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Studies on the Constituents of Palmae Plants. I. The Constituents of *Trachycarpus fortunei* (HOOK.) H. WENDL. (1)[1]

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The constituents of the leaves, stems and underground parts of *Trachycarpus fortunei* (HOOK.) H. WENDL. (Palmae) have been investigated. Glucoluteolin (**3**), luteolin 7-*O*-rutinoside (=scolymoside, **4**) and methyl proto-Pb (**6**) from the leaves, dioscin (**1**), Pb (**2**), methyl proto-dioscin (**5**) and methyl proto-Pb (**6**) from the stems, and dioscin (**1**) and methyl proto-Pb (**6**) from the underground parts were isolated and identified. This is the first report of the isolation of steroidal saponins from Palmae plants and these results are interesting from the standpoint of chemotaxonomy.

Keywords——*Trachycarpus fortunei*; Palmae; steroidal saponin; furostanol oligoside; diosgenin; proto-diosgenin; flavone glycoside; luteolin; glucoluteolin; scolymoside

Trachycarpus fortunei (HOOK.) H. WENDL. (Japanese name: Shuro) is a well-known member of the family Palmae in Japan. This plant grows widely in South Kyushu, either wild or as an ornamental plant. The constituents of this plant were investigated by Mizuno *et al.*,[2] Carter *et al.*[3] and Ferrara *et al.*,[4] who reported the isolation of leucoanthocyanins from seeds, fatty acids from seeds and sialic acids from pollen, respectively.

During the course of our screening work on molluscidal principles contained in plants,[5] the butanol extract of the title plant was found to have a remarkable activity. The purpose of the present study was to isolate the active principle. The present paper deals mainly with the isolation and structure elucidation of four steroidal glycosides, tentatively named glycosides A, B, E and F, and two flavonoidal glycosides, tentatively named glycosides C and D, in order of increasing polarity, leading to the assignment of the structures as shown in Chart 2.

The leaves, stems and underground parts of *Trachycarpus fortunei* (HOOK.) H. WENDL., harvested in Tokyo in April 1981, were separately chopped and extracted with methanol at room temperature. The methanol extract was treated by the method shown in Chart 1.

Glycoside A (**1**), $C_{45}H_{72}O_{16}$, obtained from the stems and underground parts, as well as glycoside B (**2**), $C_{51}H_{82}O_{20}$, obtained from the stems, were isolated as colorless needles. Both glycosides show a strong absorption band due to hydroxyl groups and characteristic absorption bands of the 25(*R*)-spiroketal moiety in the infrared (IR) spectra.[6] The ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrum of glycoside A shows three anomeric carbon signals at δ 100.4, 101.9 and 103.0 ppm, while that of glycoside B shows four anomeric carbon signals at δ 100.4, 102.0, 102.3 and 103.0 ppm. On acidic hydrolyses glycosides A and B afforded a common aglycone (**7**), glucose and rhamnose. Based on the properties and ^1H nuclear magnetic resonance (^1H-NMR) spectrum, compound **7** was suggested to be diosgenin, and this was confirmed by mixed fusion and by comparing the IR spectrum with that of an authentic sample. Consequently, glycosides A and B were inferred to be a diosgenin trioside and a diosgenin tetraoside, respectively. The ^{13}C-NMR spectrum of glycoside A shows two secondary methyl carbon signals due to the C_6-carbon of rhamnose,

Isolation of glycoside A－F from *Trachycarpus fortunei* (Hook.) H. Wendl.

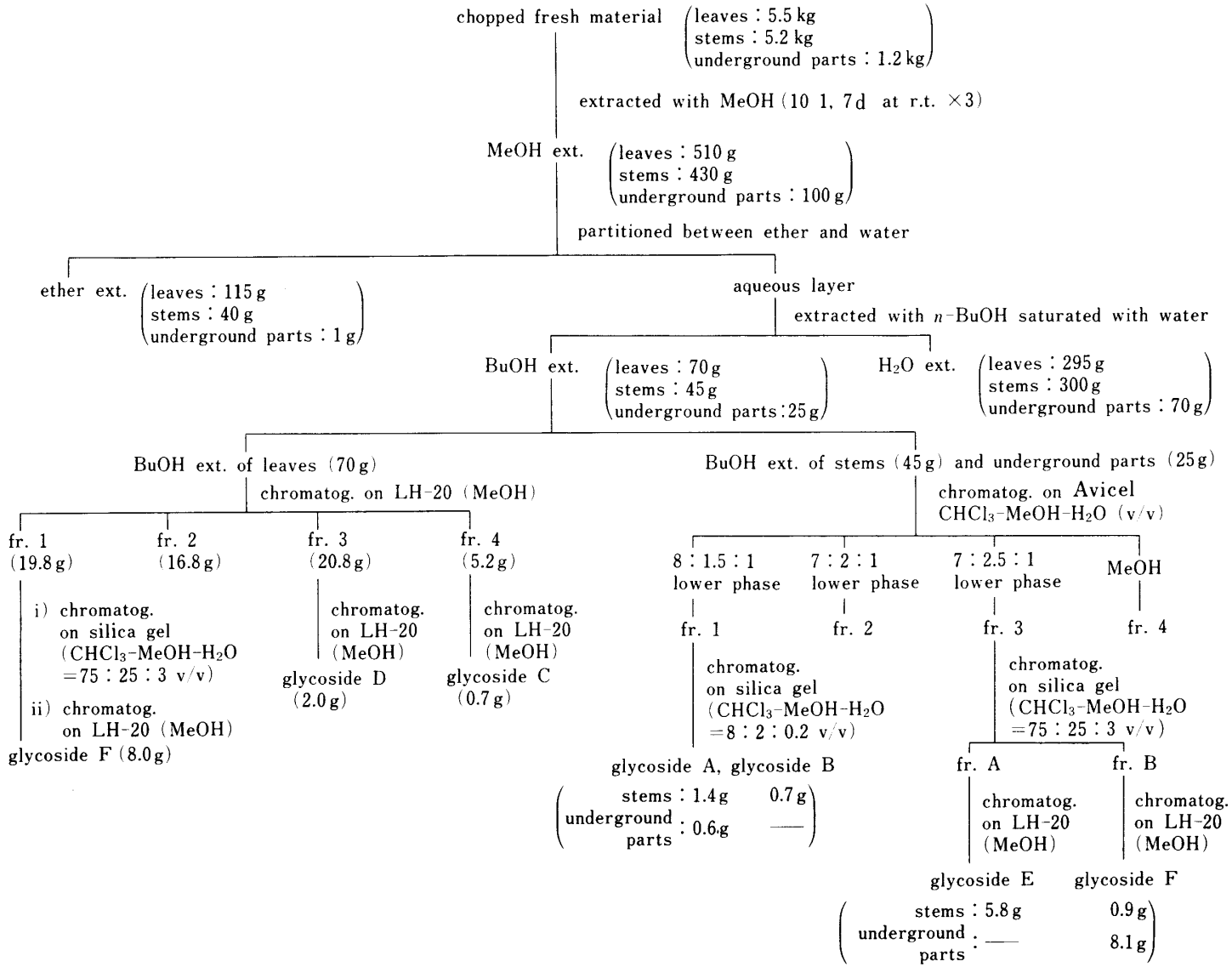

Chart 1

while that of glycoside B reveals the presence of three rhamnose moieties in the molecule. The compositions of diosgenin–glucose–rhamnose in glycosides A and B were suggested to be 1 : 1 : 2 for the former and 1 : 1 : 3 for the latter. Finally, based on the general properties and the results described above, glycosides A and B were suggested to be dioscin[7] and Pb,[8] respectively, and both glycosides were conclusively identified by comparing the IR and ^{13}C-NMR spectra with those of authentic samples.

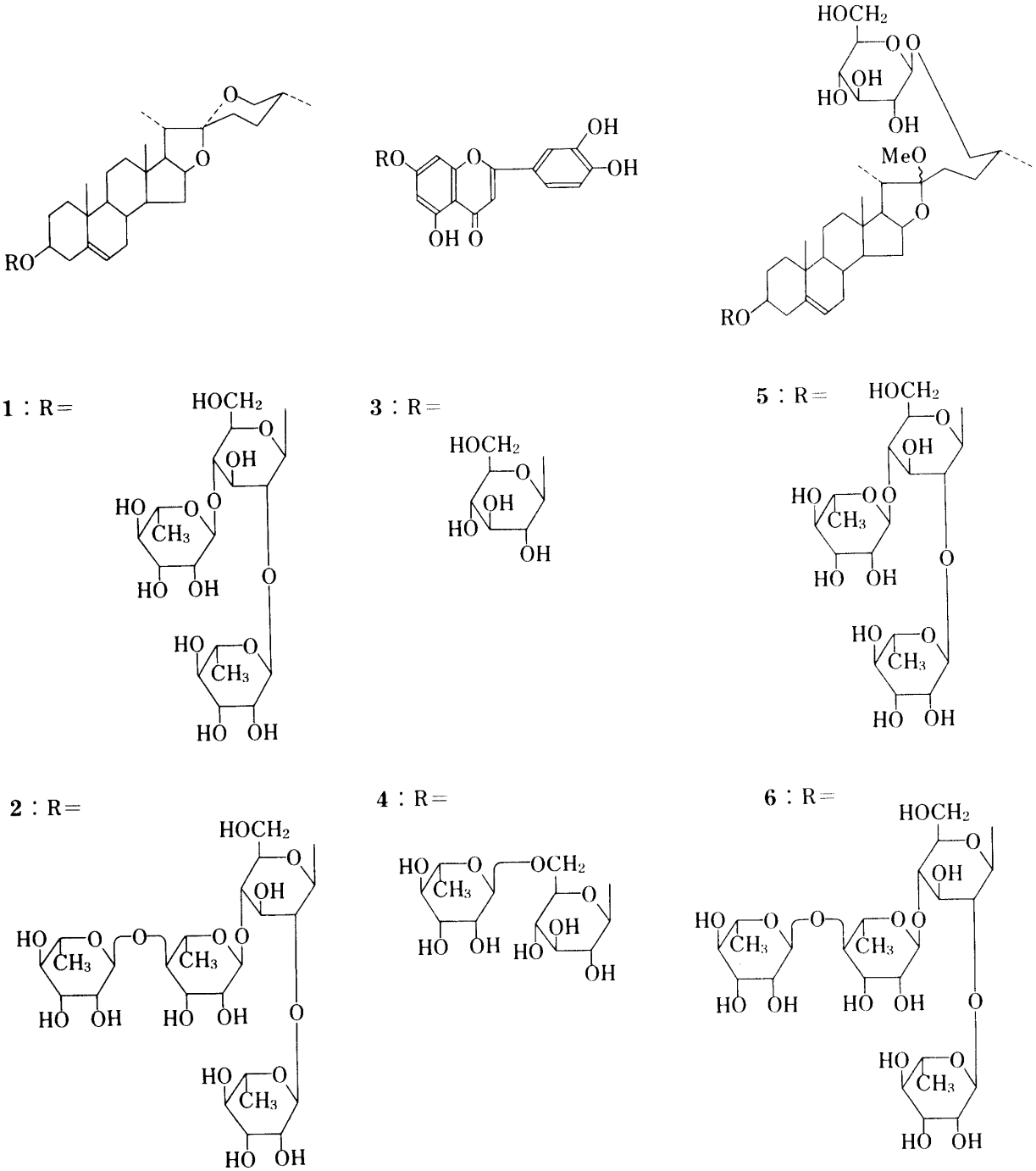

Chart 2

Both glycoside E (**5**), $C_{52}H_{86}O_{22}$, obtained from the stems, and glycoside F (**6**), $C_{58}H_{96}O_{26}$, obtained from the leaves, stems and underground parts, are positive with the Ehrlich reagent,[9] and their ^{13}C-NMR spectra reveal the presence of one methoxyl carbon in

each molecule. On refluxing with aqueous acetone, glycosides E and F each gave the des-*O*-methyl derivative (**5a** and **6a**), which regenerated the original glycosides E and F under reflux with methanol. Based on the above experimental results, glycosides E and F were assumed to be 22-*O*-methylfurostanol glycosides, and both glycosides were hydrolyzed with emulsin to afford glucose and dioscin (**1**) from glycoside E, and glucose and Pb (**2**) from glycoside F, respectively. Furthermore, the ^{13}C-NMR spectra of glycosides E and F show signals assignable to a methyl proto-diosgenin and a C_{26}-*O*-glucosyl moiety.[10] Consequently, the structures of glycosides E and F were established to be methyl proto-dioscin[11] and methyl proto-Pb,[12] respectively.

Glycoside C (**3**), $C_{21}H_{20}O_{11}$, and glycoside D (**4**), $C_{27}H_{30}O_{15}$, both obtained from the leaves, were isolated as pale yellow needles. Both glycosides are positive with aqueous ferric chloride reagent and with magnesium–hydrochloric acid reagent. The IR and ^{13}C-NMR spectra of both glycosides indicated flavonoidal mono- and diglycoside structures for **3** and **4**, respectively. On acidic hydrolysis with 10% sulfuric acid, glycoside C gave glucose and an aglycone (**8**), while glycoside D afforded glucose, rhamnose and the same aglycone (**8**) as that of glycoside C. The common aglycone (**8**) showed ultraviolet (UV) absorption shifts upon addition of sodium acetate and aluminum chloride. Furthermore, based on the results of ^{1}H-NMR spectral analyses of **8** and its acetyl derivative, the structure of **8** was inferred to be 5,7,3′,4′-tetrahydroxyflavone (=luteolin).

Based on the facts that glycosides C and D show UV absorption shifts upon addition of aluminum chloride, as in the case of luteolin, whereas no shifts upon addition of sodium acetate were observed, the sugar moieties of both glycosides were inferred to be located on the C_7-hydroxyl group of luteolin. Accordingly, the structure of glycoside C was suggested to be luteolin 7-*O*-glucoside (=glucoluteolin[13]), and the heptaacetyl derivative of glycoside C was identified as glucoluteolin heptaacetate by mixed fusion with an authentic sample.

Finally, the structure of glycoside D was established to be luteolin 7-*O*-rutinoside (=scolymoside[14]) on the basis that the enzymatic hydrolysis of glycoside D with hesperidinase afforded glycoside C, and that the C_6-carbon signal of the glucose moiety in the ^{13}C-NMR spectrum of glycoside D appeared at lower field (δ 66.2 ppm) than that of glycoside C (δ 60.8 ppm) on account of the glycosylation shift caused by conjugation with the α-rhamnopyranose moiety ($J_{C_1-H_1}$ = 168 Hz).

In this paper the isolation and the structure elucidation of two diosgenin glycosides, two proto-diosgenin bisdesmosides and two luteolin glycosides are described. The steroidal sapogenins have been found mainly in Liliaceae, Dioscoreaceae and Scrophulariaceae plants, and in some case, in Amaryllidaceae, Solanaceae, Simaroubaceae, Legminosae, Cruciferae, Bromeliaceae and Palmae plants.[15] The identification of diosgenin in *Chamaerops humilis* L. by Panizo *et al.*[16] has hitherto been the only report of the presence of spirostanol compound in Palmae plants. Thus, the isolation of steroidal saponins from Palmae plant reported in this paper must be the first finding in this field. We have examined the distribution of steroidal saponins in a number of Palmae plants from the standpoint of chemotaxonomy and the results will be reported shortly.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The optical rotations were measured with a Yanagimoto OR-50 polarimeter. The IR spectra were recorded with a JASCO IRA-1, and the UV spectra were measured with a Hitachi 340 spectrometer. The NMR spectra were recorded with a JEOL FX-100 spectrometer (100 MHz for ^{1}H-NMR and 25 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (Merck) using $CHCl_3$–MeOH–H_2O (7:3:0.4

v/v), and detection was achieved by spraying 10% H_2SO_4 or Ehrlich reagent followed by heating.

Extraction and Isolation of the Glycosides from Leaves, Stems and Underground Parts——i) The fresh leaves (5.5 kg) of *Trachycarpus fortunei* (HOOK.) H. WENDL. (Palmae) were chopped and extracted with MeOH (10 l × 3) at room temperature. The extract was evaporated to dryness *in vacuo*, then the residue (510 g) was suspended in water (2 l) and extracted with ether (500 ml × 3). The ether extract was concentrated *in vacuo* to afford the residue (115 g). On the other hand, the aqueous layer was extracted with BuOH saturated with water (500 ml × 3), and the BuOH-soluble fraction was concentrated under reduced pressure to afford a BuOH extract (70 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 eluted with MeOH to yield four fractions (Fr. 1—Fr. 4). Fr. 1 (glycoside F rich fraction) was purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (75 : 25 : 3 v/v) followed by chromatography on Sephadex LH-20 with MeOH. The eluate was refluxed for 2 h on a water bath, and the solution was concentrated to 5 ml. The concentrate (5 ml) was mixed with 200 ml of AcOEt and the precipitate (=glycoside F, 8 g) was collected by filtration. Fr. 3 (glycoside D rich fraction) and Fr. 4 (glycoside C rich fraction) were separately purified by column chromatography on Sephadex LH-20 with MeOH, and by repeated recrystallization from aqueous MeOH to afford glycoside D (2 g) from Fr. 3 and glycoside C (0.7 g) from Fr. 4.

ii) The fresh stems (5.2 kg) were treated by the same method as in the case of the leaves to afford MeOH extract (430 g), ether extract (40 g) and BuOH extract (45 g). The BuOH extract was subjected to column chromatography on Avicel to yield Fr. 1 (6 g; eluted with $CHCl_3$–MeOH–H_2O (8 : 1.5 : 1 v/v, lower phase)), Fr. 2 (3 g; eluted with $CHCl_3$–MeOH–H_2O (7 : 2 : 1 v/v, lower phase)), Fr. 3 (11 g; eluted with $CHCl_3$–MeOH–H_2O (7 : 2.5 : 1 v/v, lower phase)) and Fr. 4 (17 g; eluted with MeOH). Fr. 1 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2 v/v) to afford glycoside A (1.4 g) and glycoside B (0.7 g). Fr. 3 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (75 : 25 : 3 v/v) to provide two fractions (Fr. A and Fr. B), and each fraction was purified by column chromatography on Sephadex LH-20 with MeOH to afford glycoside E rich eluate from Fr. A and glycoside F rich eluate from Fr. B. The former eluate was refluxed for 2 h and the solution was evaporated to dryness. The residue was recrystallized from MeOH to afford glycoside E (5.8 g). The latter eluate was refluxed for 2 h and the solution was concentrated to 5 ml. The concentrate was mixed with 200 ml of AcOEt to afford a precipitate, which was collected by filtration and dried (glycoside F, 0.9 g).

iii) The fresh underground parts (1.2 kg) were extracted and fractionated by the same procedure as in the case of leaves and stems to afford MeOH extract (100 g), ether extract (1 g) and BuOH extract (25 g). The BuOH extract was treated by the same procedure as in the case of stems to afford glycoside A (0.6 g) and glycoside F (8.1 g).

Properties of Glycosides A, B, C, D, E and F——Glycoside A (**1**): colorless needles from aqueous MeOH. mp 243—246 °C (dec.), $[\alpha]_D^{16}$ −110.3° (c=0.55, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 920, 900, 864 (intensity 920 < 900, 25(*R*)-spiroketal). *Anal.* Calcd for $C_{45}H_{72}O_{16}$: C, 62.19; H, 8.35. Found: C, 61.86; H, 8.48. Glycoside A was identified as dioscin by comparisons of the IR and ^{13}C-NMR spectra with those of an authentic sample. Glycoside B (**2**): colorless needles from MeOH, mp 243—245 °C (dec.), $[\alpha]_D^{16}$ −104.7° (c=0.55, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3240 (OH), 984, 920, 902, 865 (intensity 920 < 902, 25(*R*)-spiroketal). *Anal.* Calcd for $C_{51}H_{82}O_{20} \cdot 3/2H_2O$: C, 58.77; H, 8.22. Found: C, 58.64; H, 8.25. Glycoside B was identified as Pb by comparisons of the IR and ^{13}C-NMR spectra with those of an authentic sample. Glycoside C (**3**): pale yellow needles from aqueous MeOH, mp 265—269 °C (dec.), $[\alpha]_D^{16}$ −48.0° (c=0.98, pyridine). UV λ_{max}^{MeOH} nm (log ε): 253 (4.43), 265 (4.40), 348 (4.45), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 272, 425. IR ν_{max}^{KBr} cm^{-1}: 3530—3060, 1650, 1600. ^{13}C-NMR (DMSO-d_6) δ: 100.0 (C-1 of glc), 77.2 (C-5 of glc), 76.5 (C-3 of glc), 73.2 (C-2 of glc), 69.7 (C-4 of glc), 60.8 (C-6 of glc). *Anal.* Calcd for $C_{21}H_{20}O_{11} \cdot 1/2H_2O$: C, 55.14; H, 4.63. Found: C, 55.17; H, 4.51. Glycoside D (**4**): pale yellow needles from aqueous MeOH, mp 190—192 °C (dec.), $[\alpha]_D^{16}$ −77.3° (c=0.97, pyridine). UV λ_{max}^{MeOH} nm (log ε): 257 (4.26), 265 (4.28), 342 (4.29), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 272, 420. IR ν_{max}^{KBr} cm^{-1}: 3460—3200, 1650, 1600. ^{1}H-NMR (DMSO-d_6) δ: 1.08 (3H, d, J=6 Hz, C-5 CH_3 of rha), 6.47, 6.76 (1H each, d, J=2 Hz, H-6 and 8), 6.74 (1H, s, H-3), 6.91 (1H, d, J=9 Hz, H-5′), 7.41 (1H, d, J=2 Hz, H-2′), 7.44 (1H, dd, J=2, 9 Hz, H-6′), 12.97 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 100.6 (d, $J_{C_1-H_1}$=168 Hz, α-C-1 of rha), 100.1 (d, $J_{C_1-H_1}$=159 Hz, β-C-1 of glc), 76.5 (C-3 of glc), 75.7 (C-5 of glc), 73.2 (C-2 of glc), 72.2 (C-4 of rha), 70.9 (C-3 of rha), 70.5 (C-2 of rha) 69.7 (C-4 of glc), 68.5 (C-5 of rha), 66.2 (C-6 of glc), 18.0 (C-6 of rha). *Anal.* Calcd for $C_{27}H_{30}O_{15} \cdot 1/2H_2O$: C, 53.73; H, 5.18. Found: C, 53.76; H, 5.27. Glycoside E (**5**): colorless needles from MeOH, mp 185—189 °C (dec.), $[\alpha]_D^{16}$ −102.9° (c=1.00, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3540—3220 (OH). *Anal.* Calcd for $C_{52}H_{86}O_{22} \cdot H_2O$: C, 57.76; H, 8.02. Found: C, 57.86; H, 8.33. Glycoside E was identified as methyl proto-dioscin by comparisons of the IR and ^{13}C-NMR spectra with those of an authentic sample. Glycoside F (**6**): a white powder from MeOH–AcOEt, (mp 189—190 °C (dec.)), $[\alpha]_D^{16}$ −86.4° (c=1.03, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3250 (OH). *Anal.* Calcd for $C_{58}H_{96}O_{26}$: C, 57.60; H, 8.00. Found: C, 57.28; H, 8.00.

Acidic Hydrolyses of Glycosides A and B——Glycosides A (50 mg) and B (50 mg) were separately hydrolyzed with 2 N HCl–50% dioxane (5 ml) by refluxing for 2 h on a water bath, and each reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extracts were individually washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. Each residue was repeatedly recrystallized from acetone to afford the common aglycone **7** (16 mg from glycoside A and 15 mg from glycoside B) as colorless needles from acetone, mp 205—209 °C. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 920, 900, 860 (intensity 920 < 900, 25(*R*)-spiroketal). *Anal.* Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.31; H, 10.51. The common aglycone was identified as diosgenin by mixed fusion and by comparing the IR

spectrum with that of an authentic sample.

Each aqueous layer was neutralized with $NaHCO_3$ and the neutral solution was evaporated to dryness under reduced pressure. Each residue was derivatized to the trimethylsilyl (TMS) ether and examined by GLC (column: 5% SE-52 on Chromosorb W 3 mm × 2 m; column temp. 200 °C; injection temp. 220°C; carrier gas N_2; 1.0 kg/cm^2). Hydrolysate of glycoside A: t_R (min) 3.4, 4.4 (rhamnose), 8.8, 12.0 (glucose). Hydrolysate of glycoside B: t_R (min) 3.4, 4.4 (rhamnose), 8.8, 12.0 (glucose).

Treatments of Glycosides E and F with Aqueous Acetone——Glycosides E (50 mg) and F (50 mg) were individually refluxed with 30% aqueous acetone (50 ml) for 4 h on a water bath, and each reaction mixture was concentrated to 5 ml. Each concentrate was mixed with AcOEt (100 ml) and the white precipitate was collected by filtration to afford a des-*O*-methyl derivative (**5a**, 50 mg) from glycoside E and a des-*O*-methyl derivative (**6a**, 48 mg) from glycoside F.

Compound **5a**: a white powder from aqueous acetone, (mp 190—194 °C (dec.)), $[\alpha]_D^{16}$ −81.7° (c = 1.04, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3510—3200, *Anal.* Calcd for $C_{51}H_{84}O_{22}\cdot H_2O$: C, 57.39; H, 8.12. Found: C, 57.56; H, 8.28. Compound **6a**: a white powder from aqueous acetone, (mp 187—189 °C (dec.)), $[\alpha]_D^{16}$ −94.8° (c = 1.09, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3520—3240. *Anal.* Calcd for $C_{57}H_{94}O_{26}\cdot 3/2H_2O$: C, 56.00; H, 8.00. Found: C, 56.01; H, 7.84.

Enzymatic Hydrolyses of Glycosides E and F——Aqueous solutions of glycosides E (100 mg) and F (100 mg) were separately incubated with almond emulsin (50 mg) at 37 °C for 4 h to afford glycoside A (=dioscin, 38 mg) from glycoside E as colorless needles from aqueous MeOH and glycoside B (=Pb, 30 mg) from glycoside F as colorless needles from MeOH. Each aqueous layer was evaporated to dryness *in vacuo* and the residue was derivatized to the trimethylsilyl ether, which was examined by GLC under the same conditions as described above. Sugars from glycosides E and F: t_R (min) 8.8, 12.0 (glucose).

Acidic Hydrolyses of Glycosides C and D——Glycosides C (100 mg) and D (100 mg) were individually heated on a water bath with 10% H_2SO_4 (10 ml) for 2 h. Each reaction mixture was cooled and the precipitate was collected by filtration to afford a common aglycone **8** (70 mg from glycoside C and 50 mg from glycoside D) as pale yellow needles from aqueous EtOH, mp > 300 °C (dec.). UV λ_{max}^{MeOH} nm (log ε): 250 (4.33), 262 (4.29), 288 (4.05), 345 (4.41), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 271, 300, 420, $\lambda_{max}^{MeOH+AcONa}$ nm: 304, 316, 350. IR ν_{max}^{KBr} cm^{-1}: 3400—3200, 1650, 1600. *Anal.* Calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 63.24; H, 3.54.

Each aqueous filtrate was neutralized with $NaHCO_3$ and the neutral solution was evaporated to dryness *in vacuo*. The residues were derivatized to trimethylsilyl ethers which were examined by GLC under the same conditions as described above. Hydrolysate of glycoside C: t_R (min) 8.8, 12.0 (glucose). Hydrolysate of glycoside D: t_R (min) 3.4, 4.4 (rhamnose), 8.8, 12.0 (glucose).

Acetylation of Compound 8——Acetic anhydride (2 ml) was added to a solution of **8** (50 mg) in pyridine (4 ml), and the solution was left at room temperature overnight. The reaction mixture was poured into ice water, and the precipitate was collected by filtration to afford a tetraacetate (49 mg) as colorless needles from aqueous MeOH, mp 220—222 °C, IR ν_{max}^{Nujol} cm^{-1}: 1760, 1635, 1610. *Anal.* Calcd for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 60.70; H, 4.03. The acetylation product was identified as luteolin tetraacetate by mixed fusion with an authentic sample.

Enzymatic Hydrolysis of Glycoside D——An aqueous solution of glycoside D (50 mg) was incubated with crude hesperidinase (2 mg, Seikagaku Kogyo Co., Ltd., Lot. No. 1B197) at room temperature for 3 min. The reaction mixture was extracted with BuOH saturated with water (20 ml × 3) and the extracts were combined. The BuOH extract was evaporated to dryness *in vacuo* and the residue was subjected to column chromatography on Sephadex LH-20 to afford luteolin (25 mg) and glycoside C (20 mg). The former product was identified by TLC (*Rf* 0.47), while the latter was identified by mixed fusion after acetylation.

Acetylation of Glycoside C——Acetic anhydride (2 ml) was added to a solution of glycoside C (50 mg) in pyridine (4 ml) and the solution was left at room temperature overnight. The reaction mixture was poured into ice-water and the precipitate was collected by filtration to afford colorless needles (52 mg) by repeated recrystallization from aqueous MeOH. mp 236—240 °C. IR ν_{max}^{Nujol} cm^{-1}: 1770, 1740, 1640. *Anal.* Calcd for $C_{35}H_{34}O_{18}$: C, 56.60; H, 4.62. Found: C, 56.50; H, 4.58. This acetate was identified as glucoluteolin heptaacetate by mixed fusion with an authentic sample.

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References and Notes

1) This work was presented at the 28th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, October 1981.
2) T. Mizuno and A. Uno, *Nippon Nôgeikagaku Kaishi*, **41**, 512 (1967).
3) L. Carter, *Chem. Phys. Lipids*, **4**, 96 (1970).
4) L. Ferrara and M. I. La Rotonda, *Boll. Soc. Ital. Biol. Sper.*, **51**, 696 (1975).

5) Y. Hirai, H. Koidzumi, T. Konishi, S. Isoda, S. Sanada, S. Sakuma, A. Tada, Y. Ida, J. Shoji and K. Okamoto, Abstracts of Papers, the 27th Annual Meeting of the Japanese Society of Pharmacognosy, Nagoya, September 1980, p. 35.
6) *a*) M. E. Wall, C. R. Eddy, M. L. McClennan and M. A. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); *b*) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953); *c*) R. N. Jones, K. Katzenellenbogen and K. Dohriner, *J. Am. Chem. Soc.*, **75**, 1581 (1953).
7) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.*, **10**, 703 (1962).
8) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.*, **21**, 1240 (1973).
9) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa and T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968).
10) Y. Hirai, T. Konishi, S. Sanada, Y. Ida and J. Shoji, *Chem. Pharm. Bull.*, **30**, 3476 (1982).
11) T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa and K. Mihashi, *Chem. Pharm. Bull.*, **22**, 2164 (1974).
12) *a*) M. Miyamura, K. Nakano, T. Nohara, T. Tomimatsu and T. Kawasaki, the 28th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, October 1981. (They presented the same structure for methyl proto-Pb as ours at the same meeting.); *b*) *Idem, Chem. Pharm. Bull.*, **30**, 712 (1982).
13) *a*) S. Hattori and H. Matsuda, *Acta Phytochim.* (Japan), **15**, 233 (1949) (*Chem. Abstr.*, **44**, 3214*i*); *b*) K. R. Markham, B. Ternai, R. Stanley, H. Geiger and T. J. Mabry, *Tetrahedron*, **34**, 1397 (1978).
14) H. Inouye, T. Aoki, H. Wagner, L. Hörhammer, G. Aurnhammer and W. Budweg, *Chem. Ber.*, **102**, 3009 (1969).
15) K. Takeda, T. Okanishi, A. Shimaoka and A. Akahori, *Shionogi Kenkyusho Nenpo*, **7**, 339 (1957).
16) F. M. Panizo and M. Pinar, *Chem. Ind.*, **88**, 496 (1962).