[Chem. Pharm. Bull. 22(9)2164—2175(1974)]

UDC 547.597.02.05 : 581.192

Furostanol Bisglycosides Corresponding to Dioscin and Gracillin

Toshio Kawasaki, Tetsuya Komori, Kazumoto Miyahara, Toshihiro Nohara, Ichiro Hosokawa,[1a] and Kunihide Mihashi[1b]

Faculty of Pharmaceutical Sciences, Kyushu University[1a] and Faculty of Pharmaceutical Sciences, Fukuoka University[1b]

(Received March 18, 1974)

Two compounds, positive(red) to the Ehrlich reagent and predominant in the methanol extracts of the fresh rhizomes of *Dioscorea gracillima* Miq., were isolated. Their structures were established as 26-O-β-D-glucopyranosyl 22-methoxyfurost-5-ene-3β,26-diol 3-O-β-chacotrioside (I), mp 187—191° (decomp.), $[\alpha]_D$ −95.6°, and its 22-hydroxy analog (I′), mp 190—196° (decomp.), $[\alpha]_D$ −79.8°, the furostanol bisglycosides corresponding to 25D-spirost-5-en-3β-ol (diosgenin) 3-O-β-chacotrioside (dioscin) (III), the major component of the stored materials. I is regarded as an artifact formed from I′, and I′ and I are named proto-dioscin and methyl proto-dioscin, respectively. Smilax-saponin B, which had been assigned a structure, diosgenin hexaglycoside, was identical with I and the structure is revised.

Among the Ehrlich positive compounds in the methanol extracts of the fresh rhizomes of *D. septemloba* Thunb., the major, mp 249—251° (decomp.), $[\alpha]_D$ −76.9°, was isolated and characterized as the analog (II) of I, chacotriose being replaced by gracillimtriose, corresponding to gracillin (IV). One of the minor components was identified with the 22-hydroxy compound (II′) of II, mp 235—238° (decomp.), $[\alpha]_D$ −57.8°. II′ and II are named proto-gracillin and methyl proto-gracillin, respectively. Kikuba-saponin, which had been assigned the structure, IV monoglucoside, was identical with II and the structure is revised.

In 1966 Schreiber and Ripperger[2] reported the first isolation of a furostanol glucoside jurubine and next year Tschesche, Lüdke, and Wulff[3] isolated and characterized a furostanol bisglycoside sarsaparilloside corresponding to a spirostanol glycoside parillin. These findings were in contrast to Wall's claim[4] and supported the Marker's classical suggestion[5] that a steroid sapogenin (spirostanol) is secondarily produced from a glycoside of steroid having an aliphatic chain at C-17 or/*via* a furostanol glycoside. Since then several compounds of this type have been isolated by Tschesche, *et al*,[6] Petričič[7] and Sato, *et al*.[8] The authors[9] reported

1) Location: *a) Katakasu, Fukuoka*; *b) Nanakuma, Fukuoka.*
2) K. Schreiber and H.R. Ripperger, *Tetrahedron Letters*, **1966**, 5997; H. Ripperger, H. Budzikiewicz, and K. Schreiber, *Chem. Ber.*, **100**, 1725 (1967).
3) R. Tschesche, G. Lüdke, and G. Wulff, *Tetrahedron Letters*, **1967**, 2785; *idem*, *Chem. Ber.*, **102**, 1253 (1969).
4) *a)* M.M. Krider and M.E. Wall, *J. Am. Chem. Soc.*, **74**, 3201 (1952); *b)* E.S. Rothman, M.E. Wall, and C.R. Eddy, *ibid.*, **74**, 4013 (1952); M.M. Krider and M.E. Wall, *ibid.*, **76**, 2938 (1954); M.M. Krider, T.C. Cordon, and M.E. Wall, *ibid.*, **76**, 3515 (1954).
5) 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); R.E. Marker and J. Lopez, *ibid.*, **69**, 2389 (1947).
6) R. Tschesche, B.T. Tjoa, G. Wulff, and R.V. Noronha, *Tetrahedron Letters*, **1968**, 5141; R. Tschesche, L. Seidel, S.C. Sharma, and G. Wulff, *Chem. Ber.*, **105**, 3397 (1972); R. Tschesche, K.H. Hermann, R. Langlais, B.T. Tjoa, and G. Wulff, *ibid.*, **106**, 3010 (1973); R. Tschesche and G. Wulff, "Fortschritte der Chemie organischer Naturstoffe," vol. 30, ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, Wien, New York, 1973, p. 490, 491.
7) J. Petričič and A. Radosevič, *Farmac. Glasnik*, **25**, 91 (1969) [*C.A.*, **71**, 64049 (1969)].
8) H. Sato and S. Sakamura, *Agr. Biol. Chem.*, **37**, 225 (1973).
9) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968); K. Miyahara and T. Kawasaki, Abstracts of Papers, 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April, 1968, p. 262; T. Kawasaki, T. Komori, T. Nohara, and I. Hosokawa, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Kumamoto, May, 1968.

in 1968 that a furostanol and its glycoside[10] such as pseudodiosgenin 3,26-diacetate, 3-O-glycoside acetate and 22-methoxy-5α-furostane-3,26-diol diacetate showed a characteristic red color with *p*-dimethylaminobenzaldehyde and hydrochloric acid (Ehrlich reagent[11] (E-reag.)) and yellow with anisaldehyde[11] (A-reag.), whereas the spirostanols and their glycosides, kryptogenin, β-sitosterol and digitoxin were all negative to the E-reag. By means of thin-layer chromatography (TLC) using these reagents as the detector the fresh materials of some Dioscoreaceae and Liliaceae plants were found to contain predominantly this kind of compounds, and their isolation and properties were also reported[9,12] as preliminary accounts.

This paper concerns isolation and characterization of the Ehrlich positive 22-methoxy- and 22-hydroxyfurostanol 3,26-O-bisglycosides (I, I′, II, II′) corresponding to dioscin (III)[13] and gracillin (IV),[13] two common diosgenin (25D-spirost-5-en-3β-ol) glycosides in *Dioscorea* plants.

The methanol extracts of the fresh rhizomes of *Dioscorea gracillima* Miq.[14] cultivated in the botanical garden of this institute and collected at the end of October showed on thin-layer of silica gel at least four Ehrlich positive spots (compounds A, B, C, D) accompanied by a few negative ones (Fig. 1). Among the Ehrlich positive components compound D could not be obtained due to its poor content, but A, B and C were successfully separated in nearly homogeneous state with the aid of conventional column chromatography over silica gel using a chloroform–methanol–water mixture[15] as the eluent.

Compound A was crystallized only to give a white powder, mp 142—180° (decomp.), and further purification and detailed study on the structure were prevented by small amount available. However, it was hydrolyzed with almond emulsin to afford glucose and a prosapogenin[16] of dioscin, suggesting a furostanol bisglycoside related to the latter. The B fraction was crystallized repeatedly from methanol to give an homogeneous compound B (I) as colorless needles, mp 187—191° (decomp.),[17] $[\alpha]_D$ −95.6° (pyridine),[17] −92.6° (methanol). An attempted crystallization of the C fraction from methanol seemed to cause a considerable alteration of C into I as observed on TLC, and it was crystallized from acetone–water (1: 1) and further recrystallized from water to provide chromatographically pure C (I′) as colorless needles, mp 190—196° (decomp.),[17] $[\alpha]_D$ −79.8° (pyridine).[17]

I was hydrolyzed with acid to yield diosgenin, glucose and rhamnose, and with almond emulsin to give dioscin (diosgenin 3-O-bisrhamnosyl-glucoside (chacotrioside)) (III)[13] and glucose. Its infrared (IR) spectrum (Fig. 2) showed no spiroketal absorptions[4b,18] as seen

10) Later the furostanol derivatives corresponding to pennogenin glycosides (carrying a hydroxy group at C-17) were found negative to E-reag. (T. Nohara, dissertation).
11) E. Stahl, "Dünnschicht-Chromatographie," Springer-Verlag, Berlin, 1962, p. 498, 503.
12) The furostanol 3,26-O-bisglycosides corresponding to pennogenin di-and tetraglycosides and prosapogenin A of dioscin (T. Nohara, Y. Ogata, K. Miyahara, and T. Kawasaki, Abstracts of Papers, 14th Symposium on the Chemistry of Natural Products, Fukuoka, October, 1970, p. 374) and the furostanol 26-O-monoglucoside corresponding to free diotigenin 4-acetate (S. Kiyosawa, M. Hutoh, T. Nohara, K. Miyahara, and T. Kawasaki, Abstracts of Papers, 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974, II, p. 170), have also been isolated and characterized. A glycoside, mp 235—237° (decomp.), $[\alpha]_D$ −24.1° (c=0.54, pyridine), obtained from the rhizomes pf *Dioscorea Tokoro* Makino, is most likely to be a furostanol 26-O-glucoside corresponding to free diosgenin (T. Komori, H. Miyahara, and T. Kawasaki, unpublished data).
13) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).
14) The rhizomes (air-dried and powdered) collected in Mt. Tsukuba during December contained a minor amount of gracillin along with dioscin (T. Tsukamoto and T. Kawasaki, *Yakugaku Zasshi*, **74**, 1127 (1954); T. Tsukamoto, T. Kawasaki, A. Naraki, and T. Yamauchi, *ibid.*, **74**, 984 (1954)). However, gracillin and the corresponding furostanol glycoside could not be detected in the present materials.
15) T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), **11**, 1546 (1963).
16) T. Tsukamoto, T. Kawasaki, and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **4**, 35 (1956); T. Kawasaki and T. Yamauchi, *ibid.*, **16**, 1070 (1968).
17) The melting points and optical rotations reported[9] earlier are corrected as given.
18) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); C.R. Eddy, M.E. Wall, and M.K. Scott, *ibid.*, **25**, 266 (1953).

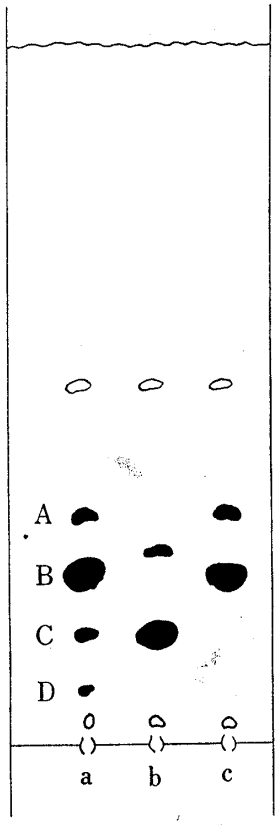

Fig. 1. Thin-layer Chromatogram of Extracts of Rhizomes of *D. gracillima*

Kieselgel G nach Stahl, solvent: $CHCl_3$–MeOH–water (65 : 35 : 10, lower phase), detector: Ehrlich reagent (●:positive (red), ○: negative)
a : MeOH extract[14]
b : dioxane extract
c : product yielded on refluxing dioxane extractives with MeOH

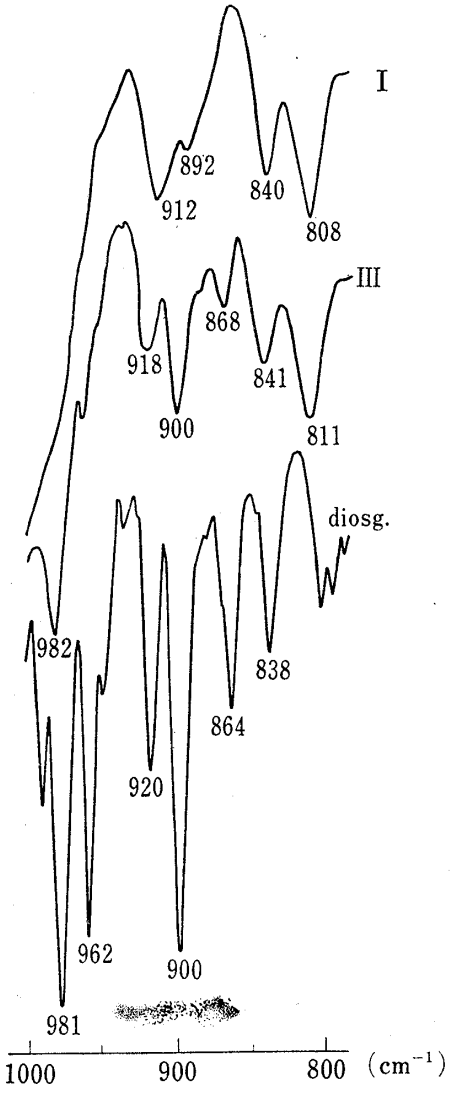

Fig. 2. IR Spectra (KBr disk) of I, III and Diosgenin (diosg.)

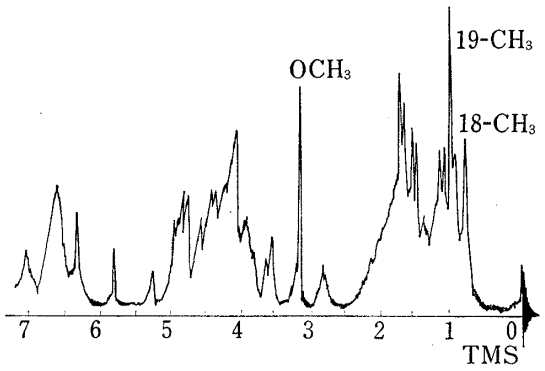

Fig. 3. NMR Spectrum of I in pyridine-d_5 (100 MHz, δ ppm) TMS

in those of diosgenin and III, and a nuclear magnetic resonance (NMR) spectrum (Fig. 3) exhibited a methoxy signal at 3.26 ppm. Acetylation of I with acetic anhydride–pyridine on heating gave an acetate (V), a white powder (from ether–pet. ether), mp 125—127°, $[\alpha]_D$ —45.1°. A NMR spectrum of V showed a methoxy signal at 3.14 ppm and a mass spectrum[19] gave the peak of highest mass number at *m/e* 1534 presumably due to the M^+—MeOH ion. A probable molecular weight of V, 1566, suggests that V contains two moles each of acetylated glucose and rhamnose residues. Based on these data, I was thought to have a glucose and a chacotriose joined respectively with 26- and 3-hydroxy groups of 22-methoxyfurost-5-ene-3β,26-diol. Confirmation of this presumption was made as follows. When V was subjected

19) T. Komori, Y. Ida, Y. Mutou, K. Miyahara, T. Nohara, and T. Kawasaki, in preparation.

to Baeyer–Villiger oxidation[20] and the product was treated with alkali, acetylated and chromatographed over silica gel, a colorless oil (VI) and a white crystalline powder (VII), mp 158—161° (decomp.), were obtained. VI showed the same NMR and mass spectra as those of methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside tetraacetate reported by Tschesche, *et al.*[3] and a compound yielded by saponification of VI was cleaved with almond emulsin to afford glucose. Another product VII from V was hydrolyzed with acid and acetylated to give colorless needles, mp 214—216°, $[\alpha]_D$ −25.4°, which was identified with 5α-pregnane-3β,5α,6β,16β,20α-pentaol tetraacetate (VIII) synthesized[20] from diosgenin.

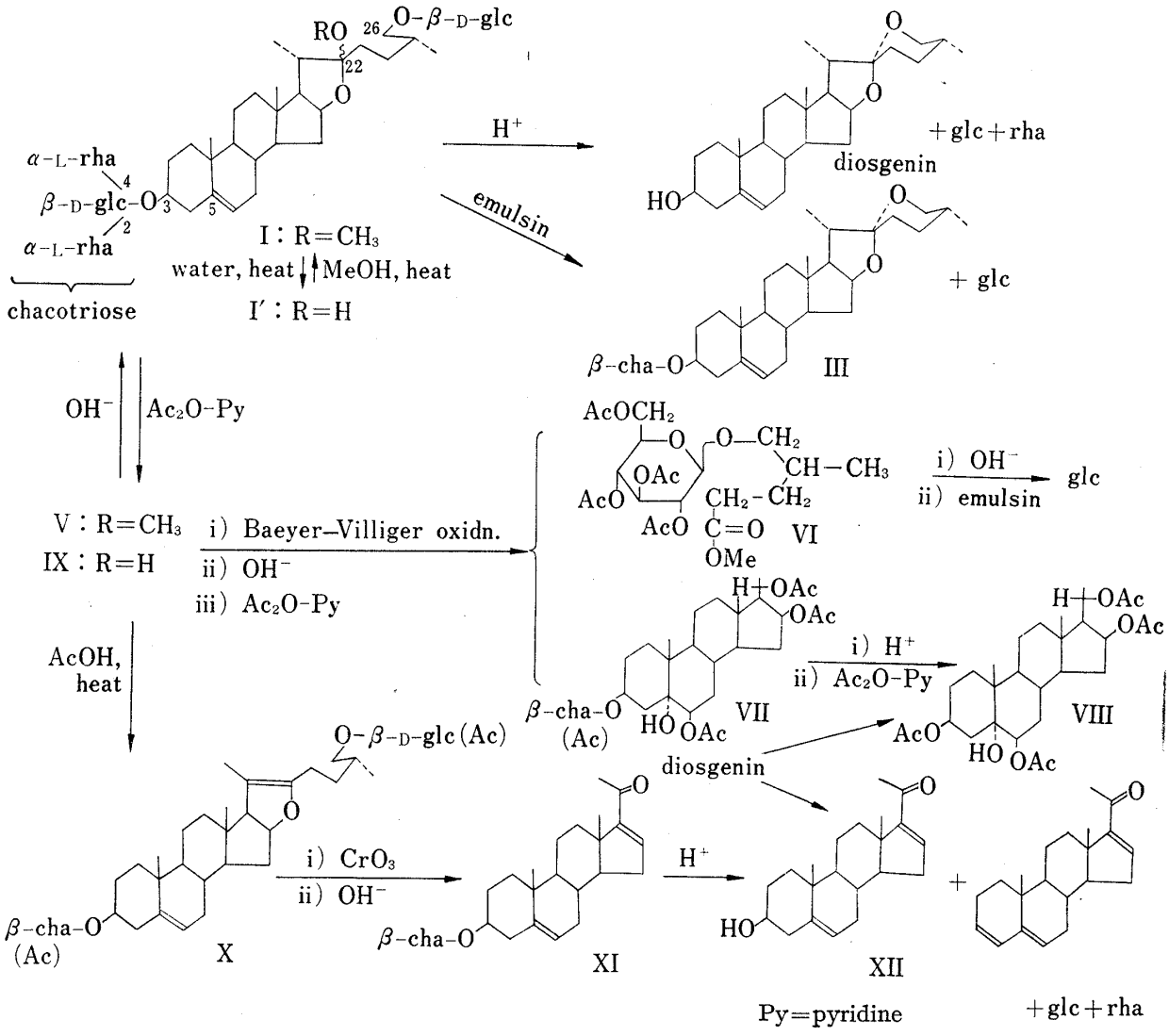

Chart 1

Consequently I is defined as 26-O-β-D-glucopyranosyl 22-methoxyfurost-5-ene-3β,26-diol 3-O-β-chacotrioside,[21] the furostanol bisglycoside corresponding to III.

20) *a*) R.E. Marker and D.L. Turner, *J. Am. Chem. Soc.*, **62**, 2540 (1940); K. Morita, S. Noguchi, H. Kono, and T. Miki, *Chem. Pharm. Bull.* (Tokyo), **11**, 90, 144 (1963); M. Uchibayashi, A. Okabori, K. Morita, and T. Miki, *ibid.*, **11**, 103, 139 (1963); *b*) T. Miki, K. Morita, S. Noguchi, T. Kishi, K. Hiraga, and H. Nawa, *ibid.*, **11**, 95 (1963).

21) According to Hirschmann and Hirschmann[22] the configurations of hydroxy and alkoxy groups at C-22 are equally regarded as α.

22) H. Hirschmann and F.B. Hirschmann, *Tetrahedron*, **3**, 243 (1958).

As suggested during the purification procedure, I′ was converted to I on refluxing with methanol and I was transformed back to I′ on boiling with aqueous acetone or water. Analogous interconversion has been reported between 22-hydroxy- and 22-methoxyfurostanol glycosides[6] as well as between 22-hydroxy- and 22-alkoxy-26-acetoxyfurostanols,[22] and hence I′ was presumed to be 22-hydroxy analog of I. Just same as I, I′ was hydrolyzed with emulsin to give III and glucose, and showed no spiroketal absorptions on an IR spectrum. I′ acetate (IX), a white powder, mp 123—124°, $[\alpha]_D$ −47.4°, prepared in the same way as V, showed no methoxy signal on a NMR spectrum. Both IX and V gave on heating with acetic acid[22] the same acetate (X),[23] mp 120—126°, 126—128°, $[\alpha]_D$ −39°, −40°, which exhibited on its NMR spectrum (Fig. 4) no methoxy but 21-methyl signal at 1.58 ppm as a singlet. According to the Marker's degradation[3] X was oxidized with chromic acid and then treated with alkali to give a glycoside (XI), mp 259—262° (decomp.), $[\alpha]_D$ −79.2°, which was hydrolyzed with acid to yield 3β-hydroxypregna-5,16-dien-20-one (XII), pregna-3,5,16-trien-20-one, glucose and rhamnose. From the above data XI was regarded as 3-O-β-chacotrioside of XII, and X as that (peracetate) of pseudodiosgenin (furosta-5,20(22)-diene-3β,26-diol).

Therefore I′ is the 22-hydroxy analog of I.

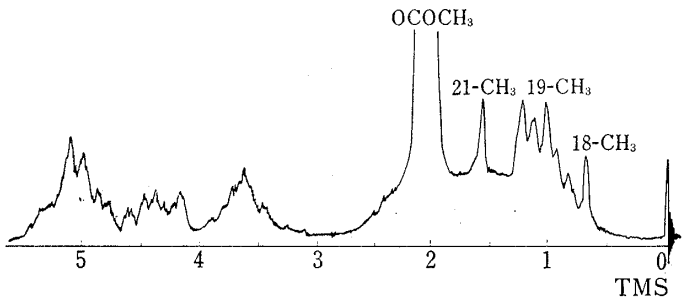

Fig. 4. NMR Spectrum of X in $CDCl_3$ (60 MHz, δ ppm)

In consideration of the extraction and isolation procedures, I is most likely to be a secondary product formed from I′ with methanol and actually the dioxane extracts of the plant revealed on TLC predominantly the spot of I′ and none of I (Fig. 1). The fact that the Ehrlich positive compounds were predominant in the extracts of fresh materials, while III was major in the air-dried and powdered ones[14] suggests I′ to be a parent glycoside from which III is yielded enzymatically in plants during the treatment and storage. The names proto-dioscin and methyl proto-dioscin are then proposed for I′ and I, respectively.

Smilax-saponin B[24] which was obtained from the rhizomes of *Smilax chinae* L. and assigned a structure having additional two glucose and one rhamnose residues combined with the chacotriose moiety of III, shows quite similar melting point, 183—186° (decomp.), and optical rotation, $[\alpha]_D$ −90° (metha-

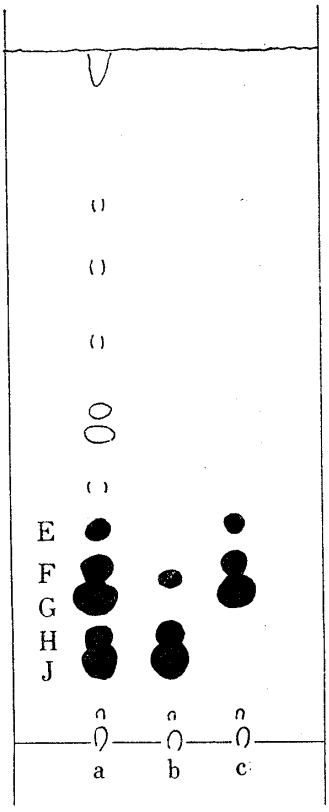

Fig. 5. Thin-layer Chromatogram of Extracts of Rhizomes of *D. septemloba*

Kieselgel G nach Stahl, solvent: $CHCl_3$–MeOH–water (65: 35: 10, lower phase), detector: Ehrlich reagent (●: positive (red), ○: negative)
a: MeOH extract
b: dioxane extract[39]
c: product yielded on refluxing dioxane extractives with MeOH

23) X and its free glycoside could not be distinguished on TLC from V and I, respectively.
24) T. Kawasaki, I. Nishioka, T. Tsukamoto, and K. Mihashi, *Yakugaku Zasshi*, **86**, 673 (1966).

nol), to those of I. It was now found to give a spot on TLC identical with that of I in *Rf* value and coloration with E-reag. Its IR and NMR spectra were also same as those of I, showing no spiroketal absorptions and exhibiting a methoxy signal, respectively. The structure of smilax-saponin B, diosgenin hexaglycoside, had been based, for one thing, on the *Rf* value[15] on TLC run in parallel with F-gitonin[25,26] and desgalactotigonin, [25,27] but I, furostanol tetraglycoside, showed much smaller *Rf* value than those of spirostanol tetraglycosides such as aspidistrin[28] and the above two glycosides. Quantitative determinations of total sugar and aglycone in an acid hydrolysate of smilax saponin B were then repeated, and the yields were found to be in good accordance respectively with those calculated for I rather than for the previous formula.[24,29]

Accordingly smilax-saponin B is considered to be nothing but methyl proto-dioscin and the structure is revised as I.[30]

Similarly to the case of *D. gracillima*, the fresh rhizomes of *Dioscorea septemloba* Thunb. were found on TLC (Fig. 5) to contain at least five polar substances (E, F, G, H, J) positive (red) to the E-reag. along with small amounts of less polar and negative ones. The former substances were thought to be furostanol bisglycosides related to the latters which could be the spirostanol monoglycosides, *e.g.* IV, III and their prosapogenins found[31] mainly in the sliced and air-dried materials. The methanol extracts were treated as described in the experimental part and three compounds, E, F and G, were isolated in chromatographically pure state. Compound E was hydrolyzed with emulsin to yield glucose and prosapogenin B[16] of dioscin, mp 220—222° (decomp.), suggesting its furostanol bisglycoside structure corresponding to the spirostanol monoglycoside. Compound F was crystallized from methanol to provide colorless needles, mp 188—192° (decomp.), and identified with I. Compound G, the major component, was crystallized from methanol to give colorless needles, mp 228—230° (decomp.), $[\alpha]_D$ −66.7°. In another run the method[32] (partitioning between butanol-ethyl acetate and water) previously employed for the separation of hederagenin 3-O-monoglycosides from 3,28-O-bisglycosides was applied, and a mixture of the Ehrlich positive compounds was easily freed from the negative ones. The mixture was subsequently subjected to direct crystallization from methanol to give G as colorless needles, mp 229—231° (decomp.). It was hydrolyzed with emulsin to give glucose and gracillin (diosgenin 3-O-gracillimatrioside (glucosyl-(rhamnosyl)-glucoside)) (IV),[13] but showed no spiroketal absorptions on an IR spectrum and a methoxy signal was observed on a NMR spectrum. Therefore G was regarded as a 22-methoxyfurostanol bisglycoside corresponding to IV. G was acetylated as usual and the product was crystallized from ethanol to give an acetate (XIII) as colorless needles, mp 160—162°, $[\alpha]_D$ −50.5°. XIII had no methoxy signal on a NMR spectrum and was once suspected to be pseudodiosgenin glycoside acetate (XIV). However, its melting point and optical rotation were different from those of XIV prepared later, and on a NMR spectrum the singlet ascribable to 21-methyl which is observed in XIV and X could not be distinguished from other

25) T. Kawasaki and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **12**, 1311 (1964).
26) T. Kawasaki, I. Nishioka, T. Komori, T. Yamauchi, and K. Miyahara, *Tetrahedron*, **21**, 299 (1965).
27) K. Miyahara, Y. Ida, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2506 (1972).
28) Y. Môri and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 224 (1973).
29) Microanalytical data (C, 56.90; H, 8.19 (reported[24]), C, 56.61; H, 8.40 (redetermined)) agree with those (C, 56.82; H, 8.25) calculated for I ($C_{52}H_{86}O_{22}\cdot 2H_2O$) rather than those (C, 56.49; H, 7.68) for the previous formula ($C_{63}H_{102}O_{30}$).
30) A saponin, mp 202—203° (decomp.), $[\alpha]_D$ −69.88°, acetate, mp 121.5—122.5°, $[\alpha]_D$ −42.54°, was isolated from *Dioscorea polystachya*, named dioscinin, and identified as a glucoside-bisrhamnoside-glucoside of 25D-22α-spirost-5-en-3β-ol by Madaeva, *et al.* (O.S. Madaeva, V.K. Ryzhkova, and V.V. Panina, *Khim. Prir. Soedin*, 3, 155 (1967) [*C.A.*, **67**, 87785 (1967)]). It could also be I.
31) T. Kawasaki, T. Yamauchi, and R. Yamauchi, *Chem, Pharm. Bull.* (Tokyo), **10**, 698 (1962); T. Tsukamoto, T. Kawasaki, and Y. Shimauchi, *Yakugaku Zasshi*, **77**, 1221 (1957).
32) R. Higuchi, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1935 (1972).

methyl signals. Accordingly XIII was assumed to be the 22-ethoxy analog formed[22] during crystallization with ethanol, and this assumption was supported by the facts that XIII was boiled with acetone–water (3: 1) to yield a white powder (XV), mp 135—139°, $[\alpha]_D$ −51.3°, being more polar than XIII and regarded as 22-hydroxy analog of XIII, and that on boiling with methanol XIII was converted to 22-methoxy analog (XVI), colorless needles, mp 147—149°, $[\alpha]_D$ −54.2°, showing methoxy signal on a NMR spectrum. XIII gave on a mass spectrum[19] the peak ascribable to M^+—EtOH ion at *m/e* 1592, suggesting two moles each of acetylated glucose and rhamnose units in the molecule. The acetate was saponified and the free glycoside was repeatedly recrystallized from methanol to give compound G′, (II) as colorless needles, mp 249—251° (decomp.), $[\alpha]_D$ −76.9°. II was identical (IR and NMR spectra, *Rf* value, enzymatic hydrolysis to IV and glucose) with G. Taking into account of the higher melting point of II than that of G, II was regarded as pure G[33] having gracillimatriose and glucose joined respectively with 3- and 26-hydroxy groups of 22-methoxyfurost-5-ene-3β,26-diol. The structure was unequivocally proved in a similar way to the Tschesche method.[3] Thus, XIII was heated with acetic acid and the product, pseudodiosgenin glycoside acetate (XIV), mp 116—119°, $[\alpha]_D$ −59.3°, was subjected to oxidation with chromic acid followed

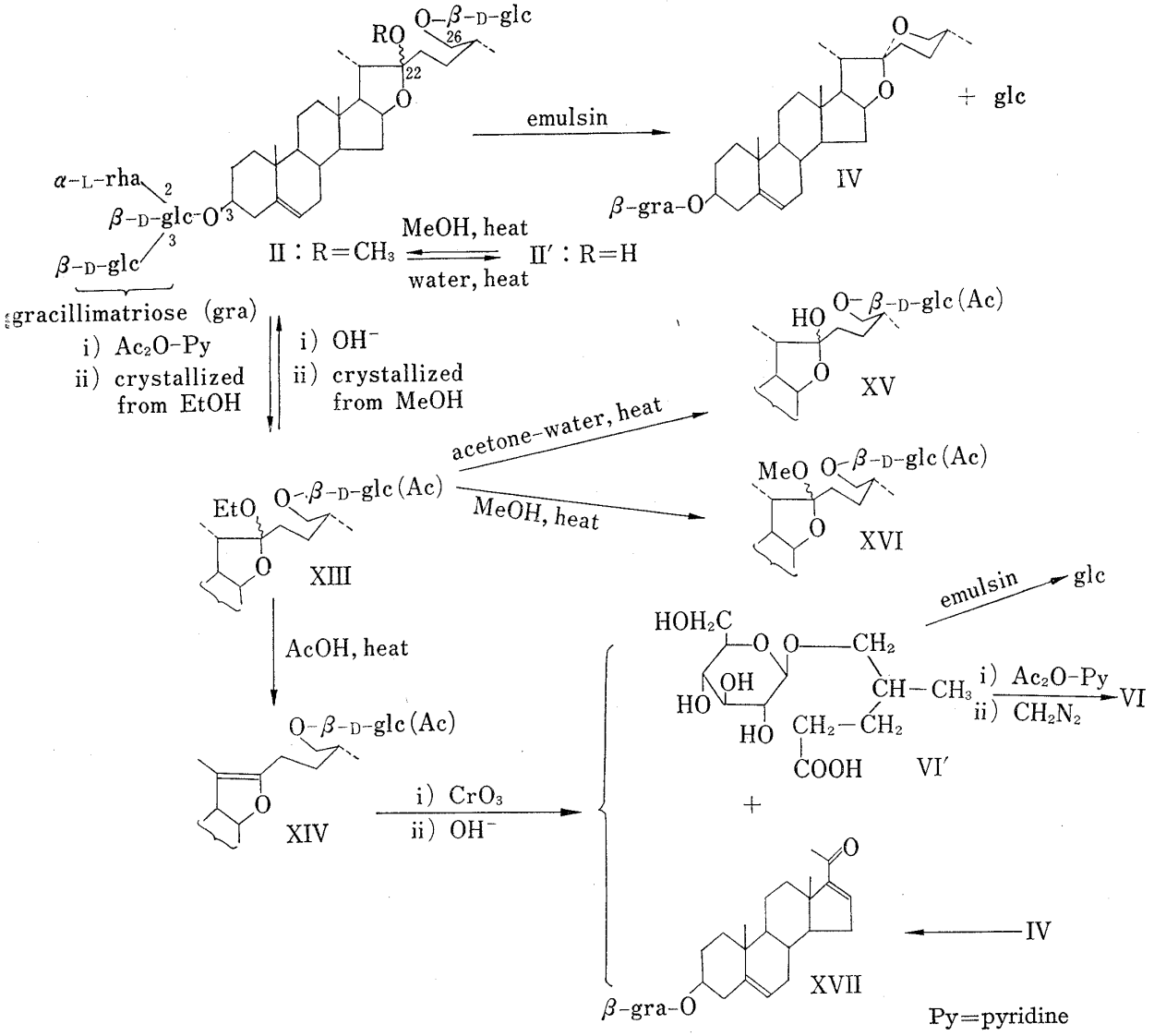

Chart 2

33) G is possibly contaminated with a small amount of I.

by treatment with alkali to give two compounds. One was identified with the 3β-hydroxy-pregna-5,16-dien-20-one glycoside (XVII)[27] derived from IV and the other was acetylated and methylated to provide VI.

Consequently II is defined as 26-O-β-D-glucopyranosyl 22-methoxyfurost-5-ene-3β,26-diol 3-O-β-gracillimatrioside,[21] the furostanol bisglycoside corresponding to IV.

When II was boiled with water a compound (II'), mp 235—238° (decomp.), $[\alpha]_D$ −57.8°, was provided. II' showed neither methoxy signal on a NMR nor spiroketal absorptions on an IR spectra. Its *Rf* value and color with E-reag. were identical with those of a minor compound J[34] in the original extracts and was converted back to II on refluxing with methanol.

Accordingly, in analogy to I', II' is considered as the 22-hydroxy compound of II.

Since the dioxane extracts of the fresh materials contained predominantly II' and no II (Fig. 5), II' is regarded as the originally existing glycoside and designated proto-gracillin, and hence II is to be named methyl proto-gracillin.

Some time ago our group isolated[31] kikuba-saponin (Ks) from the rhizomes of *D. septemloba* and assigned[13] it the structure, β-D-glucopyranoside of IV. However, some of its properties, for instance, an unusually low *Rf* value,[15,31] neither capacity of cholesteride formation[31] nor antifungal activities,[35] were unbecoming[3] to the structure as proposed, and it had remained to be reinvestigated. Now Ks was found to be positive (red) to the E-reag., and the melting points and the optical rotations of II and XIII were in good accordance respectively with those of Ks (mp 249—251° (decomp.), $[\alpha]_D$ −75°) and its acetate (mp 155—158°, $[\alpha]_D$ −51°) reported previously.[31] The identity of II with Ks was evidenced by direct comparison.

Therefore the structure of Ks is revised as II.[36]

Experimental

General Method——Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured with JASCO DIP-SL automatic polarimeter at 15—25°. IR spectra were obtained with Koken DS-201 and JASCO IR-G spectrometers. UV spectra were taken on a Shimazu SV-50-A spectrometer. NMR spectra were recorded on a JEOL C-60H (60 MHz) spectrometer unless otherwise stated and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). Mass spectra were taken on a JEOL JMS-01SG mass spectrometer.[19] TLC was performed on Kieselgel G nach Stahl (Merck) using $CHCl_3$–MeOH–water (65 : 35 : 10, lower phase)[15] as solvent unless otherwise specified and Ehrlich reagent (E-reag.),[11] anisaldehyde reagent (A-reag.)[11] and 10% H_2SO_4 as detectors. Column chromatography was carried out, unless otherwise indicated, with Kieselgel (0.05—0.2 mm) (Merck) in thirty to fifty times quantity of the material. Paper partition chromatography (PPC) for sugar was conducted on Toyo Roshi No. 50 using BuOH–AcOH–water (4 : 1 : 5) and BuOH–pyridine-water (6 : 2 : 3) (upper phase)+pyridine (1) (double ascending) as solvent and aniline hydrogen phthalate as staining agent. The ratios of solvents and reagents in mixture are given in v/v. Enzymatic hydrolysis was carried out by using commercial almond emulsin (Sigma Chem. Co.).

Extraction and Separation of Component Glycosides of Rhizomes of *D. gracillima*——The fresh rhizomes (3.15 kg) cultivated in the botanical garden of this institute and collected at the end of October were sliced and extracted at room temperature with MeOH (12 liters) for 8 days. The MeOH extracts were evaporated *in vacuo* to brown resinous mass (170 g). It showed on TLC at least four Ehrlich positive (red) spots (compounds, A, B, C, D) and a few negative to E-reag. but yellow with A-reag. (Fig. 1). A part (10 g) of the residue was placed on a silica gel (100—200 mesh, Kanto, 420 g) column and eluted with $CHCl_3$–MeOH–water 65 : 35 : 10 (lower phase): Fr. 1, III (major)+less polar substances (E-reag. −), 700 mg; Fr. 2, A (major)+B, 100 mg; Fr. 3, B (major)+C, 2.2 g; Fr. 4, C (major)+B, 1.8 g; Fr. 5, much polar substances (E-reag. −), small amount.

Compound A——Attempted crystallization of Fr. 2 from MeOH resulted only to give a white powder, mp 142—180° (decomp.). The powder (10 mg) in water (5 ml) was incubated with emulsin (*ca.* 2 mg) at

34) Compound H is presumed to be I' on the basis of its *Rf* value.

35) S. Imai, S. Fujioka, E. Murata, M. Goto, T. Kawasaki, and T. Yamauchi, *Ann. Report of Takeda Res. Lab.*, **26**, 76 (1967).

36) The previous[13] detection of a tri-O-methyl-glucose in hydrolysate of Ks permethylate might have been due to incomplete methylation.

37° for 1 day to give glucose (PPC) and a glycoside of which *Rf* value was identical with that of prosapogenin A of dioscin[16] run in parallel.

Compound B (Methyl Proto-dioscin) (I)——Fr. 3 was boiled with MeOH (100 ml) for 3 hr. The solvent being evaporated, the residue was recrystallized three times from MeOH to give homogeneous I as colorless needles, mp 187—191° (decomp.),[17] $[\alpha]_D$ −95.6°[17] (c=1.16, pyridine), −92.6° (c=1.44, MeOH). No spiroketal absorptions on an IR spectrum (Fig. 2). NMR (pyridine-d_5) (100 MHz, JEOL PS-100): 0.81 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 3.26 (3H, s, -OCH_3) (Fig. 3). Hydrolysis with 2N-HCl for 3 hr gave glucose, rhamnose (PPC) and diosgenin (mp 201°, alone and on admixture with an authentic sample). I (15 mg) in water (5 ml) was incubated with emulsin (*ca.* 2 mg) at 35° for 1 day. The precipitates were collected by filtration and crystallized from EtOH to give III as colorless needles, mp 272—275° (decomp.), $[\alpha]_D$ −105° (c=0.82, EtOH). Identified with authentic III, mp 275—277° (decomp.), $[\alpha]_D$ −110° (c=1.02, EtOH), by mixed melting point and by comparison of their IR spectra and of *Rf* values run in parallel on TLC in three different solvent systems.[15] The filtrate of the hydrolysate was examined by PPC and only glucose was detected.

Compound C (Proto-dioscin) (I′)——Fr. 4 was boiled with acetone–water (1: 1) (10 ml) for 10 hr, the solution concentrated, and a solid deposited was recrystallized twice from water to give I′ as colorless needles, mp 190—196° (decomp.),[17] $[\alpha]_D$ −79.8° (c=0.99, pyridine).[17] Hydrolyzed with emulsin as I to give III and glucose. IR: no spiroketal absorptions. NMR: no methoxy signal. Refluxing I′ (15 mg) with MeOH (5 ml) for 6 hr gave I (TLC), and I was converted to I′ (TLC) on boiling with acetone-water or water for 10 hr. The fresh rhizomes (300 g) were sliced and extracted with dioxane (1 liter) at room temperature for 2 days. The extracts were evaporated *in vacuo* and examined by TLC. (Fig. 1).

Acetate (V) of I——I (200 mg) was heated on a water-bath with acetic anhydride–pyridine (1: 1) (20 ml) for 1 hr. The residue obtained by evaporation *in vacuo* was again acetylated as above. The product was dissolved in MeOH, treated with active charcoal, filtered and the filtrate evaporated to dryness. Crystallization from ether–pet. ether gave V as a white powder, mp 125—127°, $[\alpha]_D$ −45.1° (c=0.89, $CHCl_3$). *Anal.* Calcd. for $C_{76}H_{110}O_{34}\cdot H_2O$: C, 57.56; H, 7.12. Found: C, 57.47; H, 7.28. NMR ($CDCl_3$) (100 MHz, Varian HA-100: 3.14 (3H, s, -OCH_3). Mass Spectrum[19] *m/e*: 1534 (M^+−MeOH).

Baeyer-Villiger Oxidation of V——To V (500 mg) suspended in $(CH_2Cl)_2$ (12 ml) were added 90% formic acid (15 ml) and 30% H_2O_2 (1.6 ml). The mixture was heated at 50° for 30 min and evaporated *in vacuo*. To the residue was added 3% KOH in MeOH (30 ml) and the solution was left stand at 50° for 20 min. The mixture was neutralized with dil. HCl and evaporated *in vacuo*. The residue was acetylated twice with acetic anhydride–pyridine (2: 1) (30 ml) on a water-bath for 1 hr. Solvent being removed *in cavuo*, the product was chromatographed over silica gel using hexane–AcOEt (2: 1) as eluent.

Methyl γ-Methyl-δ-hydroxypentanoate β-D-Glucopyranoside Tetraacetate (VI)——The first fraction in the above chromatography gave VI as a colorless oil (35 mg). NMR ($CDCl_3$): 0.93 (3H, d, J=6 Hz, >CH-CH_3), 2.00—2.13 (12H, 4×$OCOCH_3$), 3.70 (3H, s, -$COOCH_3$). Mass Spectrum *m/e*: 417 (M^+−CH_3COO), 331, 243, 242, 200, 169, 157, 145, 141, 140, 129.092 ($C_7H_{13}O_2^+$), 115, 109, 103, 98, 97.065 ($C_6H_9O^+$).[3] The above spectra are identical with those reported by Tschesche, *et al.*[3] VI (10 mg) was saponified with 3% KOH in MeOH (10 ml) on a water-bath for 20 min, the mixture was neutralized with 5% HCl in MeOH and evaporated. The residue was hydrolyzed with emulsin to give glucose (PPC).

5α-Pregnane-3β,5α,6β,16β,20α-pentaol Tetraacetate (VIII)——The second fraction in the chromatography of the product from V was crystallized from ether–pet.ether to provide a white crystalline powder (VII) (300 mg), mp 158—161° (decomp.). *Anal.* Calcd. for $C_{61}H_{88}O_{29}\cdot H_2O$: C, 56.21; H, 6.96. Found: C, 55.98; H 6.90. VII (200 mg) was boiled with 5% HCl in MeOH (20 ml) for 5 hr, the hydrolysate was neutralized with 5% KOH in MeOH and evaporated to dryness. The residue was acetylated on heating with acetic anhydride–pyridine (2: 1) (30 ml) for 1 hr and the mixture was poured into ice-water. The precipitates were crystallized from ether–pet.ether to give VIII as colorless needles (55 mg), mp 214—216°, $[\alpha]_D$ −25.4°. *Anal.* Calcd. for $C_{29}H_{44}O_9$: C, 64.90; H, 8.26. Found: C, 65.23; H, 8.44. It was identified by direct comparison (mixed mp, IR, NMR) with an authentic sample of VIII, mp 214—216°, synthesized from diosgenin according to the reported method.[20b]

Acetate (IX) of I′——I′ (200 mg) was acetylated in the same way as I. Crude acetate was dissolved in acetone, treated with charcoal, filtered, evaporated and the residue was crystallized from ether–pet.ether to give IX as a white powder, mp 123—124°, $[\alpha]_D$ −47.4° (c=0.73, $CHCl_3$). No methoxy signal on a NMR spectrum.

26-O-β-D-Glucopyranosyl Pseudodiosgenin 3-O-β-Chacotrioside Acetate (X)——IX (200 mg) in AcOH (15 ml) was refluxed for 1 hr and then evaporated *in vacuo* to dryness. The residue was crystallized from ether–pet.ether to give X (180 mg) as a white powder, mp 120—126°, $[\alpha]_D$ −39.0° (c=1.06, $CHCl_3$). NMR ($CDCl_3$): 1.58 (3H, s, =C(I)–CH_3), 0.67 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), no methoxy signal (Fig. 4). V (550 mg) was boiled with AcOH (20 ml) for 2 hr and the mixture was poured into ice-water. The precipitates were collected, washed with water, dried and crystallized from ether-pet.ether to give a white powder, mp 126—128°, $[\alpha]_D$ −40° (c=0.73, $CHCl_3$), which was identified with X obtained above by direct comparison (mixed mp, IR, NMR).

3-O-β-Chacotrioside (XI) of 3β-Hydroxy-pregna-5,16-dien-20-one (XII)——To the solution of X (1.4 g) and AcONa (290 mg) in AcOH (15 ml) was added chromic trioxide (600 mg) in 80% AcOH (2.2 ml) in 15 min. The reaction mixture was stirred for 1 hr, diluted with water and shaken with ether. Ether extract was washed with $NaHCO_3$ solution, water, dried over Na_2SO_4, and evaporated. The product was boiled with 3% KOH in MeOH (20 ml) for 10 min and the solution was neutralized with dil. HCl and evaporated. The residue was shaken with water and BuOH, and the BuOH layer was evaporated to a resinous mass. It was chromatographed over silica gel using $CHCl_3$–MeOH–water 7: 2: 0.1 as eluent. Fractions containing only the compound of *Rf* 0.48 (TLC, $CHCl_3$–MeOH–water 7: 3: 0.5) were combined, evaporated, and a resulting solid was crystallized from dil. MeOH to provide XI (230 mg) as colo rless needles, mp 259—262°(decomp.), $[\alpha]_D$ −79.2° (c=0.71, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1645, 1587 (α,β-unsaturated ketone). UV λ_{max}^{EtOH}: 240 nm. NMR (CD_3OD): 0.93 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3), 1.28 (6H, d, J=6 Hz, 6-CH_3 of rhamnose×2), 2.27 (3H, s, CH_3CO–), 4.57 (1H, d, J=6 Hz, C_1–H of glucose), 5.28 (2H, broad s, C_1–H of rhamnose×2), 6.99 (1H, m, olefinic H). In the same manner as reported previously,[37] it was hydrolyzed with 1N H_2SO_4 in 50% acetone and the products were identified as glucose, rhamnose, XII and pregna-3,5,16-trien-20-one.

Smilax-saponin B (=I)——An authentic sample, mp 183—186° (decom.),[24] $[\alpha]_D$ −90° (c=0.5, MeOH),[24] gave a spot (TLC, *Rf* 0.26, red with E-reag.) identical with that of I and of much lower *Rf* value than those of F-gitonin[25,26] (*Rf* 0.39), desgalactotigonin[25,27] (*Rf* 0.43) and aspidistrin[28] (*Rf* 0.43). Its IR and NMR spectra were also same as those of I.

Quantitative determination of aglycone and total sugar in acid hydrolysate: The sample (100.0 mg) in 2N HCl (20 ml) was refluxed for 5 hr. The precipitates formed on cooling were collected by filtration and boiled with 2N HCl in dioxane-water (1: 3) (10 ml) for 2 hr. The reaction mixture was diluted with water and the aglycone deposited was collected, washed with water and dried *in vacuo*. Yield, 37%, The filtrates of both hydrolysates were combined, neutralized with 2N NaOH and diluted with water to 1 liter. The amount of reducing sugar in an aliquot (2 ml) was determined by the anthrone–H_2SO_4 method.[38] Yield, 62% (Calcd. as glucose). Calcd. for $C_{52}H_{86}O_{22}\cdot 2H_2O$ (containing two moles each of glucose and rhamnose): aglycone, 37.7%; glucose, 62.6%. (Calcd. for the diosgenin hexaglycoside: glucose, 78%).[24]

Extraction and Separation of Component Glycosides of Rhizomes of *D. septemloha*——Method 1: The fresh rhizomes (1.6 kg) collected in the suburb of Fukuoka city at the end of October[39] were immediately sliced and extracted twice with MeOH (3 liters) at room temperature for 2 days. The MeOH extracts were combined and concentrated *in vacuo*. To the resulting aqueous solution BuOH was added and the mixture was further concentrated to 150 ml. It was extracted with BuOH saturated with water (aq. BuOH) (150 ml) three times. BuOH layers were combined, washed with water and evaporated to dryness. The residue (8.98 g) showed on TLC at least five Ehrlich positive spots (compounds E, F, G, H, J) and several ones negative to E-reag. (Fig. 5). It was dissolved in MeOH (20 ml), acetone (250 ml) added, and the precipitates (3.25 g) were collected by filtration. They were dissolved in MeOH (50 ml), the solution was refluxed for 4 hr and concentrated to 15 ml. A few drops of water were added and the mixture was left stand to provide compound G as crystals (1.43 g). The mother liquor of crystallization was combined with the above filtrate (MeOH+acetone), evaporated to dryness and the residue was chromatographed over silica gel (100—200 mesh, Kanto, 300 g) using $CHCl_3$–MeOH–water (70: 30: 6) as eluent: Fr. 1, III+IV+less polar substances (E-reag. −), 1.55 g; Fr. 2, compound E, 0.44 g; Fr. 3, compound F, 0.98 g; Fr. 4, more polar substances (E-reag. +), 4.4 g.

Method 2: The fresh rhizomes (17 kg) collected in the same place as in method 1 at the begining of September[39] were sliced and extracted twice with MeOH (18 liters) at room temperature for 3 days. The extracts were combined, concentrated to 2 liters and extracted with aq.BuOH four times (total aq.BuOH, 2 liters). BuOH layers were combined, washed with water and evaporated *in vacuo*. The residue (84 g) was dissolved in water (2 liters) and shaken with AcOEt–BuOH (5: 1) (500 ml). The organic layer being removed, the water layer was again shaken with the same solvent mixture. The procedure was repeated three times. The organic layers were combined and washed with water (50 ml) twice. The washings and the water layer were combined, further extracted with aq.BuOH four times, and evaporated to a solid (24.5 g) consisting of only Ehrlich positive compounds. All the organic layers were evaporated to give a residue (8.99 g) composed of Ehrlich negative substances along with a small amount of positive ones. A mixture of Ehrlich positive compounds (24.5 g) in MeOH (500 ml) was refluxed for 4 hr, concentrated to 300 ml, water (1 ml) added and the solution was left stand over night to give compound G as crystals (11.2 g).

Compound E——Fr. 2 in method 1 was crystallized from MeOH to give E as a white powder. E (100 mg) in water (15 ml) was incubated with emulsin (15 mg) at 37° for 1 day. The precipitates were collected by

37) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1240 (1973).
38) T.A. Scott, Jr. and E.H. Helvin, *Anal. Chem.*, **25**, 1656 (1953).
39) The materials collected at the end of summer contained considerable amounts of III and IV, while those in autumn and in winter contained much less and nearly trace, respectively.

filtration, crystallized from MeOH to give a white powder, mp 220—222° (decomp.), identical (mixed mp, IR, *Rf* on TLC) with an authentic sample of prosapogenin B of dioscin.[16] The filtrate was found on PPC to contain glucose.

Compound F (=Methyl Proto-dioscin) (I)——Fr. 3 in method 1 was crystallized from MeOH to give I as colorless needles, mp 188—192° (decomp.), identical (IR, NMR, *Rf*, enzymatic hydrolysis giving only III and glucose) with an authentic sample of I.

Compound G——Crystals (0.85 g) obtained by crystallization from MeOH in method 1 were recrystallized from MeOH to give colorless needles (0.62 g), mp 228—230° (decomp.), $[\alpha]_D$ −66.7° (c=0.9, pyridine). Similarly, crystals (11.2 g) in method 2 gave colorless needles (8.3 g), mp 229—231° (decomp.). No spiroketal absorptions on an IR spectrum. NMR (pyridine): 3.25 (3H, s, $-OC\underline{H}_3$). G (15 mg) was hydrolyzed with emulsin (*ca.* 2 mg) in the same way as I to give glucose (PPC) and IV (identified on TLC run in parallel with three different solvent systems).[15]

Ethyl Proto-gracillin Acetate (=Kikuba-saponin (Ks) Acetate) (XIII)——G (100 mg) was acetylated with acetic anhydride–pyridine (1: 2) (4 ml) at room temperature for 24 hr. The mixture was poured into ice-water, the precipitates were collected and recrystallized three times from EtOH to give XIII as colorless needles, mp 160—162°, $[\alpha]_D$ −50.5° (c=1.1, $CHCl_3$), identical with reported values[31] (mp 155—158°, $[\alpha]_D$ −51°) for Ks acetate. NMR ($CDCl_3$): 0.78 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3). No methoxy signal was observed and 21-CH_3 could not be distinguished. Mass Spectrum *m/e*: 1592 (M^+−EtOH).

XIII (100 mg) in acetone–water (3: 1) (20 ml) was refluxed for 10 hr. The solvent was evaporated *in vacuo* and the residue was extracted with ether. Ether-soluble substance was crystallized from ether–hexane to give a white powder (XV), mp 135—139°, $[\alpha]_D$ −51.3° (c=0.52, $CHCl_3$). NMR ($CDCl_3$); 0.81 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3). No methoxy signal was observed and 21-CH_3 could not be distinguished. *Rf* 0.22 (XIII, 0.42, TLC, solvent, hexane–AcOEt (1: 2)).

XIII (100 mg) in MeOH (30 ml) was refluxed for 2 hr, concentrated and left stand in a refrigerator. Colorless needles (XVI), mp 147—149°, $[\alpha]_D$ −54.2° (c=0.82, $CHCl_3$), were barely separated out. NMR ($CDCl_3$): 0.78 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.12 (3H, s, $-OC\underline{H}_3$).

Compound G′ (Methyl Proto-gracillin=Kikuba-saponin (Ks)) (II)——XIII (200 mg) in 5% KOH–MeOH (30 ml) was refluxed for 1 hr, water (20 ml) added and MeOH removed *in vacuo*. The aqueous solution was neutralized with AcOH and extracted with aq. BuOH. The BuOH layer was washed with water, evaporated to dryness and the residue was boiled with MeOH (50 ml) for 2 hr. The MeOH solution was concentrated and the crystals separated out were collected and recrystallized from MeOH to give II as colorless needles, mp 249—251° (decomp.), $[\alpha]_D$ −76.9° (c=0.45, MeOH) (reported for Ks,[31] mp 249—251° (decomp.), $[\alpha]_D$ −75° (MeOH)). IR spectrum, showing no spiroketal absorptions, and *Rf* value and color with E-reag. on TLC were identical with those of G and Ks. NMR (pyridine): 3.25 (3H, s, $-OC\underline{H}_3$), 0.84 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3). *Anal.* Calcd. for $C_{52}H_{86}O_{23}\cdot H_2O$: C, 56.92; H, 8.08. Found: C, 56.50; H, 7.94.

II (15 mg) was hydrolyzed with emulsin (*ca.* 2 mg) in the same way as G to give glucose and IV.

Degradation of XIII——26-O-β-D-Glucopyranosyl pseudodiosgenin 3-O-β-gracillimatrioside acetate (XIV): XIII (300 mg) in AcOH (15 ml) was refluxed for 1 hr and then evaporated *in vacuo* to dryness. The residue was crystallized from ether–hexane to give XIV as a white powder, mp 116—119°, $[\alpha]_D$ −59.3° (c=0.89, $CHCl_3$). NMR ($CDCl_3$): 1.58 (3H, s, $=C(-C\underline{H}_3)-$).

3β-Hydroxy-pregna-5,16-dien-20-one 3-O-β-gracillimatrioside (XVII) and methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside acetate (VI): To the solution of XIV (300 mg) in 80% AcOH (20 ml) was added chromic trioxide (200 mg) in 80% AcOH (1 ml) in 15 min under stirring and cooling below 20°. The mixture was stirred for another 3 hr and an excess reagent was decomposed with MeOH. The product was then extracted with ether, the ether layer was washed with water and evaporated to dryness. The residue (280 mg) was hydrolyzed on refluxing with 5% K_2CO_3 in iso-PrOH (20 ml) for 1 hr. The hydrolysate was diluted with water (20 ml) and extracted with aq.BuOH. The BuOH layer was washed with water, evaporated *in vacuo* and the residue was crystallized from dil. MeOH to give XVII as colorless needles, mp 273—275.5° (decomp.), $[\alpha]_D$ −36.5° (c=0.98, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1647, 1592 (enone). *Anal.* Calcd. for $C_{39}H_{60}O_{16}\cdot H_2O$: C, 58.34; H, 7.78. Found: C, 57.96; H, 7.66. Identified with an authentic specimen of XVII synthesized[27] from IV by mixed melting point determination and by comparison of IR spectra and *Rf* values on TLC (solvent, $CHCl_3$–MeOH–water (7: 3: 0.5)). The water layer of the above hydrolysate was made pH 6 with AcOH and extracted with aq.BuOH. The BuOH solution was evaporated and resulting solid was passed through a Sephadex LH-20 (20 ml) column using MeOH as eluent. The first eluate (containing XVII) was removed and the later fraction was crystallized from MeOH to give γ-methyl-δ-hydroxypentanoic acid glucoside (VI′) as colorless needles (25 mg), mp 266—268° (decomp.), $[\alpha]_D$ about +4° (c=0.5, H_2O), NMR (D_2O, external TMS, 100 MHz, JEOL PS-100): 0.68 (3H, d, J=6 Hz, $>CH-C\underline{H}_3$), 2.17 (2H, t, J=7 Hz, $-C\underline{H}_2COOH$), 4.39 (1H, d, J=7.5 Hz, C_1–H of glucose). Five mg was incubated with emulsin (*ca.* 1 mg) in water (pH 6) for 1 day to give glucose (PPC). Acetylation with acetic anhydride–pyridine followed by methylation in MeOH with diazomethane and chromatography of the product on silica gel column (solvent, hexane–AcOEt (1: 2)) gave VI as an oil. NMR ($CDCl_3$): 3.73 (3H, s, $-COOC\underline{H}_3$), 2.0—

2.02, 2.09, 2.13 (12H, $CH_3COO \times 4$), 0.95 (3H, d, $J=6$ Hz, $>CH-CH_3$). Mass Spectrum *m/e*: 331, 243, 242, 200, 169, 157, 145, 141, 140, 129, 115, 109, 103, 98, 97.

Compound J (Proto-gracillin) (II')——II (100 mg) was boiled with water (5 ml) for 15 hr. The solution was concentrated and left stand to give II' as a white crystalline powder, mp 235—238° (decomp.), $[\alpha]_D$ −57.8° ($c=1.2$, pyridine). IR: no spiroketal absorptions. NMR: no methoxy signal. Identical (*Rf* value and color on TLC) with compound J. On boiling with MeOH II was regenerated.

Dioxane Extract of the Rhizomes of *D. septemloba*——The fresh rhizomes (300 g) collected in the aforementioned place in the middle of February[39] were immediately sliced and extracted with dioxane (1 liter) at room temperature for 2 days. The extract was evaporated *in vacuo* and examined by TLC (Fig. 5). The residue was refluxed with MeOH for 3 hr, evaporated and examined by TLC (Fig. 5).

Acknowledgement The authors are grateful to Dr. K. Takeda of Shionogi Research Laboratory for a NMR spectrum (Varian HA-100), to Miss Y. Inatsu for the mass spectra, to Mr. Matsui and Miss Soeda for the IR (Koken DS-201) and UV spectra, and to the member of the Central Analysis Room of this University for microanalytical data. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan, which is gratefully acknowledged.