Methyl (16β,17β-Diacetoxyestra-1,3,5 (10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (IV)—To a solution of IIIb (200 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate (400 mg) in anhydrous benzene (40 ml) was added freshly prepared Ag_2CO_3 (400 mg), and the suspended solution was stirred at room temperature for 24 hr. During the continuation of stirring additional amount of Ag_2CO_3 (500 mg) was added in several portions. The precipitate was filtered off and the filtrate was evaporated to dryness *in vacuo*. The crude product thus obtained was submitted to preparative thin-layer chromatography using hexane–AcOEt (2:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.43) and recrystallization of the eluate from EtOH gave IV (66 mg) as colorless needles. mp 218—220°. $[\alpha]_D^{20}$ +31.0° (c=0.19). *Anal.* Calcd. for $C_{35}H_{44}O_{14}\cdot{}^1/_2H_2O$: C, 60.25; H, 6.50. Found: C, 59.83, 59.89; H, 6.15, 6.11. NMR (5% solution in $CDCl_3$) δ: 0.93 (3H, s, 18-CH_3), 2.03 (6H, s, 16β-, 17β-$OCOCH_3$), 3.70 (3H, s, -$COOCH_3$), 4.58 (1H, d, 17α-H), 5.25 (4H, m, 16α-H, pyranose-C<u>H</u>-$OCOCH_3$).

Sodium (16β, 17β-Dihydroxyestra-1,3,5 (10)-trien-3-yl-β-D-glucopyranosid)uronate (V)——To a solution of IV (50 mg) in MeOH (6 ml) was added 1N NaOH (1.2 ml), and the resulting solution was allowed to stand at room temperature for 24 hr. The precipitated crystalline product was collected by filtration, washed with H_2O (1 ml) and dried. Recrystallization from MeOH gave V (24 mg) as colorless prisms. mp 272—276° (decomp.). $[\alpha]_D^{22}$ 0° (c=0.11, H_2O). *Anal.* Calcd. for $C_{24}H_{31}O_9Na\cdot H_2O$: C, 57.13; H, 6.59. C, 57.18; H, 6.97.

Enzymatic Hydrolysis of V with β-Glucuronidase——To an aq. solution (2 ml) of V (*ca.* 0.1 mg) were added 0.1M acetate buffer (pH 4.7, 5 ml) and beef-liver β-glucuronidase (Tokyo Zōkikagaku Co., Ltd.) (13000 Fishman U/ml, 2 ml), and the solution was incubated at 37° for 24 hr. The incubated fluid was saturated with NaCl and extracted with ether (30 ml ×2). The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. A portion of the residue thus obtained was submitted to thin-layer chromatography employing Silica gel G (E. Merck AG) as adsorbent and ether–benzene (3:1) as developing solvent. The test sample exhibited a sport at *Rf* 0.43, which proved to be identical with that of the authentic sample (16-epiestriol).

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Isolation of Isonarthogenin from *Dioscorea quinqueloba* Thunb.[1]

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Although diosgenin (25D-spirost-5-en-3β-ol) and/or yamogenin (25L-spirost-5-en-3β-ol) are known to be contained in many species of the genus Dioscorea, other steroidal sapogenins have been isolated only from a few species of this genus. Kryptogenin, which was reported by Marker, *et al.*[3] to be contained in eleven Central American species, could not be found by other investigators[4] in these plant. Except kryptogenin, four sapogenins were isolated together with diosgenin and yamogenin from two Dioscorea species of the New World; gentrogenin (25D-spirost-5-en-3β-ol-12-one) and correllogenin (25L-epimer of gentrogenin) from

1) Studies on the steroidal components of domestic plants. LXIII. Part LXII: G. Sauer, A. Shimaoka, H. Minato, and K. Takeda, *Ann. Chem.*, **742**, 156 (1970).
2) Location: *a*) *Fukushima-ku, Osaka*; *b*) *Koga-cho, Shiga.*
3) R. E. Marker, R. B. Wagner, R. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, and C. H. Ruof, *J. Am. Chem. Soc.*, **65**, 1199 (1943).
4) M. E. Wall, C. S. Fenske, H. E. Kenney, J. J. Willaman, D. S. Correll, B. G. Schubert, and H. S. Gentry, *J. Am. Pharm. Assoc.*, **46**, 653 (1957); D. K. Cox, personal communication.

D. spiculiflora Hemsl.[5] and chiapagenin (25L-spirost-5-ene-3β,12β-diol) and isochiapagenin (25D-epimer of chiapagenin) from *D. chiapasensis* Matuda.[6]

Two Japanese plants, *D. tokoro* Makino[7,8] and *D. tenuipes* complex[9,10] contain 3α-hydroxylsapogenins besides diosgenin and yamogenin. Although several spots of minute quantities of steroidal sapogenins were detected in paperchromatograms of some of the other sapogenin-bearing Japanese Dioscorea species, only diosgenin was isolated from these plants.[9] However, a markedly different sapogenin composition was observed in the seedlings of *D. tokoro*.[8] Isodiotigenin (25D,5β-spirostane-2β,3α,4β-triol), which is not found in any part of mature plant appeared in the seedling immediately after germination and increased in quantity during the first growing season. The concentration of this sapogenin decreased in the second growing season and thereafter. It was also found in young progenies produced by rhizome-cutting.[11] This observation suggested to us that the sapogenins which are contained in trace in mature Dioscorea plants may exist in considerable amounts in younger plants. *D. quinqueloba* Thund. was therefore investigated, being one of the plants from which only diosgenin had been isolated.

Result

All rhizome pieces sprouted new shoots regardless of the presence of apical buds. Although, unlike *D. tokoro*, *D. quinqueloba* did not exhibit a marked change of sapogenin composition with the age of the plant, a second sapogenin, isonarthogenin (25D-spirost-5-ene-3β,27-diol), mp 243—245°, was isolated together with diosgenin from the hydrolysate of the saponins of the young progenies produced by rhizome-cutting. This second sapogenin was first isolated from *Metanarthecium luteo-viride* Maxim. (Liliaceae)[12] and later also found in *Aletris spicata* (Thund.) Franch. (Liliaceae).[13] It is estimated to correspond to the spot with *Rf* 0.58 observed in the paperchromatogram of the previous work.[9] This sapogenin was also detected in thin-layer chromatogram of the hydrolysate of an extract of the seedlings of this plant.

Experimental

Thin-Layer Chromatography (TLC)——Plate: Silicagel G, 250 mμ; solvent: benzene–acetone–acetic acid (70:30:3); spray reagent: 1% cinnamic aldehyde in ethanol and 25 g of $SbCl_3$ in 5 ml of nitrobenzene.

Material——Rhizomes of *D.quinqueloba* were collected at Mt. Kongō, Osaka Pref. on November 10, 1969, cut into pieces (about 5 cm long) and planted in vermiculite. A 0.05% solution of Hyponex (Hydroponic Chemical Co., Inc.) was supplied every week and water every second day. The seeds of this plant were harvested at Tonomine, Nara Pref. on November 11, 1969 and stored in a desiccator.

Extraction of the Sapogenins from Clonal Progenies produced by Rhizome-cutting——Seven pieces of rhizome planted in vermiculite were placed by the window and kept at 20°. The plants were harvested on March 10, 1970 when the shoots were about 40 cm high. After air-drying, the stems (1.226 g, dry weight) were extracted with methanol. The methanol extract was hydrolysed with 2N HCl and extracted with ether to yield 39 mg of a crude sapogenin mixture. Dried rhizomes (71.028 g) were treated as above and yield-

5) H. A. Walens, S. S. Serota, and M. E. Wall, *J. Am. Chem. Soc.*, **77**, 5196 (1955).
6) I. T. Harrison, M. Velasco, and C. Djerassi, *J. Org. Chem.*, **26**, 155 (1961).
7) M. Nishikawa, K. Morita, H. Hagiwara, and M. Inoue, *Yakugaku Zasshi*, **74**, 1165 (1954); T. Okanishi and A. Shimaoka, *Shionogi Kenkyusho Nempo*, **6**, 78 (1956); K. Takeda, T. Okanishi, and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **6**, 532 (1958); A. Akahori, I. Okuno, T. Okanishi, and T. Iwao, *ibid.*, **16**, 1994 (1968).
8) A. Akahori, F. Yasuda, I. Okuno, M. Togami, T. Okanishi, and T. Iwao, *Phytochemistry*, **8**, 45 (1969).
9) A. Akahori, *Phytochemistry*, **4**, 97 (1965).
10) A. Akahori, F. Yasuda, and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968).
11) A. Akahori, F. Yasuda, M. Togami, K. Kagawa, and T. Okanishi, *Phytochemistry*, **8**, 2213 (1969).
12) H. Minato and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **11**, 876 (1963).
13) T. Okanishi and F. Yasuda, unpublished.

ed 1321 mg of a sapogenin mixture. Because the thin-layer chromatograms of the sapogenins from both parts showed a same pattern, these sapogenin mixtures were united and chromatographed on alumina. From a benzene–chloroform (8:2) eluate (627 mg), 189 mg of diosgenin, mp 196—198° was obtained as colorless needles after crystallization from methanol. A chloroform eluate (176 mg) was subjected to preparative TLC on Silica gel G plates. The sapogenin fraction (33 mg) was crystallized from methanol to yield 2 mg of colorless needles, mp 243—245°. This was identified with isonarthogenin by comparison of its IR and mass spectra and its mobility on a thin-layer plate with those of an authentic sample. The mother liquor was evaporated and acetylated with acetic anhydride in pyridine. The acetate obtained was subjected to preparative TLC and yielded 3 mg of colorless needles, mp 140—143°. This was identified with isonarthogenin diacetate by IR and mass spectra and mobility on a thin-layer plate.

Isolation of the Sapogenin Mixture from the Seedlings——Seeds were immersed in water on January 13, 1970 and kept at 3° for a month to break dormancy and then sowed in vermiculite on February 13. Three hundred seedlings were harvested on March 16, 6 days after the emergence of their first leaves above ground. They were ground in a Waring blendor, added to 2N HCl and hydrolysed for 3 hr on a water bath. The hydrolysis product was extracted with ether to yield 225 mg of an yellow liquid. A TLC of the liquid gave two spots, the *Rf* values of which corresponded with those of diosgenin and isonarthogenin.