Phytochemistry, Vol. 26, No. 8, pp. 2357–2360, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

STRUCTURES OF COMPOUNDS DERIVED FROM THE ACYL MOIETIES OF QUILLAJASAPONIN

RYUICHI HIGUCHI and TETSUYA KOMORI*

Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, 812 Fukuoka, Japan

(*Received* 5 *December* 1986)

Key Word Index—*Quillaja saponaria*; Rosaceae; Quillaja Bark; Quillajasaponin; 3,5-dihydroxy-6-methyl-octanoic acid; 3,5-dihydroxy-6-methyl-octanoic acid 5-*O*-glycosides.

Abstracts—Three compounds, an acid and two glycosidic acids, derived from the acyl moieties of quillajasaponin were obtained from the alkaline hydrolysate of the saponin mixture. On the basis of chemical and spectral evidence, they were identified as 3,5-dihydroxy-6-methyl-octanoic acid, 3,5-dihydroxy-6-methyl-octanoic acid 5-*O*-α-L-arabinofuranoside and 5-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranoside. The first compound was, in fact, isolated and characterized as its lactone form. FDMS was useful in providing information on the M_r of the unique acid and glycosidic acids.

INTRODUCTION

A physiologically active triterpenoid saponin mixture (so-called quillajasaponin) obtained from the bark of *Quillaja saponaria* is a mixture of acylated triterpenoid oligoglycosides(acylated saponins). The isolation and the structure elucidation has been reported [1] of the two desacylsaponins (quillaic acid 3,28-*O*-bisglycosides) obtained, together with less polar compounds (eliminated acyl groups), by mild alkaline hydrolysis of the quillajasaponin. In this paper we wish to report the isolation and structure determination of the eliminated acyl groups, compounds **1**, **2** and **3**, originating from the acyl moieties of quillajasaponin.

RESULTS AND DISCUSSIONS

A mixture of the less polar compounds obtained as described in the preceding paper [1], showing three spots on thin-layer chromatography due to the eliminated acyl groups (compounds **1**, **2** and **3**), was separated by normal phase (silica gel) column chomatography as shown in the experimental section to give compounds **2**, **3** and **4**. Compound **1** was converted into **4** during the separation by the column chromatography.

Compound **4** revealed the IR absorptions due to hydroxy and carbonyl groups and was assigned the molecular formulae $C_9H_{16}O_3$ by taking the FD and EI mass spectral data into account, while the ^1H and ^{13}C NMR (Table 1) spectra suggested the presence of one primary methyl, one secondary methyl, three methylenes, one methine, two oxygen-bearing methines and one estercarbonyl group.

Compound **4** was acetylated to afford the monoacetate **5**. Since **4** contained two degrees of unsaturation, these data suggested **4** to be a monocyclic compound possessing hydroxy and ester functionalities. The characteristic fragment peak at *m/z* 57$[C_4H_9]^+$ in the EI mass spectrum and the ^{13}C NMR signals (δ11.6, 13.9, 25.0, 38.9) [2] of **4** indicated the existence of partial structure A (1-methylpropyl group).

Table 1. ^{13}C NMR spectral data (C_5D_5N) for compounds **2**, **3** and **4**

C	4*	2	3	Reference compounds
1	171.9 *s*	177.7 *s*	175.4 *s*	
2	39.6 *t*[a]	44.8 *t*[b]	44.3 *t*[c]	
3	63.8 *d*	66.0 *d*	65.7 *d*	
4	34.8 *t*[a]	39.3 *t*[b]	39.4 *t*[c]	
5	80.8 *d*	78.6 *d*	78.7 *d*	
6	38.9 *d*	38.6 *d*	38.7 *d*	
7	25.0 *t*	24.9 *t*	24.9 *t*	
8	11.6 *q*	12.3 *q*	12.4 *q*	
6-Me	13.9 *q*	14.9 *q*	15.1 *q*	
				[A]
1′		108.8 *d*	106.5 *d*	110.5 *d*
2′		83.4 *d*	88.3 *d*	83.4 *d*
3′		78.2 *d*	77.1 *d*	78.6 *d*
4′		85.7 *d*	85.0 *d*	85.5 *d*
5′		62.9 *t*	62.2 *t*	62.7 *t*
				[R]
1″			100.9 *d*	102.6 *d*
2″			72.4 *d*[d]	72.1 *d*
3″			72.5 *d*[d]	72.7 *d*
4″			73.8 *d*	73.8 *d*
5″			70.1 *d*	69.5 *d*
6″			18.5 *q*	18.6 *q*

*in $CDCl_3$, [a,b,c,d]: Assignments may be reversed in each vertical column.

[A]: Methyl α-L-arabinofuranoside.

[R]: Methyl α-L-rhamnopyranoside.

Detailed analysis of the ^1H NMR spectrum of the acetate **5**, including spin-decoupling experiments, implied

*To whom correspondence should be addressed.

the presence of the partial structure B in **4**. Irradiation of H-3 (proton next to acetoxy group) at δ5.19, which was assigned by comparison of the ^{1}H NMR spectra of **4** and **5**, collapsed two *dd* (H_2-2) at δ2.54 and 2.95 to two *d* (each J = 17 Hz) indicating C-2 was adjacent to a quarternary carbon(carbonyl group), and also collapsed two *ddd*(H_2-4) at δ1.68 and 2.30 to two *dd*. Irradiation of H-5 (proton next to ester group) at δ4.14 collapsed two *ddd*, due to H_2-4, to two *dd*. The partial structure B was also supported by a fragment peak at *m/z* 115 $[C_5H_7O_3]^+$ in the EI mass spectrum of **4**. These data established the structure of compound **4** as a monohydroxy δ-lactone.

Since **4** was an artefact of **1** and it gave **1** by alkaline treatment, compound **1**, the genuine eliminated acyl group, must be 3,5-dihydroxy-6-methyloctanoic acid though it was not isolated as its acid form.

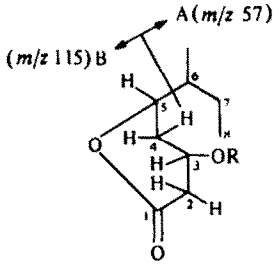
Compound **2** was hydrolysed with acid to yield compounds **1**, **4** and arabinose and revealed in the FD mass spectrum the molecular ion peak as a cationized cluster ion, $[M(C_{14}H_{26}O_8)+Na]^+$ at *m/z* 345. In the ^{13}C NMR spectrum the signals due to one anomeric carbon and a carboxylic acid were observed at δ108.8 and 177.7, respectively. The above data indicate that compound **2** is the monoarabinoside of **1**. Comparison of the ^{13}C NMR spectrum of **2** with those of **4** (artefact of **1**) and methyl arabinosides (Table 1) indicated the existence of an α-arabinofuranosyl moiety in **2**. Consideration of the glycosylation [3, 4] and esterification [5, 6] shifts revealed that the 5-hydroxy group was glycosylated. The location and the configuration of the arabinose was confirmed as follows.

When **2** was methylated by the method of ref. [7] it produced the permethylate **6** which afforded methyl 2,3,5-tri-*O*-methyl-arabinofuranoside upon methanolysis. The EI mass spectrum of **6** gave characteristic fragment peaks (*m/z* 261, 131, 117) due to the cleavage shown in Scheme 1, thus indicating the site of linkage of the arabinose together with the peaks originating from the terminal permethylated pentose (*m/z* 175) [8] and aglycone residue (*m/z* 201). The α-configuration was verified by comparison of the anomeric proton signal (δ5.08, *s*) in the ^{1}H NMR spectrum of **6** with those of methyl furanosides of 2,3,5-tri-*O*-methyl-α- and β-arabinose [9].

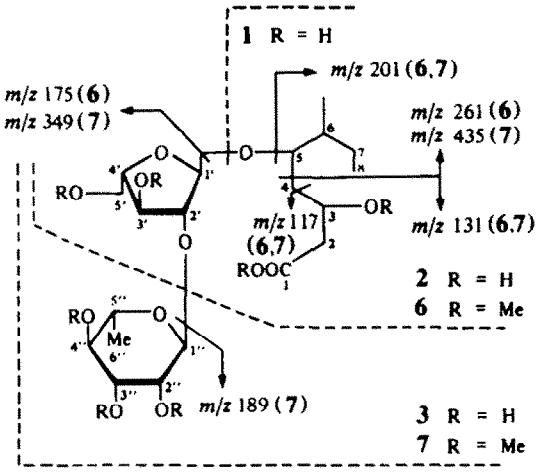
Consequently, if arabinose is assumed to be the most commonly found L-series, compound **2** is 3,5-dihydroxy-6-methyl-octanoic acid 5-*O*-α-L-arabinofuranoside.

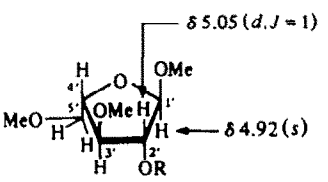
Compound **3** gave, on acid hydrolysis, **1**, **4**, arabinose and rhamnose, and showed in the FD mass spectrum the molecular ion peaks at *m/z* 469 and 491 as a cationized cluster ion, $[M(C_{20}H_{36}O_{12})+H]^+$ and $[M+Na]^+$. In the ^{13}C NMR spectrum two anomeric carbon signals were observed at δ106.5 and 100.9. Therefore, compound **3** is regarded to consist of one mol. each of **1**, arabinose and rhamnose. The FD mass spectrum of **3**, further, revealed the peak at *m/z* 345, $[M+Na-146]^+$, derived from the elimination of a terminal rhamnose residue [10]. In the ^{13}C NMR spectrum signals due to aglycone moieties of **2** and **3** showed good agreement (Table 1). The above data indicated **3** was the 5-*O*-rhamnosyl-arabinoside of **1**, and the structure of the sugar moiety was determined as follows.

When the ^{13}C NMR spectrum of **3** was compared with those of **2** and methyl rhamnosides, the glycosylation shift was observed on the signals at C-1', C-2' and C-3' of α-arabinofuranose in **3** and the α-pyranose form of a rhamnose moiety in **3** was also indicated (Table 1).



4 R = H
5 R = Ac





8 R = H
9 R = Ac

Permethylation of **3** followed by methanolysis of the product (**7**) afforded methyl 2,3,4-tri-*O*-methyl-rhamnopyranoside and **8**. Compound **8** was assigned by the ^{1}H NMR spectrum of its acetate (**9**) as methyl 3,5-di-*O*-methyl-arabinofuranoside. Since the α-configuration of rhamnose and arabinose units was confirmed by their anomeric proton signals, δ4.94 (doublet, J = 2Hz) and 5.12 (singlet) [9, 11], in the ^{1}H NMR spectrum of **7**, the sugar moiety of **3** was assignable to be the α-rhamnopyranosyl-(1 → 2)-α-arabinofuranoside.

Accordingly, compound **3** is 3,5-dihydroxy-6-methyl-octanoic acid 5-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranoside because rhamnose is also usually found as the L-series in natural products.

Investigations concerning the stereochemistry of **4** and the aglycone moiety of **2** and **3** are in progress, but, **2** and **3** are, to the authors' knowledge, novel natural glycosides. For example the aglycone moiety is especially unique in that it is the first of C_9 natural carboxylic acid and the sugar moiety of **3** is also unique in that arabinofuranose, which is usually located at a terminal, in this case is located at the internal position of the glycoside.

It is assumed that compounds **1**, **2** and **3** are combined with desacylsaponins which were described in the preceding paper [1] to form the respective parent acylated saponin, quillajasaponin.

EXPERIMENTAL

For general methods, except for those described below, refer to the preceding paper [1]. Solvent systems for TLC (silica gel and Avicel): (*a*) $CHCl_3$–MeOH–H_2O(7:3:0.3), (*b*) *n*-hexane–EtOAc(1:1), (*c*) upper layer of *n*-BuOH–pyridine–H_2O (6:2:3) + pyridine (1). Conditions of GLC(FID mode): glass column (1.2 m × 3 mm) packed with 10% 1,4-butanediol succinate on shimalite W(60–80 mesh), column temp. 135°. IR spectra were run as CCl_4 soln.

Isolation of compounds **4**, **2** *and* **3**. A mixture of less polar compounds (eliminated acyl groups) (2.2 g), obtained from quillajasaponin by mild alkaline hydrolysis [1] and showing three major spots (R_f 0.39, 0.23, 0.10) on TLC (silica gel; solvent *a*), was chromatographed on silica gel (eluant; $CHCl_3$–MeOH–H_2O, 8:2:0.2 → 7:3:0.3) to give three fractions, fraction 1 (R_f 0.90), 2(R_f 0.23) and 3 (R_f 0.10). Each fraction was passed through a Sephadex LH-20 column (eluant; MeOH) to give compound **4** (105 mg), **2** (107 mg) and **3** (169 mg), respectively.

Compound **4**. Colourless syrup, $[\alpha]_D$ + 39.5° (MeOH; *c* 1.15). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1725 (ester). FDMS *m/z*: 173[M($C_9H_{16}O_3$) + H]$^+$. EIMS *m/z*: 154.1009[M − H_2O]$^+$, calcd for $C_9H_{14}O_2$: 154.0994 (16%), 115.0391[$C_5H_7O_3$]$^+$, calcd for $C_5H_7O_3$: 115.0393 (100%), 57 [C_4H_9]$^+$ (45%). ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, *t*, J = 6 Hz, Me), 0.97 (3H, *d*, J = 6 Hz, Me), 4.16 (2H, *m*, H-3 and H-5). ^{13}C NMR ($CDCl_3$): Table 1.

Monoacetate **5** *of compound* **4**. Compound **4** (21 mg) was acetylated with Ac_2O(1.5 ml)–pyridine (1.5 ml) at 70° for 30 min and the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed, dried and evaporated. The residue was passed through a silica gel column (eluant *n*-hexane–EtOAc, 1:1) to give an acetate (**6**) (15 mg) as a colourless syrup. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1740 (ester). ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, *t*, J = 6 Hz, Me), 0.98 (3H, *d*, J = 6 Hz, Me), 2.08 (3H, *s*, OAc), 1.68 (1H, *ddd*, J = 14, 12, 9 Hz, H-4), 2.30 (1H, *ddd*, J = 14, 7, 3 Hz, H-4), 2.54 (1H, *dd*, J = 17, 7 Hz, H-2), 2.95 (1H, *dd*, J = 17, 7 Hz, H-2), 4.14 (1H, *ddd*, J = 12, 4, 3 Hz, H-5), 5.19 (1H, *dddd*, J = 9, 7, 7, 7 Hz, H-3). Irradiation of δ5.19: δ1.68 (*dd*, J = 14, 12 Hz), 2.30 (*dd*, J = 14, 3 Hz), 2.54 (*d*, J = 17 Hz), 2.95 (*d*, J = 17 Hz). Irradiation of δ4.14: δ1.68 (*dd*, J = 14, 9 Hz), 2.30 (*dd*, J = 14, 7 Hz).

Compound **2**. Amorphous hygroscopic powder, $[\alpha]_D$ −82° (MeOH; *c* 0.87). FDMS *m/z*: 345[M($C_{14}H_{26}O_8$) + Na]$^+$. ^{13}C NMR (C_5D_5N): Table 1. Compound **2** was heated with 1 N H_2SO_4 for 1 hr at 70° and diluted with H_2O. The reaction mixture was extracted with Et_2O and the Et_2O layer was washed, dried and evaporated to give crude aglycone fraction in which **4** and **1** were detected by TLC (silica gel, solvent *a*). The H_2O layer was neutralized with $Ba(OH)_2$ soln, filtered and the filtrate was concentrated. The residue was examined by TLC (Avicel, solvent *b*), and arabinose was detected.

Permethylate **6** *of compound* **2** [7] *and its methanolysis*. Compound **2** (47 mg) was treated with NaH (250 mg) and MeI (7 ml) in DMSO (10 ml). The reaction mixture was diluted with H_2O and extracted with $CHCl_3$, and the $CHCl_3$ layer was washed, dried and evapd. The residue was chromatographed on silica gel (eluant, *n*-hexane–EtOAc, 2:1) to give **6** (23 mg) as a colourless syrup. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1740 (ester). FDMS *m/z*: 392[M($C_{19}H_{36}O_8$)]$^+$. EIMS *m/z* (rel. int.): 261 (7), 201 (66), 175 (100), 131 (12), 117 (17). ^{1}H NMR($CDCl_3$): δ0.8–1.0(6H, *m*, Me × 2), 1.4–1.9 (5H, *m*, H_2-4, H-6, H_2-7), 2.53 (2H, *d*, J = 6 Hz, H_2-2), 3.38 (12H, *s*, OMe × 4), 3.68 (3H, *s*, COOMe), 5.08 (1H, *s*, anomeric H of arabinofuranose). Compound **6** was heated with 7% HCl in MeOH for 2 hr, the mixture was treated with Ag_2CO_3 and filtered. The filtrate was evapd and the residue (methanolysate) was examined by TLC (silica gel, solvent *b*) and GLC and methyl 2,3,5-tri-*O*-methyl-arabinofuranoside was detected (α-anomer, R_f 0.56, R_t 10.6 min; β-anomer, R_f 0.39, R_t 14.0 min).

Compound **3**. Amorphous hygroscopic powder, $[\alpha]_D$ −98.3° (MeOH; *c* 0.58). FDMS *m/z*: 469[M($C_{20}H_{36}O_{12}$) + H]$^+$, 491[M + Na]$^+$, 345[M + Na − 146]$^+$. ^{13}C NMR (C_5D_5N): Table 1. On hydrolysis with acid under the same conditions as for **2**, compound **3** gave **4** and **1** (detected by TLC; silica gel, solvent a) and a sugar mixture. The sugar mixture was found to consist of arabinose and rhamnose (TLC: Avicel, solvent b).

Preparation of permethylate **7**. Methylation of **3** (88 mg) in the same manner as for **2** afforded **7** (61 mg) as a colourless syrup. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1740 (ester). FDMS *m/z*: 567 [M($C_{27}H_{50}O_{12}$) + H]$^+$. EIMS *m/z* (rel. int.): 435 (1), 349 (62), 201 (79), 189 (100), 131 (10), 117 (11). ^{1}H NMR ($CDCl_3$): δ0.8–1.0 (6H, *m*, Me × 2), 1.27 (3H, *d*, J = 6 Hz, H_3-6″ of rhamnose), 1.4–1.9 (5H, *m*, H_2-4, H-6, H_2-7), 2.53 (2H, *d*, J = 6 Hz, H_2-2), 3.37, 3.39, 3.42, 3.48, 3.50, 3.53 (each *s*, 3H, OMe × 6), 3.67 (3H, *s*, COOMe), 4.94 (1H, *d*, J = 2 Hz, anomeric H of rhamnose), 5.12 (1H, *s*, anomeric H of arabinofuranose).

Methanolysis of compound **7**. Permethylate **7** (40 mg) was boiled with 10% HCl in MeOH for 2 hr and treated in the same manner as for **6**. The methanolysate was examined by TLC (silica gel, solvent *b*) and GLC and methyl 2,3,4-tri-*O*-methyl-rhamnopyranoside was detected (α-anomer, R_f 0.54, R_t 8.6 min). It was then chromatographed on silica gel (eluant, *n*-hexane-EtOAc, 1:1) to give **8** (colourless syrup) (R_f 0.20, silica gel, solvent *b*). Compound **8** yielded, by usual acetylation, a monoacetate **9** (colourless syrup). ^{1}H NMR ($CDCl_3$): δ2.10 (3H, *s*, OAc), 3.40 (3H, *s*, OMe), 3.44 (6H, *s*, OMe × 2), 3.6 (3H, *m*, H-3′, H_2-5′), 4.16 (1H, *m*, H-4′), 4.92 (1H, *s*, H-1′), 5.05 (1H, *d*, J = 1 Hz, H-2′). Irradiation of δ5.05 simplified the signal at δ3.6.

Acknowledgements—The authors are grateful to Emeritus Prof. T. Kawasaki of Kyushu University for helpful discussion and suggestion and to Mrs E. Onae (neé Uemura) for technical assistance. Thanks are also due to Mr. I. Maetani, Mr A. Tanaka and Miss K. Soeda of the Faculty of Pharmaceutical Sciences, Kyushu University, for FD- and EIMS, ^{13}C NMR and ^{1}H NMR data, respectively.

REFERENCES

1. Higuchi, R., Tokimitsu, Y., Fujioka, T., Komori, T., Kawasaki, T. and Oakenful, D. G. (1987) *Phytochemistry* **26**, 229.
2. Johnson, L. F. and Jankowski, W. C. (1972) *Carbon-13 NMR Spectra* p. 207. John Wiley, New York.
3. Kasai, R., Suzuo, M., Asakawa, J. and Tanaka, O. (1977) *Tetrahedron Letters* 175.
4. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y. (1977) *Tetrahedron Letters* 179.
5. Terui, Y., Tori, K. and Tsuji, N. (1976) *Tetrahedron Letters* 621.
6. Vigon, M. R. and Vottero, P. J. A. (1976) *Tetrahedron Letters* 2445.
7. Hakomori, S. (1964) *J. Biochem. (Tokyo)* **55**, 205.
8. Komori, T., Ida, Y., Muto (nee Inatsu), Y., Miyahara, K., Nohara, T. and Kawasaki, T. (1975) *Biomed. Mass Spectrom.* **2**, 65.
9. Nohara, T., Yabuta, H., Suenobu, M., Hida, R., Miyahara, K. and Kawasaki, T. (1973) *Chem. Pharm. Bull.* **21**, 1240.
10. Schulten, H. R., Komori, T. Nohara, T., Higuchi, R. and Kawasaki, T. (1978) *Tetrahedron* **34**, 1003.
11. Higuchi, R. and Kawasaki, T. (1972) *Chem. Pharm. Bull.* **20**, 2143.