[Chem. Pharm. Bull. 21(8)1799—1805(1973)]

UDC 547.92.02 : 581.192

Steroidal Components of a Clone of *Dioscorea tenuipes* derived from a Plant found at Hitoyoshi[1]

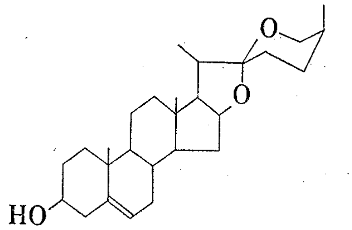
Akira Akahori, Fumio Yasuda, Kiyomi Kagawa[2a] and Toru Iwao[2b]

Shionogi Research Laboratory, Shionogi & Co., Ltd.[2a] *and Plant Science Section*
Aburahi Laboratories, Shionogi & Co., Ltd.[2b]

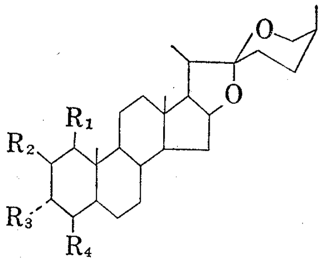
(Received February 16, 1973)

Steroidal sapogenins of the aerial parts of a clone of *D. tenuipes* complex derived from a plant collected at Hitoyoshi were found to consist almost exclusively of neotokorogenin. A small amount of neoyonogenin was also isolated from these parts, but diotigenin, tenuipegenin and 25D sapogenins were not found. Sapogenins found in the underground parts were all mixtures of 25D and 25L sapogenins, the main sapogenins being neotokorogenin and tokorogenin, yamogenin and diosgenin being found only in traces. A saponin isolated from a benzene extract of the aerial parts was confirmed to be neotokorogenin 1-O-α-L-arabinopyranoside.

Dioscorea tenuipes French. and Savat.[3] has three recognized cytotypes ($2n=20$, 30 and 40). Individuals having 20 chromosomes are distributed in eastern Japan and those having 40 chromosomes are found in western Japan. The boundary line between the distribution areas of these two forms lies in the middle of the Kinki District and plants having 30 chromosomes have been collected near this line. Plants having 20 chromosomes contain yamogenin (25L-spirost-5-en-3β-ol), diosgenin (25D-spirost-5-en-3β-ol) and tokorogenin (25D, 5β-spirostane-1β,2β,3α-triol) in their underground parts but contain no detectable amounts of sapogenins in their aerial parts.[4,5] Sapogenins are found in the aerial parts of plants having 40 chromosomes, but in varying amounts. Our investigation of the aerial parts of plants collected at Mt. Rokko, Hyogo Pref. showed that the main sapogenin contained is diotigenin (25L, 5β-spirostane-2β,3α,4β-triol), accompanied by small amounts of neoyonogenin (25L, 5β-spirostane-2β,3α-diol), neotokorogenin (25L isomer of tokorogenin) and others (Table I).[6] Although this seems to be the pattern of sapogenin composition in the aerial parts of most specimens



25L yamogenin
25D diosgenin



R1	R2	R3	R4	25	
H	OH	OH	H	L	neoyonogenin
OH	OH	OH	H	D	tokorogenin
OH	OH	OH	H	L	neotokorogenin
H	OH	OH	OH	L	diotigenin

---

[1] Studies on the steroidal components of domestic plants. LXV. Part LXIV: A. Akahori, F. Yasuda and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2409 (1971).
[2] Location: *a) Fukushima-ku, Osaka, 553, Japan*; *b) Koga-cho, Shiga, 520-34. Japan.*
[3] Y. Takeuchi, T. Iwao and A. Akahori, *Acta Phytotax. Geobot.*, **24**, 168 (1970).
[4] A. Akahori, *Phytochemistry*, **4**, 97 (1965).
[5] A. Akahori, *Acta Phytotax. Geobot.*, **21**, 149 (1965).
[6] A. Akahori, F. Yasuda and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968).

of *D. tenuipes* ($2n=40$), during our work on the relation between chromosome number and sapogenin composition we have also found instances of other sapogenin patterns in individuals which are nevertheless identified as *D. tenuipes* from a morphological standpoint.⁷⁾

The present investigation is of a clone derived from a plant found among a group of *Dioscorea asclepiadea* PRAIN et BURKILL collected by Mr. Kanjiro Maebara at Hira, near Hitoyoshi, Kyushu. Rhizomes of the plants were dug up in the winter of 1961, then planted by us at the experimental farm of Aburahi Laboratories. The components of their aerial and underground parts were investigated the next summer. One plant in the group grew a much longer vine than *D. asclepiadea* and was a deep purplish color. Although this plant bore female flowers indistinguishable from those of *D. tenuipes*, the pattern of a paperchromatogram of its steroidal sapogenins was quite different from that of the Rokko population. We have propagated this plant by rhizome cutting since 1962, and we now report an elucidation of its steroidal components.

Experimental

Material——Aerial parts of the plant were harvested in August 1970 and air-dried. Underground parts were taken up in December 1970, immediately cut into pieces, and extracted.

Isolation of Steroidal Components from the Aerial Parts——Powdered, dried aerial parts (503 g) were extracted with refluxing benzene (3×2 liters, 5 hr each), then with methanol (4×2 liters, 5 hr each). The benzene extract was stripped of solvent and the residue was warmed in 90% methanol (100 ml) containing KOH (1 g) for an hour, then extracted with ether to yield yellow crystals (746 mg) and a yellowish orange tar (4.125 g). The crystals were chromatographed on Al_2O_3. Elution with chloroform–methanol (95:5—60:40) gave a fraction (454 mg) which was washed with methanol. The undissolved portion (73 mg) was acetylated in pyridine–acetic anhydride. The acetylation product was crystallized from methanol to yield colorless needles, mp 163—165° (compound A, 77 mg). Further recrystallization raised the melting point to 169—170°. $[\alpha]_D^{21}$ $-24.6°$ ($c=0.951$, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{54}O_8$: C, 69.74; H, 9.03. Found: C, 69.83; H, 9.24. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1758, 1240 (−OAc), 1040, 907. Liebermann-Burchard reaction, red to green. Color of a spot on TLC plate, purple with anisaldehyde-$SbCl_3$. The methanol–soluble portion of the eluate (380 mg) was washed with petroleum ether. The residue (315 mg) was recrystallized from methanol to yield colorless needles, mp 262—268° (decomp.) (compound B, 227 mg). Further recrystallization from 80% methanol raised the melting point to 270—274° (decomp.). $[\alpha]_D^{24}$ $-11.5°$ ($c=0.677$, pyridine). *Anal.* Calcd. for $C_{32}H_{52}O_9 \cdot H_2O$: C, 64.19; H, 9.09. Found: C, 63.98; H, 8.83. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450—3250 (broad, OH); 988, 916>896,⁸⁾ 848 (E, F ring, 25L). Mass Spectrum *m/e*: 580 (M^+), 459, 449, 432, 414, 317, 305, 299, 287, 269, 251, 230, 139, 115. Crystals (103 mg) recovered from the recrystallization mother liquor of compound B were dissolved in acetone (50 ml) containing *p*-toluenesulfonic acid (50 mg) and refluxed for 5 hr. The acetonide mixture (115 mg) obtained was chromatographed on Al_2O_3. Elution with benzene–chloroform (9:1—5:5) gave a fraction (24 mg) which afforded colorless needles (C acetonide, 18 mg), mp 213—214° after recrystallization from methanol. $[\alpha]_D^{24}$ $-48.3°$ ($c=0.883$, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{60}O_9$: C, 69.06; H, 9.15. Found: C, 69.38; H, 9.40. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH); 1170, 1155, 1085 (shoulder), 1068, 986, 914>894, 848 (25L). Mass Spectrum *m/e*: 660 (M^+), 645, 599, 487, 459, 441, 412, 395, 345, 315, 299, 281, 269, 251, 139, 115. Elution with chloroform — chloroform–methanol (9:1) gave a fraction (68 mg) which afforded colorless platelets (36 mg), mp 160—165°, identified as a monoacetonide of compound B. The yellowish orange tar (4.125 g) was chromatographed on Al_2O_3. Elution with chloroform — chloroform–methanol (98:2) gave a fraction (292 mg) which afforded colorless needles (57 mg), mp 186>191° after recrystallization from methanol. This compound was identified as neoyonogenin. Elution with chloroform–methanol (95:5—80:20) gave a fraction (87 mg) which on recrystallization from methanol yielded colorless needles (18 mg), mp 241—243°, identified as neotokorogenin.

The methanol extract from the material was dissolved in methanol (500 ml) containing 35% HCl (60 ml) and refluxed for 5 hr. The hydrolysate (11.732 g) was obtained after extraction with ethyl acetate and chromatographed on Al_2O_3. Elution with benzene–chloroform (7:3—5:5) gave a fraction which was acetylated with acetic anhydride in pyridine. The acetylation product (322 mg) was purified by column chromatography on Al_2O_3 and crystallized from methanol to yield colorless needles (D acetate, 16 mg,) mp 197—200°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1734, 1740 (OAc); 1142 (strong), 1136 (weak), 868 (weak); 987, 917—896, 849 (25L). The mother liquor was saponified with methanol-KOH, and the saponification product was crystallized from methanol to yield colorless needles (D, 17 mg), mp 242—245°. Further recrystallization from methanol

---

7) Unpublished.

8) The intensity of the band at 916 cm^{-1} is stronger than that at 896 cm^{-1}.

raised the melting point to 248—249°. [α]$_D^{20.5}$ −37.8° (c=0.389, CHCl$_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH); 1141 (strong), 1132 (shoulder), 870 (weak); 986, 916>893, 848 (25L). Elution with methanol yielded a fraction (2201 mg) which was treated with acetone and p-toluenesulfonic acid. The reaction product was crystallized from methanol to yield colorless needles (491 mg), mp 236—238°, identified as neotokorogenin acetonide. Benzene–chloroform (5: 5) — chloroform — chloroform–methanol (100: 2) elution gave 6052 mg of a substance which was purified by repeated chromatography and recrystallization to yield two batches of colorless needles, mp 192—194° (34 mg) amd mp 242—248° (1780 mg), identified as neoyonogenin and neotokorogenin respectively by comparison of their physical properties with those of authentic samples.

Isolation of Steroidal Components from the Underground Parts——Small pieces of fresh underground parts (881 g, moisture 46.1%) were extracted with methanol (5×3 liters) for 8 hr under reflux. The methanol solution was concentrated *in vacuo*, mixed with water, then extracted with ether. The ether extract was saponified with 5% KOH in methanol. Unsaponified material was chromatographed on Al$_2$O$_3$. Elution with chloroform afforded colorless needles (2 mg), detected as a single spot on thin-layer plates. The infrared spectrum of this compound was identical with that of neoyonogenin, but a week band due to its 25D isomer was also detected. Elution with chloroform–methanol (9: 1—8: 2) gave colorless needles (4 mg), identified as neotokorogenin contaminated with a small amount of tokorogenin by infrared spectrum. Elution with chloroform–methanol (7: 3) — 1% acetic acid-chloroform–methanol (7: 3) gave a fraction which was treated with acetone and p-toluenesulfonic acid then subjected to preparative thin-layer chromatography (TLC). Colorless needles isolated (3 mg and 5 mg) were identified as acetonides of compounds C and B respectively by their infrared spectra.

The water layer was mixed with methanol (1 liter) and 35% HCl (300 ml) and refluxed for 5 hr to yield an amorphous substance (4.754 g). This was chromatographed on Al$_2$O$_3$. Elution with benzene–chloroform (9: 1) gave a fraction which afforded colorless needles (5 mg) on crystallization from methanol. This compound was identified as yamogenin by its infrared spectrum. Elution with benzene–chloroform (5: 5) and recrystallization of the fraction obtained from methanol gave colorless needles (E, 15 mg), mp 178—180°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3480 (OH); 1093 (strong), 1018, 1000; 985, 910>892, 864 (25L). Elution with chloroform gave a fraction which was purified by acetylation followed by chromarographic separation and saponification to yield colorless needles (187 mg). This compound was identified as a mixture of neoyonogenin and yonogenin by its infrared spectrum. A fraction eluted with chloroform–methanol (95: 5) —1% acetic acid-chloroform–methanol (7: 3) was recrystallized from methanol to yield colorless needles (912 mg), mp 260—261°, identified as tokorogenin. Crystals recovered from the mother liquor were treated with acetone and p-toluenesulfonic acid, then recrystallized from methanol to give two lots of colorless needles, mp 282—285° (672 mg) and mp 234—238° (34 mg). These were identified as acetonides of tokorogenin and neotokorogenin respectively. Also from the mother liquor, colorless needles (766 mg), mp 244—256° were recovered and identified as a mixture of acetonides of tokorogenin and neotokorogenin.

Acetonide of Compound B——Compound B (26 mg) was dissolved in acetone (10 ml) containing p-toluenesulfonic acid (10 mg). The solution was refluxed for 5 hr, then poured into water and extracted with ethyl acetate. The ethyl acetate solution was evaporated to dryness after being washed with 10% Na$_2$CO$_3$ and water. The reaction product (28 mg) was subjected to preparative thin-layer chromatography affording colorless needles, mp 160—164° (7 mg) as well as starting material (6 mg). [α]$_D^{21.5}$ −34.0° (c=0.638, CHCl$_3$). *Anal.* Calcd. $C_{35}H_{56}O_9 \cdot H_2O$: C, 65.80; H, 9.15. Found: C, 65.91; H, 9.20. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340—3450 (broad OH); 982, 914>892, 846 (25L).[9] Mass Spectrum m/e: 620 (M$^+$), 605, 562, 459, 432, 413, 395, 317, 297, 285, 267, 139, 115. NMR (CDCl$_3$) ppm: 0.76, 0.93, 1.13, 1.37, 1.54, 3.1—4.7.

Acid Hydrolysis of Compound B——Compound B (28 mg) was dissolved in methanol (8.5 ml) containing 35% HCl (1.5 ml). The solution was refluxed for 5 hr, then poured into water and extracted with chloroform to yield an aglycone (18 mg). This was recrystallized from methanol to yield colorless needles (8 mg), mp 234—236°, [α]$_D^{24}$ −41.5° (c=0.386, CHCl$_3$). This compound was identified as neotokorogenin. Water layer after extraction with chloroform was concentrated *in vacuo*. A syrup obtained was spotted on Toyo-Roshi No. 50, developed with n-butanol–acetic acid–water (4: 1: 5) or phenol–water (3: 1) containing formic acid (1%) and sprayed with aniline hydrogenphthalate. *Rf* values of the spots detected and their color (red) were identical with those of L-arabinose.

Compound B monoacetonide (7.66 mg) was dissolved in a small amount of methanol, 35% HCl (3 ml) was added, and the solution was warmed on a water bath for 2 hr. A precipitate which formed on cooling was collected by filtration and dissolved in chloroform (5 ml). The chloroform solution (0.03 ml) was spotted on a thin-layer plate and measured by the Okanishi and Togami method.[10] The quantity of neotokorogenin estimated was 4.5 mg in 7.16 mg of compound B. The filtrate was neutralized with 1N NaOH and the solution made up to 20 ml with water. The solution (3 ml) was analysed by the Borel, *et al.* method.[11] The quantity of L-arabinose in compound B (7.16 mg) was estimated to be 1.4 mg.

---

9) Infrared (IR) spectra of all derivatives of compound B showed four bands due to the 25L side chain of its aglycone, neotokorogenin. The data on these four bands are omitted in the following description.

10) T. Okanishi and M. Togami, *Chem. Pharm. Bull.* (Tokyo), **17**, 315 (1969).

11) E. Borel, F. Hostettler and H. Devel, *Helv. Chim. Acta*, **35**, 115 (1952).

Peracetylation of Compound B——Compound B (53 mg) was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (1 ml) and warmed on a water bath for 4 hr. The acetylation product (64 mg) was crystallized from methanol to yield a colorless powder, mp 143—146° (27 mg). $[\alpha]_D^{24.5}$ $-11.6°$ ($c=0.586$, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{62}O_{14}$: C, 63.78; H, 7.90. Found: C, 64.01; H, 7.97. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1257 (OAc). Mass Spectrum *m/e*: 790 (M^+), 772, 760, 732, 647, 515, 455, 413, 397, 281, 269, 258, 251, 199, 159, 139. NMR ($CDCl_3$) ppm: 0.200, 0.203, 0.207, 0.212 (15H, $OCOCH_3$ $\times 5$), 4.53 (anomeric H, doublet, $J=6$ cps).

Permethylation of Compound B——Compound B (112 mg) was methylated twice according to the Hakomori method[12] and subjected to preparative thin–layer chromatography. The permethylate isolated (57 mg) was a colorless powder (XI), freely soluble in methanol, acetone, dioxane, ether, *n*-hexane *etc.* but which could not be crystallized. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1093 (OMe). NMR ($CDCl_3$) ppm: 0.340—0.352 (15H, OCH_3 $\times 5$), 4.90 (anomeric H, doublet, $J=2$ cps).

Methanolysis of Compound B Permethylate——XI (36 mg) was dissolved in anhydrous methanol (5 ml) containing HCl (5%) and warmed on a water bath in a sealed tube for 5 hr. After removal of HCl through a column packed with Amberlite IR-4B, the solvent was evaporated *in vacuo*. The residue was chromatographed on silica gel. The product obtained from the benzene–chloroform eluate (18 mg) was crystallized from methanol and yielded colorless needles, mp 206—208° (III, 5 mg), $[\alpha]_D^{22}$ $-32.1°$ ($c=0.980$, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 72.96; H, 10.07. This compound was identified as neotokorogenin 2,3-dimethylether by comparison of its physical properties with those of an authentic sample. The chloroform–methanol eluate gave a colorless syrup (10 mg) which was examined by gas chromstography. Its retention time was found to be 7.8 min.[13]

Neotokorogenin 2,3-Dimethylether (II)——Neotokorogenin (I, 653 mg) was dissolved in anhydrous benzene (30 ml), added with methyl iodide (650 mg) and Ag_2O (1 g), and the mixture was stirred for 2 days in the dark at room temperature. After removal of a precipitate by filtration, the filtrate was evaporated to yield colorless crystals (650 mg). The product was chromatographed on Al_2O_3, and the fraction eluted by benzene (112 mg) was crystallized repeatedly from methanol to yield colorless platelets (II, 3 mg), mp 207—209°, $[\alpha]_D^{23.5}$ $-126.4°$ ($c=0.148$, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.54; H, 10.07. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1128, 1097, 1070, 965. The fraction eluted by benzene–chloroform (6: 4) (46 mg) was recrystallized from methanol to yield colorless needles (III, 23 mg), mp 206—208°, $[\alpha]_D^{23}$ $-31.8°$ ($c=0.987$, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 73.32; H, 10.14. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH); 1110, 1090 (OCH_3). NMR ($CDCl_3$) ppm: 3.45, 3.43 (OCH_3). The fraction eluted by chloroform was recrystallized from methanol to yield colorless platelets (IV, 127 mg), mp 236—238°, $[\alpha]_D^{26}$ $-33.7°$ ($c=1.017$, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.91; H, 10.11. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1096 (OCH_3). NMR ($CDCl_3$) ppm: 3.40 (OCH_3).

25L,5*β*-Spirostane-2*β*,3*α*-diol-1-one Dimethylether (V)——Compound III (88 mg) was dissolved in acetone (5 ml), 3 drops of Jone's reagent was added and the solution stirred for 3 minutes at room temperature. The reaction mixture was poured into water after addition of a small amount of methanol and extracted with ether to obtain the oxidation product (83 mg). This was recrystallized from methanol to yield colorless needless (V, 54 mg), mp 185—188°, $[\alpha]_D^{23.5}$ $-56.8°$ ($c=0.789$, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.57; H, 9.91. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1716 (CO), 1095 (OCH_3). NMR ($CDCl_3$) ppm: 4.00 (2C proton, doublet, $J=10$ cps); 3.49, 3.45 (OCH_3). ORD (dioxane) [M] (mμ): +4898 (281) (peak), −6527 (323) (trough). CD [θ] (mμ): −6474 (316) (shoulder), −9313 (306) (trough), −8698 (299) (shoulder).

Neotokorogenin 3-Monomethylether (IV)——Neotokorogenin acetonide (VI, 215 mg) was dissolved in N,N-dimethylformamide, Ag_2O (1 g) and methyl iodide (1 g) were added and the mixture was allowed to stand at room temperature for 20 hr, then refluxed on a water bath for 30 min. The reaction product (238 mg) was extracted with chloroform. The product was chromatographed on Al_2O_3. Elution with benzene gave a product which was recrystallized from methanol to yield colorless needles (VII, 118 mg), mp 201—202°, $[\alpha]_D^{23}$ $-26.8°$ ($c=1.038$, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.91; H, 10.07. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1094 (OCH_3), 867 (acetonide). Compound VII was dissolved in 80% acetic acid and warmed on a water bath for 5 hr. The reaction mixture was evaporated to dryness under reduced pressure. The residue was saponified in 80% methanol (30 ml) containing KOH (1 g) for 1 hr and then extracted with chloroform. The saponification product was recrystallized from methanol to yield colorless platelets (IV, 42 mg), mp 233—235°, $[\alpha]_D^{24}$ $-31.8°$ ($c=0.999$, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.89; H, 10.29.

Neotokorogenin 1,2-Dimethylether (X)——Compound VIII (440 mg, mp 224—226°), prepared from VI by acetylation with pyridine and acetic anhydride as reported in the previous paper[6] dissolved in 50%

---

[12] S. Hakomori, *J. Biochem.*, (Tokyo) **55**, 205 (1964).

[13] Shimazu Model GC-2C, FID detector. Column, 3 mm × 1.5 m glass column packed with Gaschrom Q (60—80 mesh) coated by 1,4-butane-diol succinate (5%). Carrier gas, N^2, Flow rate, 70 ml/min (1 kg/cm^2). Temperature, 130°. Retention time: methyl-tetra-O-methyl-D-glucopyranoside, 11.6 min; methyl-tri-O-methyl-L-arabinopyranoside, 7.8 min.

acetic acid. The solution was warmed on a water bath for 1 hr, then poured into water and extracted with ether. The reaction product was chromatographed on Al_2O_3. The substance eluted by benzene–chloroform (5: 5) was crystallized from methanol to yield colorless needles (IX, 185 mg), mp 259—260°, $[\alpha]_D^{23.5}$ −34.5° (c=0.974, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.72; H, 9.63. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400—3600 (OH); 1735, 1257 (OAc). A solution of IX (140 mg) in methyl iodide (3 ml) containing Ag_2O (0.5 g) was stirred at room temperature for 16 hr, then refluxed on a water bath for 30 min. Extraction with chloroform gave the reaction product (167 mg), which was chromatographed on Al_2O_3. Elution with *n*-hexane–benzene (5: 5) yielded a substance (31 mg) which was saponified in methanol containing KOH and crystallized from methanol to yield colorless needles (X, 13 mg), mp 113—115°, $[\alpha]_D^{24}$ −37.0° (c=0.838, $CHCl_3$). Mass Spectrum: 476 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1107, 1082 (OCH_3). NMR ($CDCl_3$) ppm: 0.78 (18-CH_3), 0.99 (doublet, J=2 cps, 21-CH_3), 1.08 (19-CH_3), 3.45, 3.48 (OCH_3). This product was not identical with III.

Result and Discussion

The infrared (IR) spectra of compound B, its peracetate and permethylate contain four bands characteristic of the 25L spiroketal side chain, indicating that the F ring of the aglycone is closed in these substances. Compound B afforded neotokorogenin and arabinose on acid hydrolysis, suggesting that B is neotokorogenin arabinoside. To estimate the molar ratio of aglycone to sugar, neotokorogenin and arabinose formed on hydrolysis were determined by the Okanishi and Togami[10] and Borel, *et al.*[11] methods respectively. It was found that 7.66 mg of B acetonide (7.16 mg as B) afforded 4.5 mg of neotokorogenin (10.03 μ moles) and 1.4 mg of arabinose (9.33 μ moles), a ratio not significantly different from unity. B was converted to the permethylate by the Hakomori method,[12] then subjected to methanolysis. The methylated sugar obtained was identified as methyl-2,3,4-tri-O-methyl-L-arabinopyranoside by gas-liquid chromatography. The methylated neotokorogenin was shown to be identical with one (III) of the two dimethoxy-neotokorogenins (III, X) derived from neotokorogenin, as shown in Chart 1. The rotatory dispersion and circular dichroism spectra of the oxidation product of III indicate that it is a 1-one.[14] Compound B is consequently assigned as neotokorogenin 1-O-L-arabinopyranoside. A signal assigned to an anomeric proton of the sugar moiety of B appears as a doublet (J=2 cps) at 4.90 ppm in the nuclear magnetic resonance spectrum of B permethylate and as a doublet (J=6 cps) at 4.53 ppm in that of B peracetate. This indicates that L-arabinose is α-linked to the hydroxyl group at C_1;[15,16] the difference in molecular rotations of neotokorogenin and B ($\Delta = M_D(B) - M_D$(neotokorogenin)$= -66.79 + 212.20 = +146.20°$) also supported this conclusion.[17] Compound B was thus confirmed to be neotokorogenin 1-O-α-L-arabinopyranoside. Like tokoronin of *D. tokoro*,[18] this saponin is extracted from the plant material by benzene.

Concentrations of the steroidal sapogenins isolated from this clone are summarized in Table I and compared with those of the Rokko[6] and Izu[4] populations of this species complex.[19] The total concentration of sapogenins in this clone does not greatly differ from that in the Rokko population. However, there is quite a difference in sapogenin composition between this clone and other populations. Diotigenin, the main component (81.2% of total sapogenin) in the Rokko population[6] and usually detected as the largest spot in paper or thin-layer chromatograms of free sapogenins extracted from the aerial parts of tetraploid plants,[5] and

---

[14] T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965); I. Yoshisawa, M. Tohma and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **15**, 129 (1967); M. Kimura, M. Tohma and I. Yoshizawa, *ibid.*, **16**, 1228 (1968).

[15] R.U. Lemieux, R.K. Kullnig, H.J. Berustein and W.G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958); N, Mori, S. Omura, O. Yamamoto, T. Suzuki and Y. Tsuzuki, *Bull. Chem. Soc. Jap.*, **36**, 1048 (1963).

[16] K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1369 (1969).

[17] W. Klyne, *Biochem. J.*, **47**, xli (1950).

[18] A. Akahori, *Shionogi Kenkyusho Nempo*, **10**, 1141 (1960).

[19] Weights of saponin, acetate and acetonide isolated were transformed to weights of sapogenins contained in them and summed.

tenuipegenin, isolated in small amount6) from the Rokko population, were not found in this clone. On the other hand, the clone contained much more neotokorogenin (0.487% of dry weight, 94.52% of total sapogenin) than the Rokko population (0.137% of dry weight, 16.46% of total sapogenin).6)

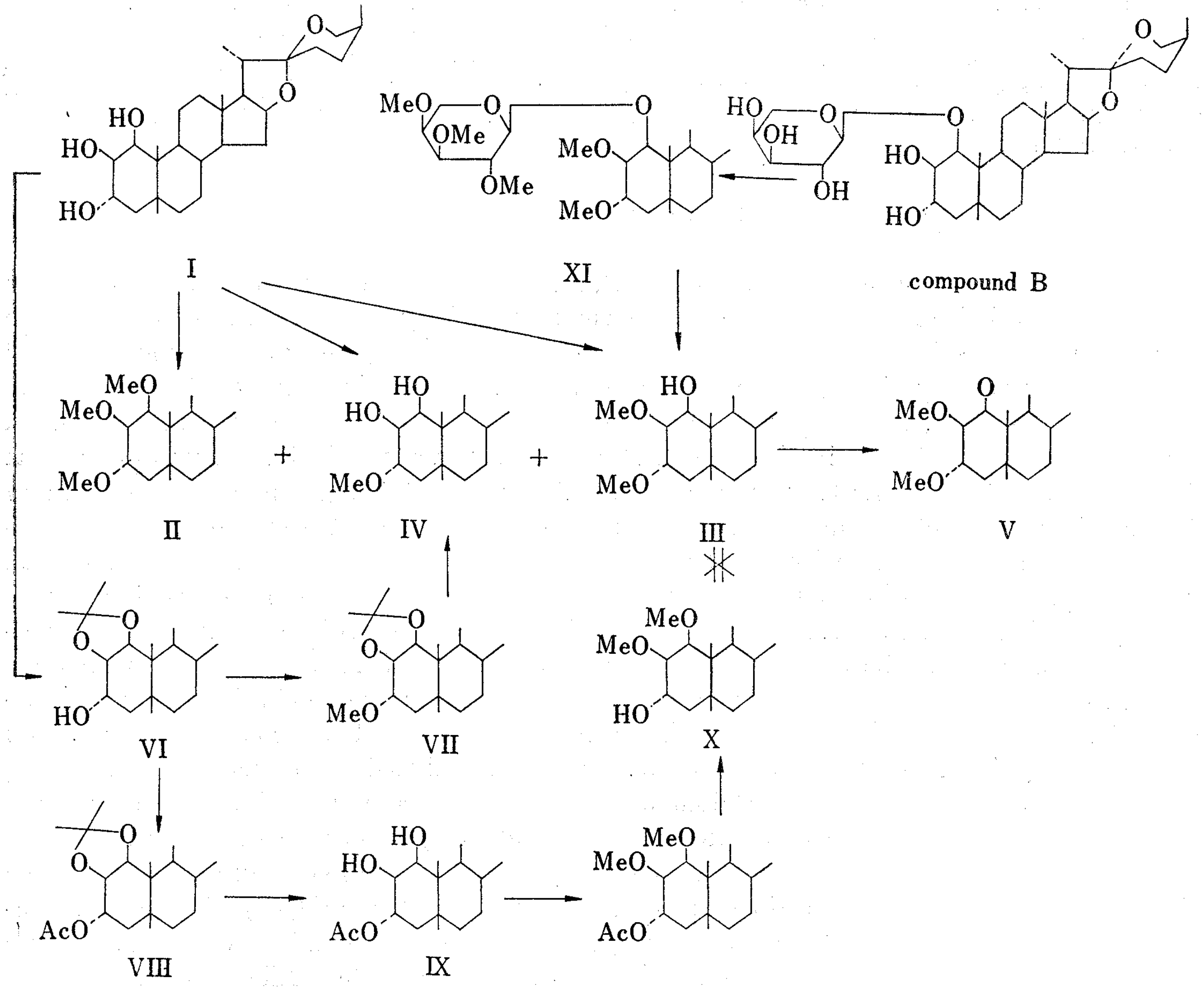


Chart 1

Table I. Concentrations of Steroidal Sapogenins contained in *D. tenuipes* Complex (% of dry weight)

	Source	Izu[a)]		Rokko[b)]		Hitoyoshi	
	2*n*	20		40		40	
Sapogenin[c)]	part	Aerial	Underground	Aerial	Uncerground	Aerial	Underground
Yamogenin		0	0.187[d)]	0.001	0.578	0	0.001
Neoyonogenin		0	0	0.005	0	0.018	0.040
Neotokorogenin		0	0.002	0.137	0.012	0.487	0.480
Diotigenin		0	0	0.676	0	0	0
Tenuipegenin		0	0	0.014	0	0	0
C		0	0	0	0	0.004	0.001
D		0	0	0	0	0.007	0
E		0	0	0	0	0	0.003

a) Calculated from data reported in the literature No. 4.
b) Calculated from data reported in the literature No. 6.
c) Some of sapogenins include their 25D isomers.
d) % of wet weight

The composition of the sapogenins contained in the underground parts of this plant is also quite different from those of other plants. Sapogenins previously isolated from the underground parts of Dioscorea plants have almost exclusively consisted of diosgenin and yamogenin, other sapogenins being either not found or found only in small amounts.[4,20,21] As seen in Table I, both the Izu ($2n=20$) and Rokko ($2n=40$) populations of this species complex contain only a small amount of neotokorogenin in addition to the large amounts of yamogenin and diosgenin; and in 10 specimens collected at Takarazuka, Hyogo Pref., these two sapogenins form between 0.12 and 0.36 per cent of wet weight.[6] In the underground parts of the present clone, however, neotokorogenin and tokorogenin form 0.48 per cent of dry weight (91.43% of total sapogenins), while yamogenin and diosgenin are only present in traces (0.20% of total sapogenins). Furthermore, neoyonogenin and yonogenin, which have never before been isolated from the underground parts of this species complex, were found in considerable amounts.

The absence of diotigenin and the presence of only minute quantities of yamogenin and diosgenin suggest that the biosynthetic pathways of these sapogenins are blocked at some stage, and it seems likely that the accumulation of neotokorogenin in the palnt is a result of such inhibition. It is also interesting that the sapogenins found in the aerial parts are all 25L sapogenins while both 25D and 25L isomers are contained together in the undeground parts. This suggests that the sapogenins are synthesized in both parts of the plant and not transferred from one part to another, or, alternatively, that conversion of the side chains occurs either in the aerial or in the underground parts.

---

20) Cf.K. Takeda, "The Steroidal Sapogenins of the Dioscoreaceae" in "Progress in Phytochemistry," Vol. 3, ed. by L. Reinhold and Y. Liwschitz, Interscience Publishers, New York, 1972.

21) A. Akahori, F. Yasuda and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **19**, 846 (1971).