) was recrystallized from MeOH to yield 2 mg of colorless needles, mp 205—208°. This was identified by mixed melting point and an infrared spectra with tenuipegenin diacetonide. From the $CHCl_3$–MeOH (19:1) fractions 23 mg of colorless needles were obtained. This was identified with starting material tenuipegenin monoacetonide.

Tenuipegenin Acetonide Diacetate——101 mg of tenuipegenin monoacetate was acetylated with 3 ml of pyridine and 1.5 ml of Ac_2O overnight at room temperature. From the reaction mixture 85 mg of amorphous substance were obtained. This was crystallized from MeOH to yield 48 mg of colorless platelets, mp 205—207°, $[\alpha]_D^{22}$ +12.2° (c=1.042, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{52}O_8$: C, 69.36; H, 8.90. Found: C, 69.42; H, 8.94. IR cm^{-1} ($CHCl_3$): (OAc) 1743; (E, F ring) 985, 912, 895, 848 (25L, 912>895).

Isolation of Yamogenin and Diosgenin Mixtures from the Individual Rhizomes——Ten rhizomes of *D. tenuipes* were collected at Takarazuka, Hyôgo Pref. during early October and then treated individually. Each rhizome was cut into small pieces and immersed immediately in 200 ml of MeOH. After refluxing for 5 hr the extractions with MeOH were carried out two more times. MeOH solutions were concentrated to 50 ml *in vacuo* and then refluxed with 10 ml of 35% HCl for 5 hr. The reaction mixtures were concentrated *in vacuo*, poured into water and extracted with ether. Ether extracts were chromatographed on 20 g of Al_2O_3 (Merck, standard). The diosgenin fractions detected by TLC were recrystallized from MeOH. Infrared

spectra of the crystalline material were measured and the existence of 25L- and 25D-isomers were estimated by comparison of the intensities of the bands at 920 cm^{-1} with those at 890 cm^{-1} and the existence of bands at 860 cm^{-1} and 850 cm^{-1}. The weights of the materials and the properties of the crystals are summarized in Table II. In this Table, D>L indicates that the amount of 25D-isomer is distinctly more than that of the 25L-isomer and (D) means that the 25D-isomer exists only in trace amounts in this sapogenin mixture.

TABLE II

No.	Fresh weights of material (g)	Yamogenin and diosgenin fractions		
		weight (g)	mp (°C)	C_{25}
1	30	0.069	176—180	D<L
2	41	0.086	177—181	D, L
3	14	0.019	182—186	D<L
4	35	0.113	183—186	D>L
5	35	0.046	181—183	D, L
6	27	0.046	183—184	D, L
7	54	0.116	178—181	D, L
8	24	0.087	177—179	D<L
9	35	0.086	168—174	D, L
10	39	0.047	181—183	(D) L