Studies on the Constituents of *Thladiantha dubia* BUNGE. I. The Structures of Dubiosides A, B and C, the Quillaic Acid Glucuronide Saponins Isolated from the Tuber

Tsuneatsu NAGAO, Hikaru OKABE,* Kunihide MIHASHI and Tatsuo YAMAUCHI

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8–19–1, Jonan-ku, Fukuoka 814–01, Japan. *Received October 11, 1988*

Three bisdesmosidic glucuronide saponins of quillaic acid, named dubiosides A, B and C, were isolated as their methyl esters from the tuber of *Thladiantha dubia* BUNGE (Cucurbitaceae). Their structures were elucidated on the basis of chemical and spectral evidence.

All the dubiosides have a common prosapogenin structure, quillaic acid-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside, and differ only in the structures of the 28-*O*-linked sugar moieties. Dubioside A is a 28-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside, dubioside B, a 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and dubioside C, a 28-*O*-β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside.

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

Thladiantha dubia BUNGE is a cucurbitaceous vine which is distributed in the east Asia, from China to the Korean peninsula. This plant is dioecious and only the male plant has become naturalized in Japan.

In the course of screening of cucurbitaceous plants for saponin constituents it was found that the tuber of the title plant contains a considerable amount of saponins. The saponin constituents of the tuber were investigated and three glucuronide saponins of quillaic acid, named dubiosides A, B and C, were isolated as their methyl esters together with three neutral saponins having the same aglycone. This paper deals with the isolation and the structures of the glucuronide saponins.

The powder of the dried tuber was percolated with 50% MeOH and MeOH, and the extracts were chromatographed on a Diaion HP-20P column. The 40% acetone eluate, which contained saponins, was passed through a column of an ion-exchange resin (Amberlite IRC-84) and treated with CH_2N_2. The product was chromatographed repeatedly on silica gel and LiChroprep RP-18 columns to give methyl esters of dubioside A (2.7% of the dried tuber), B (0.45%) and C (2%) and three non-ester saponins. The methyl ester (I) of dubioside A gave, on acid hydrolysis, D-glucuronic acid, D-galactose, L-rhamnose and L-arabinose, and it showed an $[M+Na]^+$ ion in the fast-atom bombardment mass spectrum (FAB-MS) at *m/z* 1139, indicating the molecular weight to be 1116. The elemental analysis data were consistent with the molecular formula $C_{54}H_{84}O_{24}\cdot 3H_2O$. The acetate of I showed an $[M+Na]^+$ ion in the FAB-MS at *m/z* 1643, and the electron impact mass spectra (EI-MS) showed fragment ions at *m/z* 273, 331 and 489. The first two fragment ions indicate the presence of the terminal rhamnosyl group and a galactosyl group, and the last one shows the presence of the rhamnosyl-arabinosyl group. The ^{1}H-nuclear mangetic resonance (^{1}H-NMR) spectrum showed signals of six tertiary methyl groups (δ 0.85, 1.03, 1.04, 1.15, 1.38 and 1.77), one secondary methyl group [δ 1.67 (d, $J=6$ Hz)], one carbomethoxyl group [δ 3.71 (s)], one trisubstituted olefinic proton [δ 5.59 (t-like)], one aldehydic proton [δ 9.88 (s)] and four anomeric protons [δ 4.90 (d, $J=7$ Hz), 5.21 (d, $J=8$ Hz), 5.83 (s) and 6.48 (d, $J=3$ Hz)]. The ^{13}C-NMR spectrum showed signals of six saturated C–C bonded quaternary carbons (δ 30.9, 36.3, 40.3, 42.1, 49.5 and 55.0), two ester carbons (δ 170.2 and 175.8), olefinic carbons (δ 122.4 and 144.5), an aldehydic carbon (δ 209.2) and four anomeric carbons (δ 93.6, 101.4, 103.2 and 106.3). These spectral data suggested that I is a bisdesmosidic tetraglycoside of an oleanane-type triterpene carboxylic acid in which one of the sugar moieties is linked to the carboxylic acid group with an ester linkage.

On the selective cleavage of the ester–glycoside linkage

TABLE I. ^{13}C-NMR Chemical Shifts[a] of the Aglycone Moieties of I and Related Compounds

C	IV′[b]	V[c]	III[d]	II	I
1	38.1	38.0	38.1	38.1	38.2
2	26.0	25.1	25.2	24.9	24.9
3	71.7	82.2	82.1	82.3	82.2
4	55.2	55.3	55.4	55.0	55.1
5	48.1	47.8	47.8	48.4	48.5
6	20.7	20.3	20.5	20.4	20.7
7	32.3	32.7	32.5	32.6[e]	32.8
8	39.8	39.9	40.0	40.0	40.3
9	46.7	47.8	47.0	46.9	47.1
10	35.9	36.1	36.1[f]	36.3	36.3
11	23.2	23.3	23.7	23.7	23.8
12	122.2	122.5	—[g]	122.3	122.4
13	142.8	144.1	144.5	144.5	144.6
14	41.4	42.0	41.2	41.9	42.1
15	35.4	28.0	35.9[f]	35.9	36.1
16	74.7	23.6	74.3	74.3	73.9
17	48.7	46.9	49.0	49.0	49.6
18	40.5	41.8	41.9	41.2	41.3
19	46.4	46.0	47.0	46.9	47.2
20	30.3	30.7	30.9	30.9	30.9
21	35.4	33.9	35.9	35.9	36.0
22	30.3	32.3	32.5	32.5[e]	32.1
23	207.2	206.7	206.6	209.2	209.3
24	9.0	10.4	10.4	10.9	10.9
25	15.7	15.5	15.7	15.7	15.8
26	16.9	17.0	17.1	17.1	17.5
27	27.0	26.1	27.1	27.1	27.2
28	177.2	177.8	177.6	177.7	175.8
29	32.7	33.1	33.2	33.2	33.3
30	24.6	23.6	24.6	24.6	24.8
$COO\underline{C}H_3$	51.9	51.5	51.8	51.7	

a) ^{13}C-NMR spectra were obtained on a JEOL GX-400 (100 MHz) except for III. *b*) Data reported by Tori *et al.*[3] *c*) Data reported by Okabe *et al.*[4] *d*) Data obtained on a JEOL FX-100 (25 MHz). *e, f*) The assignments may be reversed. *g*) The signal was overlapped by the pyridine signal.

Table II. ^{13}C-NMR Chemical Shifts of Sugar Moieties of I and Related Compounds

Compds.	III	V	II	GGG	I	VII	VI	
GlcAM 1	105.2	105.2	103.2	103.2	103.2	103.2		
2	74.9	74.9	83.4	83.4	83.5	83.4		
3	77.7	77.7	77.4	77.4	77.4	77.4		
4	73.0	72.9	72.5	72.5	72.5	72.6		
5	77.2	77.2	76.8	76.8	76.7	76.8		
6	170.6	170.5	179.2	170.2	170.2	170.2		
COOMe	52.0	51.9	52.0	52.0	52.0	52.0		
Gal 1			106.3	106.3	106.3	106.3		
2			74.3	74.4	74.3	74.4		
3			74.9	74.9	75.3	75.1		
4			70.1	70.1	70.1	70.1		
5			77.1	77.0	77.1	77.1		
6			62.2	62.2	62.2	62.2	α	β
Ara 1					93.6	93.3	103.7	101.2
2					74.9	74.9	76.8	78.5
3					70.4	70.2	74.2	70.7
4					66.2	66.0	69.2	70.0
5					63.2	62.9	65.8	63.2
Rha 1					101.4	101.2	102.4	104.4
2					72.3[a]	72.1	72.4	72.2
3					72.5[a]	72.4	72.8	72.7
4					73.8	73.7	74.1	74.0
5					70.4	70.2	69.8	69.2
6					18.5	18.4	18.5	18.6
OMe							56.0	55.1

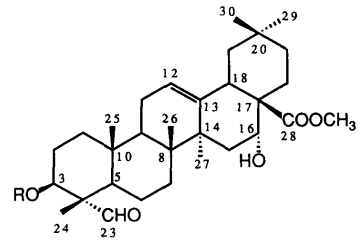
a) The assignments may be reversed. Abbreviations: GlcAM, 6-*O*-methyl glucuronopyranosyl; Gal, galactopyranosyl; Ara, arabinopyranosyl; Rha, rhamnopyranosyl; GGG, gypsogenin-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside dimethyl ester.[4]

according to the method reported by Ohtani *et al.*,[1] I gave a prosapogenin and an anomeric mixture of methyl glycosides. The methyl ester (II) of the prosapogenin showed an $[M+Na]^+$ ion in its FAB-MS at *m/z* 875 and it gave, on acid hydrolysis, D-glucuronic acid and D-galactose. The ^{1}H-NMR spectrum showed a pair of anomeric proton signals at δ 4.90 (d, *J*=7 Hz) and 5.21 (d, *J*=7 Hz), and the ^{13}C-NMR spectrum showed the corresponding anomeric carbon signals at δ 103.2 and 106.3.

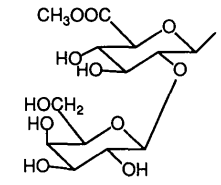
Methanolysis of II under mild conditions provided methyl D-galactoside, a monoglycoside (III) and an aglycone (IV). The aglycone was identified as quillaic acid methyl ester by direct comparison with an authentic sample[2] after conversion to a diacetate. Compound III was identified as quillaic acid 3-*O*-β-D-glucuronopyranoside dimethyl ester by comparison of the ^{13}C-NMR spectrum with those of quillaic acid methyl ester (IV′)[3] and gypsogenin-3-*O*-β-D-glucuronopyranoside dimethyl ester (V) derived from the saponin (luperoside I) which was isolated from *Luffa operculata* COGN.[4]

The structure of II was presumed to be quillaic acid-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside dimethyl ester from the facts that ^{13}C-NMR glycosylation shifts were observed at the signals of C_2 (+8.5 ppm), C_1 (−2.0 ppm) and C_3 (−0.3 ppm) of the glucuronopyranosyl group compared with those of III, and that the chemical shifts of the carbons of the sugar moiety are identical with those of gypsogenin-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside dimethyl ester.[4]

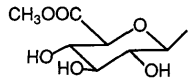
The $NaBH_4$ reduction product of II was permethylated and methanolysated to provide methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 3,4,6-tri-*O*-methyl-D-glucopyranose. These results provided unambiguous proof of the structure of II. The configurations of the sugar linkages were determined from the coupling constants (7 Hz) of the anomeric protons.



II : R =



III : R =



IV : R = H

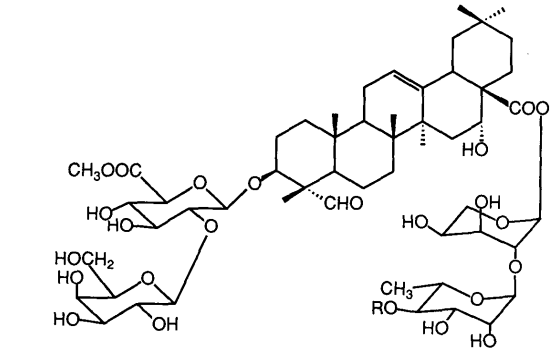
The methyl glycoside (VI) obtained from I by selective cleavage of the ester–glycoside linkage gave L-arabinose and L-rhamnose on acid hydrolysis. The field-desorption mass spectrum (FD-MS) showed an $[M+Na]^+$ ion at *m/z* 333, indicating that VI is composed of 1 mol each of L-rhamnose and L-arabinose. The ^{1}H-NMR spectrum showed the anomeric proton signals at *ca.* δ 4.60 (overlapped by other hydroxymethine protons), 5.33 (d, *J*=3 Hz), 5.68 (br s) and 6.00 (br s), indicating that VI is a mixture of two anomeric methyl glycosides. The last two signals should be those of the α-L-rhamnosyl group of each anomer and the second may be that of a β-L-arabinosyl group. The ^{13}C-NMR signals of the corresponding anom-

Table III. ^{1}H- and ^{13}C-NMR Chemical Shifts of the Anomeric Protons and Carbons of VI, IX and XI

	VI				IX				XI			
	α-Anomer		β-Anomer		α-Anomer		β-Anomer		α-Anomer		β-Anomer	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Ara	*ca.* 4.60	103.7	5.33 (d, 3)	101.2	*ca.* 4.60	103.7	5.30 (d, 3)	101.2	4.55 (d, 4)	103.7	5.30 (d, 3)	101.2
Rha	6.00 (br s)	102.4	5.68 (br s)	104.4	5.98 (br s)	102.2	5.63 (br s)	104.2	5.96 (br s)	102.2	5.61 (br s)	104.2
Xyl					5.14 (d, 8)	107.2	5.12 (d, 7)	107.3	5.17 (d, 8)	106.5	5.15 (d, 7)	106.4
Xyl									5.22 (d, 8)	106.0	5.22 (d, 8)	106.0

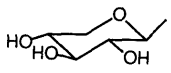
eric carbons appeared at δ 103.7, 101.2, 104.4 and 102.4. Assignments of the signals of anomeric protons and carbons of the monosaccharides of each anomeric methyl glycoside were performed considering the chemical shifts, coupling constants and peak intensities. The results are shown in Table III.

The ^{13}C-NMR spectrum showed 24 signals, which were divided into two groups of carbons of two anomers judging from the relative peak heights of the signals (Table II). The chemical shifts of carbon signals of the two groups were almost identical with those reported for methyl α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside and its β-anomer.[5] The ^{13}C-NMR chemical shifts of carbons of the ester-linked sugar moiety are in good agreement with those reported for 3-*O*-acetyl oleanolic acid-28-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (VII).[6] The structure of I is, therefore, a dimethyl ester of quillaic acid-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside-28-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside. The conformation of the arabinopyranosyl group is considered to be ^{1}C$_4$ form, as reported for VII.[6]

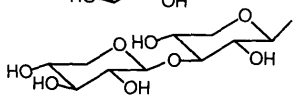


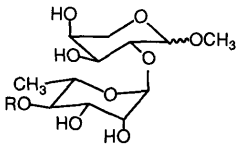
I : R = H

VIII : R =



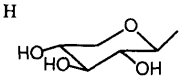
X : R =



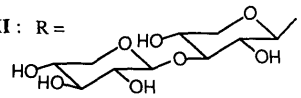


VI : R = H

IX : R =



XI : R =



The methyl ester (VIII) of dubioside B was obtained as a colorless crystalline powder and its FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1271, indicating the molecular weight to be 1248. Acid hydrolysis of VIII gave D-glucuronic acid, D-galactose, L-arabinose, L-rhamnose and D-xylose as component monosaccharides.

The ^{1}H-NMR spectrum showed a similar pattern to that of I, though the signal pattern of the hydroxymethine proton region was more complex than that of I. The signals of the anomeric protons appeared at δ 4.89 (d, J=7 Hz), 5.17 (d, J=7 Hz), 5.21 (d, J=8 Hz), 5.80 (br s) and 6.43 (d, J=3 Hz). The ^{13}C-NMR spectrum showed the corresponding anomeric carbon signals at δ 103.3, 106.8, 106.3, 101.0 and 93.7. The ^{13}C-NMR signals of the aglycone moiety were almost superimposable on those of I, suggesting that VIII is a bisdesmosidic pentaglycoside of quillaic acid. The selective cleavage of the ester–glycoside linkage provided a prosapogenin and an anomeric mixture (IX) of methyl triglycosides. The methyl ester of the former was identical with II. Compound IX gave L-arabinose, L-rhamnose and D-xylose on acid hydrolysis, and its showed in the FAB-MS an $[M+Na]^+$ ion at *m/z* 465 and fragment ions at *m/z* 279 (a pentosyl-rhamnopyronium cation) and *m/z* 133 (a pentopyronium cation). The results of FAB-MS suggested that IX is a methyl pentosyl-rhamnosyl-pentoside. The ^{1}H-NMR spectrum showed six anomeric proton signals at *ca.* δ 4.60 (overlapped by other hydroxymethine protons), 5.12 (d, J=8 Hz), 5.14 (d, J=7 Hz), 5.30 (d, J=3 Hz), 5.63 (br s) and 5.98 (br s). The corresponding anomeric carbon signals were observed at δ 103.7, 107.3, 107.2, 101.2, 104.2 and 102.2. Compound IX was fully methylated and the permethylate gave, on methanolysis, methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis. Therefore, IX is a mixture of methyl β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside and its β-anomer. The assignments of ^{1}H-NMR and ^{13}C-NMR signals of the anomeric protons and carbons of the component monosaccharides of each anomer were done as shown in Table III in the same way as for the methyl diglycoside (VI) from I. The conformation and configuration of the L-rhamnopyranosyl group are ^{1}C$_4$ and α, judged from the signal pattern of the anomeric proton (br s) and J_{CH} values (166 and 169 Hz).

The structure of VIII was reconstructed from the above data as a methyl ester of quillaic acid-3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside-28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside. The configuration and conformation of the ester-linked L-arabinopyranosyl group were determined as α and ^{1}C$_4$ from the coupling constant (3 Hz) and J_{CH} value (172 Hz).[7]

The methyl ester (X) of dubioside C gave elemental analysis data consistent with the molecular formula $C_{64}H_{100}O_{32}\cdot 2H_2O$, and its FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1403, 132 mass units more than VIII, suggesting that X has one more pentosyl group than VIII. Compound X gave, on acid hydrolysis, D-glucuronic acid, D-galactose, L-arabinose, L-rhamnose and D-xylose. The ^{1}H-NMR and ^{13}C-NMR spectra of the aglycone moiety were almost the same as those of VIII, but the signals of the sugar moiety were more complex than those of VIII. The ^{1}H-NMR spectrum showed six anomeric proton signals at δ 4.89 (d, J=8 Hz), 5.16 (d, J=7 Hz), 5.22 (2H, d, J=7 Hz), 5.71 (br s) and 6.48 (br s). The corresponding anomeric carbon signals were observed at δ 103.2, 106.0, 106.2 (×2), 101.0 and 93.5.

The selective cleavage of the ester–glycoside linkage provided a prosapogenin and an anomeric mixture (XI) of methyl glycosides. The methyl ester of the former was identical with II. The latter gave, on acid hydrolysis, D-xylose (×2), L-arabinose and L-rhamnose, and its FAB-MS showed an $[M+Na]^+$ ion at *m/z* 597, indicating that it is a methyl tetraglycoside which consists of 1 mol each of L-

rhamnose and L-arabinose, and 2 mol of D-xylose. The negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 573 and fragment ions at *m/z* 441 (573-pentosyl group), 309 (441-pentosyl group) and 183 (309-rhamnosyl group), indicating that the sugar sequence is MeO-pentose-rhamnose-pentose-pentose. The ^{1}H-NMR spectrum showed the anomeric proton signals at δ 4.55 (d, $J=4$ Hz), 5.15 (d, $J=7$ Hz), 5.17 (d, $J=8$ Hz), 5.22 (2H, d, $J=8$ Hz), 5.30 (d, $J=3$ Hz), 5.61 (br s) and 5.96 (br s). The corresponding anomeric carbon signals appeared at δ 103.7, 106.4, 106.5, 106.0 ($\times 2$), 101.2, 104.2 and 102.2. The NMR signals of the anomeric protons and carbons were compared with those of the methyl triglycoside (IX) obtained from VIII. Compound XI gave the signals of the additional xylosyl group [^{1}H-NMR δ: 5.22 (2H, d, $J=8$ Hz), ^{13}C-NMR δ: 106.0 ($\times 2$)], and the signals of the other xylosyl group were a little shifted upfield. The signals of the arabinosyl groups and rhamnosyl groups appeared at almost the same magnetic field as those of IX, suggesting that there is one xylosyl group linked to the terminal xylosyl group of IX.

The permethylate of XI gave, on methanolysis, methyl glycosides of 3,4-di-*O*-methyl-L-arabinopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose, 2,4-di-*O*-methyl-D-xylopyranose and 2,3,4-tri-*O*-methyl-D-xylopyranose, indicating XI to be methyl β-D-xylopyransoyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-L-arabinopyranoside. Therefore, the structure of IX is concluded from the above results to be as shown. The conformations and configurations of the component monosaccharides were determined on the same basis as for VIII.

Dubiosides A, B and C were isolated as their methyl esters because it was extremely difficult to separate them from the neutral saponins. They are contained in the tuber in the acidic and carboxylate forms.

The structures of the neutral saponins will be reported in the next paper.

Experimental[8]

Extraction, Fractionation and Isolation of Dubiosides A, B and C The tubers of *Thladiantha dubia* BUNGE were collected in Sapporo. The dried tuber (200 g) was powdered and percolated successively with MeOH (1 l) and 50% MeOH (1 l). TTe percolates were combined and concentrated *in vacuo*. The residue was dissolved in H_2O (500 ml) and passed through a column of a polystyrene polymer, Diaion CHP-20P (300 ml). The column was washed with H_2O (1 l) and then eluted with 40% acetone (1 l) and acetone (1 l). The 40% acetone eluate was passed through a column of an ion-exchange resin, Amberlite IRC-84 (200 ml). The acidic eluate was concentrated *in vacuo* and the residue was dissolved in 80% MeOH (200 ml). An ether solution of CH_2N_2 was added until the solution became neutral and the solvent was evaporated off to give a saponin fraction (fr. II, 20 g). Fraction II (2 g) was repeatedly chromatographed on silica gel (100 times the weight of the material) eluting with $CHCl_3$–MeOH (3:1, 2:1, 3:2). The eluates were monitored by thin layer chromatography (TLC) [Kieselgel 60 F_{254} plate; solvent, $CHCl_3$–MeOH–H_2O–AcOH (21:15:3:1)] and separated into three fractions (fr. IIa, IIb and IIc). Fraction IIa was chromatographed on an RP-18 column using 35% CH_3CN as an eluting solvent to give I (550 mg).

Fraction IIb was repeatedly chromatographed on an RP-18 column (solvent, 60% MeOH) and silica gel (100 times the weight of the material) column using $CHCl_3$–MeOH (2:1) as the eluting solvent to give VIII (90 mg) and X (150 mg). Fraction IIc contains non-ester neutral saponins and the investigation of this fraction will be reported in the next paper.

Dubioside A Methyl Ester (I): Amorphous powder, mp 210—215 °C (dec.), $[\alpha]_D^{25}$ −31.9° (*c*=2.7, MeOH). *Anal.* Calcd for $C_{54}H_{84}O_{24}\cdot 3H_2O$: C, 55.37; H, 7.75. Found: C, 55.47; H, 7.85. FAB-MS *m/z*: 1139 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; ≥CH_3; 0.85, 1.03, 1.04, 1.15, 1.38, 1.77. >C=CH–; 5.59(t-like). ≥CHO; 9.88 (s). sugar moiety; >CH–CH_3; 1.67 (d, $J=6$ Hz). anomeric H; 4.90 (d, $J=7$ Hz), 5.21 (d, $J=8$ Hz), 5.83 (br s), 6.48 (d, $J=3$ Hz). –COOC<u>H</u>$_3$; 3.71 (s). ^{13}C-NMR: shown in Tables I and II.

I-Acetate: Compound I (100 mg) was dissolved in Ac_2O–pyridine (1:1) (3 ml) and warmed at 60 °C for 5 h. The reaction mixture was poured into water and the precipitates were filtered off. The acetate was purified by silica gel column chromatography using 10% acetone in benzene as an eluting solvent. Colorless fine needles from MeOH, mp 228—230 °C. FAB-MS *m/z*: 1643 ($[M+Na]^+$). EI-MS *m/z*: 489, 331, 273.

Dubioside B Methyl Ester (VIII): Fine needles from MeOH, mp 225—226 °C, $[\alpha]_D^{24}$ −26.1° (*c*=1.00, MeOH). *Anal* Calcd for $C_{59}H_{92}O_{28}\cdot 3H_2O$: C, 54.37; H, 7.58. Found: C, 54.35; H, 7.68. FAB-MS *m/z*: 1271 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; ≥CH_3; 0.84, 1.02, 1.03, 1.14, 1.41, 1.75. >C=CH–; 5.59 (t-like). ≥C–CHO; 9.89 (s). sugar moiety; >CH–CH_3; 1.73 (d, $J=5$ Hz). –COOC<u>H</u>$_3$; 3.72 (s). anomeric H; 4.89 (d, $J=7$ Hz), 5.17 (d, $J=7$ Hz), 5.21 (d, $J=8$ Hz), 5.80 (br s), 6.43 (d, $J=3$ Hz). ^{13}C-NMR δ: aglycone moiety: the chemical shifts are almost the same as those of I. sugar moiety; anomeric C; 103.3, 106.8, 106.3, 101.0, 93.7. COO<u>C</u>H$_3$; 52.1. <u>C</u>OOCH$_3$; 170.3.

VIII-Acetate: Prisms from EtOH, mp 168—170 °C. FAB-MS *m/z*: 1859 ($[M+Na]^+$). EI-MS *m/z*: 259, 331.

Dubioside C Methyl Ester (X): Fine needles from MeOH, mp 229—231 °C, $[\alpha]_D^{24}$ −27.6° (*c*=0.80, 70% MeOH). *Anal.* Calcd for $C_{64}H_{100}O_{32}\cdot 2H_2O$: C, 54.23; H, 7.40. Found: C, 54.28; H, 7.50. FAB-MS *m/z*: 1403 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; ≥CH_3; 0.84, 1.02 1.03, 1.15, 1.41, 1.76. >C=CH–; 5.59 (t-like). ≥C–CHO; 9.89 (s). sugar moiety; >CH–CH_3; 1.70 (d, $J=5$ Hz). –COOC<u>H</u>$_3$; 3.72 (s). anomeric H; 4.89 (d, $J=8$ Hz), 5.16 (d, $J=7$ Hz), 5.22 (2H, d, $J=7$ Hz), 5.71 (br s), 6.48 (br s). ^{13}C-NMR δ: aglycone moiety; the chemical shifts of the carbons of the aglycone moiety are almost the same as those of I. sugar moiety; anomeric C; 103.2, 106.0, 106.2 ($\times 2$), 101.0, 93.5. COO<u>C</u>H$_3$; 52.0. <u>C</u>OOCH$_3$; 170.2.

Selective Cleavage of the Ester–Glycoside Linkage Compound I (140 mg) and LiI (110 mg) were added to a mixture of 2,6-lutidine (2 ml) and dry MeOH (1 ml) and the mixture was heated at 120 °C for 12 h. After cooling, 50% MeOH (10 ml) was added and the diluted solution was passed through a column of Amberlite MB-3 (12 ml). The eluate was concentrated and dissolved in MeOH (5 ml). An ether solution of CH_2N_2 was added and evaporated. The residue (120 mg) was chromatographed on Diaion CHP-20P (10 ml). The methyl glycoside (VI, 30 mg) was eluted with 20% MeOH and a prosapogenin methyl ester was eluted with MeOH. The prosapogenin methyl ester fraction (73 mg) was purified by silica gel (20 g) column chromatography [solvent, $CHCl_3$–MeOH (9:1)] to give thin-layer-chromatographically homogeneous II (49 mg). The same treatment of VIII (100 mg) gave II (47 mg) and IX (25 mg). Compound X (56 mg) gave II (25 mg) and XI (5 mg).

Prosapogenin Methyl Ester (II): Amorphous powder, $[\alpha]_D^{24}$ +12.2° (*c*=1.25, MeOH). FAB-MS *m/z*: 875 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; ≥CH_3: 0.82, 0.87, 1.03, 1.10, 1.41, 1.74. COOC<u>H</u>$_3$; 3.68 (s). >C=CH–; 5.51 (t-like). ≥C–CHO; 9.94 (s). sugar moiety; anomeric H; 4.90 (d, $J=7$ Hz), 5.21 (d, $J=7$ Hz). COOC<u>H</u>$_3$; 3.72 (s). ^{13}C-NMR: shown in Tables I and II.

II-Acetate: Colorless prisms from EtOH, mp 146—149 °C. FAB-MS *m/z*: 1169 ($[M+Na]^+$). EI-MS *m/z*: 605, 482, 436, 331, 295, 260, 201, 169.

VI: FAB-MS *m/z*: 333. ^{1}H-NMR: chemical shifts of the anomeric protons are shown in Table III. ^{13}C-NMR: shown in Table II.

IX: FAB-MS *m/z*: 465 ($[M+Na]^+$), 279 (a xylosyl-rhamnopyronium cation). ^{1}H-NMR δ: anomeric H; *ca.* 4.60, 5.12 (d, $J=8$ Hz), 5.14 (d, $J=7$ Hz), 5.30 (d, $J=3$ Hz), 5.63 (br s), 5.98 (br s). >CH–C<u>H</u>$_3$; 1.68 (d, $J=6$ Hz), 1.70 (d, $J=6$ Hz). OCH$_3$; 3.39 (s) 3.50 (s). ^{13}C-NMR δ: anomeric C; 103.7, 107.3, 107.2, 101.2, 104.2, 102.2. >CH–<u>C</u>H$_3$; 18.3, 18.5. OCH$_3$: 55.1, 56.0.

XI: FAB-MS *m/z*: 597 ($[M+Na]^+$). Negative FAB-MS *m/z*: 573 ($[M-H]^-$), 441 (573-xylosyl group), 309 (441-xylosyl group), 183 (309-rhamnosyl group). ^{1}H-NMR δ: anomeric H; 4.55 (d, $J=4$ Hz), 5.15 (d, $J=7$ Hz), 5.17 (d, $J=8$ Hz), 5.22 (2H, d, $J=8$ Hz), 5.30 (d, $J=3$ Hz), 5.61 (br s), 5.96 (br s). >CH–C<u>H</u>$_3$; 1.63 (d, $J=6$ Hz), 1.65 (d, $J=6$ Hz). OCH$_3$; 3.41 (s), 3.51 (s). ^{13}C-NMR δ: anomeric C; 103.7, 106.4, 106.5, 106.0 ($\times 2$), 101.2, 104.2, 102.2. >CH–<u>C</u>H$_3$; 18.3, 18.5. OCH$_3$: 55.2, 56.0.

Mild Acid Hydrolysis of II Compound II (500 mg) obtained by alkaline hydrolysis of the crude saponin fraction (fr. II) was dissolved in 2 N HCl–MeOH (5 ml) and stirred at room temperature for 7 h. Acid was neutralized with Ag_2CO_3 and the mixture was filtered. The filtrate was concentrated *in vacuo* and the residue was extracted with H_2O. A part of the water-soluble material (87 mg) was trimethylsilylated and checked by gas liquid chromatography (GLC) for component monosaccharides. Methyl glucuronate and methyl galactoside were detected. The water-insoluble

material (350 mg) was chromatographed on silica gel (100 g) using $CHCl_3$ and $CHCl_3$–MeOH as eluting solvents. The $CHCl_3$ eluate gave an aglycone fraction (43 mg), and the 10% MeOH in $CHCl_3$ eluate gave a monoglycoside fraction (127 mg). By elution with 20% MeOH in $CHCl_3$, II (155 mg) was recovered. The aglycone fraction was again chromatographed on a silica gel (8 g) column eluting with benzene–acetone (9 : 1) to give an aglycone (IV) as a solid (34 mg). Compound IV was acetylated in a mixture (0.2 ml) of pyridine–Ac_2O at room temperature. Chromatography of the acetylation product on silica gel (solvent, 10% AcOEt in hexane) gave a diacetate (IV-acetate-1, 12 mg, prisms from MeOH, mp 200—206 °C) and a monoacetate (IV-acetate-2, 12 mg, prisms from MeOH, mp 204—207 °C). Compound IV-acetate-1 was identified as quillaic acid methyl ester 3,16-di-*O*-acetate, and IV-acetate-2, as a corresponding 3-*O*-monoacetate, by comparison of the IR spectra with those of authentic samples derived from saponins which were isolated from *Momordica cochinchinensis* SPRENG.[2]

The monoglycoside fraction (127 mg) was again chromatographed on silica gel (40 g) eluting with 3% MeOH in $CHCl_3$ to give III as an amorphous powder, mp 163—166 °C. FAB-MS *m/z*: 713 ($[M+Na]^+$). ^{1}H-NMR (100 MHz) δ: ≻CH_3; 0.83, 0.90, 1.02, 1.10, 1.30, 1.76. $COOCH_3$: 3.69 (s), 3.74 (s). ≻C=CH–; 5.52 (br s). ≻C–CHO; 9.73 (s). ^{13}C-NMR (25 MHz): shown in the Tables I and II.

$NaBH_4$ Reduction of II Compound II (40 mg) was dissolved in MeOH (3 ml). $NaBH_4$ (20 mg) was added and the reaction mixture was stirred for 6 h. After neutralization with AcOH and evaporation of the solvent, the residue was chromatographed on silica gel (solvent, 10% MeOH in $CHCl_3$) to give a thin-layer-chromatographically homogeneous reduction product (26 mg). The ^{1}H-NMR spectrum did not show the signals of the aldehydic proton and the carbomethoxyl proton assigned to the methyl ester of the glucuronopyranosyl group. After acid hydrolysis of the reduction product with 1 N HCl, a GLC check revealed the presence of D-galactose and D-glucose.

Permethylation of $NaBH_4$ Reduction Product of II, and Identification of the Component Methylated Monosaccharides by Gas Chromatography–Chemical Ionization-Mass Spectrometry (GC-CI-MS) The reduction product (20 mg) was fully methylated by Hakomori's method[9] and the methylation product was purified by silica gel column chromatography [10 g; solvent, hexane–AcOEt (1 : 1)] to give a permethylate (10 mg). The permethylate was dissolved in 1 N HCl–MeOH (0.5 ml) and the solution was refluxed for 3 h. After neutralization with Ag_2CO_3, the product was acetylated with Ac_2O–pyridine (1 : 1) (0.2 ml) at room temperature. The solvent was blown off by an N_2 stream and the residue was checked by GC-CI-MS. The measurement conditions for GC-CI-MS are shown below. Methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 3,4,6-tri-*O*-methyl-2-*O*-acetyl-D-glucopyranose were identified by comparison of retention times and CI-MS patterns with those of authentic samples.

Permethylation of IX and XI, and Identification of the Component Methylated Monosaccharides Compound IX and XI (10 mg each) were fully methylated according to Hakomori's method, and each product was purified by silica gel column chromatography (2 g; solvent, benzene and 10% acetone in benzene). The thin-layer-chromatographically homogeneous product (5 mg from IX and 6 mg from XI) was dissolved in 1 N HCl–MeOH (0.3 ml) and refluxed for 2 h. The reaction mixture was neutralized with Ag_2CO_3, acetylated with Ac_2O–pyridine and checked by GC-CI-MS. The permethylate of IX gave methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3-di-*O*-methyl-4-*O*-acetyl-L-rhamnopyranose and 3,4-di-*O*-methyl-2-*O*-acetyl-L-arabinopyranose. The permethylate of XI gave methyl glycosides of above methylated monosaccharides and 2,4-di-*O*-methyl-3-*O*-acetyl-D-xylopyranose.

GC-CI-MS Conditions: GC part: column, 2% OV-17 on Chromosorb W (80—100 mesh) packed in a glass column (1.0 m × 3 mm i.d.); column temperature, 130—190 °C (elevation rate, 3 °C/min); carrier gas, He 20 ml/min; injection port temperature, 250 °C. CI-MS part: reagent gas, isobutane; pressure of the reagent gas, less than 1.0×10^{-5} Torr; ionization source temperature, 280 °C; ionizing electron energy, 150 eV; scan range, *m/z* 100—400 (4 s/scan).

Identification of the Component Monosaccharides of I, II, III, V, VII, VIII, IX and X Each compound (10 mg each of quillaic acid glycosides and 5 mg each of methyl glycosides) was dissolved in 1 N HCl–MeOH (2 ml) and refluxed for 2 h. After neutralization with Ag_2CO_3 and filtration, one half of the filtrate was concentrated *in vacuo*. The residue was trimethylsilylated with trimethylsilylimidazole and checked by GLC. GLC conditions were as follows: column, G-SCOT OV-17 on Silanox (50 m × 0.3 mm i.d.); column bath temperature, 130 °C (for pentose and methylpentose derivatives) and 150 °C (for hexose and glucuronic acid derivatives); injection port temperature, 250 °C; carrier gas, He 0.8 ml/min; split ratio, 1/75; make-up gas, He at 50 ml/min.

The other half of the filtrate was used for the determination of the absolute configuration (D/L) of the component sugars according to Hara *et al.*[10] Thus, the filtrate was concentrated *in vacuo* and dissolved in 1 N HCl (1 ml). The solution was heated at 90 °C for 1 h. The acid was neutralized with Ag_2CO_3 and the mixture was filtered. H_2S was bubbled through the filtrate to remove the silver ion and the solution was concentrated *in vacuo* to give a free sugar (mixture). The sugar fraction was converted into the polyhydroxyalkyl-thiazolidine derivative, trimethylsilylated and subjected to GLC. By comparison of the t_R values with those of the thiazolidine derivatives of authentic sugars, the absolute configurations of galactose and xylose were determined to be D, and those of arabinose and rhamnose were determined to be L. The absolute configuration of glucuronic acid was determined to be D by GLC of the hydrolysate of the $NaBH_4$ reduction product of II in the same manner.

The GLC conditions for the determination of the absolute configurations of the component monosaccharides were the same as above except for the column bath temperature (200 C) and the injection port temperature (270 °C).

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References and Notes

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8) The instruments and materials used in this work were as follows: Yanaco micromelting point apparatus (melting points), JASCO DIP-360 digital polarimeter (specific rotations), JEOL JNM FX-100 and GX-400 spectrometers (^{1}H- and ^{13}C-NMR spectra), Hitachi R-22 (90 MHz) spectrometer (^{1}H-NMR spectra), JEOL JMS DX-300 mass spectrometer (MS), Shimadzu GC-8A gas chromatograph (GLC), Shimadzu Auto GCMS-6020 gas chromatograph-mass spectrometer with a GC-MSPAC 500 FDG data analyzer (GC-CI-MS), Kiesel gel 60, 70—230 mesh (E. Merck), MCI Gel CHP-20P, 150—300 μm (Mitsubishi Chemical Industries Ltd.), LiChroprep RP-18, 25—40 μm (E. Merck). NMR spectra were measured in pyridine-d_5 and chemical shifts were shown on the δ-scale using tetramethylsilane as an internal standard.
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