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AN ACYLATED TRITERPENOID SAPONIN FROM *QUILLAJA SAPONARIA*

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Key Word Index—*Quillaja saponaria*, Rosaceae, quillaja bark, quillajasaponin, acylated triterpenoid saponin, quillaic acid, 3,5-dihydroxy-6-methyl-octanoic acid

Abstract—A new major component in the acylated triterpenoid saponin mixture (called quillajasaponin) obtained from the bark of *Quillaja saponaria* was isolated in a pure state It was characterized on the basis of chemical and spectral data 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)-[*β*-D-glucopyranosyl- (1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-3-[5-*O*-[5-*O*-α-L-rhamnopyranosyl- (1 → 2)-α-L-arabinofuranosyl-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoyl]-*β*-D-fucopyranoside The diazomethane cleavage method providing selectively 28-*O*-glycoside from 3,28-*O*-bisglycoside was effective for the structure elucidation Negative fast atom bombardment mass spectrometry was useful in providing information on the molecular weight and the structure of the complex acylated oligoglycoside.

INTRODUCTION

Quillajasaponin, which has been obtained from the bark of *Quillaja saponaria* Molina (Rosaceae) and known to be the physiologically active triterpenoid saponin mixture showing strong adjuvant activity [1–4] and plasma cholesterol lowing effect [5, 6], was found to be a mixture of acylated triterpenoid saponins [7]. The structures of the desacylsaponins and the acyl moieties obtained by the mild alkaline hydrolysis of the saponin mixture (quillajasaponin) have already been reported [7, 8]. In a continuation of these studies we pursued the isolation and structure elucidation of the genuine components of the quillajasaponin, which has been used for a long time in a crude state It was hoped to find new biologically active compounds and determine the structure–activity correlation for the compounds We wish to report in this paper the structure of a new major component, acylated quillaic acid 3,28-*O*-bisglycoside, of quillajasaponin

RESULTS AND DISCUSSIONS

A glycoside mixture (quillajasaponin) obtained from the methanol extract of the bark was fractionated as shown in the experimental section by droplet counter-current chromatography (DCCC) and reversed phase column chromatography to give one of the major components named QS-III (**1**) Compound **1** showed a single spot on normal- and reversed-phase TLC and the homogeneity of **1** was also suggested by the negative FABMS and ^{13}C NMR spectral data.

Compound **1** revealed the strong IR absorptions due to hydroxy and ester groups and was hydrolysed with sodium bicarbonate to yield 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 (**2**) (desacylsaponin) [7], 3,5-dihydroxy-6-methyl-octanoic acid (**3**) and 5-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoic acid (**4**) [8]. The ^{13}C NMR spectrum of **1** showed the carbon signals due to three ester carbonyls at *δ* 172 3 and 176.0 and the negative FABMS spectrum of **1** revealed the molecular ion peak at *m/z* 2295[M($C_{104}H_{168}O_{55}$)−H]$^-$ These data indicate **1** to consist of one mol each of **2**, **3** and **4** and each of the components were apparently combined with ester linkages. The negative FABMS spectrum of **1** showed, beside the molecular ion peak, notable fragment peaks at *m/z* 467 (base peak) and 639 originating from the terminal acyl residue and the acyloyl acyl residue, respectively These facts suggested that **2**, **3** and **4** were linked linearly

Since the attempts to determine the sites of linkage of the acyl moieties by chemical and spectroscopic methods were unsuccessful because of the existence of labile acyl functionalities and the relatively large molecular size (M_r = 2296) of **1**, selective cleavage of the sugar-aglycone linkages under mild conditions followed by spectral examination of the products were required As described in the preceding paper [7], an important product (modified 28-*O*-glycoside) (**5**) was obtained from **2** by the selective cleavage of the 3-*O*-glycosidic linkage using the diazomethane degradation method [9] When this mild cleavage reaction (diazomethane degradation) was applied to compound **1**, a useful product for structure elucidation of **1** was obtained

Treatment of **1** with diazomethane–ether in methanol afforded a less polar compound **6** which was different from **5**. Compound **6** revealed a molecular ion peak at *m/z* 1839 [M−H]$^-$ together with the peaks derived from terminal acyl and acyloyl acyl moieties at *m/z* 467 and 639 in the negative FABMS spectrum, and gave with mild alkaline hydrolysis **5**, **3** and **4**. These data indicated that **6** was the acylated derivative of **5** and the acyloyl

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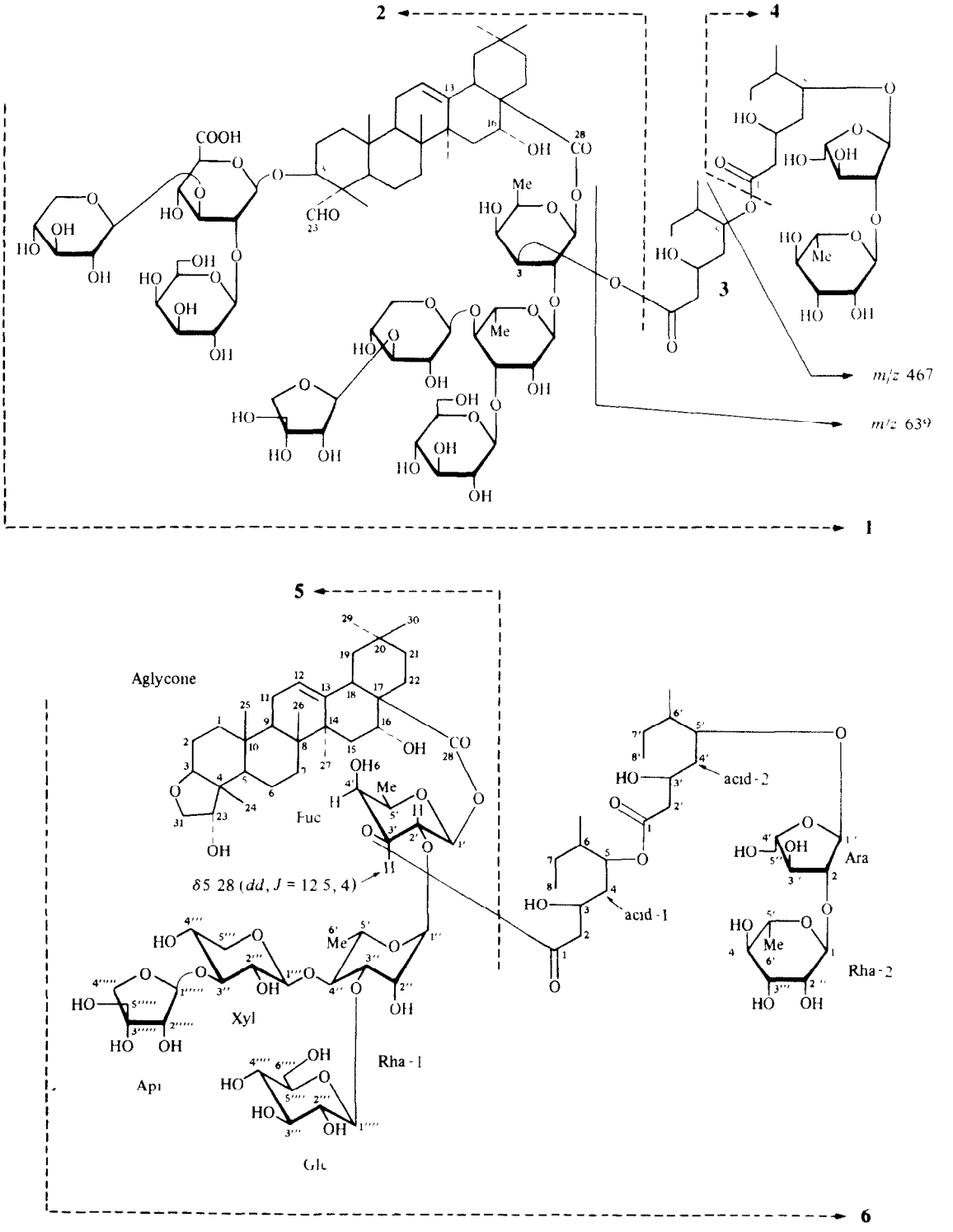

acyl moiety was located at the aglycone part or the oligosaccharide part combined with C-28 of the aglycone. The location of **4** and the acyloyl acyl moiety were determined by analyses of the ^{1}H NMR and ^{13}C NMR spectra of compound **6** as follows

In the ^{13}C NMR spectrum signals ascribable to the acyloyl acyl moiety of **6** which were assigned by comparison of the spectra of **6** and **4** (Table 1) The signals due to C-2, C-3 and C-4 of the inner acyl moiety (acid-1) were in agreement with those of the aglycone (acid-2) of the terminal acyl moiety and the esterification shifts [10, 11] were not observed Therefore, moiety **4** was indicated to be linked at the 5-hydroxy group of **3**.

The ^{13}C NMR signals assignable to the aglycone part

Table 1 ^{13}C NMR spectra of compounds **5** and **6** (in C_5D_5N)

C	5	6		C	5	6		C	4[8]	6
1	39.7	39 6		1′	95 1	94.9		1		172 3
2	22.7	22 6		2′	76 1	72 8[a]		2		43 9[h]
3	82.9	82 8	Fuc	3′	72 8	74 2[d]		3		64 9[i]
4	48 0	47 9		4′	72 3	70 7	acid-1	4		39 0[j]
5	47 4	47 3		5′	75 0	75 4		5		†
6	20 7	20 8		6′	16 7	16 7		6		39 2
7	33 3	33 1						7		25 7
8	40 9	40 7		1″	102 0	102 2		8		12 1
9	47 8	47 6		2″	71 0	70 9		6-Me		14 9[k]
10	38 1	38 0	Rha-1	3″	78 1	78 3				
11	24 3	24 5		4″	82 6	82 8		1′	175 4	172 3
12	122 4	122 3		5″	69 0	69 1		2′	44 3	43.5[h]
13	144 6	144 6		6″	18 8	19 0		3′	65 7	65 3[i]
14	42 2	42 2					acid-2	4′	39 4	39 6[j]
15	36 0[a]	36 1[b]		1‴	104 8[c]	105 0[e]		5′	78 7	78 3
[A] 16	74 3	74 2		2‴	75 4	75 4[f]		6′	38 7	38 5
17	49 4	49 3	Xyl	3‴	85 9	85 6		7′	24 9	24 7
18	41 7	41 5		4‴	69 8	69 8		8′	12.4	12 5
19	47 6	47 6		5‴	66 5	67 1		6′-Me	15.1	15 1[k]
20	30 7	30 7								
21	36 4[a]	36 4[b]		1⁗	105 2[c]	105 2[e]		1″	106 5	106 3
22	31 5	32 0		2⁗	75 4	75 7[f]		2″	88 3	88 2
23	77 5	77 0	Glc	3⁗	78 5	78 3	Ara	3″	77 1	77 0
24	14 9	14 6		4⁗	72 0	71 7		4″	85 0	84 8
25	17 8	17 7		5⁗	78 5	78 3		5″	62 2	62 1
26	17 6	17 7		6⁗	63.0	62 8				
27	27 3	27 2						1‴	100 9	100 9
28	175 9	176.0		1⁗′	111 2	111 1		2‴	72 4[g]	72 5
29	33 1	33 1		2⁗′	77 7	77 6	Rha-2	3‴	72 5[g]	72 5
30	24 9	24.6	Api	3⁗′	80 1	80 4		4‴	73 8	73 8
31	76 3	76 6		4⁗′	75 0	75 2		5‴	70 1	70 1
				5⁗′	65 6	65 3		6‴	18 5	18 5

[A] aglycone, a–k Signals may be reversed in each vertical columns.

Fuc D-fucopyranose, Rha L-rhamnopyranose, Xyl D-xylopyranose Glc D-glucopyranose, Api D-apiofuranose, Ara L-arabinofuranose

† The signal was not assigned by overlapping with other signals

of **6** were in good agreement with those of **5** (Table 1), and indicated that the acyloyl acyl moiety was not combined with C-16 hydroxy group of aglycone but with 28-*O*-oligosaccharide part The carbon signals due to the 28-*O*-oligosaccharide moiety of **6** were assigned by taking the glycosilation shift [12, 13] into account and by comparing with the spectra of ester glycoside [14] and platicodin A [15, 16] When these signals were compared with those of **5** (Table 1), the signals due to C-2, C-3 and C-4 of the fucopyranose in **6** were observed to shift to lower (C-3) and upper field (C-2, C-4) on esterification [10, 11]. This suggested that the C-3 hydroxy group of fucopyranose was acylated by the acyloyl acyl moiety.

The location of the acyloyl acyl moiety was confirmed by comparison of the ^{1}H NMR spectra of **6** and **5**. A signal of a one proton quartet ($J = 12.5$, 4 Hz) at δ 5 28 was observed in the spectrum of **6**. This signal was attributable to the proton next to an acylated hydroxy group, and the splitting pattern and the J values of the signal suggested that the proton had axial–axial and axial–equatorial relationships to its neighbouring protons [17] When seeking for such relationships in the 28-*O*-sugar moiety of **6**, H-2, H-3 and H-3-H-4 of the fucopyranose were applicable to the relationships, since the conformations of fucose (Cl), xylose (Cl), glucose (Cl) and rhamnose-1 (1C) were characterized by the J values of the anomeric proton signals [18] in the ^{1}H NMR spectra of **6** and permethylate of **5** [7].

Based on the above evidence obtained from the ^{13}C NMR and ^{1}H NMR spectra of compound **6**, QS-III(**1**) was identified 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)-[β-D-glucopyranosyl- (1 → 3)]-α-L-rhamnopyranosyl- (1 → 2)-3-[5-*O*-[5-*O*-α-L-rhamnopyranosyl- (1 → 2)-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoyl]--β-D-fucopyranoside Recently there have been reported interesting acylated triterpenoid oligoglycosides, gleditsia saponins [19, 20], which were isolated from *Gleditsia Japonica* and possessing two monoterpene carboxylic acids Compound **1**, identified in the present study is equally interesting since it contains an unique organic acid (or normonoterpene carboxylic acid) (**3**) and an

organic acid glycoside (or normonoterpene carboxylic acid glycoside) (**4**) while the two acyl moieties are linked linearly with the parent oligoglycoside (**2**) The stereochemistry of the organic acids remains to be elucidated

EXPERIMENTAL

For general methods, except for those described below, refer to the preceding paper [7] Conditions of DCCC (descending method) column, glass column (40 cm × 1 5 mm) × 300, mobile phase, lower layer of $CHCl_3$–MeOH–AcOH–H_2O (5 6 1 4), stationary phase, upper phase of $CHCl_3$ MeOH–AcOH–H_2O (5 6 1 4) The negative FABMS spectra were obtained from glycerol–HMPA soln Solvent systems for TLC (i) $CHCl_3$–MeOH–AcOH–H_2O (15 9 1 2), (ii) $CHCl_3$–MeOH–H_2O (7 3 0 3), (iii) MeOH–Me_2CO–H_2O (6 5 9), (iv) MeOH–H_2O (6 4)

Isolation of QS-III (**1**) A crude glycoside mixture (quillajasaponin) (14 g) obtained from the MeOH extract of the bark of *Quillaja saponaria*, showing three major spots (R_f 0 27, 0 19, 0 13) on TLC (silica gel, solvent *i*), was separated by DCCC to give three fractions, fraction 1 (R_f 0 27) 2 (R_f 0 19) and 3 (R_f 0 13) Each fraction was passed through a Sephadex LH-20 column (eluant MeOH) Fraction 1 showed five spots on TLC [C-8 (reversed phase), solvent *iii*] and the isolation of the major component (QS-1) is in progress Fraction 2 revealed four spots on TLC (C-8, solvent *iii*) and was separated by reversed phase CC (C-8) (eluant MeOH H_2O, 6 5 3 5) followed by freeze-drying the eluate to give the major component (QS-II) (0 64 g) as an amorphous powder QS-II gave a single spot on TLC (silica gel and C-8) and one peak on HPLC (column, C-8, solvent, MeOH–H_2O, 5 5 4 5) However, ^{13}C NMR and negative FABMS spectra of QS-II could not be regarded as those of pure glycoside Fraction 3 showed three spots on TLC (C-8 solvent *iii*) and the major component (QS-III) (0 41 g, R_f 0 19 (C-8)) was obtained in the same manner as in QS-II QS-III was considered to be pure glycoside by TLC, NMR and MS

QS-III (**1**) Amorphous powder, mp 203–206 (decomp), $[\alpha]_D$ −37 5° (MeOH, c 1 23) IR ν_{max}^{KBr} cm^{-1} 3420 (OH), 1730 (ester), 1630 (COO^-) ^{13}C NMR (C_5D_5N) δ84 8 (C-3 of aglycone), 94 8 (anomeric C of fucose), 100 9 (anomeric C of rhamnose-2), 101 9 (anomeric C of rhamnose-2), 103 9 (anomeric C), 104 2 (anomeric C), 105 0 (anomeric C × 2), 105 5 (anomeric C), 106 3 (anomeric C of arabinose), 111 0 (anomeric C of apiose), 144 5 (C-13 of aglycone), 172 3 (ester carbonyl C × 2), 176 0 (C-28 of aglycone), 210 1 (C-23 of aglycone) Negative FABMS *m/z* 2295 $[M(C_{104}H_{168}O_{55})-H]^-$ (44%), 639 $[C_{29}H_{52}O_{15}-H]$ (77%), 467 $[C_{20}H_{36}O_{12}-H]^-$ (100%) (Found C, 51 26, H, 7 51 $C_{104}H_{168}O_{55}$ $8H_2O$ requires C, 51 15, H, 7 54%)

Compound **1** (2 mg) was boiled with 12 mg $NaHCO_3$ in MeOH (2 ml) for 10 min, and the reaction mixture was neutralized with Dowex 50W-X8 and filtered The filtrate was evapd and the residue was examined by TLC (silica gel, solvent (i), C-8, solvent (iv) and **2**, **3** and **4** were detected (silica gel, R_f 0 09 (**2**), 0 46 (**4**), 0 79 (**3**), C-8, R_f 0 62 (**2**))

Diazomethane degradation of **1** A soln of CH_2N_2 in Et_2O (170 ml) was poured into a soln of **1** (323 mg) in MeOH (180 ml) while cooling with ice The mixture was left standing for 30 min at room temp, the excess of CH_2N_2 was decomposed with AcOH and the solvents were removed by dist The crude reaction mixture revealing four spots (R_f 0 34, 0 26 0 23, 0 19) on TLC (silica gel, solvent i) was chromatographed on silica gel [eluant, $CHCl_3$–MeOH–H_2O (7 3 0 3 → 25 17 3)] to give **6** (R_f 0 34) (43 mg), 3-*O*-trisaccharide residues in **1** (R_f 0 26) (11 mg) [9] and the related compounds of methyl ester of **1** (R_f 0 23, 0 19) [9]

Compound **6** Amorphous powder, mp 174–177 (decomp), $[\alpha]_D$ −47 3 (MeOH, c 1 58) ^{13}C NMR (C_5D_5N) Table 1 ^1H NMR (C_5D_5N + D_2O) (70°) δ5 28 (1H, *dd*, *J* = 12 5, 4 Hz, H-3 of fucose), 5 43 (1H, *d*, *J* = 1 5 Hz, anomeric H of rhamnose-2), 5 52 (1H, *br* s, anomeric H of rhamnose-1), 5 57 (1H *m*, H-12 of aglycone), 5 61 (1H, *d*, *J* = 1 5 Hz anomeric H of arabinose), 5 87 (1H, *d*, *J* = 4 Hz, anomeric H of apiose), 5 88 (1H, *d*, *J* = 7 Hz, anomeric H of fucose) [anomeric H signals in the ^1H NMR of permethylate of **5** [7] δ4 68 (*d*, *J* = 7 Hz, xylose), 4 82 (*d*, *J* = 7 Hz, glucose), 5 05 (*d*, *J* = 2 Hz, rhamnose-1), 5 39 (*d*, *J* = 8 Hz, fucose), 5 41 (*d*, *J* = 2 Hz, apiose)] Negative FABMS *m/z* 1839 $[M(C_{88}H_{144}O_{40})-H]$ (5%), 639 (29%), 499 [aglycone $(C_{31}H_{48}O_5-H]$ (100%), 467 (91%)

On hydrolysis with $NaHCO_3$ under the same conditions as for **1**, compound **6** gave **5**, **3** and **4** which were detected by TLC (silica gel) [solvent **i**, R_f 0 83 (**3**), 0 56 (**5**), 0 52 (**4**), solvent ii, R_f 0 43 (**3**) 0 36 (**5**), 0 15 (**4**)]

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