Resin Glycosides. XIV.[1] Quamoclins I—IV, New Ether-Soluble Resin Glycosides (Jalapin) from the Seeds of *Quamoclit pennata*

Masateru ONO, Katsumi KUWABATA, Toshio KAWASAKI and Kazumoto MIYAHARA*

Faculty of Pharmaceutical Sciences, Setsunan University, 45–1 Nagaotoge-cho, Hirakata, Osaka 573–01, Japan.

Received April 20, 1992

Four new resin glycosides, quamoclins I—IV, were isolated from the jalapin fraction of the seeds of *Quamoclit pennata*, and their structures have been determined on the basis of chemical and spectral data. They are the first examples of jalapins with organic acid (2*S*-methylbutyric acid) and fatty acid (*n*-decanoic or *n*-dodecanoic acid) groups coexisting in the molecules.

Keywords resin glycoside; *Quamoclit pennata*; quamoclinic acid A; convolvulinolic acid; quamoclin I; quamoclin II; quamoclin III; quamoclin IV

As a part of our studies on the resin glycosides, which are characteristic of Convolvulaceae plants, the seeds of *Quamoclit pennata* BOJER. were examined. This paper deals with the isolation and structure elucidation of four new ether-soluble resin glycosides (jalapin) named quamoclins I (**1**), II (**2**), III (**3**) and IV (**4**).

The MeOH extractives (12.9%) of the seeds were partitioned between ether and H_2O. The aqueous layer was extracted with *n*-BuOH, and the extractive was chromatographed over MCI gel CHP 20P and Sephadex LH-20 to afford jalapin (0.4%) and ether-insoluble resin glycoside (convolvulin)[2] (2.3%) fractions.

First, the chemical components of the jalapin fraction were examined. The alkaline hydrolysis product was fractionated into organic acid and glycosidic acid fractions. Gas chromatography (GC) of the former revealed the presence of 2-methylbutyric, *n*-decanoic and *n*-dodecanoic acids. The configuration at C-2 of 2-methylbutyric acid was determined to be *S* by Helmchen's method.[3]

The latter fraction gave a new glycosidic acid named quamoclinic acid A (**5**), $C_{44}H_{78}O_{24}$. Acidic hydrolysis of **5** provided an aglycone and a monosaccharide mixture. Methylation of the former with diazomethane furnished methyl convolvulinolate (methyl 11-hydroxytetradecanoate).[4] Its chirality at C-11 was defined as *S* by Mosher's method.[5] Thin layer chromatography (TLC) of the monosaccharide mixture revealed the presence of fucose, rhamnose and glucose, which were disclosed to be of D-, L- and D-form, respectively, on the basis of GC analysis according to Hara *et al.*[6]

The negative ion fast atom bombardment mass spectrum (negative FAB-MS) of **5** revealed the $[M-H]^-$ ion peak at *m/z* 989 and fragment ion peaks at *m/z* 843 $[989-146$ (deoxyhexose unit)$]^-$, 827 $[989-162$ (hexose unit)$]^-$, 681 $[843-162$ and/or $827-146]^-$, 535 $[681-146]^-$, 389 $[535-146]^-$, 243 $[389-146$, convolvulinolic acid$-H]^-$ (Fig. 1) suggesting a branched chain pentaose structure composed of 1 mol of hexose and 4 mol of 6-deoxyhexose.

The ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) signals due to the sugar moieties of **5** were superimposable on those of the methyl ester of operculinic acid A (**6**) previously obtained from Jalapae Braziliensis[2]

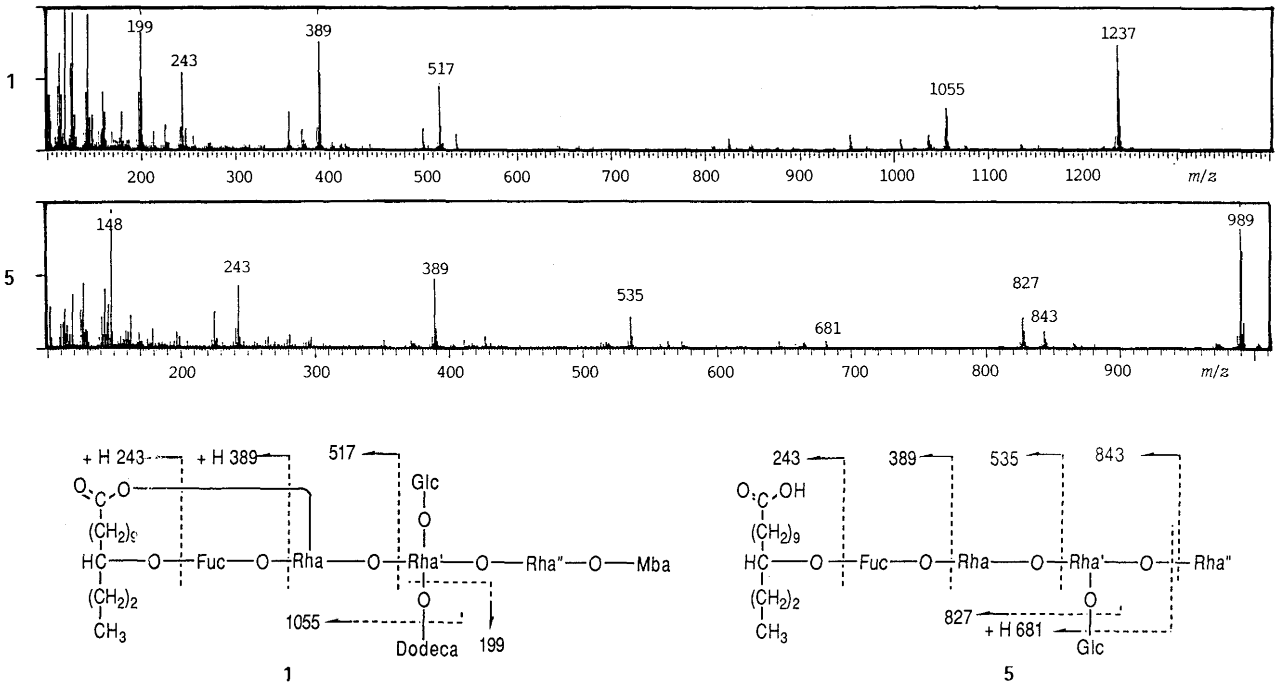


Fig. 1. Negative FAB-MS of **1** and **5**

Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Dodeca, *n*-dodecanoyl; Mba, 2-methylbutyryl.

TABLE I. ^{1}H-NMR Spectral Data for **1**—**5** (in Pyridine-d_5)

	1$^{a)}$	**2**$^{a)}$	**3**$^{b)}$	**4**$^{a)}$	**5**$^{a)}$
Fuc -1	4.69, d (7.3)	4.82, d (7.7)	4.69, d (7.3)	4.71, d (7.7)	4.77, d (7.7)
2	4.13, dd (7.3, 9.5)	4.52, dd (7.7, 9.2)	4.10, dd (7.3, 9.0)	4.16, d (7.7, 9.2)	4.46, dd (7.7, 9.5)
3	4.03, dd (9.5, 4.0)	4.21, dd (9.2, 3.3)	4.02, dd (9.0, 3.0)	4.05, dd (9.2, 3.6)	4.13, dd (9.5, 3.7)
4	*ca.* 3.94	3.94, d (3.3)	*ca.* 3.94	*ca.* 3.96	3.93, dd (3.7, 0.7)
5	3.71, dq (0.7, 6.4)	3.82, q (6.6)	3.71, q (6.4)	3.74, q (6.5)	3.78, dq (0.7, 6.4)
6	1.48, d (6.4)	1.52, d (6.6)	1.48, d (6.4)	1.50, d (6.5)	1.50, d (6.4)
Rha -1	5.48, d (1.8)	6.34, s	5.48, d (1.5)	5.50, d (1.6)	6.19, d (1.1)
2	5.90, dd (1.8, 3.3)	5.24, d (2.6)	5.89, dd (1.5, 3.4)	5.93, dd (1.6, 3.2)	4.65, dd (1.1, 3.3)
3	4.99, dd (3.3, 9.2)	5.67, dd (2.6, 9.5)	4.98, dd (3.4, 9.3)	5.02, dd (3.2, 9.4)	4.59, dd (3.3, 9.3)
4	4.15, dd (9.2, 9.2)	4.67, dd (9.5, 9.5)	4.14, dd (9.3, 9.3)	4.17, dd (9.4, 9.4)	4.19, dd (9.3, 9.3)
5	4.47, dq (9.2, 5.9)	4.98, dq (9.5, 6.2)	4.47, dq (9.3, 6.1)	4.49, dq (9.4, 6.4)	4.83, dq (9.3, 6.2)
6	1.63, d (5.9)	1.56, d (6.2)	1.63, d (6.1)	1.65, d (6.4)	1.57, d (6.2)
Rha′ -1	5.88, d (1.8)	5.63, d (1.5)	5.88, d (1.5)	5.91, d (1.6)	5.86, d (2.2)
2	6.30, dd (1.8, 3.3)	6.00, dd (1.5, 3.3)	6.29, dd (1.5, 3.0)	6.31, dd (1.6, 3.4)	5.15, dd (2.2, 2.9)
3	4.76, dd (3.3, 9.2)	4.65, dd (3.3, 9.6)	4.75, dd (3.0, 9.0)	4.78, dd (3.4, 9.3)	4.70, dd (2.9, 9.2)
4	4.33, dd (9.2, 9.2)	4.33, dd (9.6, 9.6)	4.32, dd (9.0, 9.0)	4.35, dd (9.3, 9.3)	4.47, dd (9.2, 9.2)
5	*ca.* 4.38	*ca.* 4.37	*ca.* 4.37	*ca.* 4.38	4.38, dq (9.2, 6.2)
6	1.66, d (5.9)	1.63, d (5.5)	1.65, d (5.5)	1.66, d (5.6)	1.58, d (6.2)
Rha″-1	6.22, d (1.8)	6.22, s	6.21, d (1.7)	6.25, d (1.6)	6.18, d (1.5)
2	4.92, dd (1.8, 3.3)	4.92, d (3.3)	4.92, dd (1.7, 3.3)	4.95, dd (1.6, 3.2)	4.87, dd (1.5, 3.5)
3	4.51, dd (3.3, 9.2)	4.46, dd (3.3, 9.2)	4.51, dd (3.3, 9.3)	4.54, dd (3.2, 9.3)	4.42, dd (3.5, 9.2)
4	5.74, dd (9.2, 9.2)	5.74, dd (9.2, 9.2)	5.73, dd (9.3, 9.3)	5.77, dd (9.3, 9.3)	4.21, dd (9.2, 9.2)
5	*ca.* 4.38	*ca.* 4.35	*ca.* 4.37	*ca.* 4.38	4.30, dq (9.2, 6.1)
6	1.40, d (6.2)	1.41, d (6.2)	1.40, d (6.1)	1.41, d (6.4)	1.57, d (6.1)
Glc -1	5.06, d (7.7)	5.09, d (7.7)	5.05, d (7.6)	5.07, d (7.7)	5.20, d (7.7)
2	3.96, dd (7.7, 9.0)	3.97, dd (7.7, 9.2)	3.95, dd (7.6, 9.0)	3.98, dd (7.7, 9.3)	3.97, dd (7.7, 9.0)
3	4.04, dd (9.0, 9.0)	4.14, dd (9.2, 9.2)	4.04, dd (9.0, 9.0)	4.06, dd (9.3, 9.3)	4.17, dd (9.0, 9.0)
4	3.94, dd (9.0, 9.0)	4.18, dd (9.2, 9.2)	3.93, dd (9.0, 9.0)	*ca.* 3.96	4.09, dd (9.0, 9.0)
5	3.75, ddd (2.6, 5.8, 9.0)	3.88, ddd (2.2, 4.0, 9.2)	3.75, ddd (2.1, 6.0, 9.0)	3.76, ddd (2.0, 6.0, 9.3)	*ca.* 3.94
6	4.09, dd (5.8, 11.7)	4.39, dd (4.0, 11.7)	4.09, dd (6.0, 11.5)	4.11, dd (6.0, 11.7)	4.26, dd (5.5, 11.7)
	4.39, dd (2.6, 11.7)	4.49, dd (2.2, 11.7)	4.39, dd (2.1, 11.5)	4.41, dd (2.0, 11.7)	4.51, dd (2.4, 11.7)
Ag -2	2.27, ddd (4.0, 6.6, 15.0)	2.29, ddd (2.4, 6.6, 15.0)	2.27, ddd (4.5, 8.0, 14.5)	2.27, ddd (4.0, 8.0, 14.5)	2.51, t (7.3)
	2.44, ddd (4.0, 8.8, 15.0)	2.70, ddd (1.8, 10.8, 15.0)	2.45, ddd (4.0, 8.0, 14.5)	2.45, ddd (4.0, 8.0, 14.5)	
11	3.83, m	3.88, m	3.82, m	3.84, m	*ca.* 3.98
CH_3	0.91, t (7.3)	0.99, t (7.0)	0.91, t (7.3)	0.87, t (7.4)	0.98, t (7.3)
Mba -2	2.52, ddq (7.0, 7.0, 7.0)	2.50, ddq (7.0, 7.0, 7.0)	2.52, ddq (7.0, 7.0, 7.0)	2.53, ddq (6.9, 6.9, 6.9)	
4	0.94, t (7.3)	0.93, t (7.5)	0.94, t (7.0)	0.94, t (7.5)	
5	1.20, d (7.0)	1.20, d (7.0)	1.20, d (7.0)	1.21, d (6.9)	
Org -2	2.34, m	2.43, m	2.34 m	2.34, m	
CH_3	0.87, t (7.2)	0.87, t (7.1)	0.85, t (7.3)	0.87, t (7.1)	

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses); Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Ag, aglycone ((*S*)-convolvulinolic acid or (*S*)-jalapinolic acid); Mba, 2*S*-methylbutyryl; Org, *n*-dodecanoyl or *n*-decanoyl. Assignments are based on the ^{1}H–^{1}H COSY and NOESY spectral data. *a*) 600 MHz, *b*) 400 MHz.

(Fig. 2, Tables I and II).

Consequently, quamoclinic acid A (**5**) was concluded to be 11*S*-convolvulinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, as shown in Fig. 3.

Quamoclin I (**1**), $C_{61}H_{106}O_{25}$, furnished, on alkaline hydrolysis, 2-methylbutyric acid, *n*-dodecanoic acid and **5**, and exhibited the signals due to three ester carbonyl (δ 176.3, 173.6 and 173.1) and five anomeric carbons (δ 105.4, 104.1, 103.2, 100.0 and 98.4) in the ^{13}C-NMR spectrum, indicating that **1** consists of 1 mol each of 2-methylbutyric acid, *n*-dodecanoic acid and **5** (Fig. 2, Table II).

The $[M-H]^-$ ion peak at *m/z* 1237 in the negative FAB-MS and the nonequivalent 2-H_2 signals (δ 2.27 and 2.44) of the convolvulinolic acid group of **1** in the ^{1}H-NMR indicated that the carboxy group of the convolvulinolic acid is intramolecularly linked with a hydroxy group of the sugar moiety to form a macrocyclic ester structure, as in all the jalapins so far isolated.[1,2,7-11]

Comparing the chemical shifts of ^{1}H-NMR signals between **5** and **1**, downfield shifts of 1.25, 1.15 and 1.53 ppm were observed at 2-H of the first rhamnose (Rha), 2-H of the second rhamnose (Rha′) and 4-H of the third rhamnose (Rha″), respectively. Therefore, the ester linkages are located at 2-OH of Rha, 2-OH of Rha′ and 4-OH of Rha″ (Fig. 2, Table I).

The negative FAB-MS of **1** exhibited, besides the same fragment peaks at *m/z* 243 and 389 as those of **5**, a peak at *m/z* 517 in place of that at *m/z* 535 [convolvulinolic acid + 2 × (6 − deoxyhexose unit) − H]$^-$ observed in the spectrum of **5** (Fig. 1). The difference of 18 mass units suggested the intramolecular ester linkage to be located in Rha.[1]

The EI-MS of the peracetate of **1** (**7**) revealed fragment ion peaks at *m/z* 315, 331, 625, 627 and 973 ascribable to the fragments a, b, c, e and g in Fig. 4. The ester linkages of the *n*-dodecanoic and 2-methylbutyric acids are therefore located at 2-OH of Rha′ and 4-OH of Rha″, respectively.

Accordingly, the structure of quamoclin I (**1**) was defined as (*S*)-convolvulinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[4-*O*-2(*S*)-methylbutyryl-α-L-rhamnopyranosyl-

TABLE II. ^{13}C-NMR Spectral Data for **1**—**5** (in Pyridine-d_5)

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]		**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
Fuc -1	104.1	101.5	104.1	104.3	101.0	Rha″-4	75.2	75.3	75.2[c]	75.2	73.9
2	80.0	73.5	80.0	79.9	75.2	5	68.2	68.1[c]	68.2	68.1	70.4
3	73.4	76.5	73.4	73.4	76.5	6	18.0	18.0	18.0	18.0	18.4
4	72.9	73.6	72.9	72.9	73.5	Glc -1	105.4	104.8	105.4	105.4	105.5
5	70.8	71.2	70.8	70.8	71.2	2	75.2	75.2	75.3[c]	75.2	75.1
6	17.3	17.2	17.3	17.3	17.2	3	78.4	78.4	78.4	78.4	78.4
Rha -1	98.4	100.0	98.4	98.5	101.5	4	71.5	70.8	71.5	71.4	71.7
2	73.7	69.9	73.7	73.6	72.6	5	78.1	78.1	78.1	78.2	78.4
3	69.4	77.8	69.4	69.4	72.6	6	62.9	62.6	62.9	62.9	62.9
4	81.5	76.9	81.5	81.4	82.3	Ag -2	34.2	34.2	34.2	34.2	35.0
5	68.9	68.1	68.9	68.9	67.6	11	82.0	79.3	82.0	82.3	77.6
6	19.1	19.1	19.1	19.1	18.7	CH_3	14.6	14.7	14.6	14.3	14.6
Rha′-1	100.0	99.3	100.0	100.0	103.4	C=O	173.1	173.6	173.1	173.1	176.3
2	73.2	72.2	73.2	73.2	71.9		173.6	174.6	173.6	173.6	
3	80.1	80.2	80.1	80.1	82.8		176.3	176.3	176.3	176.3	
4	78.7	78.6	78.7	78.6	78.6	Mba -2	41.6	41.5	41.6	41.5	
5	68.5	68.0[c]	68.5	68.5	68.5	3	27.2	27.1	27.2	27.2	
6	18.9	18.7	18.9	18.9	18.9	4	11.8	11.7	11.8	11.8	
Rha″-1	103.2	103.3	103.2	103.2	103.1	5	17.0	17.0	17.0	17.0	
2	72.5	72.3	72.5	72.4	72.6	Org -2	34.6	34.5	34.6	34.5	
3	70.3	70.3	70.3	70.3	72.8	CH_3	14.3	14.3	14.3	14.3	

δ in ppm from TMS. Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Ag, aglycone ((*S*)-convolvulinolic acid or (*S*)-jalapinolic acid); Mba, 2*S*-methylbutyryl; Org, *n*-dodecanoyl or *n*-decanoyl. *a*) 100 MHz, *b*) 150 MHz. *c*) Assignments may be interchanged in each column. All data are based on the HETCOR spectra.

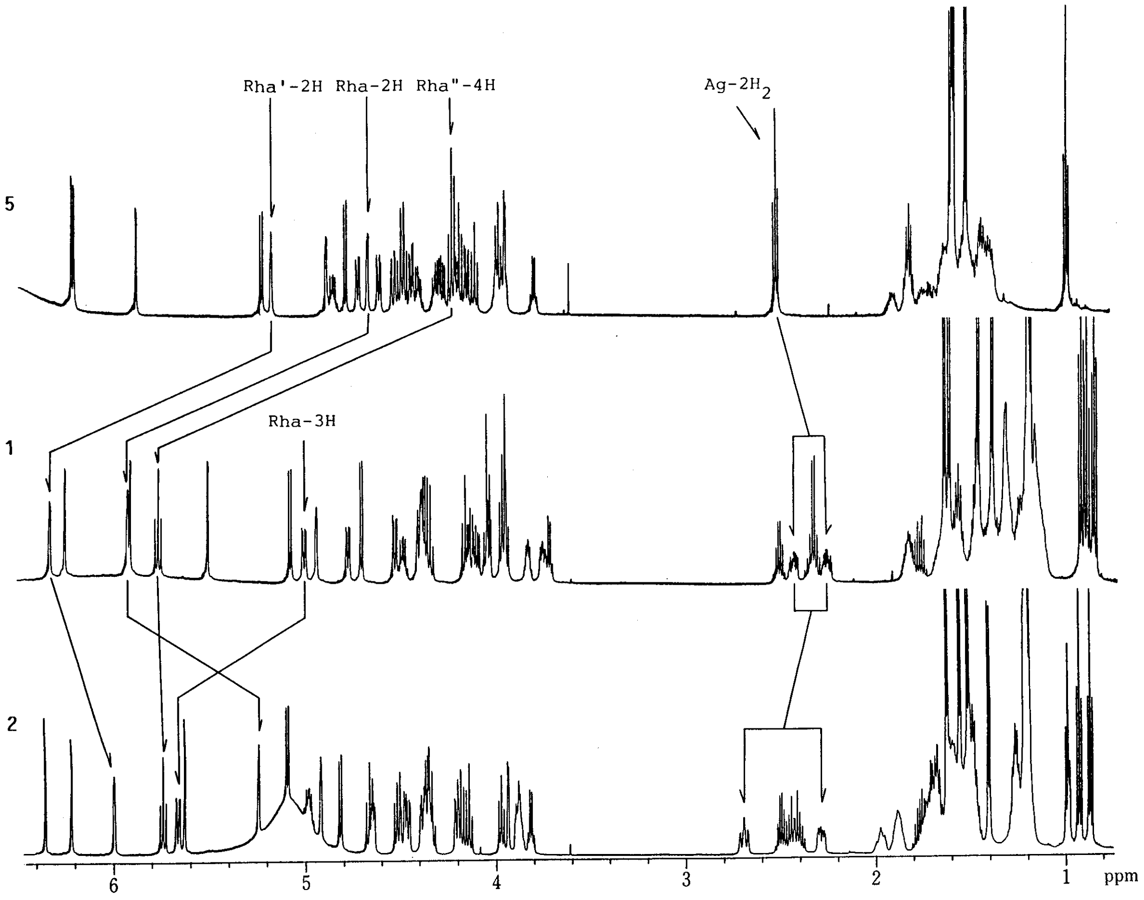
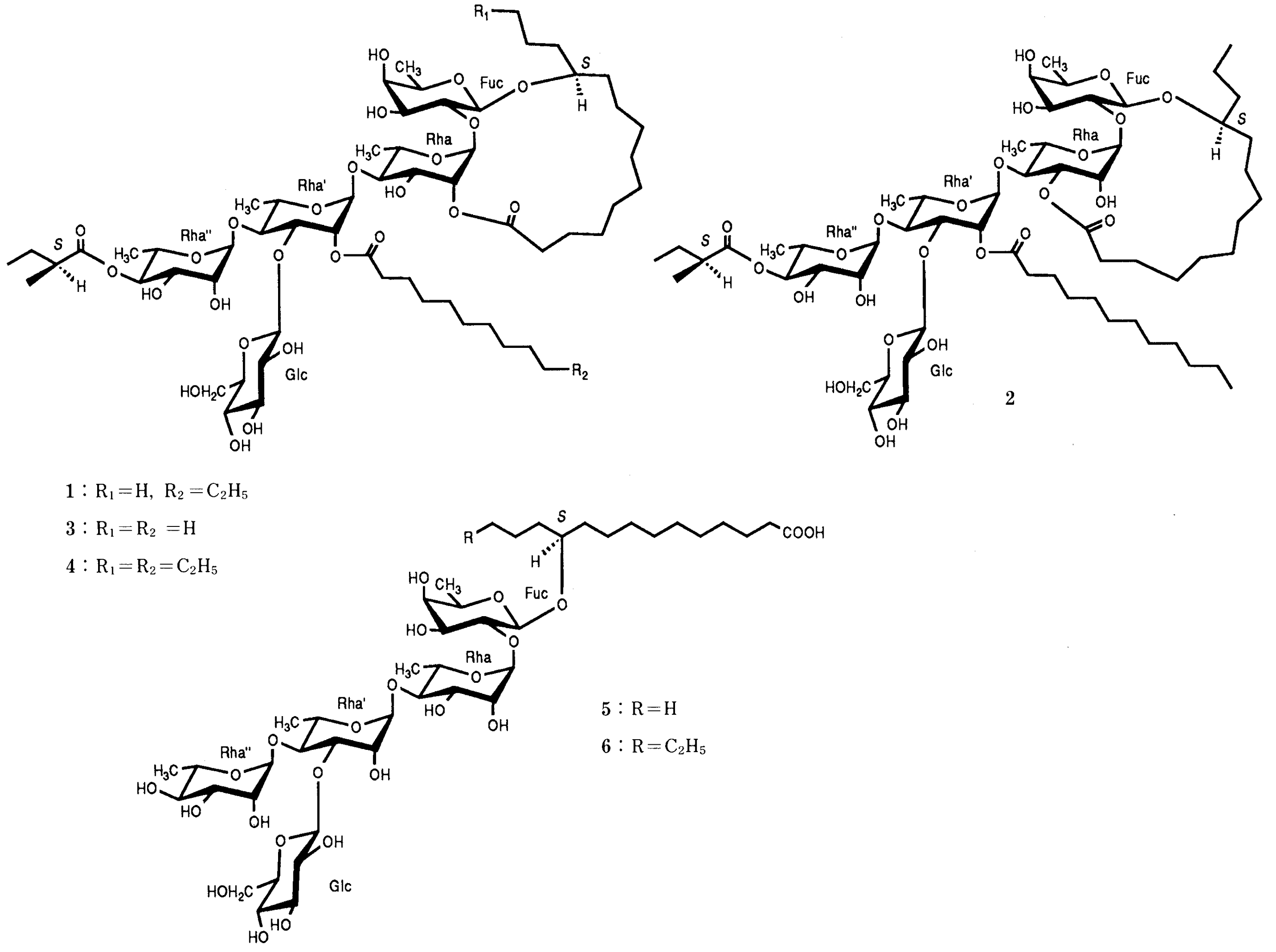


Fig. 2. ^{1}H-NMR Spectra of **1**, **2** and **5** (in Pyridine-d_5, 600 MHz)

(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, intramolecular 1, 2″-ester, as shown in Fig. 3.

Quamoclin II (**2**), $C_{61}H_{106}O_{25}$, yielded the same components as those of **1** on alkaline hydrolysis, and its negative FAB-MS was almost superimposable on that of **1**, suggesting **2** to be a positional isomer of **1** as to the ester linkage.



1 : $R_1=H$, $R_2=C_2H_5$

3 : $R_1=R_2=H$

4 : $R_1=R_2=C_2H_5$

5 : R=H

6 : $R=C_2H_5$

Fig. 3. Structures of **1**—**6**

In the ^{1}H-NMR spectrum of **2**, compared with that of **1**, the signal of 2-H of Rha was shifted upfield by 0.66 ppm, whereas the signal of 3-H of Rha exhibited a downfield shift of 0.68 ppm. Further, the peracetate of **2** (**8**) revealed the same fragment peaks at *m*/*z* 315 (a), 331 (b), 625 (c), 627 (e) and 973 (g) as those of **7** (Fig. 4).

Consequently, **2** was determined to be the positional isomer in which the cyclic ester group at 2-OH of Rha in **1** is transferred to 3-OH in the same sugar unit (Fig. 3).

Quamoclin III (**3**), $C_{59}H_{102}O_{25}$, yielded 2-methylbutyric acid, *n*-decanoic acid and **5**, and showed the $[M-H]^-$ ion peak at *m*/*z* 1209 along with the same fragment peaks as those of **1** at *m*/*z* 243, 389 and 517, in the negative FAB-MS. The ^{1}H-NMR spectrum of **3** was quite similar to that of **1**, though the deshielded signals due to 2-H of Rha, 2-H of Rha′ and 4-H of Rha″ suggested **3** to be a homologue of **1** which differs only in the fatty acid ester group (Table II).

The EI-MS of the peracetate of **3** (**9**) showed fragment peaks at *m*/*z* 315, 331, 597, 627 and 945 assignable to the fragments a, b, d, e and h (Fig. 4).

Accordingly, the structure of **3** was concluded to be (*S*)-convolvulinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-2(*S*)-methylbutyryl-*α*-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-decanoyl)-*α*-L-rhamnopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, intramolecular 1, 2″-ester, as shown in Fig. 3.

Quamoclin IV (**4**), $C_{63}H_{110}O_{25}$, afforded, on alkaline hydrolysis, 2-methylbutyric acid, *n*-dodecanoic acid and operculinic acid A (**6**).[2)]

The ^{1}H- and ^{13}C-NMR signals due to the sugar moieties of **4** were almost superimposable on those of **1**, suggesting **4** to be a homologue of **1**, which has the same structure as that of **1** except for the aglycone in the glycosidic acid moiety. This suggestion was confirmed by the $[M-H]^-$ ion peak at *m*/*z* 1265 and the fragment peaks at *m*/*z* 1083, 545, 417 and 271, all of which were 28 mass units larger than those of **1**, in the negative FAB-MS, and the fragment peaks at *m*/*z* 315, 331, 625, 655 and 973, owing to the fragments a, b, c, f and g in the EI-MS of the peracetate of **4** (**10**) (Fig. 4).

Consequently, quamoclin IV (**4**) was characterized as (11*S*)-jalapinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-2(*S*)-methylbutyryl-*α*-L-rhamnopyranosyl(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-*α*-L-rhamnopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, intramolecular 1, 2″-ester, which is an analog of operculin I[10a)] of Jalapae Braziliensis in that the 2*S*-methylbutyric acid group at 4-OH of Rha″ of **4** is replaced by an *n*-dodecanoic acid group.

Although quamoclins I—IV in the present study have

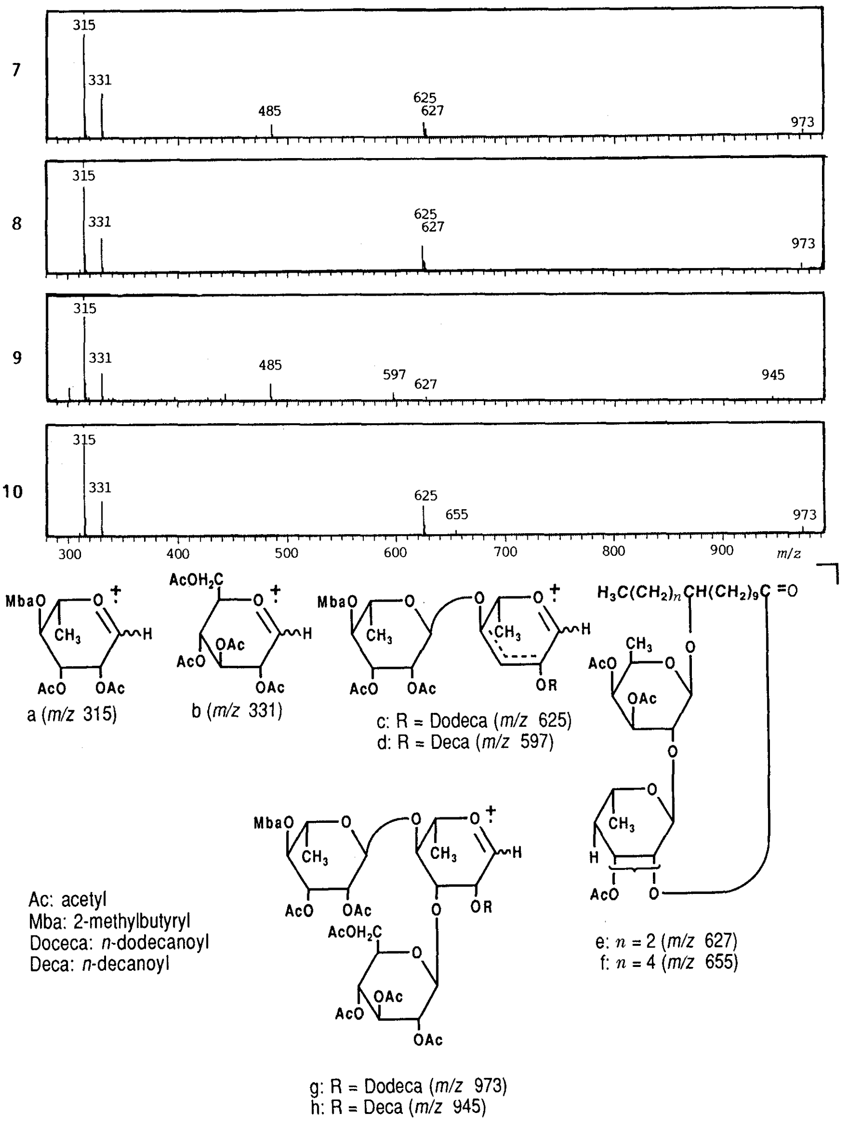


Fig. 4. EI-MS of **7**—**10**

intramolecular ester structures like other ether-soluble resin glycosides (jalapin) so far isolated,[1,2,7-11] they are the first examples of jalapins with organic acid (2*S*-methylbutyric acid) and fatty acid (*n*-decanoic or *n*-dodecanoic acid) moieties coexisting in the molecule. Further, it is noteworthy that 11*S*-convolvulinolic acid of quamoclinic acid A, the glycosidic acid of quamoclins I—III, differs from jalpinolic acid hitherto found in jalapins.

Experimental

All melting points (mp) were determined on a Yanaco MP-S3 apparatus and are uncorrected. IR spectra were taken on a JASCO A-302 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM GSX-400 and GE Omega 600 spectrometers. MS were obtained on a JEOL JMS DX-300 spectrometer equipped with a JMA 3500 data system. The analytical GC was carried out with a Shimadzu GC-8A chromatograph with a flame ionizing detector for organic acids, and a Hitachi G-3000 gas chromatograph equipped with a 30:1 splitter and a flame ionizing detector for sugars. Optical rotations were determined with a JASCO DIP-140 polarimeter. HPLC separation was run on a JASCO TWINCLE equipped with a Shodex RI SE-11 or a JASCO UVIDEC-100-IV detector. For column chromatography, Silica gel 60 (Merck, Art 9385), MCI gel CHP 20P (100—200 mesh, Mitsubishi Chemical Industries) and Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals) were used.

Extraction and Preparation of Jalapin and Convolvulin Fractions The seeds of *Quamoclit pennata* (672.3 g) purchased from Heiwaen in Nara prefecture were powdered and percolated with MeOH (8 l) at room temperature, and the solvent was removed under reduced pressure to afford a brown syrup (86.69 g). This was suspended in H_2O (150 ml) and then extracted successively with ether (4 × 200 ml) and *n*-BuOH (4 × 200 ml). Chromatography of the *n*-BuOH extractives (37.2 g) over MCI gel CHP 20P (45 mm i.d. × 160 mm; 70% MeOH→MeOH→acetone) gave fr. 1 (9.7 g), fr. 2 (22.4 g) and fr. 3 (2.7 g). Chromatography of fr. 2 on a Sephadex LH-20 column (MeOH) furnished convolvulin (15.3 g) and jalapin (3.0 g) fractions.

Alkaline Hydrolysis of Jalapin Fraction The jalapin fraction (117 mg) was suspended in 1 N KOH (3 ml) and heated at 95 °C for 2 h. After cooling, the mixture was adjusted to pH 3 with 1 N HCl and shaken with ether (3 × 3 ml). The combined ether layer was washed with H_2O and evaporated to give an oil (15 mg, organic acid fraction), an aliquot of which was analyzed by GC (condition 1: column, 3.2 mm i.d. × 2 m glass column packed with Unisole 30T (5%); carrier gas N_2, 1.0 kg/cm^2; column temperature, 120 °C); t_R (min), 4.35 (2-methylbutyric acid).

A part of the oil was methylated with diazomethane and then analyzed by GC (condition 2: column, 3.2 mm i.d. × 2 m glass column packed with Unisole 3000; carrier gas N_2, 1.25 kg/cm^2; column temperature, 160 °C); t_R (min), 4.17 (methyl *n*-decanoate), 8.28 (methyl *n*-dodecanoate).

The H_2O layer was desalted over MCI gel CHP 20P (H_2O→acetone) to give a white powder (82 mg, glycosidic acid fraction).

Determination of the Absolute Configuration of 2-Methylbutyric Acid The organic acid fraction (13 mg) in dry acetone (10 ml) was neutralized with triethylamine, then evaporated *in vacuo*. A mixture of the residue and *p*-bromophenacyl bromide (100 mg) in dry acetone was left to stand at room temperature overnight. After removal of the solvent, H_2O (10 ml) was added and the mixture was extracted with ether (3 × 10 ml). The ether fraction was chromatographed over silica gel (*n*-hexane–AcOEt (4 : 1)→AcOEt) to give fr. 4 (65 mg), fr. 5 (12 mg) and fr. 6 (1 mg). Preparative HPLC [Kusano CIG Si gel, 22 mm i.d. × 100 mm, *n*-hexane–AcOEt (6 : 1)] of fr. 4 afforded fr. 7 (2 mg), fr. 8 (46 mg) and fr. 9 (12 mg). Fraction 8 (37 mg) was heated with 1 N KOH (1 ml) at 95 °C for 1 h. The mixture was acidified (pH 3) with 1 N HCl, diluted with H_2O (5 ml), then extracted with ether (3 × 5 ml). A mixture of the ether fraction (30 mg) and $SOCl_2$ (45 mg) in benzene (4 ml) was refluxed for 15 min and then *S*-1-phenylethylamine (100 mg) was added. The solution was refluxed for 1 h, then diluted with H_2O (15 ml) and extracted with AcOEt (20 ml). The organic layer was washed with 0.1 N HCl (15 ml), 5% aqueous $NaHCO_3$ (15 ml) and H_2O (15 ml), successively. The organic layer was concentrated, and the residue was subjected to HPLC (Chemcosorb 5 Si, 6 mm i.d. × 250 mm) to furnish colorless needles (*n*-hexane–AcOEt) (5 mg), mp 85—88 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.62 (1H, br s, NH), 5.15 (1H, dq, J=7.0, 7.0 Hz, NCH), 2.08 (1H, ddq, J=7.0, 7.0, 7.0 Hz, 2-H_2), 1.65 (1H, ddq, J=13.5, 7.5, 7.0 Hz, 3-H_a), 1.49 (3H, d, J=7.0 Hz, NHCHC$\underline{H}_3$), 1.42 (1H, ddq, J=13.5, 7.5, 7.0 Hz, 3-H_b), 1.14 (3H, d, J=7.0 Hz, 5-H_3), 0.86 (3H, dd, J=7.5, 7.5 Hz, 4-H_3). The t_R value of 13.0 min was identical with that of an authentic sample of (*S*)-*N*-1-phenylethyl-2(*S*)-methylbutyrylamide [*cf.* (*S*)-*N*-1-phenylethyl-2(*R*)-methylbutyrylamide, t_R: 11.3 min].

Isolation of Quamoclinic Acid A (5) The glycosidic acid fraction (82 mg) was subjected to a combination of silica gel ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) and Sephadex LH-20 (MeOH) chromatography to afford **5** (67 mg), a white powder, mp 185—190 °C, $[\alpha]_D^{21}$ −64.3° (c=5.5, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1700 (C=O). Negative FAB-MS *m*/*z*: see Fig. 1. ^{1}H- and ^{13}C-NMR δ: see Fig. 2, Tables I and II. *Anal.* Calcd for $C_{44}H_{78}O_{24} \cdot 2H_2O$: C, 51.45; H, 8.05. Found: C, 51.33; H, 7.99.

Acidic Hydrolysis of 5 A solution of **5** (145 mg) in 1 N HCl (2 ml) was heated at 95 °C for 2 h. The reaction mixture was diluted with H_2O (10 ml) and extracted with ether (4 × 10 ml). The ether layer was treated with diazomethane. The mixture was evaporated and the residue was crystallized from *n*-hexane–AcOEt to furnish methyl convolvulinolate[4] (33 mg), colorless needles (*n*-hexane–AcOEt), mp 33—34 °C. ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 174.4, 71.7, 51.4, 39.7, 37.5, 34.1, 29.7, 29.5, 29.4, 29.2, 29.1, 25.6, 25.0, 18.8, 14.1.

The aqueous layer was neutralized with 1 N KOH then evaporated. The residue was desalted by chromatography on Sephadex LH-20 (MeOH) to furnish a syrup (104 mg). TLC on Avicel SF (Funakoshi Pharm. Co.), *n*-BuOH–pyridine–H_2O (6 : 2 : 3) top layer + pyridine (1)]; *Rf* 0.39 (glucose), 0.54 (fucose), 0.64 (rhamnose).

The syrup (2 mg) was converted into the trimethylsilyl ether of the thiazolidine 4(*R*)-carboxylate derivative and subjected to GC-analysis according to Hara *et al.*[6] GC (GL Sciences, column, fused silica capillary column Bonded MPS-50 (Quadrex), 0.25 mm i.d. × 50 m, film thickness 0.25 μm; carrier gas, He (30 ml/min); column temperature, 220 °C); t_R (min), 19.34 (L-rhamnose), 20.74 (D-fucose), 26.62 (D-glucose).

Isolation of Quamoclins I (1), II (2), III (3) and IV (4) The jalapin fraction (3.0 g) was subjected to chromatography over silica gel ($CHCl_3$–MeOH–H_2O, 14 : 2 : 0.1) to give fr. 10 (320 mg), fr. 11 (889 mg) and fr. 12 (488 mg). Preparative HPLC on Inertsil ODS (GL Sciences, 20 mm i.d. × 250 mm, MeOH) of fr. 10 afforded fr. 13 (12 mg), fr. 14 (23 mg), fr. 15 (17 mg), **1** (123 mg) and **4** (23 mg). Fraction 11 was chromatographed over Cosmosil 140 C18-OPN (Nacalai Tesque Inc., 90% MeOH→93% MeOH→95% MeOH→98% MeOH→MeOH) to afford fr. 16 (26 mg), fr. 17 (29 mg), fr. 18 (326 mg), fr. 19 (429 mg) and fr. 20 (55 mg). Fraction 18 was subjected to HPLC (Cosmosil 5 C18-Ar, 6 mm i.d. × 250 mm, 95% MOH) to give fr. 21 (10 mg), fr. 22 (15 mg), fr. 23 (23 mg), fr. 24 (12 mg), fr. 25 (20 mg), fr. 26 (9 mg), fr. 27 (2 mg), **3** (28 mg), fr. 28 (4 mg), fr. 29 (48 mg), fr. 30 (3 mg) and **1** (105 mg). Similar HPLC of fr. 19 as for fr. 10 gave fr. 31 (1 mg), fr. 32 (13 mg), **1** (244 mg), fr. 33 (16 mg), fr. 34 (26 mg), fr. 35 (14 mg) and fr. 36 (13 mg). Preparative HPLC (Inertsil ODS, 95% MeOH) of fr. 14 afforded **2** (19 mg).

1: A white powder, mp 133—137 °C, $[\alpha]_D^{24}$ −22.7° (c=5.9, MeOH). Negative FAB-MS *m*/*z*: see Fig. 1. ^{1}H- and ^{13}C-NMR δ: see Fig. 2, Tables I and II. *Anal.* Calcd for $C_{61}H_{106}O_{25} \cdot 7/2H_2O$: C, 56.25; H, 8.74. Found: C, 56.23; H, 8.47.

2: A white powder, mp 127—132 °C, $[\alpha]_D^{27}$ −57.8° (c=2.3, MeOH). Negative FAB-MS *m*/*z* (%): 1237 (88) $[M-H]^-$, 1055 (33) $[1237-182$ (dodecanoic acid unit)$]^-$, 517 (53) $[1055-146$ (deoxyhexose unit) $\times 2-162$ (hexose unit) -84 (2-methylbutyric acid unit)$]^-$, 389 (100) $[517-128\ (146-18)]^-$, 243 (89) $[389-146]^-$. ^{1}H- and ^{13}C-NMR δ: see Fig. 2, Tables I and II. *Anal.* Calcd for $C_{61}H_{106}O_{25}$: C, 59.11; H, 8.62. Found: C, 58.90; H, 8.79.

3: A white powder, mp 125—130 °C, $[\alpha]_D^{20}$ −27.4° (c=1.0, MeOH). Negative FAB-MS *m*/*z* (%): 1209 (63) $[M-H]^-$, 1055 (35) $[M-H-154$ (decanoic acid unit)$]^-$, 517 (46) $[1055-146\times 2-162-84]^-$, 389 (100) $[517-128]^-$, 243 (56) $[389-146]^-$. ^{1}H- and ^{13}C-NMR δ: see Tables I and II. *Anal.* Calcd for $C_{59}H_{102}O_{25} \cdot 1/2H_2O$: C, 58.06; H, 8.51. Found: C, 58.10; H, 8.66.

4: A white powder, mp 116—120 °C, $[\alpha]_D^{27}$ −23.0° (c=2.4, MeOH). Negative FAB-MS *m*/*z* (%): 1265 (100) $[M-H]^-$, 1083 (49) $[1265-182]^-$, 545 (77) $[1083-146\times 2-162-84]^-$, 417 (100) $[545-128]^-$, 271 (100) $[417-146$, jalapinolic acid$-H]^-$. ^{1}H- and ^{13}C-NMR δ: see Tables I and II. *Anal.* Calcd for $C_{63}H_{110}O_{25} \cdot 1/2H_2O$: C, 59.28; H, 8.76. Found: C, 59.38; H, 8.99.

Alkaline Hydrolysis of 1—4 Suspensions of **1** (15 mg), **2** (7 mg), **3** (7 mg) and **4** (7 mg) in 1 N KOH (1 ml) were each heated at 95 °C for 1 h. The reaction mixture was adjusted to pH 3 with 1 N HCl, diluted with H_2O (5 ml) then extracted with ether (3 × 4 ml). The ether layer was washed with H_2O (10 ml). After removal of the solvent, the residue was subjected to GC analysis (condition 1), t_R (min), 4.34 (2-methylbutyric acid) for each of compounds **1**—**4**. An aliquot of each residue was treated with diazomethane in ether and analyzed by GC (condition 2), t_R (min) 8.32 (methyl *n*-dodecanoate) for **1**, **2** and **4**; 4.19 (methyl *n*-decanoate) for **3**.

The aqueous layer was desalted by chromatography over MCI gel CHP 20P to give a white powder (glycosidic acid) (5 mg from **1**, 3 mg from **2**, 3 mg from **3**, 3 mg from **4** ($[\alpha]_D^{22}$ −69.7° (c=0.5, MeOH)). The ^{1}H-NMR spectra of the glycosidic acids of **1**, **2** and **3** were each superimposable on that of **5**, whereas the glycosidic acid of **4** was identified as operculinic acid A (**6**)[2] on the basis of ^{13}C-NMR spectral data.

Acetylation of 1—4 Solutions of **1** (10 mg), **2** (3 mg), **3** (3 mg) and **4** (3 mg) in Ac_2O–pyridine (1 : 1, 1 ml) were each left to stand at room temperature overnight. After removal of the reagent, the residue was partitioned between ether (0.5 ml) and H_2O (0.5 ml). The ether layer was reduced to afford a white powder, **7** (11 mg from **1**), **8** (3 mg from **2**), **9** (3 mg from **3**) and **10** (3 mg from **4**).

7: A white powder, mp 85—93 °C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.19, 2.17, 2.10, 2.07, 2.04, 2.01, 2.00, 1.99, 1.95 ($COCH_3$). EI-MS *m*/*z*: see Fig. 3. High resolution EI-MS *m*/*z*: 315.14344. Calcd for $C_{15}H_{23}O_7$ (a): 315.14434; *m*/*z*: 331.10283. Calcd for $C_{14}H_{19}O_9$ (b): 331.10286; *m*/*z*: 625.35767. Calcd for $C_{33}H_{53}O_{11}$ (c): 625.35873; *m*/*z*: 627.33874. Calcd for $C_{32}H_{51}O_{12}$ (e): 627.33799.

8: A white powder, mp 86—92 °C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.20, 2.15, 2.08, 2.07, 2.00, 2.00, 1.98, 1.98, 1.96 ($COCH_3$). EI-MS *m*/*z*: see Fig. 4.

9: A white powder, mp 87—92 °C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.19, 2.17, 2.10, 2.07, 2.04, 2.01, 2.00, 1.99, 1.95 ($COCH_3$). EI-MS *m*/*z*: see Fig. 4.

10: A white powder, mp 68—72 °C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.19, 2.17, 2.10, 2.07, 2.04, 2.00, 2.00, 1.99, 1.95 ($COCH_3$). EI-MS *m*/*z*: see Fig. 4.

Acknowledgment The authors are indebted to Mr. M. Nishi and Mr. S. Inoue of this university for measurements of the NMR spectra, MS and elemental analyses.

References and Notes

1) Part XIII: M. Ono, K. Fujimoto, M. Kawata, T. Fukunaga, T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.*, **40**, 1400 (1992).
2) M. Ono, T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.*, **37**, 3209 (1989).
3) G. Helmchen, H. Völter and W. Schühle, *Tetrahedron Lett.*, **16**, 1417 (1977).
4) T. Kawasaki, *Proc. Jpn. Acad.*, **25**, 15 (1949); *idem*, *Yakugaku Zasshi*, **70**, 485 (1949).
5) M. Ono, N. Noda, F. Yamada, K. Miyahara and T. Kawasaki, Abstracts of Papers, 38th Meeting of the Japanese Society of Pharmacognosy, Kobe, Sept. 1991, p. 1C-4. Details of determination of the configuration will be published elsewhere.
6) S. Hara, H. Okabe and K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986); *idem*, *ibid.*, **35**, 501 (1987).
7) N. Noda, M. Ono, K. Miyahara, T. Kawasaki and M. Okabe, *Tetrahedron*, **43**, 3889 (1987).
8) N. Noda, H. Kobayashi, K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.*, **36**, 920 (1988); N. Noda, M. Nishi, K. Miyahara and T. Kawasaki, *ibid.*, **36**, 1707 (1988).

9) I. Kitagawa, H. Shibuya, Y. Yokokawa, N. I. Baek, K. Ohashi, M. Yoshikawa, A. Nitta and H. Wiriadinata, *Chem. Pharm. Bull.*, **36**, 1618 (1988); I. Kitagawa, N. I. Baek, K. Ohashi, M. Sakagami, M. Yoshikawa and H. Shibuya, *ibid.*, **37**, 1131 (1989).

10) *a*) M. Ono, M. Nishi, T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.*, **38**, 2986 (1990); *b*) M. Ono, T. Kawasaki and K. Miyahara, *ibid.*, **93**, 2534 (1991).

11) N. Noda, H. Kogetsu, T. Kawasaki and K. Miyahara, *Phytochemistry*, **29**, 3565 (1990); H. Kogetsu, N. Noda, T. Kawasaki and K. Miyahara, *ibid.*, **30**, 957 (1991).