Chemical and Chemotaxonomical Studies of Ferns. LXXXVII.[1] Constituents of *Trichomanes reniforme*

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Received August 11, 1994; accepted November 12, 1994

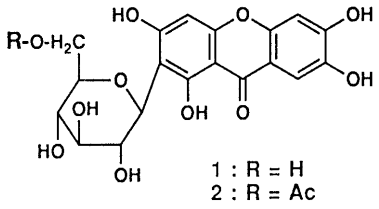
Five new glycosides, 3,4-dihydroxyphenethyl alcohol 4-*O*-caffeoyl-β-D-allopyranoside, (6*S*,13*S*)-13-β-D-fucopyranosyloxy-6-{β-D-fucopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene, (6*S*,13*S*)-13-β-D-fucopyranosyloxy-6-{β-D-quinovopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene, (6*S*,13*S*)-13-α-L-arabinopyranosyloxy-6-{β-D-fucopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene and (6*S*,13*S*)-13-α-L-arabinopyranosyloxy-6-{β-D-quinovopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene, were isolated, together with mangiferin and 6′-*O*-acetylmangiferin, from the fronds of a New Zealand fern, *Trichomanes reniforme*.

Key words *Trichomanes reniforme*; diterpene glycoside; phenylpropanoid; fern; clerodane; xanthone

Trichomanes reniforme FORST. f (Hymenophyllaceae), kidney fern, is endemic to New Zealand. The fronds were used as a medicine to heal ulcers.[2] Two xanthone *C*-glucosides, mangiferin (**1**) and 6′-*O*-acetylmangiferin (**2**), have been isolated from this fern.[3] In our detailed study, five more constituents (**3**—**7**) were isolated, together with **1** and **2**, from the methanol extract of the dried fronds.

Compound **3**, a colorless amorphous solid, $[\alpha]_D$ +4° (c=1.0, MeOH), was formulated as $C_{23}H_{26}O_{11}$ from the secondary ion mass spectrum (SI-MS) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. The proton (^{1}H) and ^{13}C-NMR data revealed the presence of a caffeoyl, a 3,4-dihydroxyphenethyl and a hexose group in the molecule (see Experimental). The hexose was confirmed to be D-allose on acid hydrolysis. The ^{1}H-NMR data of the allosyl part were assigned by coupling patterns as shown in Fig. 1. Considering the large coupling constant of the anomeric proton signal and the down field shift of the C-4 proton signal, the glycosidic linkage was determined as β and the esterified position as C-4. Thus, the structure of **3** was established as 3,4-dihydroxyphenethyl alcohol 4-*O*-caffeoyl-β-D-allopyranoside.

Compound **4**, colorless needles, mp 186—189 °C, $[\alpha]_D$ −46° (c=1.0, MeOH), was formulated as $C_{44}H_{74}O_{18}$ from the high resolution mass spectrum (HR-MS). By ^{13}C–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{1}H COSY, the presence of three fucopyranosyl groups and one rhamnopyranosyl group was revealed (Table II and Experimental). The remaining signals indicated that the aglycone was a diterpene with a secondary and a tertiary hydroxyl group, a vinyl group and a secondary, three tertiary and an olefinic methyl group (Table I and



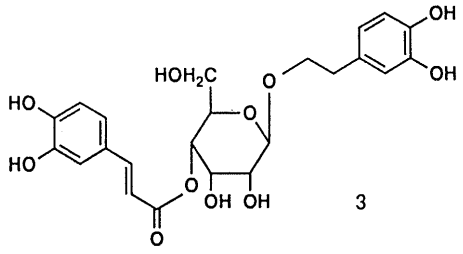


Chart 1

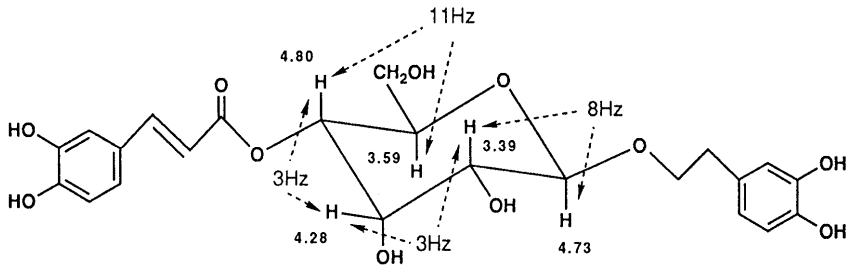


Fig. 1. ^{1}H-NMR Data of β-D-Allosyl Part of **3** in CD_3OD
Chemical shifts (ppm) and coupling constants.

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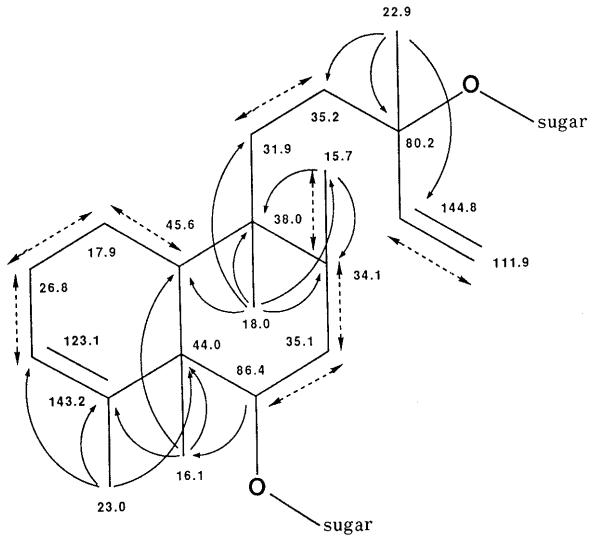

Fig. 2. ^{13}C-NMR Data in C_5D_5N, and ^{1}H–^{1}H COSY (←——→) and Long Range ^{13}C–^{1}H COSY (^{1}H⌒^{13}C) Connections for **4**

TABLE I. ^{13}C-NMR Data of Aglycone Moieties in C_5D_5N

	4	4a	4b	5	5a	6	7
1	17.9	17.9	18.2	17.9	18.0	17.9	17.9
2	26.8	26.9	27.1	26.9	27.0	27.0	26.9
3	123.1	122.8	121.8	123.2	123.1	123.2	123.2
4	143.2	143.6	145.3	143.4	143.6	143.3	143.3
5	44.0	44.1	44.5	44.1	44.2	44.1	44.1
6	86.4	86.3	75.0	86.5	86.5	86.5	86.5
7	35.1	35.3	38.9	35.3	35.4	35.2	35.2
8	34.1	34.2	34.8	34.2	34.3	34.2	34.2
9	38.0	37.9	38.3	38.1	38.0	38.1	38.1
10	45.6	45.9	46.0	45.7	46.0	45.8	45.8
11	31.9	32.5	32.6	32.0	32.6	32.0	32.0
12	35.2	35.8	35.8	35.2	35.8	35.1	34.9
13	80.2	72.5	72.5	80.3	72.6	80.3	80.3
14	144.8	147.2	147.3	145.0	146.3	144.8	145.1
15	111.9	111.3	111.3	114.9	111.3	114.9	111.9
16	22.9	28.6	28.7	22.9	28.7	23.0	23.0
17	15.7	15.9	15.9	15.9	15.9	15.9	15.9
18	23.0	22.9	23.3	23.0	22.9	23.1	23.1
19	16.1	16.2	15.9	16.2	16.2	16.2	16.2
20	18.0	18.3	18.4	18.1	18.2	18.1	18.0

Experimental). By ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and long range ^{13}C–^{1}H COSY, a plane structure of the aglycone was deduced as shown in Fig. 2.

On acid hydrolysis, **4** gave L-rhamnose and D-fucose, but the aglycone was degraded in this condition. On enzymatic hydrolysis with a glycosidase mixture of turbo, **4** gave a mono-rhamnoside of the aglycone (**4a**), a colorless amorphous powder, $[\alpha]_D$ −80° (c=1.0, MeOH). The product **4a** was then subjected to Smith's degradation,[4] and the aglycone (**4b**), colorless needles, mp 149—151 °C, $[\alpha]_D$ −15° (c=1.3, $CHCl_3$), was obtained successfully. The physical properties and spectral data were practically the same as those of (6*S*,13*S*)-cleroda-3,14-dien-6,13-diol.[5-7] The sequence of the sugar moiety was determined by nuclear Overhauser effect correlation spectroscopy (NOESY), where the correlation between H-6 and the anomeric proton of the rhamnosyl group, between CH_3-13 and the anomeric proton of the fucosyl group (C), between H-2 of the rhamnosyl group and the anomeric proton of the fucosyl group (B) and between H-4 of the rhamnosyl group and the anomeric proton of the fucosyl group (A) was observed. Thus, the structure of **4** was determined to be (6*S*,13*S*)-13-β-D-fucopyranosyloxy-6-{β-D-fucopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene.

TABLE II. ^{13}C-NMR Data of Sugar Moieties in C_5D_5N

	4	4a	5	5a	6	7
	Rha	Rha	Rha	Rha	Rha	Rha
1	102.3	103.7	102.4	102.7	102.4	102.4
2	80.8	72.5	80.5	82.2	80.8	80.5
3	72.8	73.9	72.2	72.9	72.9	72.7
4	84.3	72.7	84.6	74.4	84.5	84.5
5	67.9	69.9	68.0	69.6	68.0	68.0
6	17.9	18.1	18.1	18.1	18.0	17.9
	Fuc(A)		Fuc(A)		Fuc(A)	Fuc(A)
1	107.2		106.8		107.3	106.8
2	72.5		73.6		72.6	73.6
3	75.2		75.4		75.3	75.4
4	72.5		72.6		72.8	72.6
5	71.6		71.7		71.7	71.5
6	17.4		17.2		17.4	17.2
	Fuc(B)		Qui	Qui	Fuc(B)	Qui
1	106.6		106.6	107.0	106.6	106.6
2	73.5		76.0	75.8	73.6	76.0
3	75.3		78.1	78.1	75.4	78.0
4	72.6		76.8	76.7	72.8	76.7
5	71.4		73.3	73.3	71.5	73.2
6	17.2		18.7	18.6	17.2	18.7
	Fuc(C)		Fuc(C)		Ara	Ara
1	99.9		100.3		100.3	100.3
2	72.3		72.5		72.8	72.8
3	75.4		75.5		74.7	74.7
4	72.6		72.7		69.6	69.6
5	70.8		71.0		66.8	66.8
6	17.1		17.6			

Rha, rhamnosyl; Fuc, fucosyl; Qui, quinovosyl; Ara, arabinosyl.

Compound **5**, colorless needles, mp 192—194 °C, $[\alpha]_D$ −40° (c=1.0, MeOH), was formulated as $C_{44}H_{74}O_{18}$ from the HR-MS. The ^{1}H- and ^{13}C-NMR spectra coincided with those of **4** except for the signals corresponding to one quinovosyl unit (Tables I and II). The sugars of **5** were confirmed to be L-rhamnose, D-fucose and D-quinovose on acid hydrolysis. On enzymatic hydrolysis with a glycosidase mixture of turbo, **5** gave a partially hydrolyzed product, **5a**, a colorless amorphous powder, $[\alpha]_D$ −25° (c=1.0, MeOH). The structure of **5a** was determined to be a 2′-*O*-β-D-quinovoside of **4a** by comparison of their ^{13}C-NMR data. The differences in chemical shifts between **5a** and **4a** are ascribable to glycosylation at C-2 of the rhamnosyl moiety of **4a**.[8] By application of this glycosylation shift rule between **5** and **5a**, the structure of **5** was determined to be (6*S*,13*S*)-13-β-D-fucopyranosyloxy-6-{β-D-quinovopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene. The sequence of the sugar moiety was also supported by NOESY.

Compound **6**, colorless needles, mp 189—191 °C, $[\alpha]_D$ −32° (c=1.0, MeOH), was formulated as $C_{43}H_{72}O_{18}$ from the HR-MS. The ^{1}H- and ^{13}C-NMR data coincided with those of **4** except for the signals corresponding to an

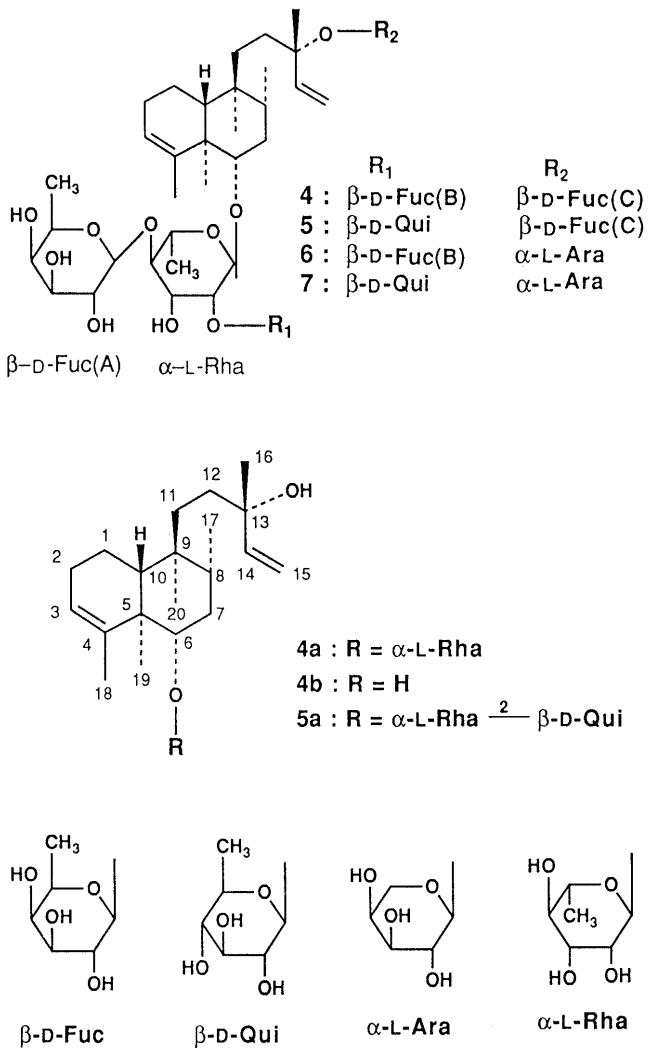

Chart 2

arabinose unit. The sugars were identified as L-rhamnose, D-fucose and L-arabinose on acid hydrolysis. On enzymatic hydrolysis with a glycosidase mixture of turbo and crude hesperidinase, **6** gave **4a**. By application of the glycosylation shift rule and NOESY, the structure of **6** was determined as (6*S*,13*S*)-13-α-L-arabinopyranosyloxy-6-{β-D-fucopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene.

Compound **7**, a colorless amorphous solid, $[\alpha]_D$ −38° (c=1.0, MeOH), was formulated as $C_{43}H_{72}O_{18}$ from the HR-MS, and showed similar NMR spectra to those of **5** except for the signals corresponding to an arabinose unit. The sugars were identified as L-rhamnose, D-fucose, D-quinovose and L-arabinose on acid hydrolysis. On enzymatic hydrolysis with a glycosidase mixture of turbo and crude hesperidinase, **7** gave **5a**, and the structure of **7** was determined, in the same manner mentioned above, to be (6*S*,13*S*)-13-α-L-arabinopyranosyloxy-6-{β-D-quinovopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)-α-L-rhamnopyranosyloxy]}-cleroda-3,14-diene.

Recently, Suga and his co-workers reported the presence of similar clerodane-type glycosides from the two Gleicheniaceous ferns, *Dicranopteris pedata* NAKAIKE and *Gleichenia japonica* SPRENG.[5] Though a taxonomical affinity of *Trichomanes* for Gleicheniaceous ferns has not been found at present, the chemical data suggests a close relationship between the two.

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic polarimeter. The ^{13}C-NMR spectra were measured with a JEOL GSX-500 spectrometer. The ^{1}H-NMR spectra were measured with a JEOL GSX-500 (500 MHz) and a FX-100 (100 MHz) spectrometer (multiplicity, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, NC: coupling patterns were not confirmed because of overlapping signals). Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer and infrared (IR) spectra on a Shimadzu IR-460 spectrometer. Mass spectra were measured with a JEOL SX-102 spectrometer (FAB-MS) or a Hitachi M-80A spectrometer (SI-MS). Gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) were run on a Shimadzu GC-8A apparatus using an HR-1701 capillary column (0.3 mm i.d. × 30 m) and a Shimadzu LC-9A apparatus with a UV detector (Shimadzu SPD-6AV), respectively.

Isolation The air-dried fronds (120 g) of *Trichomanes reniforme* collected in July in Auckland, New Zealand, were extracted twice with 2 l of MeOH under reflux for 6 h. The extracts and then 5 l of MeOH were passed over a column of activated charcoal (20 g). The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel using $CHCl_3$ and MeOH and on Sephadex LH-20 using MeOH and H_2O. The fractions containing **1** and **2** were collected and chromatographed on polyamide using EtOAc and MeOH to give **1** (70 mg) and **2** (18 mg). The fractions containing **3** were collected and chromatographed on silica gel using a mixture of $CHCl_3$, MeOH and H_2O (6:4:1) to give **3** (27 mg). The fractions containing **4**, **5**, **6** and **7** were subjected to HPLC on TSK GEL Carbon 500 [Tosoh, 4.6 mm i.d. × 250 mm, MeCN–H_2O (1:1)] to give **4** (130 mg), **5** (65 mg), **6** (70 mg) and **7** (68 mg).

Compound 1 (Mangiferin) Pale yellow needles from MeOH, mp 278—280 °C, $[\alpha]_D$ +27° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 243 (4.56), 259 (4.61), 318 (4.26), 368 (4.22). IR (KBr) ν_{max} cm^{-1}: 3355, 1648, 1611, 1514, 1484, 1050, 845, 818. ^{1}H-NMR (DMSO-d_6) δ: 7.36 (1H, s), 6.86 (1H, s), 6.37 (1H, s), 4.64 (1H, d, J=8 Hz), 3.8—3.0 (6H). ^{13}C-NMR (DMSO-d_6) δ: 179.2 (C), 163.9 (C), 161.8 (C), 156.3 (C), 154.1 (C), 150.9 (C), 143.8 (C), 111.8 (C), 108.2 (CH), 107.7 (C), 102.7 (CH), 101.4 (C), 93.4 (CH), 81.6 (CH), 79.0 (CH), 73.2 (CH), 70.7 (CH), 70.4 (CH), 61.6 (CH_2). Its physical properties and spectral data were the same as those of an authentic sample obtained from *Hypodematium fauriei*.[9]

Compound 2 (6′-*O*-Acetylmangiferin) A pale yellow amorphous solid, $[\alpha]_D$ +32° (c=0.25, C_5H_5N). UV (MeOH) λ_{max} nm (log ε): 243 (4.46), 259 (4.48), 317 (4.17), 368 (4.14). ^{1}H-NMR (DMSO-d_6) δ: 7.34 (1H, s), 6.82 (1H, s), 6.34 (1H, s), 4.60 (1H, d, J=8 Hz), 3.0—4.0 (6H), 1.98 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 179.1 (C), 170.5 (C), 163.9 (C), 161.9 (C), 156.3 (C), 154.3 (C), 150.9 (C), 143.9 (C), 111.7 (C), 108.1 (CH), 107.4 (C), 102.7 (C), 101.4 (C), 93.4 (CH), 79.0 (CH), 78.8 (CH), 73.2 (CH), 70.5 (CH), 70.0 (CH), 64.6 (CH_2), 20.8 (CH_3). FAB-MS m/z: 464 (M^+). Its physical properties and spectral data were the same as those reported.[3] On acid hydrolysis, **2** gave mangiferin (**1**).

Compound 3 (3,4-Dihydroxyphenethyl Alcohol 4-*O*-Caffeoyl-β-D-alloside) A colorless amorphous solid, $[\alpha]_D$ +4° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 252 (4.12), 293 (4.08), 333 (4.21). ^{1}H-NMR (100 MHz, CD_3OD) δ: 7.60 (1H, d, J=16 Hz), 7.04 (1H, d, J=2 Hz), 6.95 (1H, dd, J=2, 8 Hz), 6.77 (1H, d, J=8 Hz), 6.69 (1H, d, J=2 Hz), 6.66 (1H, d, J=8 Hz), 6.56 (1H, dd, J=2, 8 Hz), 6.30 (1H, d, J=16 Hz), 4.80 (1H, dd, J=11, 3 Hz), 4.73 (1H, d, J=8 Hz), 4.28 (1H, t, J=3 Hz), 4.01 (2H, m), 3.71 (2H, d, J=6 Hz), 3.59 (1H, dt, J=11, 6 Hz), 3.39 (1H, dd, J=8, 3 Hz), 2.78 (2H, m). ^{13}C-NMR (CD_3OD) δ: 3,4-dihydroxyphenethyloxy moiety; 131.6 (C-1), 116.6 (C-2), 144.7 (C-3), 146.1 (C-4), 117.1 (C-5), 121.3 (C-6), 72.2 (C-7), 36.7 (C-8), caffeoyl moiety; 127.8 (C-1), 114.8 (C-2), 149.7 (C-3), 146.9 (C-4), 116.3 (C-5), 123.1 (C-6), 147.6 (C-7), 115.2 (C-8), 168.1 (C-9), allosyl moiety; 100.2 (C-1), 72.0 (C-2), 70.7 (C-3), 70.5 (C-4), 73.4 (C-5), 62.5 (C-6). The chemical shifts of 3,4-dihydroxyphenethyloxy and caffeoyl moieties are the same as those reported.[10] SI-MS m/z: 479 $[M+H]^+$.

Acid Hydrolysis of 3 Compound **3** (10 mg) was hydrolyzed with 3% HCl (5 ml) under reflux for 1 h. The reaction mixture was concentrated and chromatographed on silica gel using 20% MeOH in $CHCl_3$ as an eluent to yield 3 mg of D-allose, $[\alpha]_D$ +10° (c=0.3). Its trimethylsilyl

ether was identical to an authentic sample on GLC.

Compound 4 Colorless needles from MeOH, mp 186—189 °C, $[\alpha]_D$ −46° (c=1.0, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1630, 1370, 1065, 950, 900. ^{1}H-NMR (C_5D_5N) δ: aglycone, 0.65 (3H, s, H_3-20), 0.69 (3H, d, J=6.6 Hz, H_3-17), 1.04 (3H, s, H_3-19), 1.58 (3H, s, H_3-16), 1.90 (3H, s, H_3-18), 1.32 (1H, d, J=11.7 Hz, H-10), 2.20 (1H, dt, J=13.6, 4.4 Hz, H-7), 3.15 (1H, dd, J=11.0, 4.4 Hz, H-6), 5.12 (1H, br s, H-3), 5.28 (1H, dd, J=11.0, 1.1 Hz, H-15), 5.42 (1H, dd, J=17.6, 1.1 Hz, H-15), 6.25 (1H, dd, J=17.6, 11.0 Hz, H-14); fucosyl (A), 5.05 (1H, d, J=7.7 Hz, H-1), 4.45 (1H, dd, J=7.7, 8.0 Hz, H-2), 4.07 (1H, dd, J=8.0, 3.3 Hz, H-3), 4.03 (1H, dd, J=3.3, 0.7 Hz, H-4), 3.78 (1H, dq, J=0.7, 6.6 Hz, H-5), 1.57 (3H, d, J=6.6 Hz, H_3-6); fucosyl (B), 4.73 (1H, d, J=7.7 Hz, H-1), 4.30 (1H, dd, J=7.7, 9.2 Hz, H-2), 3.99 (1H, dd, J=9.2, 3.3 Hz, H-3), 4.02 (1H, dd, J=3.3, 1.1 Hz, H-4), 3.55 (1H, dq, J=1.1, 6.6 Hz, H-5), 1.51 (3H, d, J=6.6 Hz, H_3-6); fucosyl (C), 4.96 (1H, d, J=8.1 Hz, H-1), 4.40 (1H, dd, J=8.1, 7.7 Hz, H-2), 4.04 (1H, dd, J=7.7, 3.3 Hz, H-3), 3.95 (1H, dd, J=3.3, 1.0 Hz, H-4), 3.71 (1H, dq, J=1.0, 6.2 Hz, H-5), 1.40 (3H, d, J=6.2 Hz, H_3-6); rhamnosyl, 5.49 (1H, br s, H-1), 4.46 (1H, d, J=1.5 Hz, H-2), 4.48 (1H, dd, J=1.5, 9.6 Hz, H-3), 4.21 (1H, dd, J=9.6, 9.6 Hz, H-4), 4.11 (1H, dq, J=9.6, 6.2 Hz, H-5), 1.64 (1H, d, J=6.2 Hz, H_3-6). HR-FAB-MS (negative mode) m/z: 889.481 $[M-H]^-$, Calcd for $C_{44}H_{73}O_{18}$: 889.480.

Acid Hydrolysis of 4 Compound **4** (30 mg) was hydrolyzed with 3% HCl (7 ml) under reflux for 1 h. The reaction mixture was concentrated under reduced pressure and chromatographed on silica gel using $CHCl_3$ and MeOH to obtain sugar fractions. The sugar fractions were collected and subjected to HPLC (ODS, 20% H_2O/MeOH) to yield D-fucose (7 mg, $[\alpha]_D$ +75°) and L-rhamnose (2 mg, $[\alpha]_D$ +10°). Their trimethylsilyl ethers were identified with authentic samples on GLC.

Enzymatic Hydrolysis of 4 A solution of **4** (70 mg) and a glycosidase mixture of turbo (100 mg, Seikagaku Kogyo Co., Ltd.) in 0.05 M citrate buffer (pH 4.0, 30 ml) was stirred at 40 °C for 17 h. The reaction mixture was extracted with n-BuOH (100 ml). The extract was washed with water and concentrated and chromatographed on silica gel using $CHCl_3$ and MeOH to get **4a** (32 mg).

Compound 4a A colorless amorphous powder, $[\alpha]_D$ −40° (c=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ : aglycone, 0.71 (3H, s, H_3-20), 0.78 (3H, d, J=6.6 Hz, H_3-17), 1.08 (3H, s, H_3-19), 1.50 (3H, s, H_3-16), 1.78 (3H, d, J=1.1 Hz, H_3-18), 1.32 (1H, d, J=11.7 Hz, H-10), 2.20 (1H, dt, J=13.6, 4.6 Hz, H-7), 3.43 (1H, dd, J=11.2, 4.6 Hz, H-6), 5.12 (1H, br s, H-3), 5.18 (1H, dd, J=10.6, 2.0 Hz, H-15), 5.57 (1H, dd, J=17.2, 2.0 Hz, H-15), 6.17 (1H, dd, J=17.2, 10.6 Hz, H-14); rhamnosyl, 5.38 (1H, d, J=1.1 Hz, H-1), 4.50 (1H, dd, J=1.1, 3.3 Hz, H-2), 4.44 (1H, dd, J=3.3, 8.8 Hz, H-3), 4.29 (1H, dd, J=8.8, 8.8 Hz, H-4), 4.30 (1H, dq, J=8.8, 5.5 Hz, H-5), 1.66 (3H, d, J=5.5 Hz, H_3-6). FAB-MS (negative mode) m/z: 451 $[M-H]^-$.

Smith's Degradation of 4a A solution of $NaIO_4$ (180 mg) in H_2O (18 ml) was added to a solution of **4a** (30 mg) in MeOH (45 ml) and stirred at room temperature for 24 h. The reaction mixture was extracted with EtOAc and the extract was washed, dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in MeOH (10 ml) and reduced with $NaBH_4$ (100 mg) for 1 h. The reaction mixture was adjusted to pH 2 with 2% H_2SO_4 and allowed to stand at room temperature for 24 h. The product was extracted with EtOAc, washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using $CHCl_3$ and EtOAc to get **4b** (12 mg).

Compound 4b Colorless needles from MeOH, mp 149—151 °C, $[\alpha]_D$ −14° (c=1.2, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3350, 1630, 1380, 1080, 920. ^{1}H-NMR ($CDCl_3$) δ: 0.71 (3H, s, H_3-20), 0.79 (3H, d, J=6.6 Hz, H_3-17), 1.00 (3H, s, H_3-19), 1.27 (3H, s, H_3-16), 1.83 (3H, dd, J=4.0, 1.1 Hz, H_3-18), 1.25 (1H, dd, J=11.9, 2.4 Hz, H-10), 3.52 (1H, dd, J=11.0, 5.3 Hz, H-6), 5.06 (1H, dd, J=10.8, 1.2 Hz, H-15), 5.20 (1H, dd, J=17.4, 1.2 Hz, H-15), 5.23 (1H, br s, H-3), 5.87 (1H, dd, J=17.4, 10.8 Hz, H-14); (C_5D_5N) δ: 0.77 (3H, s, H_3-20), 0.82 (3H, d, J=6.6 Hz, H_3-17), 1.29 (3H, s, H_3-19), 1.51 (3H, s, H_3-16), 2.20 (3H, br s, H_3-18), 1.45 (1H, dd, J=11.9, 1.2 Hz, H-10), 3.75 (1H, dd, J=8.2, 7.6 Hz, H-6), 5.18 (1H, dd, J=10.7, 2.1 Hz, H-15), 5.26 (1H, br s, H-3), 5.58 (1H, dd, J=17.4, 2.1 Hz, H-15), 6.17 (1H, dd, J=17.4, 10.7 Hz, H-14). ^{13}C-NMR ($CDCl_3$) δ: 17.7 (C-1), 26.2 (C-2), 122.3 (C-3), 143.7 (C-4), 44.0 (C-5), 75.7 (C-6), 37.8 (C-7), 34.4 (C-8), 38.0 (C-9), 45.4 (C-10), 31.8 (C-11), 34.9 (C-12), 73.4 (C-13), 145.1 (C-14), 111.9 (C-15), 27.9 (C-16), 14.9 (C-17), 22.4 (C-18), 15.5 (C-19), 18.0 (C-20). FAB-MS (negative mode) m/z: 305 $[M-H]^-$.

Compound 5 Colorless needles from MeOH, mp 192—194 °C, $[\alpha]_D$ −40° (c=1.0, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1630, 1370, 1065, 950, 900. ^{1}H-NMR (C_5D_5N) δ: 0.67 (3H, s, H_3-20), 0.71 (3H, d, J=6.7 Hz, H_3-17), 1.07 (3H, s, H_3-19), 1.61 (3H, s, H_3-16), 1.93 (3H, s, H_3-18), 1.18 (1H, d, J=11.9 Hz, H-10), 2.02 (1H, dd, J=13.4, 4.0 Hz, H-7), 3.37 (1H, dd, J=11.3, 4.0 Hz, H-6), 5.18 (1H, br s, H-3), 5.28 (1H, dd, J=11.0, 1.2 Hz, H-15), 5.45 (1H, dd, J=17.7, 1.2 Hz, H-15), 6.28 (1H, dd, J=17.7, 11.0 Hz, H-14); fucosyl (A), 5.02 (1H, d, J=7.9 Hz, H-1), 4.43 (1H, dd, J=7.9, 8.2 Hz, H-2), 4.02 (1H, dd, J=8.2, 3.2 Hz, H-3), 3.98 (1H, dd, J=3.2, 0.8 Hz, H-4), 3.60 (1H, dq, J=0.8, 6.4 Hz, H-5), 1.48 (3H, d, J=6.4 Hz, H_3-6); fucosyl (B), 4.80 (1H, d, J=7.6 Hz, H-1), 4.33 (1H, dd, J=7.6, 9.2 Hz, H-2), 4.08 (1H, dd, J=9.2, 3.5 Hz, H-3), 4.28 (1H, dd, J=3.5, 0.8 Hz, H-4), 3.74 (1H, dq, J=0.8, 6.4 Hz, H-5), 1.54 (3H, d, J=6.4 Hz, H_3-6); quinovosyl, 5.22 (1H, d, J=7.6 Hz, H-1), 4.11 (1H, NC, H-2), 4.17 (1H, NC, H-3), 3.78 (1H, NC, H-4), 3.79 (1H, q, J=6.4 Hz, H-5), 1.68 (3H, d, J=6.4 Hz, H_3-6); rhamnosyl, 5.51 (1H, br s, H-1), 4.52 (1H, br s, H-2), 4.52 (1H, d, J=9.5 Hz, H-3), 4.28 (1H, t, J=9.5 Hz, H-4), 4.14 (1H, NC, H-5), 1.48 (3H, d, J=6.4 Hz, H_3-6). HR-FAB-MS (negative mode) m/z: 889.481 $[M-H]^-$, Calcd for $C_{44}H_{73}O_{18}$: 889.480.

Acid Hydrolysis of 5 Compound **5** (20 mg) was hydrolyzed with 3% HCl (7 ml) and the sugars were isolated in the same manner as **4**; D-fucose, $[\alpha]_D$ +70° (c=0.2, H_2O); L-rhamnose, $[\alpha]_D$ +10° (c=0.1, H_2O); D-quinovose, $[\alpha]_D$ +26° (c=0.2, H_2O). Their trimethylsilyl ethers were identified with authentic samples on GLC.

Enzymatic Hydrolysis of 5 Compound **5** (25 mg) was hydrolyzed in the same way as **4** to yield **5a** (11 mg).

Compound 5a A colorless amorphous powder, $[\alpha]_D$ −25° (c=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.71 (3H, s, H_3-20), 0.76 (3H, d, J=6.7 Hz, H_3-17), 1.10 (3H, s, H_3-19), 1.52 (3H, s, H_3-16), 1.96 (3H, br s, H_3-18), 1.33 (1H, d, J=12.2 Hz, H-10), 2.10 (1H, dt, J=12.8, 3.8 Hz, H-7), 3.42 (1H, dd, J=11.3, 4.6 Hz, H-6), 5.19 (1H, dd, J=10.7, 1.8 Hz, H-15), 5.23 (1H, br s, H-3), 5.59 (1H, dd, J=17.4, 1.8 Hz, H-15), 6.19 (1H, dd, J=17.4, 10.7 Hz, H-14); rhamnosyl, 5.56 (1H, br s, H-1), 4.50 (1H, d, J=3.3 Hz, H-2), 4.45 (1H, dd, J=3.3, 8.8 Hz, H-3), 4.20 (1H, t, J=8.8 Hz, H-4), 4.24 (1H, dq, J=8.8, 5.8 Hz, H-5), 1.61 (3H, d, J=5.8 Hz, H_3-6); quinovosyl, 5.19 (1H, d, J=8.4 Hz, H-1), 4.08 (1H, t, J=8.4 Hz, H-2), 4.15 (1H, t, J=8.4 Hz, H-3), 3.71 (1H, t, J=8.4 Hz, H-4), 3.77 (1H, dq, J=8.4, 5.8 Hz, H-5), 1.66 (3H, d, J=5.8 Hz, H_3-6). FAB-MS (negative mode) m/z: 597 $[M-H]^-$.

Compound 6 Colorless needles from MeOH, mp 189—191 °C, $[\alpha]_D$ −32° (c=1.0, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1630, 1370, 1065, 950, 900. ^{1}H-NMR (C_5D_5N) δ: 0.69 (3H, s, H_3-20), 0.72 (3H, d, J=6.7 Hz, H_3-17), 1.07 (3H, s, H_3-19), 1.60 (3H, s, H_3-16), 1.93 (3H, s, H_3-18), 1.18 (1H, d, J=12.2 Hz, H-10), 2.01 (1H, dt, J=9.6, 4.4 Hz, H-7), 3.34 (1H, dd, J=11.1, 4.4 Hz, H-6), 5.19 (1H, br s, H-3), 5.28 (1H, d, J=11.0 Hz, H-15), 5.43 (1H, d, J=17.7 Hz, H-15), 6.24 (1H, dd, J=17.7, 11.0 Hz, H-14); fucosyl (A), 5.08 (1H, d, J=7.8 Hz, H-1), 4.50 (1H, dd, J=7.8, 8.9 Hz, H-2), 4.12 (1H, dd, J=8.9, 3.4 Hz, H-3), 4.06 (1H, dd, J=3.4, 1.0 Hz, H-4), 3.81 (1H, dq, J=1.0, 6.4 Hz, H-5), 1.60 (3H, d, J=6.4 Hz, H_3-6); fucosyl (B), 4.97 (1H, d, J=7.9 Hz, H-1), 4.42 (1H, dd, J=7.9, 9.5 Hz, H-2), 4.01 (1H, dd, J=9.5, 3.4 Hz, H-3), 3.97 (1H, dd, J=3.4, 0.9 Hz, H-4), 3.56 (1H, dq, J=0.9, 6.4 Hz, H-5), 1.47 (3H, d, J=6.4 Hz, H_3-6); arabinosyl, 4.81 (1H, d, J=7.0 Hz, H-1), 4.40 (1H, dd, J=7.0, 8.9 Hz, H-2), 4.14 (1H, NC, H-3), 4.29 (1H, NC, H-4), 3.72 (1H, NC, H-5), 4.26 (1H, NC, H-5); rhamnosyl, 5.51 (1H, br s, H-1), 4.49 (1H, br s, H-2), 4.50 (1H, d, J=10.6 Hz, H-3), 4.22 (1H, t, J=10.6 Hz, H-4), 4.13 (1H, dq, J=10.6, 5.8 Hz, H-5), 1.67 (3H, d, J=5.8 Hz, H_3-6). HR-FAB-MS (negative mode) m/z: 875.464 $[M-H]^-$, Calcd for $C_{43}H_{71}O_{18}$: 875.464.

Acid Hydrolysis of 6 Compound **6** (30 mg) was hydrolyzed in the same manner as **4** to get D-fucose (4 mg, $[\alpha]_D$ +70°), L-rhamnose (2 mg, $[\alpha]_D$ +10°) and L-arabinose (2 mg, $[\alpha]_D$ +80°).

Enzymatic Hydrolysis of 6 Compound **6** (25 mg) was hydrolyzed with a glycosidase mixture of turbo (100 mg) and crude hesperidinase (100 mg) in 0.05 M citrate buffer (pH 4.0, 30 ml) to get **4a** (11 mg).

Compound 7 A colorless powder, $[\alpha]_D$ −38° (c=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.68 (3H, s, H_3-20), 0.72 (3H, d, J=6.7 Hz, H_3-17), 1.07 (3H, s, H_3-19), 1.60 (3H, s, H_3-16), 1.92 (3H, s, H_3-18), 1.19 (1H, d, J=12.2 Hz, H-10), 2.01 (1H, dd, J=9.8, 4.6 Hz, H-7), 3.36 (1H, dd, J=11.3, 4.6 Hz, H-6), 5.19 (1H, br s, H-3), 5.28 (1H, d, J=10.7 Hz, H-15), 5.43 (1H, d, J=17.7 Hz, H-15), 6.23 (1H, dd, J=17.7, 10.7 Hz, H-14); quinovosyl, 5.20 (1H, d, J=7.6 Hz, H-1), 4.11 (1H, NC, H-2), 4.15 (1H, NC, H-3), 3.76 (1H, NC, H-4), 3.77 (1H, NC, H-5), 1.68 (3H, d, J=6.6 Hz, H_3-6); fucosyl, 5.02 (1H, d, J=7.9 Hz, H-1), 4.43 (1H, dd, J=7.9, 9.3 Hz, H-2), 4.02 (1H, dd, J=9.3, 3.2 Hz, H-3), 3.98 (1H, dd,

J=3.2, 1.0 Hz, H-4), 3.60 (1H, dq, J=1.0, 6.4 Hz, H-5), 1.48 (3H, d, J=6.4 Hz, H_3-6); arabinosyl, 4.81 (1H, d, J=7.0 Hz, H-1), 4.40 (1H, NC, H-2), 4.13 (1H, NC, H-3), 4.28 (1H, NC, H-4), 3.71 (1H, dd, J=12.2, 6.2 Hz, H-5), 4.25 (1H, dd, J=12.2, 4.0 Hz, H-5); rhamnosyl, 5.50 (1H, br s, H-1), 4.51 (1H, br s, H-2), 4.51 (1H, d, J=9.1 Hz, H-3), 4.29 (1H, t, J=9.1 Hz, H-4), 4.14 (1H, dq, J=9.1, 6.4 Hz, H-5), 1.48 (3H, d, J=6.4 Hz, H_3-6). HR-FAB-MS (negative mode) m/z: 875.464 $[M-H]^-$, Calcd for $C_{43}H_{71}O_{18}$: 875.464.

Acid Hydrolysis of 7 Compound **7** (25 mg) was hydrolyzed in the same manner as **4** to get D-fucose (2 mg, $[\alpha]_D$ +75°), L-rhamnose (2 mg, $[\alpha]_D$ +10°), D-quinovose (1 mg, $[\alpha]_D$ +30°) and L-arabinose (2 mg, $[\alpha]_D$ +70°).

Enzymatic Hydrolysis of 7 Compound **7** (25 mg) was hydrolyzed in the same manner as **6** to get **5a** (9 mg).

References and Notes

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