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STRUCTURE OF DESACYLSAPONINS OBTAINED FROM THE BARK OF *QUILLAJA SAPONARIA*

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Key Word Index –*Quillaja saponaria*; Rosaceae; quillaja bark; quillajasaponin; triterpenoid saponin; desacylsaponin; diazomethane degradation; quillaic acid 3,28-*O*-bisglycoside; quillaic acid.

Abstract—A triterpenoid saponin mixture (so-called quillajasaponin) obtained from the bark of *Quillaja saponaria* was treated with weak alkali and two major desacylsaponins were isolated. On the basis of chemical and spectral evidence, they were determined as 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-D-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside and 28-*O*-β-D-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside. Diazomethane degradation providing selectively the 28-*O*-glycoside from the 3,28-*O*-bisglycoside was a useful method for the structure elucidation.

INTRODUCTION

The bark of *Quillaja saponaria* Molina, named quillaja bark (cortex quillajae), is known as a saponin crude drug and has been used as a detergent, dentifrice and expectorant [1]. The existence of a saponin mixture (designated as quillajasaponin), which was recently reported to have a strong adjuvant activity [2, 3] and a plasma cholesterol lowering effect [4], was recognized but as for the constituents of the crude saponin, little was known except for quillaic acid [5, 6] and its monoglucuronide [7], which were obtained upon acid hydrolysis of the saponin. A study on the constituents of the quillajasaponin has been conducted in an attempt to isolate the physiologically active triterpenoid compounds. We report in this paper the structures of two desacylsaponins (quillaic acid 3,28-*O*-bisglycosides), DS-1 (**1**) and DS-2 (**2**), obtained by mild alkaline hydrolysis of the quillajasaponin.

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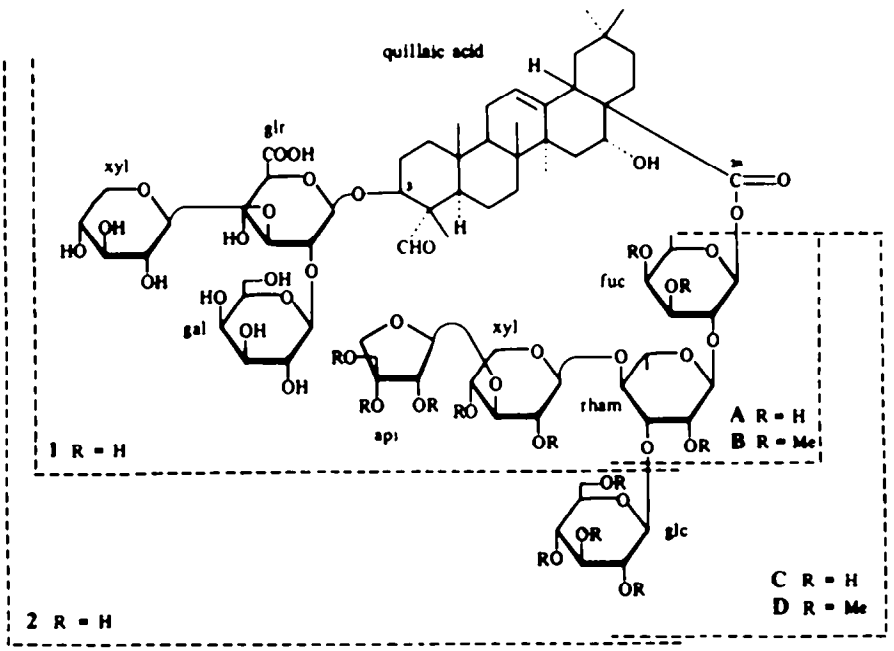


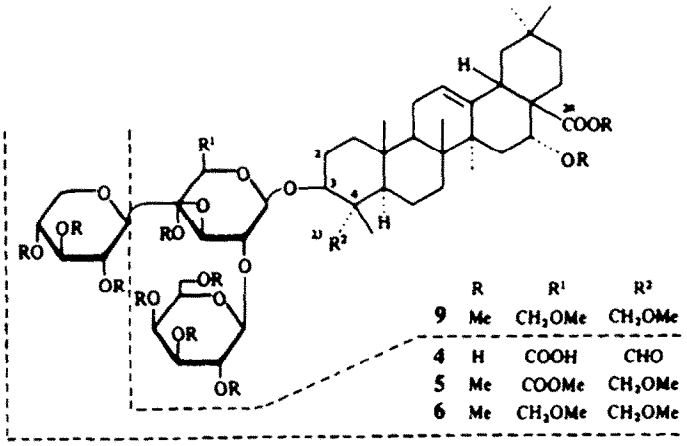
RESULTS AND DISCUSSION

The methanol extract of the bark was fractionated by the ordinary procedure, as described in the Experimental, to give a saponin fraction (quillajasaponin). On treatment with 6% $NaHCO_3$ in 50% methanol, the fraction afforded a desacylsaponin mixture, which was separated by normal and reverse phase column chromatography to give two major compounds, DS-1 (1) and DS-2 (2). Compound 1, as well as 2, showed a single spot on normal and reverse phase TLC, respectively.

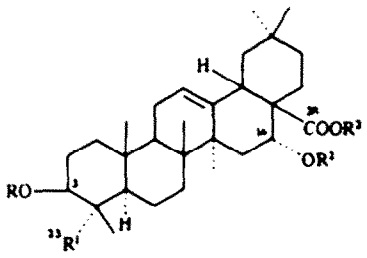
Compound 1 was hydrolysed with acid to yield glucuronic acid (Glr), galactose (Gal), xylose (Xyl), fucose (Fuc), rhamnose (Rham), apiose (Api) and quillaic acid (3). Compound 2 yielded the same products as those of 1 and in addition glucose (Glc). The ^{13}C NMR spectra of 1 and 2 showed seven and eight anomeric carbon signals respectively. The FAB mass spectra of 1 and 2 revealed the molecular ion peaks as a cationized cluster ion at m/z 1589 $[M+2K-H]^+$ and 1751 $[M+2K-H]^+$, respectively. These data indicate 1 to consist of 1 mol each of 3, Glr, Gal, Xyl, Fuc, Rham, Api and another one, Xyl or Api, and 2 to consist of the same components as those of 1 and 1 mol of Glc. Both compounds were suggested to have a 28-*O*-glycosidic linkage since in their ^{13}C NMR spectra the signals due to C-28 of the aglycone part were observed at δ176.0 (in 1) and 176.3 (in 2) [8].

When compounds 1 and 2 were treated with 2% potassium hydroxide in 50% ethanol, they afforded the same prosapogenin (4), which was hydrolysed with acid to yield 3, Glr, Gal and Xyl. Compound 4 showed in the ^{13}C NMR spectrum three anomeric carbon signals and three carbon signals at δ24.6, 54.9 and 84.2 due to C-2, C-4 and C-3 of quillaic acid having a sugar moiety at its 3-position [9], and showed the molecular ion peak at m/z 995 $[M+K]^+$ in the FAB mass spectrum. These data indicate that 4 is the 3-*O*-glycoside of 3 and consists of 1 mol each of 3, Glr, Gal and Xyl. Since the aldehyde group and Glr unit in 4 were thought to complicate its structure elucidation, compound 4 was converted to the more stable compound 6 as follows.

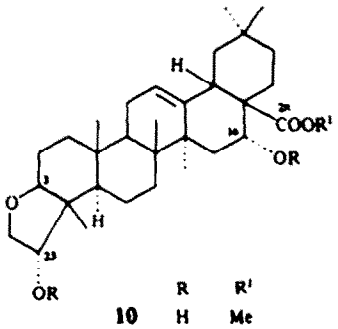
Reduction of 4 with sodium borohydride followed by methylation of the product by the Hakomori method [10] afforded compound 5. Compound 5 was again reduced and methylated to yield compound 6, which was methanolysed to give an aglycone (7) and three methylated sugars. An acetate (8) of 7 showed in the ^1H NMR spectrum the signals of one acetoxyl and three methoxyl groups, together with triplet-like signals at δ4.92 ascribable to the proton at C-3 bearing the acetoxyl group



	R	R¹	R²
9	Me	CH_2OMe	CH_2OMe
4	H	COOH	CHO
5	Me	COOMe	CH_2OMe
6	Me	CH_2OMe	CH_2OMe



	R	R¹	R²
3	H	CHO	H
7	H	CH_2OMe	Me
8	Ac	CH_2OMe	Me



	R	R¹
10	H	Me
11	H	A
12	H	H
13	Me	B
14	Me	H
16	H	C
17	Me	D

[11]. Therefore **7** and **8** were regarded as the 16,23-di-*O*-methyl ether of 16α-hydroxyhederagenin methyl ester and its 3-*O*-acetate, respectively. The methylated sugars were identified as methyl pyranosides of 2,3,4-tri-*O*-methyl-xylose (S-1), 2,3,4,6-tetra-*O*-methyl-galactose (S-2) and 4,6-di-*O*-methyl-glucose (S-3). Therefore, compound **4** has a branched trisaccharide, xylopyranosyl-[galactopyranosyl]-glucuronopyranose, combined with the 3-hydroxyl group of **3**, and the Xyl and Gal units are attached to the 2- and 3-, or 3- and 2-hydroxyl groups of Glr, respectively.

When compound **6** was treated with dilute hydrochloric acid in methanol, a major product was obtained. Methylation of the product afforded compound **9**, which gave on methanolysis compound **7**, S-2 and methyl 3,4,6-tri-*O*-methyl-glucopyranose (S-4). This indicated compound **9** to be the permethylate of the galactosyl-(1 → 2)-glucoside of 16α-hydroxyhederagenin. Therefore, if Xyl, Gal and Glr are assumed to be the most commonly found D-series, the oligosaccharide moiety of **4** is D-galactopyranosyl-(1 → 2)-[D-xylopyranosyl-(1 → 3)]-D-glucuronopyranose. The ^{1}H NMR spectrum of **5** showed three anomeric proton signals of sugar units as doublets with large J values (7, 7 and 8 Hz) indicating their β-linkage [12].

Consequently, compound **4** is quillaic acid 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranoside, and therefore compounds **1** and **2** are thought to be 28-*O*-glycosides of **4**.

In the course of experiments concerning the structure elucidation of **4**, we found and reported [13] that the sugar aglycone linkage of **4** was cleaved to give the aglycone **10** (methyl 3β,16α,23α-trihydroxy-3-*O*,23-methylenolean-12-en-28-oate) and the corresponding oligosaccharide residue by only treatment of **4** with diazomethane ether in methanol. If this procedure (diazomethane degradation) is applied to compounds **1** and **2**, the 28-*O*-glycoside of **12** must be obtained.

Treatment of compound **1** with diazomethane ether in methanol afforded a less polar compound (**11**). Compound **11** revealed an ester carbonyl absorption (1735 cm^{-1}) in the IR spectrum, the signals of four anomeric carbons in the ^{13}C NMR spectrum and a molecular ion peak at m/z 1079 $[M + Na]^+$ in the FAB mass spectrum, and gave on acid hydrolysis Fuc, Rham, Xyl, Api and an aglycone (**12**), which was converted to **10** by methylation with diazomethane. These data indicated that **11** is the 28-*O*-tetraglycoside of **12** obtained by cleavage of the 3-*O*-glycosidic linkage in **1**, and that the sugar moiety of **11** consisted of 1 mol each of Fuc, Rham, Xyl and Api.

Methanolysis of the permethylate (**13**) of **11** gave **14** (the 16,23-di-*O*-methyl ether of **12**), methyl 2,3,5-tri-*O*-methyl-apiofuranoside (S-5) and methyl pyranosides of 2,4-di-*O*-methyl-xylose (S-6), 2,3-di-*O*-methyl-rhamnose (S-7) and 3,4-di-*O*-methyl-fucose (S-8). This indicated that the sugar moiety of **11** is linear in structure and that apiofuranose is located at the terminal. Compound **11** was treated with 2% hydrochloric acid in methanol to yield **15** together with **12** and methyl apiofuranoside. Compound **15** showed a molecular ion peak at m/z 479 $[M + Na]^+$ in the FAB mass spectrum and revealed one methoxyl and three anomeric carbon signals in the ^{13}C NMR spectrum, and afforded Xyl, Rham and Fuc on acid hydrolysis. These data indicated that **15** was a methyl glycoside of a trisaccharide consisting of Xyl, Rham and Fuc. The detailed assignment of the ^{13}C NMR spectrum of **15** was made by taking the glycosylation shift [14, 15] into account and by comparison with the spectra of methyl fucopyranoside and gleditsia saponin [16] as shown in Table 1. The result of methanolysis of **13** indicated that if Xyl, Fuc and Rham are assumed to be the most commonly found D, D and L-series, compound **15** is the methyl glycoside of β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-D-fucopyranose. The above facts suggested that the sugar moiety of **11** is D-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-D-fucopyranose since apiose in glycosides is also usually found as the D-series.

The configuration of the D-fucopyranose unit was regarded to be β by the J values of its anomeric proton signal (doublet, J = 8 Hz) [12] in the ^{1}H NMR spectrum of **13**. The D-apiofuranose unit was considered to have the β-configuration by comparison of its anomeric carbon signal (δ 111.1) in the ^{13}C NMR spectrum of **11** with those of the methyl β- and α-D-apiofuranosides (β-anomer: δ 111.3; α-anomer: δ 104.4). Therefore, it follows that compound **11** is the 28-*O*-β-D-apiofuranosyl-(1 → 3)-β-D-

Table 1. ^{13}C NMR spectral data (C_5D_5N) of compounds **15**, **18** and reference compounds

C	**15**	**18**	Reference compounds*
			[F]
1′	100.5	100.4	101.6
2′	78.4	77.9†	70.0
3′	69.9	69.9	71.6
4′	73.3	73.2	73.2
5′	66.6	66.6	66.9
6′	17.0	17.0	17.1
OMe	54.9	54.9	55.1
			[R]
1″	104.2	104.0	101.2
2″	71.6	71.1	71.9
3″	72.7	77.7†	72.6
4″	84.5	82.7	83.9
5″	68.1	68.1	68.4
6″	18.4	18.7	18.7
			[X]
1‴	107.0	105.1‡	106.9
2‴	76.0	75.4§	76.0
3‴	78.5	78.7	78.6
4‴	70.9	71.1	71.0
5‴	67.4	67.0	67.4
			[G]
1‴		104.7‡	105.5
2‴		75.2§	74.9
3‴		78.7	78.3
4‴		71.1	71.6
5‴		78.7	78.3
6‴		62.2	62.7

*[F]: Methyl α-D-fucopyranoside; [R] and [X]: rhamnose and xylose parts in β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl moiety in gleditsia saponin [16]; [G]: methyl β-D-glucopyranoside.

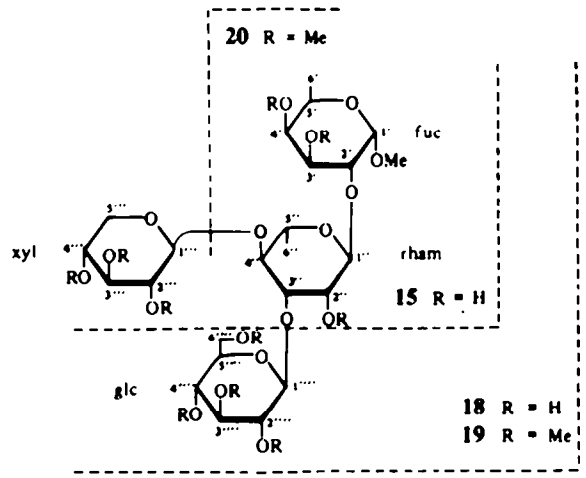
†,‡,§Assignments may be reversed in each vertical column.

xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside of **12**.

Consequently, due to the structures of compounds **4** and **11**, compound **1** is characterized as 3-*O*-β-D-galactopyranoysl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-D-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside.

The structure of the 28-*O*-oligosaccharide moiety in compound **2** was also determined by using the diazomethane degradation method as follows. On treatment with diazomethane, compound **2** afforded a less polar compound (**16**) which was presumed to be the 28-*O*-pentaglycoside of **12**. On acid hydrolysis **16** gave **12**, Fuc, Rham, Xyl, Api and Glc, and showed the molecular ion peak at *m/z* 1241 $[M + Na]^+$ in the FAB mass spectrum, five anomeric carbon signals in the ^{13}C NMR spectrum and an ester carbonyl absorption (1735 cm^{-1}) in the IR spectrum. These data indicated **16** is the 28-*O*-glycoside of **12** consisting of 1 mol each of **12**, Fuc, Rham, Xyl, Api and Glc. Methanolysis of the permethylate (**17**) of **16** afforded **14** and five methylated sugars, S-5, S-6, S-8 and the methyl pyranosides of 2-*O*-methyl-rhamnose (S-9) and 2,3,4,6-tetra-*O*-methyl-glucose (S-10). These facts and the co-existence of **1** and **2** in the same plant material suggested that the sugar moiety of **16** is a pentasaccharide in which a glucopyranose is located on the 3-hydroxyl group of the rhamnopyranose unit in the sugar moiety of **11**.

When compound **16** was treated with 2% hydrochloric acid in methanol in the same manner as **11**, compound **18** was obtained together with **12** and methyl apiofuranoside. Since **18** gave on acid hydrolysis Xyl, Rham, Fuc and Glc and showed the signals of one methoxyl and four anomeric carbons in the ^{13}C NMR spectrum and the molecular ion peak at *m/z* 619 $[M + H]^+$ in the FAB mass spectrum, compound **18** was regarded as the methyl glycoside of tetraose derived from the sugar moiety of **16**. The ^{13}C NMR signals of **18** were assigned as shown in Table 1 by taking the glycosylation shift into account and by comparison with the spectra of **15** and methyl glucopyranoside. The permethylate (**19**) of **18** was methanolysed to give S-8, S-9, S-10 and S-1. Therefore, if Xyl, Glc, Fuc and Rham are assumed to be the commonly found D, D, D and L-series, compound **18** must be methyl β-D-xylopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-α-D-fucopyranoside. The site of linkage of the Glc and Xyl units to the 3- and 4-hydroxyl groups of the rhamnose unit in **18** was confirmed as follows. Compound **19** was hydrolysed with 5% hydrochloric acid in methanol and the major product was methylated to give a compound (**20**) which showed no hydroxyl absorption in the IR spectrum and three anomeric proton signals in the ^1H NMR spectrum. Methanolysis of **20** afforded three methylated sugars, S-8, S-10 and methyl 2,4-di-*O*-methyl-rhamnopyranoside (S-11), which indicated that **20** is the permethylate of glucosyl-(1 → 3)-rhamnosyl-(1 → 2)-fucose.



Accordingly, compound **16** is the 28-*O*-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-D-fucopyranoside of **12**. The configurations of both D-fucopyranose and D-apiofuranose were suggested to be β by the anomeric proton signal of fucopyranose (doublet, $J = 8$ Hz) [12] in the ^1H NMR spectrum of **17** and the anomeric carbon signals of apiofuranose (δ 111.2) in the ^{13}C NMR spectrum of **16**.

Based on the structure of **16**, compound **2** was regarded to be 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-D-apiofuranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside.

To our knowledge, two quillaic acid 3,28-*O*-bisglycosides have been reported [9, 17], but the two desacylsaponins, **1** and **2**, reported here are different in their sugar moieties from those described so far. Diazomethane degradation was a useful method for structure elucidation of the triterpene 3,28-*O*-bisglycoside as described in this paper.

EXPERIMENTAL

All mps are uncorr. Optical rotations were recorded at 18–28° using a 1 dm cell. ^1H NMR spectra were taken at 100 MHz in $CDCl_3$ soln unless otherwise specified, using TMS as internal standard. ^{13}C NMR spectra were recorded at 25 MHz in C_5D_5N (TMS as internal standard) unless otherwise noted, employing the FT mode. The EI- and FABMS were measured on a double focusing mass spectrometer. The former were taken with an accelerating potential of 3–6.5 kV and an ionizing potential of 30–75 eV, and the latter at 1.5–3 kV for the ion source and 6 kV for an Ar beam source; the spectra were obtained from glycerol solns unless otherwise specified. FDMS were taken at 2–3 kV for the field anode and at −5 kV for the slotted cathode plate, at an ion source pressure of *ca* 10^{-6} Torr and an emitter heating current of 18–24 mA. Conditions of GLC (FID mode): (a) glass column (1.2 m × 3 mm) packed with 10% 1,4-butanediol succinate on Shimalite W (60–80 mesh), column temp. 155°; (b) glass column (1.2 m × 3 mm) packed with 1% neopentyl glycol succinate polyester on Chromosorb W(AW) DMCS (60–80 mesh), column temp. 130°. Solvent systems of TLC [silica gel, C-8 (reversed phase) and Avicel]: (a) $CHCl_3$–MeOH–HOAc–H_2O (15:9:1:2); (b) EtOAc–MeOH–H_2O (8:1:0.1); (c) $CHCl_3$–MeOH (10:1); (d) C_6H_6–Me_2CO (2:1); (e) C_6H_6–Me_2CO (4:1); (f) *n*-hexane–EtOAc (2:1); (g) 60% MeOH; (h) upper layer of *n*-BuOH–pyridine–H_2O (6:2:3) + pyridine (1); (i) upper layer of *n*-BuOH–HOAc–H_2O (4:1:5).

Isolation of quillajasaponin. Commercial quillaja bark (2.5 kg) (a voucher specimen of the crude drug is on file at the Faculty of Pharmaceutical Sciences, Kyushu University) was extracted with MeOH and the MeOH was evaporated *in vacuo* to leave the MeOH extract (420 g). The latter was partitioned between EtOAc

and H_2O, and the H_2O layer was passed through an Amberlite XAD-2 column and eluted with H_2O and MeOH. Crude glycosides (85 g) obtained by evaporation of the MeOH eluate were treated with Sephadex LH-20 CC (eluate MeOH) to give two fractions, fraction 1 (39 g) (quillajasaponin) and 2 (42 g). Fraction 1 showing 7 spots (giving a dark-green colour after spraying with 5% H_2SO_4–MeOH followed by heating) on TLC [silica gel (a)] was refluxed with 3.5% HCl in 50% EtOH for 3 hr and the reaction mixture revealed a spot of quillaic acid (R_f 0.40) on TLC [silica gel (c)] (identified with an authentic sample). Fraction 2 showed two major spots (yellow colour by 5% H_2SO_4 MeOH and heating) on TLC [silica gel (a)] and further investigation was not carried out.

Alkaline hydrolysis of quillajasaponin. Quillajasaponin (18 g) was boiled with 6% $NaHCO_3$ in 50% MeOH (300 ml) for 1 hr, and the reaction mixture was neutralized with Dowex 50W-X8 and filtered. The filtrate showing spots of less polar and polar compounds on TLC [silica gel (a)] was evaporated *in vacuo* and the residue was chromatographed on Sephadex LH-20 (MeOH) to give two fractions, fractions 1 (5.0 g) (polar compounds, desacylsaponins) and 2 (2.2 g) (less polar compounds, eliminated acyl groups). Fraction 1 showing two major spots (R_f 0.11, 0.07) on TLC [silica gel (a)] was chromatographed on silica gel (eluant $CHCl_3$ MeOH HOAc H_2O, 15:9:1:2) to give two fractions, fractions 1′ (R_f 0.11) and 2′ (R_f 0.07). Each fraction showed a major and a few minor spots on TLC [C-8(g)] and each was chromatographed on C-8 (eluant 50% MeOH) to give a major component, DS-1 (1, 1.4 g) and DS-2 (2, 2.0 g) [R_f 0.11 (1) and 0.07 (2), silica gel (a); R_f 0.29 (1) and 0.35 (2), C-8 (g)].

DS-1 (1). White powder (from *n*-BuOH–H_2O MeOH), mp 255–258° (decomp.), $[\alpha]_D$ −19.6° (H_2O; *c* 0.67). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (carbonyl), 1610 (COO$^-$). FABMS (using KI as additive) *m/z*: 1589 $[M(C_{69}H_{108}O_{36}) + 2K - H]^+$ = $[(C_{68}H_{10}\text{-}O_{34}\text{·}COOK) + K]^+$. ^{13}C NMR: δ 94.8, 101.0, 103.1, 103.9, 104.5, 106.9, 111.0 (each *d*, anomeric C × 7), 176.0 (*s*, C-28), 209.8 (*d*, C-23). Compound 1 (40 mg) was refluxed with 2 N H_2SO_4 in 50% EtOH for 8 hr and then diluted with H_2O, and the ppt. was collected by filtration. The ppt. (crude aglycone) (10 mg) was chromatographed on silica gel ($CHCl_3$ MeOH, 30:1 → 15:1) to provide 3 as colourless needles (from MeOH–H_2O), mp 256–260°, $[\alpha]_D$ +66.7° (MeOH; *c* 1.02). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1730, 1715 (carbonyl). EIMS *m/z*: 486[$M(C_{30}H_{46}O_5)$]$^+$, 264, 246. Compound 3 was identified with an authentic sample of quillaic acid, by mmp, TLC, IR and ^{13}C NMR. The H_2O layer was neutralized with $Ba(OH)_2$ soln, filtered and the filtrate was concentrated. The residue was examined by TLC [Avicel (h) and (i)], and Glr, Gal, Xyl, Fuc, Rham and Api were detected.

DS-2 (2). White powder (from *n*-BuOH–H_2O MeOH), mp 258–261° (decomp.), $[\alpha]_D$ −24.7° (H_2O; *c* 1.00). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (carbonyl), 1610 (COO$^-$). FABMS (using KI as additive) *m/z*: 1751 $[M(C_{75}H_{118}O_{41}) + 2K - H]^+$ = $[(C_{74}H_{11}\text{-}O_{39}\text{·}COOK) + K]^+$. ^{13}C NMR: δ 95.0, 101.9, 103.1, 103.6, 104.3, 104.5, 104.9, 110.8 (each *d*, anomeric C × 8), 176.3 (*s*, C-28), 210.2 (*d*, C-23). On hydrolysis with acid under the same conditions as those for 1, compound 2 gave 3 and a sugar mixture. The sugar mixture was found to consist of Glr, Gal, Xyl, Fuc, Rham, Api and Glc [TLC, Avicel (h) and (i)].

Alkaline hydrolysis of 1 and 2 providing 4. Compound 1 (200 mg) was refluxed with 2% KOH in 50% EtOH (10 ml) for 7 hr and the reaction mixture was neutralized with dilute HCl and concentrated *in vacuo*. The residue showing a spot (R_f 0.40) on TLC [silica gel (a)] was passed through a Sephadex G-15 column (H_2O) and a silica gel column ($CHCl_3$ MeOH HOAc H_2O, 15:9:1:2) to give a white powder (4) (from *n*-BuOH–H_2O MeOH) (80 mg), mp > 300° (decomp.), $[\alpha]_D$ +18.6° (H_2O; *c* 0.73). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (carbonyl), 1610 (COO$^-$). FABMS *m/z*: 995 $[M(C_{47}H_{72}O_{20}) + K]^+$. ^{13}C NMR ($C_5D_5N$ + D_2O): δ 24.6 (*t*, C-2), 54.9 (*s*, C-4), 84.2 (*d*, C-3), 102.7, 102.9, 103.6 (each *d*, anomeric C × 3), 180.1 (*s*, C-28), 210.1 (*d*, C-23). Compound 4 was hydrolysed with acid in the same manner as for 1 to provide 3 and sugars. The sugars were identified as Glr, Gal and Xyl [TLC, Avicel (h) and (i)]. Compound 2 (500 mg) was hydrolysed with alkali and worked up in the same manner as that for 1 to provide a white powder (200 mg) which was identical with 4 (mmp, TLC, IR and ^{13}C NMR).

Preparation of 6 from 4. $NaBH_4$ (1.6 g) was added to a soln of 4 (250 mg) in 50% MeOH (25 ml) under ice-cooling and the reaction mixture was stirred for 2 hr at room temp. Me_2CO (2 ml) was added to the mixture and concentrated *in vacuo*, and the residue was passed through a Diaion HP20AG column (eluant H_2O → MeOH). The MeOH eluate showing a spot (R_f 0.36) on TLC [silica gel (a)] was evaporated *in vacuo* to give a white powder (230 mg), mp 275–277° (decomp.), $[\alpha]_D$ +3.8° (H_2O; *c* 0.9). ^{13}C NMR: δ 64.2 (*t*, C-23), 175.0 (*s*, COOH of Glr), 182.4 (*s*, C-28), no CHO. The product (200 mg) was treated with NaH (500 mg) and MeI (5 ml) in DMSO (20 ml) (Hakomori method) and the reaction mixture was diluted with H_2O, extracted with Et_2O and the Et_2O layer was washed, dried and evaporated. The residue was chromatographed on silica gel (C_6H_6–Me_2CO, 7:1) to give a major product as a white powder (5) (150 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1760 (Glr-COOMe) [11], 1720 (28-COOMe) [18], no OH. ^{1}H NMR: δ 4.35 (1H, *d*, *J* = 7 Hz, anomeric H), 4.87 (1H, *d*, *J* = 7 Hz, anomeric H), 4.96 (1H, *d*, *J* = 8 Hz, anomeric H). FDMS *m/z*: 1127 $[M(C_{59}H_{98}O_{20}) + H]^+$. Compound 5 (100 mg) in MeOH (10 ml) was reduced with $NaBH_4$ (1.2 g) as for 4. Me_2CO (2 ml) and H_2O (2 ml) were added to the mixture and evaporated *in vacuo*, and the residue was diluted with excess H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed, dried and concentrated, and the residue showing a spot (R_f 0.53) (R_f of 5, 0.77) on TLC [silica gel (d)] was methylated by the Hakomori method. The crude methylated product was chromatographed on silica gel (C_6H_6–Me_2CO, 7:1) to give 6 as a white powder (23 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1720 (28-COOMe), no OH. ^{1}H NMR: δ 3.30 (6H, *s*, OMe × 2), 3.35, 3.37, 3.46 (each *s*, 3H, OMe × 3), 3.50 (9H, *s*, OMe × 3), 3.53, 3.55 (each *s*, 3H, OMe × 2), 3.59 (6H, *s*, OMe × 2), 4.24 (1H, *d*, *J* = 7 Hz, anomeric H), 4.92 (1H, *d*, *J* = 7 Hz, anomeric H), 5.02 (1H, *d*, *J* = 8 Hz, anomeric H).

Methanolysis of 6. Compound 6 (35 mg) was boiled with 10% HCl in MeOH (4 ml) for 2 hr, the mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated and the residue (methanolysate) was examined by TLC [silica gel (f) and (d)] and GLC [conditions (a) and (b)]; one aglycone (7) and three methylated sugars were detected. The sugars were identified as S-1, S-2 and S-3 by direct comparison with authentic samples. The methanolysate was chromatographed on silica gel (C_6H_6–Me_2CO, 80:1 → Me_2CO) to give 7 (10 mg). Compound 7 was acetylated with Ac_2O–pyridine as usual to give an acetate (8) (5 mg) as colourless needles (from MeOH–Me_2CO), mp 207–210°. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730 (ester), no OH. EIMS *m/z*: 572 $[M(C_{35}H_{56}O_6)]^+$, 292. ^{1}H NMR: δ 2.04 (3H, *s*, OAc), 2.93 and 3.10 (1H each, *d*, *J* = 10 Hz, H_2-23), 3.25, 3.29, 3.59 (each *s*, 3H, OMe × 3), 3.91 (1H, *br s*, H-16), 4.92 (1H, *t*-like, *J* = 8 Hz, H-3).

Preparation and methanolysis of 9. Compound 6 was heated with 2% HCl–MeOH for 8 hr at 60° and worked up as before. The mixture showing two major spots [R_f 0.28(6), 0.14] on TLC (silica gel; C_6H_6–Me_2CO, 5:1) was chromatographed on silica gel (*n*-hexane–Me_2CO, 5:1) to give a compound (R_f 0.14) which was methylated by the Hakomori method and worked up as before to yield 9 as a white powder. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1720 (ester), no OH. ^{1}H NMR: δ 3.28 (6H, *s*, OMe × 2), 3.32, 3.35, 3.39 (each *s*, 3H, OMe × 3), 3.51 (9H, *s*, OMe × 3), 3.60, 3.65 (each *s*, 3H, OMe × 2),

4.24 (1H, *d*, J = 8 Hz, anomeric H), 4.62 (1H, *d*, J = 7 Hz, anomeric H). Compound **9** was subjected to methanolysis as for **6**, and 7: S-2 and S-4 were obtained [TLC silica gel (f), GLC (a)].

Diazomethane degradation of **1** *providing* **11**. A soln of CH_2N_2 in Et_2O (150 ml) was poured into a soln of **1** (1.1 g) in MeOH (300 ml) under cooling with ice. The mixture was left to stand for 1 hr at room temp., excess CH_2N_2 was decomposed with HOAc and the solvents were removed by distillation. The crude reaction mixture revealing two major spots (R_f 0.51 and 0.27) in TLC [silica gel (a)] was chromatographed on silica gel ($CHCl_3$–MeOH–H_2O, 8:3:0.3) to give a substance (white powder, R_f 0.27) (a mixture of the related compounds of the methyl ester of **1** and trisaccharides derived from the 3-*O*-sugar residue in **1** [13]) and **11** (white powder, 164 mg, R_f 0.51), mp 198–201° (decomp.), $[\alpha]_D$ −30.4° (MeOH; *c* 1.65). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1735 (ester). FABMS *m/z*: 1079 $[M(C_{53}H_{84}O_{21}) + Na]^+$. ^{13}C NMR: δ94.8, 101.2, 106.9, 111.1 (each *d*, anomeric C × 4), 176.1 (*s*, C-28). Compound **11** was boiled with 2 N H_2SO_4 for 2 hr and the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer showing a spot (R_f 0.21) on TLC [silica gel (e)] was washed, dried and evaporated to give **12**, which was methylated with CH_2N_2 Et_2O in MeOH affording a compound corresponding with an authentic sample of **10** [TLC (e), IR, ^1H NMR and ^{13}C NMR]. The aq. layer was treated as for **1** and Fuc, Rham, Xyl and Api were detected [TLC, Avicel (h) and (i)].

Synthesis and methanolysis of the permethylate (**13**) *of* **11**. Compound **11** (40 mg) was methylated by the Hakomori method as before, and the reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ layer was washed, dried and evaporated. The residue was passed through silica gel (*n*-hexane–Me_2CO, 2:1) and a Sephadex LH-20 (MeOH) column to give **13** as a white powder (16 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750 (ester), no OH. FDMS *m/z*: 1210 $[M(C_{64}H_{106}O_{21})]^+$. ^1H NMR: δ4.63 (1H, *d*, J = 8 Hz, anomeric H of Xyl), 5.24 (1H, *d*, J = 1 Hz, anomeric H of Rham), 5.37 (1H, *d*, J = 8 Hz, anomeric H of ester glycosidic fucose [19]), 5.46 (1H, *d*, J = 2 Hz, anomeric H of Api [20]) (anomeric H signals were assigned by comparison with those observed in the ^1H NMR spectrum of **17**, and with the reported δ and J values [19, 20]). Compound **13** was methanolysed and worked up in the same manner as that for **6**. The methanolysate was examined by TLC [silica gel (d)] and GLC [condition (a)], and S-5, S-6, S-7 and S-8 were detected. Silica gel CC of the methanolysate (*n*-hexane–Me_2CO, 6:1) afforded an aglycone (**14**). EIMS *m/z*: 528 $[M(C_{33}H_{52}O_5)]^+$, 278.

Partial methanolysis of **11** *yielding* **15**. Compound **11** (300 mg) in 2% HCl MeOH (20 ml) was left to stand for 2.5 hr at 40°, and the reaction mixture was treated with Ag_2CO_3, filtered and evaporated. The residue giving three spots (R_f 0.84(**12**), 0.61 (methyl β-D-apiofuranoside) and 0.31] on TLC [silica gel (a)] was chromatographed on silica gel ($CHCl_3$–MeOH–HOAc–H_2O, 15:8:1:1) and Sephadex LH-20 (MeOH) to yield **15** (R_f 0.31) (31 mg) as a hygroscopic powder, $[\alpha]_D$ +11.8° (MeOH; *c* 1.00). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). FAB MS *m/z*: 479 $[M(C_{18}H_{32}O_{13}) + Na]^+$. ^{13}C NMR: see Table 1. Compound **15** was boiled with 2 N H_2SO_4 for 2 hr and treated as usual. The hydrolysate was examined by TLC [Avicel (h) and (i)], and Xyl, Rham and Fuc were detected.

Diazomethane degradation of **2** *affording* **16**. Compound **2** (2.0 g) in MeOH (150 ml) was treated with CH_2N_2 Et_2O (130 ml) and worked up as for **1**. The crude reaction mixture showing three spots (R_f 0.42, 0.27 and 0.21) on TLC [silica gel (a)] was chromatographed on silica gel ($CHCl_3$ MeOH–H_2O, 6:4:0.3) to give substances of R_f 0.27 (3-*O*-trisaccharide residues in **2**), R_f 0.21 (related compounds of the methyl ester of **2**) and R_f 0.42 (**16**) (a white powder, 508 mg), mp 211–214° (decomp.), $[\alpha]_D$ −40.4° (MeOH; *c* 3.23). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1735 (ester) FAB MS *m/z*: 1241 $[M(C_{59}H_{94}O_{26}) + Na]^+$. ^{13}C NMR: δ95.1, 102.0, 104.8, 105.2, 111.2 (each *d*, anomeric C × 5), 175.9 (*s*, C-28). On hydrolysis with acid under the same conditions as for **11**, **16** gave **12** and a sugar mixture. The sugar mixture was found to consist of Fuc, Rham, Xyl, Api and Glc [TLC, Avicel (h) and (i)].

Preparation and methanolysis of **17**. Compound **16** (300 mg) was methylated by the Hakomori method and worked up in the same manner as for **11** to provide a permethylate (**17**) (88 mg) as a white powder. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755 (ester), no OH. FDMS *m/z*: 1414 $[M(C_{73}H_{122}O_{26})]^+$. ^1H NMR: δ4.68 (1H, *d*, J = 7 Hz, anomeric H of xylose), 4.82 (1H, *d*, J = 7 Hz, anomeric H of Glc), 5.05 (1H, *d*, J = 2 Hz, anomeric H of Rham), 5.39 (1H, *d*, J = 8 Hz, anomeric H of Fuc), 5.41 (1H, *d*, J = 2 Hz, anomeric H of Api) (anomeric H signals were assigned by comparison with the ^1H NMR spectrum of **13**). Compound **17** was methanolysed and the methanolysate was examined as for **13**. Compound **14**, S-5, S-6, S-8, S-9 and S-10 were detected [TLC silica gel (d), GLC (a)].

Partial methanolysis of **16** *yielding* **18**. Compound **16** (450 mg) was treated with 2% HCl–MeOH for 1.5 hr and worked up as for **11**. The residue showing the spots of **12**, methyl apiofuranoside and **18** (R_f 0.22) on TLC [silica gel (a)] was chromatographed on silica gel (EtOAc–MeOH–H_2O, 6:4:0.4) and Sephadex LH-20 (MeOH) to give **18** (145 mg) as a white powder, mp 178–182°, $[\alpha]_D$ −3.2° (MeOH; *c* 3.73). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH). FABMS *m/z*: 619 $[M(C_{24}H_{42}O_{18}) + H]^+$. ^{13}C NMR: see Table 1. Compound **18** was hydrolysed with acid and worked up as for **15**; Xyl, Glc, Rham and Fuc were obtained [TLC Avicel (h) and (i)].

Synthesis of **19** *and its methanolysis*. In the same manner as that for **11**, compound **19** (63 mg) was prepared from **18** (120 mg) by the Hakomori method. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH. EIMS *m/z*: 758 $[M(C_{34}H_{62}O_{18})]^+$. Compound **19** was methanolysed as for **13** to give S-8, S-9, S-10 and S-1 [TLC silica gel (d), GLC (a)].

Preparation of **20** *and its methanolysis*. Compound **19** (50 mg) was methanolysed with 5% HCl–MeOH (3 ml) for 3 hr at 50° and worked up as before, and the residue (partial methanolysate) was chromatographed on silica gel (*n*-hexane–Me_2CO, 3:1) to give a major product (R_f 0.09) [TLC silica gel (*n*-hexane–Me_2CO, 3:1)] (R_f of **19**, 0.18). The major product was methylated by the Hakomori method and treated as usual to yield **20** (8 mg) as a colourless syrup. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH. ^1H NMR: δ3.33, 3.34, 3.47, 3.53, 3.57, 3.60, 3.62 (each *s*, 3H, OMe × 7), 3.52 (6H, *s*, OMe × 2), 4.45 (1H, *d*, J = 7 Hz, anomeric H), 4.77 (1H, *d*, J = 4 Hz, anomeric H), 4.95 (1H, *d*, J = 2 Hz, anomeric H). Methanolysis of **20** as before afforded S-8, S-10 and S-11 [TLC silica gel (d), GLC (a)].

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