Studies on the Constituents of Palmae Plants. VI.[1a] Steroid Saponins and Flavonoids of Leaves of *Phoenix canariensis* hort. ex CHABAUD, *P. humilis* ROYLE var. *hanceana* BECC., *P. dactylifera* L., and *Licuala spinosa* WURMB.

Akitoshi ASAMI, Yasuaki HIRAI, and Junzo SHOJI*

School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142, Japan. *Received February 12, 1991*

Steroid saponins and flavonoids of the leaves of *Phoenix canariensis* hort. ex CHABAUD, *P. humilis* ROYLE var. *hanceana* BECC., *P. dactylifera* L., and *Licuala spinosa* WURMB. have been investigated. Tricin 7-*O*-β-D-glucopyranoside (1), isorhamnetin 3-*O*-β-D-glucopyranoside (2), isoquercitrin (3), isorhamnetin 3-*O*-β-rutinoside (4), rutin (5), and methyl (25*S*)-proto-Pb (6) from *P. canariensis*, 1, 6, glucoluteolin (7), tricin 7-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (8), and methyl (25*S*)-proto-loureiroside (9) from *P. humilis* var. *hanceana*, 1, 2, 8, methyl proto-prosapogenin A of dioscin (10), methyl proto-reclinatoside (11) and methyl proto-Pb (12) from *P. dactylifera*, and vitexin (13) and methyl (25*S*)-proto-dioscin (14) from *Licuala spinosa* have been isolated and identified.

Keywords *Phoenix canariensis*; *Phoenix humilis* var. *hanceana*; *Phoenix dactylifera*; *Licuala spinosa*; Palmae; steroid saponin; furostanol oligoside; diosgenin; flavone glycoside; flavonol glycoside

A series of chemotaxonomical studies on plants of the Palmae family was undertaken,[1] and the constituents of steroid saponin and flavonoids from Palmae plants, *Phoenix rupicola* T. ANDERSON, *P. loureirii* KUNTH, *P. reclinata* N. J. JACQUIN, and *Arecastrum romanzoffianum* BECCARI reported.[1a] The present paper is mainly concerned with studies on the constituents of *Phoenix canariensis* hort. ex CHABAUD, *P. humilis* ROYLE var. *hanceana* BECC., *P. dactylifera* and *Licuala spinosa* WURMB.

The habitat of *P. canariensis* (Japanese name: kanariiyashi) is the Canary Islands, and the plant is cultivated in warm areas of Japan as a shade tree. The habitat of *P. humilis* var. *hanceana* (Sotetsujuro) is Formosa; that of *P. dactylifera* (Natsumeyashi) is along the bay of Persia and the fruit is used for food. Finally, the habitat of *L. spinosa* (Togegoheiyashi) is Malaya.

The leaves of *Phoenix canariensis*, *P. humilis* var. *hanceana*, and *P. dactylifera* harvested in Hachijo island, off the mainland from Tokyo, in January 1986 and *Licuala spinosa* harvested in Okinawa in January 1987 were individually chopped and extracted with methanol at room temperature. Each methanol extract was treated by the method described in the Experimental section.

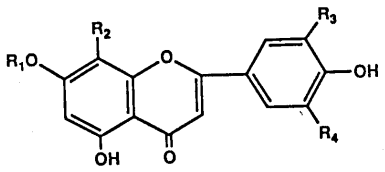
Six compounds (**1**—**6**) were separated from the methanol extract of *P. canariensis*. Compounds **1**—**5** were identified as tricin 7-*O*-β-D-glucopyranoside,[2] isorhamnetin 3-*O*-β-D-glucopyranoside,[3] isoquercitrin,[4] isorhamnetin 3-*O*-β-rutinoside[2] and rutin[5] by comparing the thin layer chromatographic (TLC) behavior, and the infrared (IR) and nuclear magnetic resonance (NMR) spectra with those of respective authentic samples.

6 was positive in the Liebermann–Burchard reaction and in the Ehrlich reaction.[6] The IR spectrum of **6** shows a strong absorption band due to hydroxyl groups and the ^{13}C-NMR spectrum of **6** shows five anomeric carbon signals and methoxyl group signal. Accordingly, **6** was suggested to be a furostanol pentaoside. On enzymatic hydrolysis with almond emulsion, **6** afforded D-glucose and a prosapogenin (**6a**). On acidic hydrolysis, **6a** gave the aglycone, D-glucose and L-rhamnose. The absolute configuration of these sugars was determined using the method of Oshima *et al.*[7] On acetylation, the aglycone of **6a** afforded yamogenin acetate and diosgenin acetate. It is well known that the methyl group at C-25 of yamogenin is easily isomerized under an acidic condition to afford diosgenin.[8] Finally, the genuine aglycone was concluded to be yamogenin by examination of IR and ^{13}C-NMR spectra. The ^{13}C-NMR spectrum of **6a** showed four anomeric carbon signals and three methyl signals corresponding to C-6 methyl group of L-rhamnose. By comparison of ^{13}C-NMR spectra, the structure of sugar moiety of **6a** was deduced to be the same as that of Pb, which has been isolated from the Palmae plant.[1] Finally, **6a** was characterized to be the (25*S*)-isomer of Pb and **6** was the (25*S*)-isomer of methyl proto-Pb. Both these compounds are new steroid saponins hitherto isolated from Palmae plants.

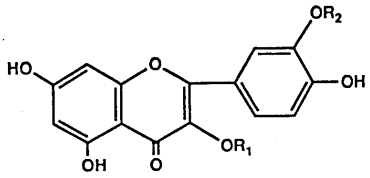
Five compounds, **1** and **6**—**9**, were isolated from the methanol extract of *P. humilis* var. *hanceana*. **7** and **8** were deduced to be glucoluteolin[1e] and tricin 7-neohesperidoside[9] by comparing the TLC behavior, and the IR and NMR spectra with those of the respective authentic sample. The latter compound, **8**, is a known compound, but this is the first report of its isolation from the palmae plant.

Compound **9** was positive in the Liebermann–Burchard reaction and in the Ehrlich reaction. Based on the IR and ^{13}C-NMR spectra, **9** was suggested to be a furostanol pentaoside. On enzymatic hydrolysis with almond emulsion, **9** afforded D-glucose and a prosapogenin (**9a**), which was suggested to be (25*S*)-spirostanol tetraglycoside by IR and ^{13}C-NMR spectra. On acidic hydrolysis, **9a** gave L-arabinose, D-glucose, L-rhamnose and an aglycone, which was deduced to be a mixture of yamogenin and diosgenin. The ^{13}C-NMR spectrum of **9a** showed four anomeric carbon signals and one methyl signal corresponding to C-6 methyl group of L-rhamnose. By comparison of ^{13}C-NMR spectra, the structure of sugar moiety of **9a** was deduced to be the same as that of loureiroside which has been isolated from a palmae plant.[1a] Finally, the structures of new steroid saponins **9a** and **9** were established to be (25*S*)-isomer of loureiroside and (25*S*)-isomer of methyl proto-loureiroside, respectively.

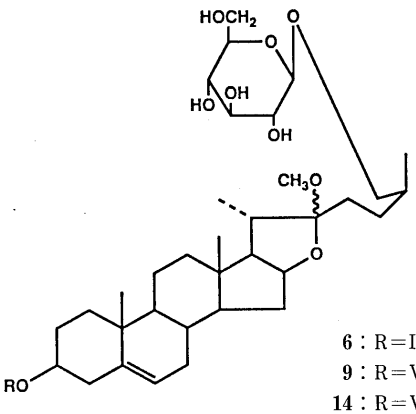
Six compounds, **1**, **2**, **8**, **10**, **11** and **12**, were separated from the methanol extract of *P. dactylifera*; **10**, **11** and **12** were identified as methyl proto-prosapogenin A of dio-



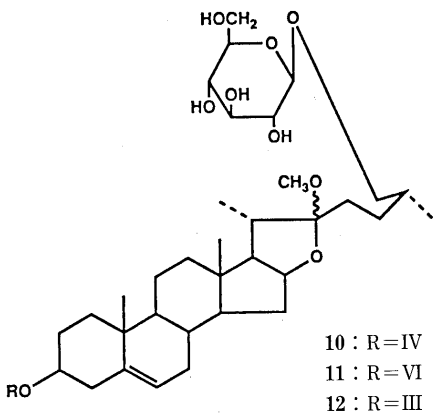
1 : R_1=I, R_2=H, R_3=R_4=OCH_3
7 : R_1=I, R_2=R_4=H, R_3=OH
8 : R_1=IV, R_2=H, R_3=R_4=OCH_3
13 : R_1=R_3=R_4=H, R_2= I



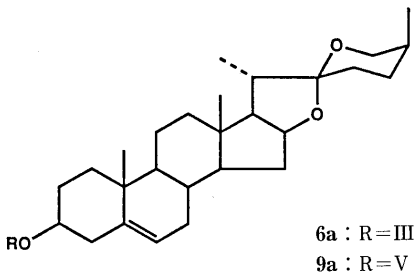
2 : R_1=I, R_2=CH_3
3 : R_1=I, R_2=H
4 : R_1=II, R_2=CH_3
5 : R_1=II, R_2=H



6 : R=III
9 : R=V
14 : R=VII

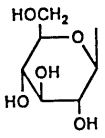


10 : R=IV
11 : R=VI
12 : R=III



6a : R=III
9a : R=V
14a : R=VII

I :



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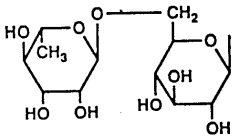
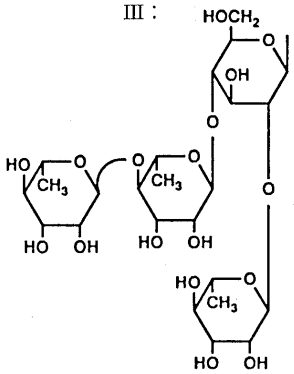
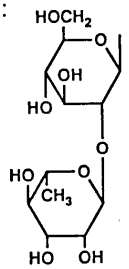


III :



IV :



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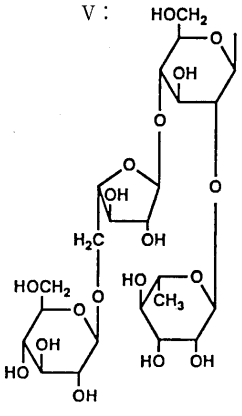

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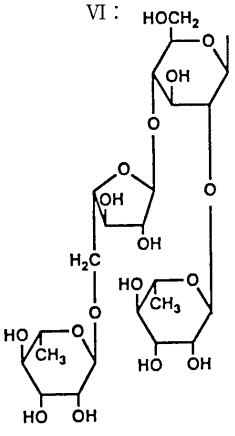

VII :

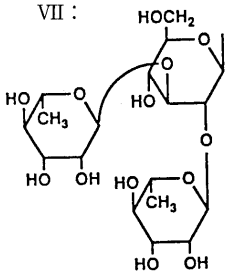

Chart 1

scin,[1d] methyl protoreclinatoside[1a] and methyl proto-Pb,[1e] respectively.

Two compounds, **13** and **14**, were separated from the methanol extract of *Licuala spinosa*, and **13** was identified as vitexin[1d] by direct comparison.

14 was positive in the Liebermann–Burchard reaction and in the Ehrlich reaction. Based on the IR and ^{13}C-NMR spectra, **14** was suggested to be a furostanol tetraoside. On enzymatic hydrolysis with almond emulsin, **14** afforded D-glucose and a prosapogenin (**14a**), which was suggested to be (25*S*)-spirostanol triglycoside by IR and ^{13}C-NMR spectra. On acidic hydrolysis, **14a** gave D-glucose, L-rhamnose and an aglycone, which was suggested to be a mixture of yamogenin and diosgenin. The ^{13}C-NMR spectrum of **14a** showed three anomeric carbon signals and two methyl signals corresponding to C-6 methyl group of L-rhamnose. By comparison of ^{13}C-NMR spectra, the structure of sugar moiety of **13a** was deduced to be the same as that of dioscin, which has been obtained from the Palmae plant, *Trachycarpus fortunei*.[1e] Finally, the structure of **14a** was established to be as the (25*S*)-isomer of dioscin,[10] and that of **14** was the (25*S*)-isomer of methyl proto-dioscin.

We are reporting the isolation of three new steroid saponins, methyl (25*S*)-proto-Pb, methyl (25*S*)-proto-loureiroside and methyl (25*S*)-proto-dioscin, along with three known steroid saponins and eight known flavonoid glycosides. We also described the isolation of tricin 7-neohesperidoside from *P. humilis* var. *hanceana* and *P. dactylifera*. In 1974, Williams and Harborne carried out a chemotaxonomical study on the leaf flavonoids of Saccharum and related genera,[9] and they reported that tricin 7-rutinoside, the isomer of **8**, was found in palms, while 7-neohesperidoside (**8**) was found only in the Saccharinae of the Gramineae. This conclusion is incompatible with our present study and our investigation on the Palmae plants will be continued.

Experimental

All melting points were determined on a Yanaco micro-melting point apparatus (hot-stage type) and are uncorrected. The optical rotations were measured with a JASCO DIP-140 polarimeter at room temperature. The IR spectra were recorded with a JASCO IR-810 and the NMR spectra were recorded with a JEOL GX-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Experimental conditions for sugars: (a) column, 5% SE-52 on Chromosorb W 3 mm × 2 m; column temp., 180 °C; injection temp., 200 °C; carrier gas N_2, 1.2 kg/cm^2; samples, trimethylsilyl (TMS) ether. (b) column, 5% SE-52 on Chromosorb W 3 mm × 2 m; column temp., 170 °C; injection temp., 180 °C; carrier gas N_2, 1.1 kg/cm^2; samples, TMS ether. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) using the following solvents: solvent 1 (solvent for the identification of glycosides); $CHCl_3$–MeOH–H_2O (7:3:0.4, v/v), solvent 2; CH_2Cl_2–acetone (99:1, v/v). Detection was made by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation of the Compounds from the Leaves Fresh leaves of *P. canariensis* hort. ex CHABAUD (5.66 kg), *P. dactylifera* L. (3.74 kg) and *P. humilis* ROYLE var. *hanceana* BECC. (2.4 kg) harvested in Hachijo island, in Tokyo, in January 1986 and dried leaves of *Licuala spinosa* WURMB. (2.40 kg) harvested in Okinawa in January 1987 were respectively chopped and extracted with MeOH at room temperature. a) *P. canariensis*: The MeOH extract was evaporated to dryness *in vacuo*. The residue (294.36 g) was suspended in water (800 ml) and partitioned with ether (200 ml × 5). The ether layer was concentrated *in vacuo* to afford the ether extract (12.38 g), and the aqueous layer was partitioned with BuOH saturated with water (200 ml × 5). The BuOH-soluble layer was concentrated under reduced pressure to afford the BuOH extract (25.49 g) and the aqueous layer was concentrated *in vacuo* to give the water extract (224.86 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to afford four fractions, fr. I (0.18 g), fr. II (2.56 g), fr. III (19.20 g), and fr. IV (0.99 g). Fraction II was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7:3:0.4, v/v) followed with $CHCl_3$–MeOH–H_2O (75:25:3, v/v) to yield **6** (0.11 g). Fraction III was rechromatographed on Sephadex LH-20 with MeOH to fractionate into three fractions (fr. I′ (7.78 g), fr. 2′ (8.92 g), fr. 3′ (0.23 g)). Fraction 2′ was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (7:3:1, v/v, lower phase) to afford five fractions (fr. A′ (2.29 g), fr. B′ (1.94 g), fr. C′ (1.79 g), fr. D′ (0.66 g), fr. E′ (1.91 g)). Fraction B′ was crystallized from MeOH to afford **1** (0.16 g), and the filtrate was subjected to column chromatography on octadecyl silica (ODS) with 60% MeOH to afford **2** (0.68 g). Fraction C′ was chromatographed on Avicel with $CHCl_3$–MeOH–H_2O (75:25:10, v/v, lower phase) followed by ODS with 60% MeOH to afford **3** (0.54 g) and **4** (0.20 g). Fraction D′ was chromatographed on Avicel with $CHCl_3$–MeOH–H_2O (75:25:10, v/v, lower phase) to afford **5** (0.28 g).

b) *P. humilis* var. *hanceana*: The MeOH extract (426.81 g) was treated by the same method to afford the ether extract (9.80 g), the BuOH extract (68.05 g) and the water extract (147.95 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to give four fractions, fr. I (0.15 g), fr. II (7.19 g), fr. III (42.82 g) and fr. IV (14.64 g). Fraction II was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (a: 13:7:1, v/v; b: 7:3:0.4, v/v; c: 13:7:1, v/v) to afford **6** (0.14 g) and **9** (0.95 g). Fraction III was subjected column chromatography on Sephadex LH-20 (MeOH) followed by Avicel ($CHCl_3$–MeOH–H_2O = 7:3:1, v/v, lower phase) to give **1** (0.10 g), **7** (0.25 g) and **8** (0.30 g).

c) *P. dactylifera*: The MeOH extract (156.27 g) was treated in the same way as described in a), and afforded the ether extract (6.57 g), the BuOH extract (11.55 g) and the water extract (137.87 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to give four fractions, fr. I (0.03 g), fr. II (5.34 g), fr. III (6.04 g) and fr. IV (0.61 g). Fraction II was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (a: 13:7:1, v/v; b: 75:25:3, v/v; c: 7:3:0.4, v/v) to afford **10** (0.04 g), **11** (0.95 g) and **6** (0.41 g). Fraction III was subjected to column chromatography on Sephadex LH-20 (MeOH), Avicel ($CHCl_3$–MeOH–H_2O = 7:3:1, v/v, lower phase) and ODS (60% MeOH) to give **1** (0.01 g), **2** (0.02 g) and **8** (0.03 g).

d) *Licuala spinosa*: The MeOH extract (401.33 g) was treated in the same way as described in a), and afforded the ether extract (84.49 g), the BuOH extract (46.57 g) and the water extract (215.90 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to afford five fractions, fr. I (0.25 g), fr. II (11.17 g), fr. III (19.79 g), fr. IV (4.66 g) and fr. V (6.09 g). Fraction II was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (a: 7:3:0.4, v/v; b: 75:25:3, v/v; c: 8:2:0.2, v/v) to afford **14** (0.26 g). Fraction IV was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O = 7:3:1, v/v, lower phase) to afford **13** (0.11 g).

Properties of 1—14 **1**: Yellow needles from MeOH, mp 228—231 °C (dec.), $[\alpha]_D$ −50.7° (c=0.34, pyridine). **2**: Yellow prisms from MeOH, mp 161—163 °C (dec.), $[\alpha]_D$ −10.6° (c=0.39, MeOH). **3**: Yellow needles from aqueous MeOH, mp 181—183 °C (dec.), $[\alpha]_D$ −9.0° (c=0.34, MeOH). **4**: A pale yellow powder from MeOH–AcOEt, (mp 178—180 °C (dec.)), $[\alpha]_D$ −12.4° (c=0.37, MeOH). **5**: Pale yellow needles from H_2O, mp 183—185 °C (dec.), $[\alpha]_D$ +3.8° (c=0.45, EtOH). **7**: Pale yellow needles from aqueous MeOH, mp 239—242 °C (dec.), $[\alpha]_D$ −46.9° (c=0.37, MeOH). **8**: Pale yellow needles from aqueous MeOH, mp 179—181 °C (dec.), $[\alpha]_D$ −84.8° (c=0.34, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3520—3300, 1660, 1620. UV λ_{max}^{MeOH} nm (log ε): 250 (4.21), 271 (4.18), 349 (4.31). $\lambda_{max}^{MeOH+AlCl_3}$ nm: 276, 300, 394. $\lambda_{max}^{MeOH+CH_3COONa}$ nm: 250, 271, 349. *Anal.* Calcd for $C_{29}H_{34}O_{16}\cdot 3/2H_2O$: C, 52.33; H, 5.60. Found: C, 52.08; H, 5.28. The properties of tricin 7-*O*-neohesperidoside were not reported in detail in the literature.[9] **13**: Yellow needles from MeOH, mp 255—258 °C (dec.) $[\alpha]_D$ −84.8° (c=0.34, MeOH). **6**: A white powder from MeOH–AcOEt, (mp 177—179 °C (dec.)), $[\alpha]_D$ −87.2° (c=0.35, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: aglycone; 38.1 (C_1), 30.7 (C_2), 78.6 (C_3), 39.5 (C_4), 141.0 (C_5), 121.8 (C_6), 32.9 (C_7), 32.3 (C_8), 50.9 (C_9), 37.7 (C_{10}), 21.7 (C_{11}), 40.3 (C_{12}), 41.0 (C_{13}), 57.1 (C_{14}), 32.8 (C_{15}), 81.7 (C_{16}), 63.4 (C_{17}), 16.7 (C_{18}), 20.0 (C_{19}), 41.3 (C_{20}), 18.1 (C_{21}), 112.9 (C_{22}), 35.0 (C_{23}), 32.7 (C_{24}), 31.5 (C_{25}), 64.6 (C_{26}), 16.8 (C_{27}), 47.8 (OCH_3). Sugar moiety; glucose (→^{3}aglycone) 100.6 (C_1), 80.6 (C_2), 78.4

(C_3), 77.2 (C_4), 78.9 (C_5), 61.9 (C_6); rhamnose ($\rightarrow^2$glucose) 102.2 (C_1), 72.7 (C_2), 73.1 (C_3), 74.5 (C_4), 69.8 (C_5), 19.3 (C_6); rhamnose ($\rightarrow^4$glucose) 103.2 (C_1), 73.1 (C_2), 70.6 (C_3), 78.0 (C_4), 68.8 (C_5), 19.1 (C_6); rhamnose ($\rightarrow^4$rhamnose) 102.5 (C_1), 72.9 (C_2), 73.0 (C_3), 74.3 (C_4), 73.4 (C_5), 18.9 (C_6); glucose ($\rightarrow^{26}$aglycone) 105.1 (C_1), 75.5 (C_2), 78.9 (C_3), 72.3 (C_4), 78.6 (C_5), 64.6 (C_6). *Anal.* Calcd for $C_{58}H_{96}O_{26}\cdot 2H_2O$: C, 55.93; H, 0.89. Found: C, 55.69; H, 7.68. **9**: A white powder from MeOH–AcOEt, (mp 185—187 °C (dec.)), $[\alpha]_D$ −78.3° ($c=0.37$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: Each signal of the aglycone moiety of **9** was analogous to that of **6**. Sugar moiety; glucose ($\rightarrow^3$aglycone) 100.5 (C_1), 78.9 (C_2), 78.1 (C_3), 76.9 (C_4), 78.5 (C_5), 62.0 (C_6); rhamnose ($\rightarrow^2$glucose) 102.2 (C_1), 73.1 (C_2), 72.6 (C_3), 74.5 (C_4), 69.8 (C_5), 19.1 (C_6); arabinose ($\rightarrow^4$glucose) 109.9 (C_1), 83.4 (C_2), 78.6 (C_3), 84.6 (C_4), 70.6 (C_5); glucose ($\rightarrow^4$arabinose) 105.4 (C_1), 75.3 (C_2), 78.3 (C_3), 72.1 (C_4), 77.9 (C_5), 63.4 (C_6); glucose ($\rightarrow^{26}$aglycone) 105.1 (C_1), 75.5 (C_2), 78.9 (C_3), 72.3 (C_4), 78.6 (C_5), 64.6 (C_6). *Anal.* Calcd for $C_{57}H_{94}O_{27}\cdot H_2O$; C, 55.69; H, 7.87. Found: C, 55.45; H, 7.89. **10**: A white powder from MeOH–AcOEt, (mp 173—177 °C (dec.)), $[\alpha]_D$ −87.6° ($c=0.53$, pyridine). **11**: A white powder from MeOH–AcOEt, (mp 150—152 °C (dec.)), $[\alpha]_D$ −86.7° ($c=0.43$, pyridine). **12**: A white powder from MeOH–AcOEt, (mp 176—179 °C (dec.)), $[\alpha]_D$ −83.1° ($c=0.43$, pyridine). **14**: A white powder from MeOH–AcOEt, (mp 166—168 °C (dec.)), $[\alpha]_D$ −73.0° ($c=0.69$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: Each signal of the aglycone moiety of **9** was analogous to that of **6**. Sugar moiety; glucose ($\rightarrow^3$aglycone) 100.6 (C_1), 79.7 (C_2), 78.2 (C_3), 77.1 (C_4), 78.3 (C_5), 62.0 (C_6); rhamnose ($\rightarrow^2$glucose) 102.1 (C_1), 72.7 (C_2), 73.1 (C_3), 74.5 (C_4), 69.7 (C_5), 19.1 (C_6); rhamnose ($\rightarrow^4$glucose) 103.3 (C_1), 73.2 (C_2), 70.6 (C_3), 78.0 (C_4), 68.8 (C_5), 19.1 (C_6); glucose ($\rightarrow^{26}$aglycone) 105.2 (C_1), 75.3 (C_2), 78.9 (C_3), 72.3 (C_4), 78.7 (C_5), 64.6 (C_6). *Anal.* Calcd for $C_{52}H_{86}O_{22}\cdot 3/2H_2O$: C, 57.28; H, 8.23. Found: C, 57.13; H, 7.82.

Enzymatic Hydrolysis of 6, 9 and 14 Each aqueous solution (30 ml) of **6** (50 mg), **9** (50 mg) and **14** (100 mg) was incubated with almond emulsin (50 mg) at 37 °C for 12 h. The precipitate was collected by filtration and dried. Crude hydrolysates of **6** and **9** were purified on silica gel column chromatography with $CHCl_3$–MeOH–H_2O (75:25:3 v/v) to afford **6a** (28 mg) and **9a** (12 mg), while that of **14** was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8:2:0.2 v/v) to afford **14a** (43 mg). **6a**: Colorless needles from aqueous MeOH, mp 243—245 °C (dec.), $[\alpha]_D$ −115.2° ($c=0.42$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3580—3300 (OH), 988, 920, 900, 842 (intensity 920 > 900, (25*S*)-spiroketal). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: aglycone; 38.1 (C_1), 30.7 (C_2), 78.6 (C_3), 39.6 (C_4), 141.0 (C_5), 121.8 (C_6), 32.9 (C_7), 32.3 (C_8), 50.9 (C_9), 37.7 (C_{10}), 21.8 (C_{11}), 40.5 (C_{12}), 41.0 (C_{13}), 57.2 (C_{14}), 32.8 (C_{15}), 81.5 (C_{16}), 63.3 (C_{17}), 16.9 (C_{18}), 20.0 (C_{19}), 43.0 (C_{20}), 15.4 (C_{21}), 109.9 (C_{22}), 28.1 (C_{23}), 27.1 (C_{24}), 26.8 (C_{25}), 65.5 (C_{26}), 16.9 (C_{27}). Each signal of the sugar moiety attached to C_3 hydroxyl group of the aglycone was analogous to that of **6**. *Anal.* Calcd for $C_{51}H_{82}O_{20}\cdot 3/2H_2O$: C, 58.77; H, 8.22. Found: C, 58.80; H, 8.27. **9a**: Colorless needles from aqueous MeOH, mp 237—239 °C (dec.), $[\alpha]_D$ −85.7° ($c=0.19$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 970, 922, 900, 856 (intensity 922 > 900, (25*S*)-spiroketal). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: Each signal of the aglycone moiety of **9a** was analogous to that of **6a**, and each signal of the sugar moiety attached to C_3 hydroxyl group of the aglycone was analogous to that of **9**. *Anal.* Calcd for $C_{50}H_{80}O_{21}\cdot 3H_2O$: C, 56.06; H, 8.09. Found: C, 56.29; H, 7.81. **14a**: Colorless needles from aqueous MeOH, mp 278—282 °C (dec.), $[\alpha]_D$ −111.0° ($c=0.33$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3500—3310 (OH), 990, 920, 898, 840 (intensity 920 > 900, (25*S*)-spiroketal). ^{13}C-NMR (in C_5D_5N at 50 °C) δ: Each signal of the aglycone moiety of **14a** was analogous to that of **6a**, and each signal of the sugar moiety attached to C_3 hydroxyl group of the aglycone was analogous to that of **14**. *Anal.* Calcd for $C_{45}H_{72}O_{16}\cdot 3/2H_2O$: C, 60.32; H, 8.44. Found: C, 60.14; H, 8.24.

Each filtrate was evaporated to dryness *in vacuo* and the residue was examined by GLC (condition a). GLC t_R (min) 7.5, 11.3 (TMS-glucose).

Acidic Hydrolysis of 6a, 9a and 14a **6a** (10 mg), **9a** (8 mg) and **14a** (10 mg) were separately heated with 2 N HCl–50% dioxane (0.2 ml/mg prosapogenin) for 3 h in a boiling water bath. Each reaction mixture was cooled and neutralized with $NaHCO_3$. The mixture was then diluted with water (1 ml/mg prosapogenin) and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness *in vacuo* and the residue was acetylated in the usual way. Each acetate was compared by TLC with solvent 2. The aqueous layer was evaporated to dryness *in vacuo* and the residue was examined by GLC. **6a** and **14a**: Aglycone; TLC *Rf* 0.73 (diosgenin acetate), 0.70 (yamogenin acetate). Sugars: GLC (condition a) t_R (min) 2.4, 3.2 (TMS-rhamnose), 7.5, 11.3 (TMS-glucose). **9a**: Aglycone; TLC *Rf* 0.73 (diosgenin acetate), 0.70 (yamogenin acetate). Sugars: GLC (condition b) t_R (min) 3.2, 3.7, 4.1 (TMS-arabinose), 3.3, 4.4 (TMS-rhamnose), 11.0, 17.1 (TMS-glucose).

Acidic Hydrolysis of 8 **8** (10 mg) was heated in a water bath with 10% H_2SO_4 (10 ml) for 2 h. The reaction mixture was cooled and the precipitate was collected by filtration to afford an aglycone (**8a**; 4 mg) as pale yellow needles from MeOH, mp 264—267 °C (dec.). The aqueous layer was evaporated to dryness *in vacuo* and the residue was examined by GLC. GLC (condition a) t_R (min) 2.4, 3.2 (TMS-rhamnose), 7.5, 11.3 (TMS-glucose).

Determination of Absolute Configurations of Sugars by HPLC Each solution of **6a**, **8**, **9a** and **14a** (1 mg) in 2 N HCl–50% dioxane (2 ml) was heated in a sealed tube for 3 h at 100 °C. The reaction mixture was diluted with water and evaporated to remove dioxane. The solution was neutralized with Amberlite IRA-93ZU (OH^- form) and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. Each component sugar in the solution was reduced to 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetate using the method of Asada *et al.*[11] and analyzed by HPLC. Condition: column, supermicro bead Silica gel B-5, 5 μm (10 × 250 mm); solvent, hexane–EtOH (9:1); flow rate, 4 ml/min; detection, ultraviolet (UV) (230 nm). t_R (min) **6a**, **8** and **14a**: L-rhamnose 22.8, D-glucose 37.7. **9a**: L-arabinose 33.0, L-rhamnose 22.8, D-glucose 37.7.

Enzymatic Hydrolysis of 8 **8** (5 mg) was dissolved in McIlvaine buffer (pH 4) and incubated with hesperidinase (1 mg) at 37 °C for 0.5 h to afford **1** (2 mg), which was identified by direct comparison.

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