Studies on the Constituents of *Thladiantha dubia* BUNGE. II.[1] Structures of Dubiosides D, E and F, Neutral Saponins of Quillaic Acid Isolated from the Tuber

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Three neutral 3,28-*O*-bisdesmosidic glycosides of quillaic acid, named dubiosides D, E and F, were isolated from the tuber of *Thladiantha dubia* BUNGE (Cucurbitaceae). Their structures were elucidated on the basis of chemical and spectral evidence.

All dubiosides have a common prosapogenin structure, 3-*O*-[*O*-β-D-glucopyranosyl-(1→3)-[*O*-β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl]-quillaic acid, and differ only in the structures of the ester-linked sugar moieties. Dubioside D is a 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, dubioside E, a 28-[*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester and dubioside F, a 28-[*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester of the prosapogenin.

Keywords *Thladiantha dubia*; Cucurbitaceae; triterpene glycoside; dubioside; quillaic acid 3,28-*O*-bisdesmoside; quillaic acid

The tuber of *Thladiantha dubia* BUNGE (Cucurbitaceae) contains a considerable amount of saponins. Three glucuronide saponins named dubiosides A, B and C were isolated as their methyl esters, and their structures were reported in our preceding paper.[1] These glucuronide saponins were accompanied by neutral saponins having a similar polarity. Thin-layer chromatography (TLC) (normal and reversed-phase TLC plates) of the neutral glycoside fraction revealed the presence of three saponins, and they were named dubiosides D, E and F in order of increasing polarity. They were isolated by chromatography on a reversed-phase material (LiChroprep RP-18) and silica gel. This paper gives details of their structures.

Dubioside D (I) was obtained as a white amorphous powder. Its fast-atom bombardment mass spectrum (FAB-MS) revealed an $[M+Na]^+$ ion at *m/z* 1273 and an $[M-H]^-$ ion at *m/z* 1249, indicating the molecular weight to be 1250. The results of elemental analysis were consistent with the molecular formula $C_{59}H_{94}O_{28}\cdot 2H_2O$. Compound I yielded D-glucose, D-galactose, L-rhamnose and L-arabinose on acid hydrolysis.

The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum of I showed signals of six tertiary methyl groups (δ 0.83, 1.03×2, 1.14, 1.41 and 1.74), one secondary methyl group [δ 1.66 (d, *J*=6 Hz)], one trisubstituted olefinic proton (δ 5.59, br s), one aldehydic proton (δ 9.86, s) and five anomeric protons [δ 4.71 (d, *J*=8 Hz); 5.25 (d, *J*=8 Hz); 5.52 (d, *J*=8 Hz); 5.79 (s) and 6.46 (d, *J*=2 Hz)].

The ^{13}C-NMR spectrum (Table I) revealed signals of six C–C bonded quaternary carbons (δ 30.9, 36.3, 40.3, 42.1, 49.5 and 55.1), one ester carbonyl carbon (δ 175.8), a pair of olefinic carbons (δ 122.4 and 144.6), an aldehydic carbon (δ 209.8) and five anomeric carbons (δ 93.6, 101.4, 103.3, 104.2 and 104.6). The NMR signals of the aglycone moiety were quite similar to those of dubioside A. These spectral data indicated that I is a bisdesmosidic pentaglycoside of quillaic acid in which the sugar moieties are linked to the C_3-hydroxyl group and the carboxylic acid group.

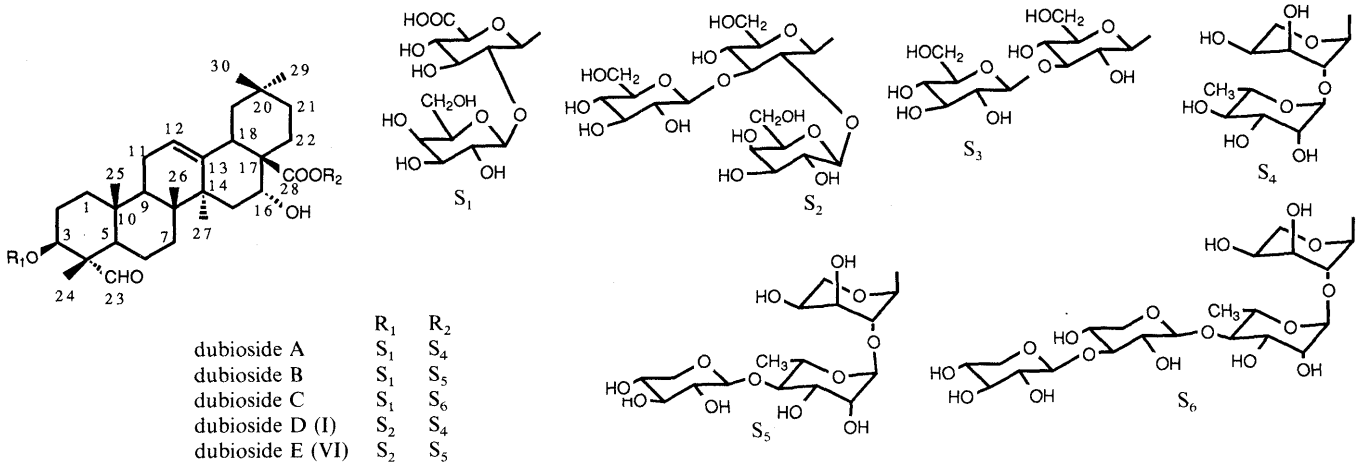
On selective cleavage of the ester-glycoside linkage according to the method reported by Ohtani *et al.*,[2] I gave a prosapogenin and an anomeric mixture of methyl glycosides. The methyl glycosides afforded L-arabinose and L-rhamnose on acid hydrolysis. The anomers were separated by high performance liquid chromatography (HPLC) to give a methyl diglycoside (II) ($[\alpha]_D$ −47.0°) and its anomer (III) ($[\alpha]_D$ +72.8°). The ^{13}C-NMR chemical shifts of II and III were in good agreement with those of the methyl *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer, respectively, reported by Mizutani *et al.*[3]

The methyl ester (IV) of the prosapogenin revealed an $[M+Na]^+$ ion at *m/z* 1009 in the FAB-MS, and it gave D-glucose and D-galactose on acid hydrolysis. The ^{1}H-NMR

TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Dubiosides and Their Degradation Products (in Pyridine-d_5)

C	Dubioside A	D (I)	E (VI)	F (IX)	IV	V
1	38.2	38.2	38.1	38.1	38.1	38.1
2	24.9	25.1	25.1	25.1	25.0	25.0
3	82.2	83.9	83.8	83.9	83.9	81.6
4	55.1	55.1	55.0	55.1	55.0	55.4
5	48.5	48.4	48.4	48.4	48.3	47.7
6	20.7	20.4	20.4	20.4	20.4	20.4
7	32.8	32.7	32.7	32.7	32.6	32.6
8	40.3	40.3	40.2	40.3	40.0	40.0
9	47.1	47.0[a]	47.0	47.0	46.9	47.0
10	36.3	36.3	36.2[a]	36.2	36.2	36.1
11	23.8	23.7	23.7	23.7	23.6	23.6
12	122.4	122.4	122.4	122.4	122.3	122.3
13	144.6	144.6	144.5	144.5	144.5	144.5
14	42.1	42.1	42.0	42.1	41.9	41.9
15	36.1[a]	36.0[b]	36.0	36.1[a]	35.7[a]	35.9
16	73.9	c)	c)	c)	c)	74.0
17	49.6	49.5	49.4	49.5	49.0	49.0
18	41.3	41.2	41.2	41.2	41.2	41.2
19	47.2	47.1[a]	47.1	47.0	46.9	47.0
20	30.9	30.9	30.8	30.9	30.8	30.8
21	36.0[a]	36.1[b]	36.1[a]	35.9[a]	35.9[a]	35.9
22	32.1	32.0	32.0	32.0	32.4	32.4
23	209.3	209.8	209.8	209.9	209.8	206.7
24	10.9	10.9	11.0	11.0	10.9	10.4
25	15.8	15.8	15.7	15.8	15.7	15.6
26	17.5	17.4	17.5	17.5	17.1	17.1
27	27.2	27.1	27.1	27.1	27.1	27.1
28	175.8	175.8	175.8	175.8	177.6	177.7
29	33.3	33.2	33.2	33.2	33.1	33.2
30	24.8	24.8	24.7	24.7	24.5	24.6
OCH_3					51.7	51.7

a, b) The assignments may be reversed in each column. *c*) The signal could not be differentiated from the signals of the sugar carbons.



	R_1	R_2
dubioside A	S_1	S_4
dubioside B	S_1	S_5
dubioside C	S_1	S_6
dubioside D (I)	S_2	S_4
dubioside E (VI)	S_2	S_5
dubioside F (IX)	S_2	S_6
IV	S_2	CH_3
V	S_3	CH_3

spectrum showed the signals of three anomeric protons [δ 4.72 (d, J=8 Hz); 5.26 (d, J=8 Hz) and 5.54 (d, J=8 Hz)] and the ^{13}C-NMR spectrum showed the corresponding anomeric carbon signals at δ 103.3, 104.6 and 104.2.

The permethylate of IV afforded methyl glycosides of 2,3,4,6-tetra-*O*-methyl D-glucopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 4,6-di-*O*-methyl-D-glucopyranose on methanolysis.

Compound IV was heated in 2 N CF_3COOH–MeOH for 7 h to give a diglycoside (V) (FAB-MS *m/z*: 847 ($[M+Na]^+$) and 823 ($[M-H]^-$)), which yielded D-glucose on acid hydrolysis. The permethylate of V afforded methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose and 2,4,6-tri-*O*-methyl-D-glucopyranose. Therefore, V and IV comprise a 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside and a 3-*O*-β-D-glucopyranosyl-(1→3)-[*O*-β-D-galactopyranosyl-(1→2)]-β-D-glucopyranoside, respectively, of quillaic acid methyl ester. The configurations of the glucopyranosyl and galactopyranosyl groups were determined from the coupling constants (8 Hz) of the anomeric protons.

Determination of the configuration and conformation of the ester-linked arabinopyranosyl group in I was difficult because the ^{1}H-NMR signals of the protons at C_2, C_3 and C_4 overlapped and prevented the splitting pattern of the 3-H from being revealed. The $J_{H1,H2}$ values (3 Hz) and $J_{C1,H1}$ values (173 Hz) indicated that the L-arabinopyranosyl group is in α-configuration and $^1C_4$ conformation, or in a β-configuration and $^4C_1$ conformation.[4] The chemical shifts and patterns of the ^{1}H-NMR signals of the arabinopyranosyl group closely resembled those of the α-L-arabinopyranosyl group of 3-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-bayogenin 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.[5] The arabinopyranosyl group of dubioside D (I) was therefore presumed to take an α-configuration and $^1C_4$ conformation.

From all the above-mentioned results, the structure of I is proposed to be as shown.

Dubioside E (VI), an amorphous white powder, revealed an $[M+Na]^+$ ion at *m/z* 1405 and the elemental analysis data were in good agreement with the molecular formula, $C_{64}H_{102}O_{32}\cdot 3/2H_2O$.

The NMR spectra showed the signals of six anomeric protons [δ 4.70 (d, J=8 Hz), 5.15 (d, J=7 Hz), 5.25 (d, J=8 Hz), 5.52 (d, J=8 Hz), 5.77 (br s) and 6.41 (d, J=3 Hz)] and the corresponding carbon signals (δ 103.3, 106.7, 104.7, 104.2, 100.9 and 93.6). The signals of the aglycone moiety were identical with those of dubioside D (I).

Selective cleavage of the ester-linked glycoside moiety gave the prosapogenin methyl ester (IV) and methyl glycosides, the latter of which were separated by HPLC to afford a methyl triglycoside (VII, $[\alpha]_D$ −58.4°) and its anomer (VIII, $[\alpha]_D$ +37.1°). Compounds VII and VIII gave D-xylose, L-arabinose and L-rhamnose on acid hydrolysis. Positive FAB-MS of VII showed an $[M+Na]^+$ ion at *m/z* 465, and negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 441 and fragment ions at *m/z* 309 and 163. The fragmentation in the negative FAB-MS indicated that VII and VIII are methyl pentosyl-rhamnosyl-pentosides. The permethylate of VII afforded methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 3,4-di-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis. The configuration of the sugar linkages and conformation of each sugar in VII were determined by examination of the splitting patterns of the ^{1}H-NMR signals to be α and $^4C_1$ for the L-arabinopyranosyl group, α and $^1C_4$ for the L-rhamnopyranosyl group and β and $^4C_1$ for the D-xylopyranosyl group. Compound VII is thus methyl *O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, and VIII is the β-anomer. The structure of VI was therefore determined to be as shown.

Dubioside F (IX), an amorphous white powder, revealed an $[M+Na]^+$ ion at *m/z* 1537 in the FAB-MS and the elemental analysis data were consistent with the molecular formula $C_{69}H_{110}O_{36}\cdot 5/2H_2O$. The NMR spectra showed the signals of seven anomeric protons and carbons. The NMR signals of the aglycone moiety were almost identical with those of dubioside E (VI) indicating that IX is a 3,28-*O*-bisdesmosidic heptaglycoside of quillaic acid.

On selective cleavage of the ester-glycoside linkage, IX gave a prosapogenin methyl ester (IV) and methyl glycosides, the latter of which were separated by HPLC to afford a methyl tetraglycoside (X, $[\alpha]_D$ −59.6°) and its anomer (XI, $[\alpha]_D$ +16.7°). Compound X gave D-xylose, L-arabinose and L-rhamnose on acid hydrolysis. Compound X showed an $[M+Na]^+$ ion at *m/z* 597 in its positive FAB-MS, while

the negative FAB-MS revealed an $[M-H]^-$ ion at *m/z* 573 and fragment ions at *m/z* 441 and 309, suggesting that the sugar sequence is methyl pentosyl-pentosyl-rhamnosyl-pentoside. The permethylate of XI afforded methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose, 2,4-di-*O*-methyl-D-xylopyranose and 3,4-di-*O*-methyl-L-arbinopyranose on methanolysis. Compound XI gave xylose, rhamnose, methyl arabinopyranoside, III and VIII on enzymatic hydrolysis with cellulase. Compound XI is thus methyl *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-L-arabinopyranoside, and X is its *α*-anomer.

The structure of dubioside F (IX) was therefore determined to be as shown.

Experimental[6]

Fractionation and Isolation of Dubiosides D (I), E (VI) and F (IX) The MeOH and 50% MeOH extracts of the dried tuber (200 g) were dissolved in H_2O and passed through a column of a polystyrene polymer (Diaion CHP-20P). The column was washed with water and then eluted with 40% acetone. The 40% acetone eluate (10 g) was dissolved in H_2O and passed through an ion-exchange resin Amberlite IRC-84 column (100 ml). The eluate was concentrated *in vacuo* and the acidic residue was dissolved in MeOH and treated with CH_2N_2. The product was repeatedly chromatographed on silica gel [AcOEt–MeOH–H_2O (6:2:1)] to give the dubioside D-containing fraction (fr. I, 318 mg) and the dubioside E, F-containing fraction (fr. II, 1.13 g). Fraction I was chromatographed on YMC gel (67% MeOH) to afford dubioside D (I) (224 mg). Fraction II was chromatographed on YMC gel (67% MeOH) and then separated by HPLC (Shiseido Capcell Pak C18, 70% MeOH) to give dubioside E (VI) (82 mg) and F (IX) (443 mg).

Dubioside D (I): Amorphous white powder from EtOH. $[\alpha]_D^{24}$ −20.0° (*c*=1.00, MeOH). *Anal.* Calcd for $C_{59}H_{94}O_{28}\cdot 2H_2O$: C, 55.04; H, 7.67. Found: C, 55.06; H, 7.72. FAB-MS *m/z*: 1273 ($[M+Na]^+$), 1249 ($[M-H]^-$). ^{1}H-NMR *δ*: aglycone moiety; ⇾CH_3; 0.83 (25-Me), 1.03×2 (26, 29-Me), 1.14 (30-Me), 1.41 (24-Me), 1.74 (27-Me). >C=CH–; 5.59 (br s)⇾CHO; 9.86 (s). C_{16}-OH; 6.25 (br s). C_{16}-H; 5.22 (br s). sugar moiety; >CH–CH_3; 1.66 (3H, d, *J*=6 Hz). anomeric H; 4.71 (d, *J*=8 Hz), 5.25 (d, *J*=8 Hz), 5.52 (d, *J*=8 Hz), 5.79 (s), 6.46 (d, *J*=2 Hz). ^{13}C-NMR *δ*: aglycone moiety; shown in Table I. sugar moiety; anomeric C; 103.3, 104.6, 104.2, 101.4, 93.6.

Dubioside E (VI): Amorphous white powder. $[\alpha]_D^{24}$ −16.9° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{64}H_{102}O_{32}\cdot 3/2H_2O$; C, 54.50; H, 7.50. Found: C, 54.46; H, 7.26. FAB-MS *m/z*: 1405 ($[M+Na]^+$). ^{1}H-NMR *δ*: aglycone moiety; ⇾CH_3; 0.83 (25-Me), 1.02 (6H, 26-Me, 29-Me), 1.13 (30-Me), 1.44 (24-Me), 1.73 (27-Me). C_{16}-H; 5.22 (br s). >C=CH–; 5.59 (t-like). ⇾CHO; 9.87 (s). sugar moiety; anomeric H; 4.70 (d, *J*=8 Hz), 5.15 (d, *J*=7 Hz), 5.25 (d, *J*=8 Hz), 5.52 (d, *J*=8 Hz), 5.77 (br s), 6.41 (d, *J*=3 Hz). >CH–CH_3; 1.71 (3H, d, *J*=6 Hz). ^{13}C-NMR *δ*: aglycone moiety; shown in Table I. sugar moiety; anomeric C; 103.3, 106.7, 104.7, 104.2, 100.9, 93.6.

Dubioside F (IX): Amorphous white powder. $[\alpha]_D^{22}$ −17.3° (*c*=1.0, 80% MeOH). *Anal.* Calcd for $C_{69}H_{110}O_{36}\cdot 5/2H_2O$; C, 53.10; H, 7.43. Found: C, 53.24; H, 7.67. FAB-MS *m/z*: 1537 ($[M+Na]^+$). ^{1}H-NMR *δ*: aglycone moiety; ⇾CH_3; 0.82 (25-Me), 1.01, 1.02 (each 3H, s, 26-Me, 29-Me), 1.14 (30-Me), 1.43 (24-Me), 1.74 (27-Me). >C=CH–; 5.59 (t-like). C_{16}-OH; 6.20 (d, *J*=5 Hz). C_{16}-H; 5.20 (br s). ⇾CHO; 9.88 (s). sugar moiety; anomeric H; 4.70 (d, *J*=8 Hz), 5.13 (d, *J*=7 Hz), 5.18 (d, *J*=8 Hz), 5.25 (d, *J*=8 Hz), 5.52 (d, *J*=8 Hz), 5.77 (br s), 6.44 (br s). ^{13}C-NMR *δ*: aglycone moiety: shown in Table I. sugar moiety; anomeric C; 103.3, 106.2, 105.9, 104.6, 104.2, 100.9, 93.5.

Identification of the Component Sugars Identification of the component monosaccharides in the dubiosides and their degradation products was performed in the same manner as reported in our preceding paper.[1] The results are shown in the text.

Selective Cleavage of the Ester-Glycoside Linkage The ester-glycoside linkages of dubiosides D, E and F were cleaved according to the method reported by Ohtani *et al.*,[2] employing the procedure described in our preceding paper. Dubioside D (I) (205 mg) gave a prosapogenin methyl ester (IV) (106 mg) and methyl glycosides (58 mg), the latter of which were preparatively separated by HPLC (Shiseido Capcell Pak C18, 10% MeOH) to afford II (10 mg) and III (20 mg).

II: A colorless syrup, $[\alpha]_D^{26}$ −47.0° (*c*=0.5, 10% MeOH). High resolution FAB-MS *m/z*: 333.116. $C_{12}H_{22}NaO_9$ requires *m/z* 333.115. ^{1}H-NMR *δ*: arabinopyranosyl moiety; 4.58 (d, *J*=6 Hz, 1-H), 4.51 (t, *J*=6 Hz, 2-H), 4.18 (dd, *J*=6, 3 Hz, 3-H), 4.19 (br s, 4-H), 3.68 (dd, *J*=10, 3 Hz, 5-H), 4.20 (dd, *J*=3, 10 Hz, 5-H). rhamnopyranosyl moiety; 5.98 (s, 1-H), 4.69 (br d, *J*=2 Hz, 2-H), 4.53 (dd, *J*=3, 9 Hz, 3-H), 4.25 (t, *J*=9 Hz, 4-H), 4.59 (dq, *J*=9, 6 Hz, 5-H), 1.62 (3H, d, *J*=6 Hz, 6-H). OCH_3; 3.51 (s). ^{13}C-NMR *δ*: arabinopyranosyl moiety; 103.6 (C_1), 76.7 (C_2), 74.1 (C_3), 69.1 (C_4), 65.7 (C_5). rhamnopyranosyl moiety; 102.3 (C_1), 72.4 (C_2), 72.8 (C_3), 74.1 (C_4), 69.7 (C_5), 18.4 (C_6). OCH_3; 55.9.

III: A colorless syrup. $[\alpha]_D^{27}$ +72.8° (*c*=1.0, 10% MeOH). FAB-MS *m/z*: 333 ($[M+Na]^+$). ^{1}H-NMR *δ*: arabinopyranosyl moiety; 5.32 (d, *J*=3 Hz, 1-H), 4.59 (dd, *J*=3, 10 Hz, 2-H), 4.49 (dd, *J*=3, 10 Hz, 3-H), 4.28 (br s, 4-H), 3.96 (2H, br s, 5-H). rhamnopyranosyl moiety; 5.66 (s, 1-H); 4.64 (br s, 2-H), 4.52 (dd, *J*=3, 9 Hz, 3-H), 4.21 (t, *J*=9 Hz, 4-H), 4.42 (dq, *J*=9, 6 Hz, 5-H), 1.60 (3H, *J*=6 Hz, 6-H). OCH_3; 3.56 (s). ^{13}C-NMR *δ*: arabinopyranosyl moiety *δ*: 101.2 (C_1), 78.5 (C_2), 69.2 (C_3), 70.7 (C_4), 63.5 (C_5). rhamnopyranosyl moiety; 104.4 (C_1), 72.2 (C_2), 72.7 (C_3), 74.0 (C_4), 70.0 (C_5), 18.6 (C_6). OCH_3; 55.1.

IV: Amorphous white powder. $[\alpha]_D^{28}$ +25.9° (*c*=1.1, MeOH). FAB-MS *m/z*: 1009 ($[M+Na]^+$), 985 ($[M-H]^-$). ^{1}H-NMR *δ*: aglycone moiety; ⇾CH_3; 0.82 (26-Me), 0.86 (25-Me), 1.02 (29-Me), 1.09 (30-Me), 1.46 (24-Me), 1.73 (27-Me). $COOCH_3$; 3.68 s. C_{16}-OH; 5.68 (br s). C_{16}-H; 4.98 (d, *J*=3 Hz), C_{12}-H; 5.51 (t-like), C_{16}-OH; 6.35 (d, *J*=4 Hz). ⇾CHO; 9.91 (s), sugar moiety; anomeric H; 4.72 (d, *J*=8 Hz), 5.26 (d, *J*=8 Hz), 5.54 (d, *J*=8 Hz). ^{13}C-NMR *δ*: aglycone moiety; shown in Table I. sugar moiety; anomeric C; 103.3, 104.6, 104.2.

Selective cleavage of the ester-glycoside linkage of dubioside E (VI) (268 mg) gave IV (170 mg) and methyl glycosides (46 mg). The methyl glycosides were separated by HPLC (Shiseido Capcell Pak C18, 15% MeOH) to afford VII (12 mg) and VIII (22 mg).

VII: Amorphous white powder. $[\alpha]_D^{28}$ −58.4° (*c*=0.6, MeOH). High-resolution FAB-MS *m/z*: 465.159 ($[M+Na]^+$). $C_{17}H_{30}NaO_{13}$ requires *m/z* 465.158. ^{1}H-NMR *δ*: arabinopyranosyl moiety; 4.55 (d, *J*=7 Hz, 1-H), 4.45 (dd, *J*=7, 8 Hz, 2-H), 4.14 (dd, *J*=8, 3 Hz, 3-H), *ca.* 4.18 (4-H), 3.67 (dd, *J*=2, 13 Hz, 5-H), 4.20 (dd, *J*=4, 13 Hz, 5-H). rhamnopyranosyl moiety; 5.94 (br s, 1-H), 4.67 (dd, *J*=2, 3 Hz, 2-H), 4.64 (dd, *J*=3, 9 Hz, 3-H), 4.34 (t *J*=9 Hz, 4-H), *ca.* 4.56 (m, 5-H), 1.68 (3H, d, *J*=6 Hz, 6-H). xylopyranosyl moiety; 5.12 (d, *J*=7 Hz, 1-H), 4.04 (t, *J*=9 Hz, 2-H), 4.07 (t, *J*=9 Hz, 3-H), *ca.* 4.15 (4-H), 3.52 (t, *J*=11 Hz, 5-H), 4.25 (dd, *J*=5, 11 Hz). OCH_3; 3.49 (s). ^{13}C-NMR *δ*: arabinopyranosyl moiety; 103.6 (C_1), 76.8 (C_2), 74.2 (C_3), 69.2 (C_4), 65.9 (C_5). rhamnopyranosyl moiety; 102.1 (C_1), 72.0 (C_2), 72.7 (C_3), 84.8 (C_4), 67.9 (C_5), 18.2 (C_6). xylopyranosyl moiety; 107.2 (C_1), 76.1 (C_2), 78.6 (C_3), 70.9 (C_4), 67.5 (C_5). OCH_3; 55.9.

VIII: Amorphous white powder. $[\alpha]_D^{28}$ +37.1° (*c*=1.1, MeOH). FAB-MS *m/z*: 465 ($[M+Na]^+$), 441 ($[M-H]^-$). ^{1}H-NMR *δ*: arabinopyranosyl moiety; 5.28 (br s, 1-H), 4.55 (d, *J*=10 Hz, 2-H), 4.46 (d, *J*=10 Hz, 3-H), *ca.* 4.28 (4-H), 3.95 (2H, br s, 5-H). rhamnopyranosyl moiety; 5.61 (s, 1-H), 4.62 (2H, br s, 2,3-H), 4.32 (d, *J*=10 Hz, 4-H), 4.40 (m, 5-H), 1.66 (3H, d, *J*=6 Hz, 6-H). xylopyranosyl moiety; 5.10 (d, *J*=7 Hz, 1-H), 4.01 (t, *J*=8 Hz, 2-H), 4.06 (t, *J*=8 Hz, 3-H), 4.14 (m, 4-H), 3.51 (t, *J*=10 Hz, 5-H), 4.23 (dd, *J*=5, 10 Hz, 5-H). OCH_3; 3.39 (s). ^{13}C-NMR *δ*: arabinopyranosyl moiety; 101.1 (C_1), 78.7 (C_2), 69.1 (C_3), 70.7 (C_4), 63.5 (C_5). rhamnopyranosyl moiety; 104.1 (C_1), 71.7 (C_2), 72.7 (C_3), 84.5 (C_4), 68.1 (C_5), 18.5 (C_6). xylopyranosyl moiety; 107.1 (C_1), 76.1 (C_2), 78.6 (C_3), 71.0 (C_4), 67.5 (C_5), OCH_3; 55.1.

Similar treatment of dubioside F (IX) (386 mg) afforded IV (115 mg), X (14 mg) and XI (23 mg).

X: Amorphous white powder. $[\alpha]_D^{28}$ −59.6° (*c*=0.7, MeOH). High-resolution FAB-MS *m/z*: 597.203. $C_{22}H_{38}NaO_{17}$ requires *m/z* 597.201. ^{1}H-NMR *δ*: 4.55 (d, *J*=7 Hz, Ara 1-H), 4.44 (dd, *J*=7, 8 Hz, Ara 2-H), 5.93 (s, Rha 1-H), 4.66 (br s, Rha 2-H), 4.61 (dd, *J*=3, 9 Hz, Rha 3-H), 4.34 (t, *J*=9 Hz, Rha 4-H), 4.54 (m, Rha 5-H), 1.65 (3H, d, *J*=7 Hz, Rha 6-H), 5.16 (d, *J*=8 Hz, Xyl 1-H), 5.21 (d, *J*=7 Hz, Xyl 1-H), 3.51 (s, OCH_3). ^{13}C-NMR *δ*: 103.6 (Ara, C_1), 76.8 (Ara, C_2), 102.1 (Rha, C_1), 72.0 (Rha, C_2), 72.8 (Rha, C_3), 84.3 (Rha, C_4), 67.8 (Rha, C_5), 18.2 (Rha, C_6), 105.9 (Xyl, C_1), 106.4 (Xyl, C_1), 56.0 (OCH_3).

XI: Amorphous white powder. $[\alpha]_D^{28}$ +16.7° (*c*=1.15, MeOH). FAB-MS *m/z*: 597 ($[M+Na]^+$), 573 ($[M-H]^-$). ^{1}H-NMR *δ*: 5.28 (d, *J*=4 Hz, Ara 1-H), 4.54 (dd, *J*=4, 10 Hz, Ara 2-H), 4.45 (dd, *J*=2, 10 Hz, Ara 3-H), 3.95 (2H, br s, Ara 5-H), 5.59 (s, Rha 1-H), 4.59 (2H, br s, Rha 2,3-H), 4.31 (dd, *J*=9, 11 Hz, Rha 4-H), *ca.* 4.38 (m, Rha 5-H), 1.63 (3H, d, *J*=6 Hz, Rha 6-H), 5.14 (d, *J*=8 Hz, Xyl 1-H), 5.20 (d, *J*=7 Hz, Xyl 1-H), 3.40 (s,

$OC\underline{H}_3$). ^{13}C-NMR δ: 101.1 (Ara, C_1), 78.7 (Ara, C_2), 69.1 (Ara, C_3), 63.5 (Ara, C_5), 104.1 (Rha, C_1), 71.7 (Rha, C_2), 72.7 (Rha, C_3), 84.0 (Rha, C_4), 68.0 (Rha, C_5), 18.4 (Rha, C_6), 105.9 (Xyl, C_1), 106.3 (Xyl, C_1), 55.1 ($O\underline{C}H_3$).

Methylation of IV and Methyl Glycosides, and Identification of the Component Methylated Monosaccharides Compound IV (110 mg) was methylated by Hakomori's method.[7] Purification of the product by column chromatography on silica gel (solvent: hexane–AcOEt, 2 : 1) gave a thin-layer chromatographically homogeneous permethylate (36 mg). The permethylate (7 mg) was dissolved in 1 N HCl–MeOH (0.3 ml) and heated at 80 °C for 2 h. The reaction mixture was treated in the same manner as reported in our preceding paper, and the product was analyzed by gas-liquid chromatography-chemical ionization-mass spectrometry (GC-CI-MS) after acetylation. Methyl glycosides (10—20 mg) were also treated in the same manner. The component sugars were identified by comparison of the relative retention times and mass spectral patterns with those of the authentic samples. The results are shown in the text, and the conditions for the GC-CI-MS are described in our preceding paper.[1]

Partial Methanolysis of IV Compound IV (280 mg) was dissolved in 2 N CF_3COOH–MeOH (4 ml) and heated at 80 °C for 7 h. After evaporation of the solvent, the residue was dissolved in 50% MeOH and passed through a column of polystyrene polymer (20 ml). The column was washed with 50% MeOH and then eluted with MeOH. The MeOH eluate (183 mg) was chromatographed on silica gel (35 g) using AcOEt–MeOH–H_2O (9 : 2 : 0.3) as an eluting solvent to give V (53 mg).

V: Amorphous white powder. FAB-MS *m/z*: 847 ($[M+Na]^+$), 823 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety; 0.84 (26-Me), 0.89 (25-Me), 1.03 (29-Me), 1.10 (30-Me), 1.32 (24-Me), 1.75 (27-Me). >C=CH–; 5.53 (t, J=3 Hz). →CHO; 9.71 s. $COOC\underline{H}_3$; 3.68 (s). C_{16}-O$\underline{H}$; 6.33 (d, J=5 Hz). C_{16}-H; 4.99 (br s). C_3-H; 4.18 (dd, J=3, 12 Hz). sugar moiety; inner glucopyranosyl moiety; 4.77 (d, J=8 Hz, 1-H), 3.91 (dd, J=8, 9 Hz, 2-H), 4.10 (t, J=9 Hz, 3-H), 4.02 (t, J=9 Hz, 4-H), 3.98 (m, 5-H), 4.27 (dd, J=6, 12 Hz, 6-H), 4.50 (dd, J=2, 12 Hz, 6-H). outer glucopyranosyl moiety; 5.21 (d, J=8 Hz, 1-H), 4.02 (t, J=8 Hz, 2-H), *ca.* 4.18 (2H, m, 3-H, 4-H), 3.85 (m, 5-H), 4.25 (dd, J=5, 12 Hz, 6-H), 4.45 (dd, J=2, 12 Hz, 6-H). ^{13}C-NMR δ: aglycone moiety; shown in Table I. sugar moiety; inner glucopyranosyl moiety; 104.1 (C_1), 74.0 (C_2), 88.7 (C_3), 69.7 (C_4), 78.0 (C_5), 62.5 (C_6). outer glucopyranosyl moiety; 105.8 (C_1), 75.5 (C_2), 78.2 (C_3), 71.6 (C_4), 78.6 (C_5), 62.5 (C_6).

Methylation of V, and Identification of the Methylated Monosaccharides Compound V (8 mg) was fully methylated by Hakomori's method to afford a permethylate (*ca.* 1 mg) of V. The permethylate was methanolyzed and the methanolysate was checked by TLC (solvent: benzene–acetone, 2 : 1). Spots having *Rf* values of 0.45 and 0.23 were detected. (*Rf* values of the standard samples: methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside, 0.45; methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside, 0.29; methyl 2,4,6-tri-*O*-methyl-α-D-glucopyranoside, 0.23).

Enzymatic Hydrolysis of XI with Cellulase Compound XI (120 mg) and cellulase (124 mg) were dissolved in H_2O and the mixture was shaken at 37 °C for 38 h. The solvent was evaporated off and the residue was extracted with MeOH. The MeOH-soluble material was dissolved in H_2O and subjected to reversed-phase column chromatography (Fuji gel, 100 ml, solvent: 10—20% MeOH) to give a mixture (23 mg) of D-xylose and L-rhamnose, methyl β-L-arabinopyranoside (14 mg), III (15 mg) and VIII (22 mg). Compound XI (23 mg) was recovered. Xylose, rhamnose and methyl arabinoside were identified by GC as TMS ethers and III and VIII were identified by comparison of the NMR spectra.

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References and Notes

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