[Chem. Pharm. Bull. 34(1) 82—87 (1986)]

Studies on the Constituents of Palmae Plants. III.[1a] The Constituents of *Chamaerops humilis* L. and *Trachycarpus wagnerianus* BECC.[2]

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(Received May 30, 1985)

The constituents of the leaves, stems and underground parts of *Chamaerops humilis* L. and *Trachycarpus wagnerianus* BECC. (Palmae) have been investigated. We isolated and identified methyl proto-dioscin and methyl proto-Pb from the stems, dioscin, Pb, methyl proto-dioscin, methyl proto-Pb and methyl proto-rhapissaponin from the underground parts, and tricin 7-*O*-rutinoside and methyl proto-Pb from the leaves of *C. humilis* L. On the other hand, we isolated and identified methyl proto-dioscin and pseudoproto-dioscin from the stems, Pb, Tg, methyl proto-Pb and pseudoproto-Pb from the underground parts, and glucoluteolin, scolymoside, methyl proto-Pb and pseudoproto-Pb from the leaves of *T. wagnerianus* BECC. This is the first report of the isolation of tricin 7-*O*-β-rutinoside, Tg and pseudodiosgenin oligosides from Palmae plants, and also the first discovery of pseudosteroidal saponin from natural sources.

Keywords——*Chamaerops humilis*; *Trachycarpus wagnerianus*; Palmae; steroidal saponin; pseudoproto-diosgenin; diosgenin; pennogenin; flavone glycoside; luteolin; tricin

In the previous paper[1] we reported the isolation and structure elucidation of steroidal glycosides and flavonoids from Palmae plants, *Trachycarpus fortunei* (HOOK.) H. WENDL., *Rhapis exelsa* HENRY, and *R. humilis* BL. A series of chemotaxonomical studies on plants of the Palmae family has been undertaken by us, and the present paper is mainly concerned with studies on the constituents of *Chamaerops humilis* L. and *Trachycarpus wagnerianus* BECC.

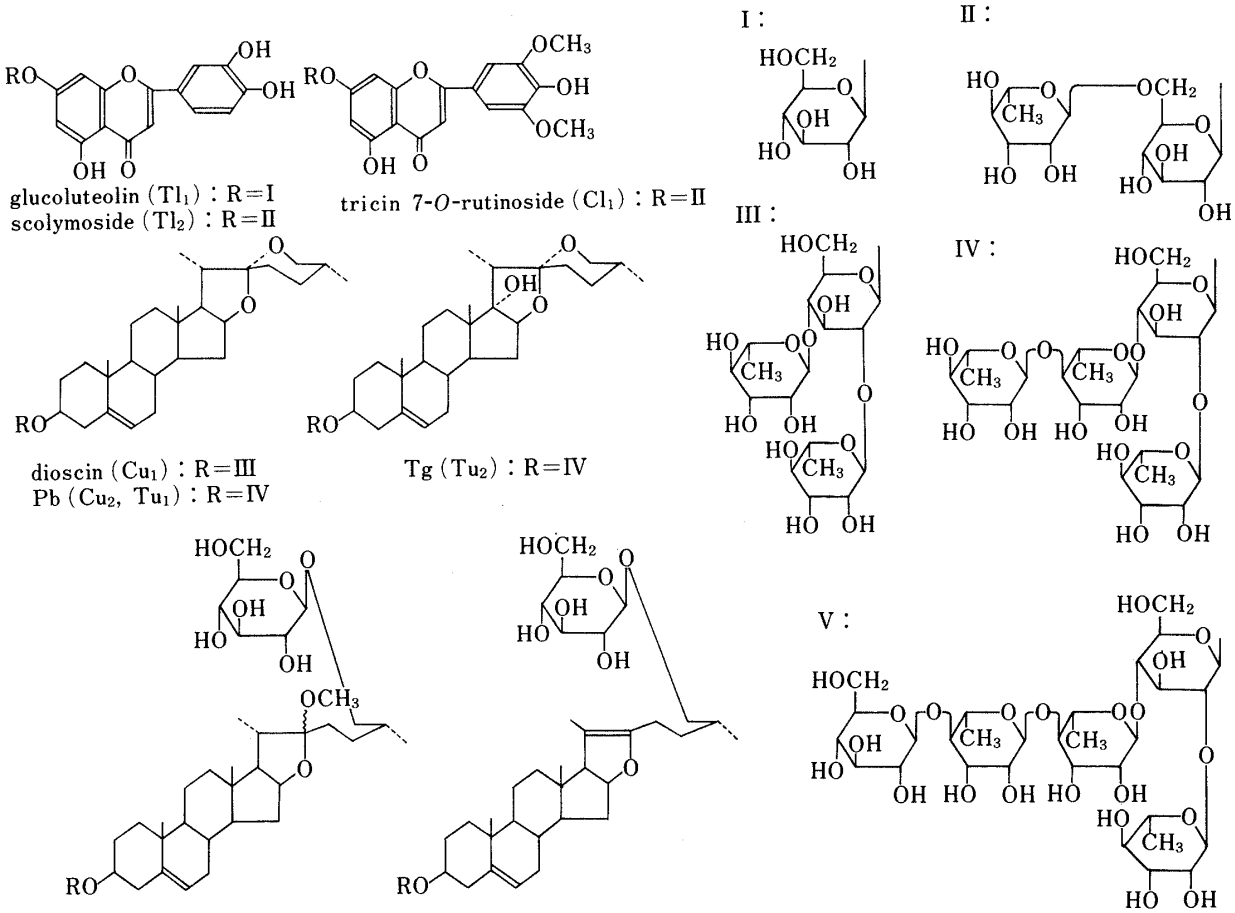
The habitat of *C. humilis* L. (Japanese name: Chabotojuro) is the Mediterranean coast, and only a few plants are cultivated in Japan, for ornamental purposes. The constituents of this plant were investigated by Williams *et al.*[3] and they reported the isolation of leucoanthocyanidin, flavone *C*-glycoside, tricin and tricin 5-*O*-glycoside from the leaves, and rutin, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside and caffeylshikimic acid from the flowers. Monache *et al.*[4] also reported the isolation of (+)-epicatechin and two kinds of procyanidins from the fruits, and Panizo and Pinar[5] reported the isolation of diosgenin from the leaves and roots.

On the other hand, the habitat of *T. wagnerianus* BECC. (Japanese name: Tojuro) is the middle southern region of China. This plant is widely cultivated in Japan as an ornamental plant, but it has been used as a tonic, hemostatic and antidiarrheic crude drug in Chinese medicine. As regards the constituents of *T. wagnerianus* BECC., only the presence of leucoanthocyanins has been reported.[6]

The leaves, stems and underground parts of *C. humilis* L. harvested in Tokyo in July 1982, were separately chopped and extracted with methanol at room temperature. The methanol extract was separated into three fractions, namely ether-soluble, butanol-soluble and water-soluble fractions. The butanol-soluble fraction of the leaves was chromatographed on a Sephadex LH-20 column eluted with methanol to yield two fractions, of which one gave

a positive Ehrlich reaction[7] on a thin layer chromatographic (TLC) plate (Fr. 1) and the other gave a positive ferric chloride reaction (Fr. 2). A compound, tentatively named Cl_2, was isolated from Fr. 1 by silica gel column chromatography and another compound named Cl_1 was isolated from Fr. 2 by Avicel column chromatography. The butanol-soluble fraction of the stems was treated in the same way as described for the leaves, and two compounds tentatively named Cst_1 and Cst_2 were isolated. Furthermore, the butanol-soluble fraction of the underground parts was treated by the same method as described above, and five compounds tentatively named Cu_1, Cu_2, Cu_3, Cu_4 and Cu_5 were isolated.

The leaves, stems and underground parts of *T. wagnerianus* BECC. harvested in Tokyo in June 1983, were separately chopped, extracted and fractionated by the same procedures as described in the case of *C. humilis*. Four compounds named Tl_1, Tl_2, Tl_3 and Tl_4 from the butanol-soluble fraction of the leaves, two compounds named Tst_1 and Tst_2 from that of the stems, and four compounds named Tu_1, Tu_2, Tu_3 and Tu_4 from that of the underground parts were isolated.



Chart

The identities of Cu_1 with dioscin,[1] Cu_2 and Tu_1 with Pb,[1] Cst_1, Cu_3 and Tst_1 with methyl proto-dioscin,[1] Tl_3, Tu_3, Cl_2, Cst_2 and Cu_4 with methyl proto-Pb,[1] Cu_5 with methyl proto-rhapissaponin,[1b] Tl_1 with glucoluteolin,[1] and Tl_2 with scolymoside[1] were demonstrated by infrared (IR) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectral com-

parisons of these compounds with authentic samples reported in the previous papers.

Among the constituents of the underground parts of *T. wagnerianus*, Tu_2 was positive in the Liebermann–Burchard reaction and it showed characteristic absorption bands of a 25(*R*)-spiroketal moiety in the IR spectrum.[8] On hydrolysis with 2 N hydrochloric acid in 50% dioxane, Tu_2 gave glucose and rhamnose as sugar components. Based on the ^{13}C-NMR spectrum of Tu_2, which showed four anomeric carbon signals at δ 100.5, 102.0, 102.3, 103.1 and three C_6-carbon signals of rhamnose at δ 18.4, 18.6, 18.8, the sugar moiety of Tu_2 was elucidated to have the same structure as that of Tu_1 (Pb), which consists of diosgenin, one glucose and three rhamnoses. However, some of the carbon chemical shifts of the steroidal aglycones of Tu_1 and Tu_2, namely C_{12}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}, and C_{21}, differed by 3—7 ppm from each other. Concerning the C_{17}-carbon signals of Tu_1 and Tu_2, a doublet signal of the former appeared at δ 63. 1 ppm, while the latter showed a singlet signal at δ 90.2 ppm. Based on the ^{13}C-NMR analysis, Tu_2 was deduced to be pennogenin glycoside and, finally, Tu_2 was identified as Tg[9] by comparing its ^{13}C-NMR spectrum with that of an authentic sample.

Tst_2 was suggested to be a furostanol derivative by the Liebermann–Burchard reaction and the Ehrlich reaction. On hydrolysis with 2 N hydrochloric acid in 50% dioxane, Tst_2 gave glucose and rhamnose as sugar components. The ^{13}C-NMR spectrum of Tst_2 showed four anomeric carbon signals at δ 100.4, 102.0, 102.9 and 104.8 ppm and two C_6-carbon signals of rhamnose at δ 18.4 and 18.6 ppm. Based on the foregoing data, Tst_2 was suggested to be a furostanol glycoside having a sugar moiety made up of two moles each of glucose and rhamnose, *i.e.*, the same components as those of Tst_1 (=methyl proto-dioscin). The ^{13}C-NMR signal pattern of the sugar moiety of Tst_2 agreed well with that of Tst_1. As regards the aglycones, the former showed C_{20} and C_{22} carbon signals at δ 40.5 and 112.7 ppm, but the latter showed olefinic carbon signals due to C_{20} and C_{22} at δ 103.6 and 152.6 ppm, respectively. Based on the ^{13}C-NMR analysis, Tst_2 was deduced to be a pseudo-derivative of proto-dioscin. Finally, Tst_2 was identified as pseudo-protodioscin by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample derived from methyl proto-dioscin by refluxing in acetic acid.

Tl_4 (=Tu_4), like Tst_2, was positive in the Liebermann–Burchard reaction and the Ehrlich reaction. On hydrolysis with 2 N hydrochloric acid in 50% dioxane, Tl_4 gave glucose and rhamnose as sugar components. The ^{13}C-NMR spectrum of Tl_4 showed five anomeric carbon signals at δ 100.6, 102.1, 102.4, 103.2 and 104.9 ppm and three C_6-carbon signals of rhamnose at δ 18.5, 18.7 and 18.9 ppm. By comparing the ^{13}C-NMR spectra of Tl_4 and methyl proto-Pb (=Tl_3, Tu_3), which was isolated from the leaves and the underground parts of *T. wagnerianus* and is a furostanol glycoside having the same sugar components as Tl_4, it was proved that the structure of the sugar moiety of Tl_4 is the same as that of methyl proto-Pb, and that of the aglycone is the same as that of pseudoproto-dioscin. Consequently, Tl_4 was confirmed to be identical with pseudoproto-Pb derived from methyl proto-Pb by refluxing in acetic acid, based on comparisons of the IR and ^{13}C-NMR spectra. This is the first report of the isolation and structure elucidation of pseudo-type steroidal glycoside from natural sources.

Cl_1, isolated from the leaves of *C. humilis*, was obtained as pale yellow needles. Based on the color reactions with ferric chloride reagent and with magnesium–hydrochloric acid reagent and analysis of the ^{13}C-NMR spectrum, which showed two anomeric carbon signals at δ 100.0 and 100.6 ppm, Cl_1 was deduced to be a flavonoidal *O*-glycoside. Furthermore, the ^{1}H-NMR spectrum of Cl_1 showed an AB quartet at δ 6.49 and 6.87 ppm (J=2 Hz), a singlet at δ 7.05 ppm, a singlet corresponding to two equivalent protons at δ 7.36 ppm, and two equivalent *O*-methyl signals at δ 3.58 ppm. Based on the NMR analyses, Cl_1 was suggested to be a tricin bioside, and this was supported by hydrolysis of Cl_1 with 10% sulfuric acid to afford tricin, glucose and rhamnose. Further comparison of the ^{13}C-NMR spectra of Cl_1 and scolymoside (=Tl_2, luteolin 7-*O*-rutinoside) suggested that the sugar moieties of these

compounds might be identical. The ultraviolet (UV) spectrum of Cl_1 indicated the presence of a 5-hydroxyl group, since a bathochromic shift was observed on the addition of aluminum chloride, and the position of rutinose was suggested to be at C_7, because no bathochromic shift was observed on addition of sodium acetate. Consequently, the structure of Cl_1 was concluded to be tricin 7-*O*-*β*-rutinoside.[10]

In this paper the isolation and structure elucidation are reported of a diosgenin oligoside, namely Pb, two proto-diosgenin bisdesmosides, methyl proto-dioscin and methyl proto-Pb, one pennogenin oligoside, Tg, two pseudoproto-diosgenin bisdesmosides, pseudoproto-dioscin and pseudoproto-Pb, and two flavone glycosides, glucoluteolin and scolymoside, from *T. wagnerianus*, as well as two diosgenin oligosides, namely dioscin and Pb, three proto-diosgenin bisdesmosides, methyl proto-rhapissaponin, methyl proto-dioscin and methyl proto-Pb, and one flavone glycoside, tricin 7-*O*-rutinoside, from *C. humilis*. Tricin 7-*O*-*β*-rutinoside isolated from *C. humilis* has not been found in *T. fortunei* (HOOK.) H. WENDL., *T. wagnerianus*, *Rhapis excelsa* HENRY, or *R. humilis* BL. hitherto studied. Almost all of the constituents of *Trachycarpus wagnerianus* are identical with those of *T. fortunei* (HOOK.) H. WENDL. except Tg, pseudoproto-dioscin and pseudoproto-Pb, which have not been isolated from the latter. This is the first report of the isolation of pseudoproto-diosgenin bisdesmosides from natural sources.

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 JASCO DIP-140 polarimeter. The IR spectra were recorded with a Hitachi EPI-2 and the UV spectra were recorded with a Hitachi 340 spectrophotometer. The NMR spectra were recorded with a JEOL JNM-FX 100 (100 MHz for ^{1}H-NMR and 25 MHz for ^{13}C-NMR) and the chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. 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 or by spraying ferric chloride reagent. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector.

Extraction and Isolation of the Compounds from the Leaves, Stems and Underground Parts of *Chamaerops humilis* L. and *Trachycarpus wagnerianus* BECC.——I-1) Leaves of *C. humilis*: The fresh leaves (1.8 kg) were chopped and extracted with MeOH (5 l × 3) at room temperature. The MeOH extract was evaporated to dryness *in vacuo*. The residue (486 g) was suspended in water (1 l) and partitioned with ether (500 ml × 3). The ether layer was concentrated *in vacuo* to afford the ether extract (71 g), and the aqueous layer was partitioned with BuOH saturated with water (500 ml × 3). The BuOH layer was concentrated under reduced pressure to afford the BuOH extract (31 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to afford two fractions, Fr. 1 (10.0 g) and Fr. 2 (28.2 g). Fr. 1 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (75 : 25 : 3, v/v) to provide the fraction rich in Cl_2, which was refluxed with MeOH (50 ml) for 2 h on a water bath. Then the solution was concentrated to 5 ml under reduced pressure. The concentrate was mixed with 200 ml of AcOEt and the precipitate was collected by filtration and dried to afford Cl_2 (0.3 g). Fr. 2 was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (8 : 1.5 : 1, v/v; lower layer) followed with (7 : 2 : 1, v/v; lower layer), (7 : 2.5 : 1, v/v; lower layer) and MeOH to yield four fractions (Fr. 1—Fr. 4). Fr. 2 was purified by repeated recrystallization to afford pure Cl_1 (0.1 g).

I-2) Stems of *C. humilis*: Extraction and isolation of the compounds from the fresh stems (13.0 kg) were carried out according to procedure I-1 to afford MeOH extract (1.0 kg), ether extract (29 g) and BuOH extract (68 g). Cst_1 (0.4 g) and Cst_2 (2.0 g) were isolated from Fr. 1 of the BuOH extract.

I-3) Underground Parts of *C. humilis*: The fresh underground parts (5.6 kg) were treated as described above to afford MeOH extract (230 g), ether extract (40 g) and BuOH extract (47 g). Cu_1 (0.04 g), Cu_2 (0.01 g), Cu_3 (0.3 g), Cu_4 (2.6 g) and Cu_5 (0.3 g) were isolated from Fr. 1 of the BuOH extract.

II-1) Leaves of *T. wagnerianus*: The fresh leaves (6.1 kg) were treated according to procedure I-1 to afford MeOH extract (661 g), ether extract (92 g) and BuOH extract (41 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to provide two fractions, namely a fraction containing Tl_3 and Tl_4 (20.3 g) and a fraction containing Tl_1 and Tl_2 (18.1 g). The former fraction was subjected to column chromatography on ODS with 50% MeOH to afford two fractions, namely a fraction rich in Tl_3 and a fraction rich in Tl_4. Each fraction was purified by column chromatography on Sephadex LH-20 with MeOH. The respective eluates were refluxed for 2 h on a water bath and concentrated to 5 ml under reduced pressure. The concentrates were each mixed

with 200 ml of AcOEt and the precipitate was collected by filtration to afford Tl_3 (0.4 g) and Tl_4 (0.1 g), respectively. The latter fraction, a fraction containing Tl_1 and Tl_2, was rechromatographed on Sephadex LH-20 with MeOH to provide two fractions, namely Fr. A and Fr. B. Each fraction was purified by repeated crystallization from aq. MeOH to afford pure Tl_2 (0.4 g) from Fr. A and Tl_1 (0.2 g) from Fr. B, respectively.

II-2) Stems of *T. wagnerianus*: The fresh stems (35 kg) were treated according to procedure I-2 to afford MeOH extract (3.4 kg), ether extract (68 g) and BuOH extract (313 g). The BuOH extract was purified according to procedure II-1 to provide Tst_1 (32.0 g) and Tst_2 (8.0 g).

II-3) Underground Parts of *T. wagnerianus*: The fresh underground parts (7.5 kg) were treated according to procedure I-3 to afford MeOH extract (1.97 kg), ether extract (21 g) and BuOH extract (103 g). The BuOH extract was purified according to procedure II-1 to afford Tu_1 (0.1 g), Tu_2 (0.01 g), Tu_3 (0.4 g) and Tu_4 (0.1 g).

Properties of the Constituents of *C. humilis* and *T. wagnerianus* ——Cl_1: Pale yellow needles from EtOH, mp 192—196 °C (dec.), $[\alpha]_D^{20}$ −93.7° ($c=1.00$, pyridine). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 241 (4.34), 262 (4.24), 346 (4.43), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 268, 304, 397, $\lambda_{max}^{MeOH+AcONa}$ nm: 241, 262, 346. IR ν_{max}^{KBr} cm^{-1}: 3400—3200, 1650, 1610. ^{1}H-NMR (DMSO-d_6) δ: 1.08 (3H, d, $J=6$ Hz, C-5 CH_3 of rha), 3.58 (6H, s, OCH_3 of C-3′,5′), 6.49 (1H, d, $J=2$ Hz, H-6), 6.87 (1H, d, $J=2$ Hz, H-8), 7.05 (1H, s, H-3), 7.36 (2H, s, H-2′,6′), 12.97 (1H, s, OH).

Cl_2, Cst_2, Cu_4, Tl_3 and Tu_3: 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). ^{13}C-NMR (pyridine-d_5) δ: 18.3 (C_6 of rha), 18.5 (C_6 of rha), 18.8 (C_6 of rha), 47.4 (OCH_3), 100.4 (C_1 of glc), 102.0 (C_1 of rha), 102.3 (C_1 of rha), 103.0 (C_1 of rha), 104.7 (C_1 of glc). Cl_2, Cst_2, Cu_4, Tl_3 and Tu_3 were identified as methyl proto-Pb by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Cst_1, Cu_3 and Tst_1: 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). ^{13}C-NMR (pyridine-d_5) δ: 18.5 (C_6 of rha), 18.6 (C_6 of rha), 47.3 (OCH_3), 100.3 (C_1 of glc), 102.0 (C_1 of rha), 102.9 (C_1 of rha), 104.8 (C_1 of glc). Cst_1, Cu_3 and Tst_1 were identified as methyl proto-dioscin by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Cu_1: Colorless needles from aq. 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). ^{13}C-NMR (pyridine-d_5) δ: 18.4 (C_6 of rha), 18.6 (C_6 of rha), 100.4 (C_1 of glc), 101.9 (C_1 of rha), 103.0 (C_1 of rha). Cu_1 was identified as dioscin by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Cu_2 and Tu_1: 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). ^{13}C-NMR (pyridine-d_5) δ: 18.4 (C_6 of rha), 18.6 (C_6 of rha), 18.8 (C_6 of rha), 100.4 (C_1 of glc), 102.0 (C_1 of rha), 102.3 (C_1 of rha), 103.0 (C_1 of rha). Cu_2 and Tu_1 were identified as Pb by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Cu_5: A white powder from MeOH–AcOEt, (mp 196—199 °C (dec.)), $[\alpha]_D^{20}$ −92.4° ($c=1.02$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3460—3320 (OH). ^{13}C-NMR (pyridine-d_5) δ: 18.2 (C_6 of rha), 18.5 (C_6 of rha), 18.8 (C_6 of rha), 47.3 (OCH_3), 100.4 (C_1 of glc), 102.0 (C_1 of rha), 102.3 (C_1 of rha), 102.6 (C_1 of rha), 104.8 (C_1 of glc), 106.5 (C_1 of glc). Cu_5 was identified as methyl proto-rhapissaponin by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Tl_1: Pale yellow needles from aq. MeOH, mp 265—269 °C (dec.), $[\alpha]_D^{16}$ −48.0° ($c=0.98$, pyridine). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 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. Tl_1 was identified as glucoluteolin by comparing the IR and ^{13}C-NMR spectra with those of an authentic sample.

Tl_2: Pale yellow needles from aq. MeOH, mp 190—192 °C (dec.), $[\alpha]_D^{16}$ −77.3° ($c=0.97$, pyridine). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 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. Tl_2 was identified as scolymoside by comparing the IR and ^{13}C-NMR with those of an authentic sample.

Tl_4 and Tu_4: A white powder from MeOH–AcOEt, (mp 181—183 °C (dec.)), $[\alpha]_D^{20}$ −84.4° ($c=0.90$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3570—3210 (OH). ^{13}C-NMR (pyridine-d_5) δ: aglycone C_1—C_{27}: 37.7, 30.3, 78.4, 39.2, 141.1, 121.8, 32.6, 31.7, 50.6, 37.3, 21.5, 39.9, 43.6, 55.2, 34.7, 84.6, 64.8, 14.3, 19.5, 103.6, 11.8, 152.6, 33.7, 23.9, 31.7, 75.3, 17.4; sugars: 18.5 (C_6 of rha), 18.7 (C_6 of rha), 18.9 (C_6 of rha), 100.6 (C_1 of glc), 102.1 (C_1 of rha), 102.4 (C_1 of rha), 103.2 (C_1 of rha), 104.9 (C_1 of glc). *Anal.* Calcd for $C_{57}H_{92}O_{25}\cdot 3/2H_2O$: C, 56.84; H, 7.95. Found: C, 56.93; H, 8.03.

Tst_2: A white powder from MeOH–AcOEt, (mp 174—176 °C (dec.)), $[\alpha]_D^{20}$ −80.4° ($c=1.01$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3470—3280 (OH). ^{13}C-NMR (pyridine-d_5) δ: aglycone C_1—C_{27}: 37.7, 30.3, 78.2, 39.1, 141.0, 121.8, 32.6, 31.6, 50.6, 37.3, 21.3, 39.9, 43.6, 55.2, 34.6, 84.6, 64.7, 14.3, 19.5, 103.6, 11.8, 152.6, 33.6, 23.8, 31.6, 75.0, 17.4; sugars: 18.4 (C_6 of rha), 18.6 (C_6 of rha), 100.4 (C_1 of glc), 102.0 (C_1 of rha), 102.9 (C_1 of rha), 104.8 (C_1 of glc). *Anal.* Calcd for $C_{51}H_{82}O_{21}$: C, 59.40; H, 8.02. Found: C, 58.95; H, 7.91.

Tu_2: Colorless needles from MeOH, mp 248—250 °C (dec.), $[\alpha]_D^{20}$ −140.3° ($c=1.00$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400—3200 (OH), 980, 920, 900, 890 (intensity 920 < 900, 25(*R*)-spiroketal). ^{13}C-NMR (pyridine-d_5) δ: aglycone C_1—C_{27}: 37.7, 30.3, 78.4, 39.1, 141.0, 121.8, 32.5, 31.9, 50.4, 37.3, 21.1, 32.1, 45.1, 53.2, 32.5, 90.3, 90.2, 17.2, 19.5, 44.9, 9.5, 109.9, 32.1, 28.9, 30.5, 66.9, 17.3; sugars: 18.4 (C_6 of rha), 18.6 (C_6 of rha), 18.8 (C_6 of rha), 100.5 (C_1 of glc), 102.0 (C_1 of rha), 102.3 (C_1 of rha), 103.1 (C_1 of rha). *Anal.* Calcd for $C_{51}H_{82}O_{21}\cdot 3/2H_2O$: C, 57.88; H, 8.10. Found: C, 57.64; H, 8.09. Tu_2 was identified as Tg by comparing the ^{13}C-NMR spectrum with that of an authentic sample.

Acidic Hydrolyses of Tl_4, Tst_2 and Tu_2——Tl_4 (5 mg), Tst_2 (5 mg) and Tu_2 (5 mg) were separately hydrolyzed with 2 N HCl–50% dioxane (2 ml) by refluxing for 2 h on a water bath, then each reaction mixture was neutralized with $NaHCO_3$ and 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; t_R (min): 3.4, 4.4 (TMS-rhamnose), 8.8, 12.0 (TMS-glucose).

Treatments of Tl_3 and Tst_1 with Acetic Acid——Tl_3 (100 mg) and Tst_1 (100 mg) were individually dissolved in acetic acid (30 ml) and refluxed for 30 min. Each reaction mixture was cooled and evaporated to dryness under reduced pressure. The residues were subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.4, v/v) to provide pseudoproto-Pb (25 mg) from Tl_3 and pseudoproto-dioscin (77 mg) from Tst_1 by repeated precipitation from MeOH–AcOEt.

Acknowledgement The authors are grateful to Prof. Toshihiro Nohara of Kumamoto University for providing valuable ^{13}C-NMR data. Thanks are also due to Mr. Susumu Isoda of the Medicinal Plant Garden of Showa University for his kind supply of the plant materials and to the staff of the Analytical Laboratory of this School for elemental analysis and NMR spectral measurement. One of the authors (J. S.) is grateful to the Ministry of Education, Science and Culture of Japan for a Grant-in-Aid for Scientific Research (No. 58570885).

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