Resin Glycosides. XXV.[1] Multifidins I and II, New Jalapins, from the Seed of *Quamoclit* x *multifida*

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Received July 2, 1997; accepted July 30, 1997

Alkaline hydrolysis of the ether-soluble resin glycoside fraction of seeds of *Quamoclit* (*Q.*) x *multifida*, a hybrid between *Q. pinnata* and *Q. coccinea*, gave new glycosidic acids, multifidinic acids A and B, along with two known glycosidic acids, quamoclinic acid A and operculinic acid A, and three organic acids, (2*S*)-2-methylbutyric acid, *n*-decanoic acid and *n*-dodecanoic acid. Further, as major ether-soluble resin glycosides, new jalapins named multifidins I and II, were isolated accompanied by quamoclins I—IV, which were previously obtained from seeds of *Q. pinnata*. The structures of multifidins I and II, and multifidinic acids A and B have been determined on the basis of chemical and spectral data.

Key words *Quamoclit* x *multifida*; resin glycoside; jalapin; Convolvulaceae; multifidin I-II; multifidinic acid A, B

Quamoclit (*Q.*) x *multifida* RAF. (*Q. sloteri* NIEUWEL) (Convolvulaceae) is a decorative plant that was hybridized from *Q. pinnata* (L.) BOJER (*Ipomoea* (*I.*) *quamoclit* L.) and *Q. coccinea* AUCT. non (L.) MOENCH (*I. hederifolia* L.) in the U.S. in 1917.[3] In the preceding study, we isolated four jalapins,[4] quamoclins I—IV, from the ether-soluble resin glycoside fraction of the seed of *Q. pinnata*.[5] We are interested in the resin glycosides of *Q. multifida* from the chemotaxonomical point of view, and we have isolated six jalapins from the ether-soluble fraction of the seed. This paper deals with the isolation and structure elucidation of two new jalapins named multifidins I (**11**) and II (**12**), as well as two new glycosidic acids named multifidinic acids A (**3**) and B (**6**).

The pulverized seeds of *Q. multifida* were extracted with MeOH and the extractive was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble portion was defatted with *n*-hexane and the residue was subjected to chromatography over DIAION HP-20 and Sephadex LH-20, successively, to afford ether-soluble (0.4%, jalapin)[4] and ether-insoluble (1.6%) fractions.

Before isolation and structural study of genuine resin glycosides, the component organic acids and glycosidic acids of the jalapin fraction were examined. Alkaline hydrolysis of the fraction gave organic acid and glycosidic acid fractions. The former was shown by gas chromatography (GC) to be composed of 2-methylbutyric acid, *n*-decanoic acid and *n*-dodecanoic acid. The absolute configuration of 2-methylbutyric acid was identified as *S* by Helmchen's method.[6]

On acidic hydrolysis followed by methylation with diazomethane, the glycosidic acid fraction afforded aglycone and monosaccharide fractions. The former was chromatographed to give methyl convolvulinolate (**1**, methyl 11-hydroxytetradecanoate) and methyl jalapinolate (**2**, methyl 11-hydroxyhexadecanoate),[7] both of which were determined to have *S* configuration by Mosher's method.[8] The monosaccharide fraction was found to contain L-rhamnose, D-glucose, D-fucose and D-quinovose, the configurations of which were determined by Hara's method.[9]

The glycosidic acid fraction was esterified with diazomethane and the product was subjected successively to chromatography on a silica gel column and reversed-phase HPLC to give four methyl esters (**3a**—**6a**). Compounds **4a** and **5a** were identical with quamoclinic acid A[5] from the seed of *Q. pinnata* and operculinic acid A[9] from Brazilian Jalap (root of *I. operculata*), respectively.

Compound **3a**, on basic hydrolysis, gave a new glycosidic acid named multifidinic acid A (**3**), powder, mp 187—191 °C, $[\alpha]_D$ −91°. On acidic hydrolysis, it furnished glucose, rhamnose and quinovose in the ratio of about 1:3:1 (GC) and convolvulinolic acid (**1**). The ^{1}H-NMR spectrum of **3a** showed the signals ascribable to five anomeric protons (δ 4.85, 5.20, 5.86, 6.18, 6.28), and an ester methyl group (δ 3.63), an equivalent methylene next to the ester carbonyl group (δ 2.32) and a terminal methyl group (δ 1.00) of the aglycone moiety. Negative ion FAB-MS exhibited the quasi molecular ion peak at m/z 1003 $[M-H]^-$, and the fragment ions at m/z 857 $[1003-146$ (deoxyhexose unit)$]^-$, 841 $[1003-162$ (hexose unit)$]^-$, 549 $[857-162$ and/or $841-146]^-$ and 403 $[549-146]^-$. These observations indicated that the sugar moiety of **3a** consists of a branched chain pentasaccharide having terminal glucose and rhamnose or quinovose units.

Detailed assignment of ^{1}H- and ^{13}C-NMR spectral data of **3a** was carried out with the aid of correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY spectra (Table I). In the ^{13}C-NMR spectrum, glycosylation shifts were observed at 2-C of quinovose (Qui), 4-C of the first rhamnose (Rha) counted from the aglycone, as well as 3-C and 4-C of the second rhamnose (Rha′) amounting to 3.6, 9.5, 10.1 and 4.8 ppm, respectively, compared with those of corresponding methyl glycosides in the literature.[12] The nuclear Overhauser and exchange spectroscopy (NOESY) spectrum exhibited correlations between 1-H of Rha and 4-H of Qui, 1-H of Glc and 3-H of Rha, as well as 1-H of Rha″ and 4-H of Rha′. Furthermore, the J_{C1-H1} and J_{H-H} values of the signals in the sugar moiety indicated that the glycosidic linkages and conformations of Glc and Qui are β in $^4C_1$, and those of Rha, Rha′ and Rha″ are α in $^1C_4$.

Consequently, the structure of multifidinic acid A (**3**) was defined as (11*S*)-convolvulinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-

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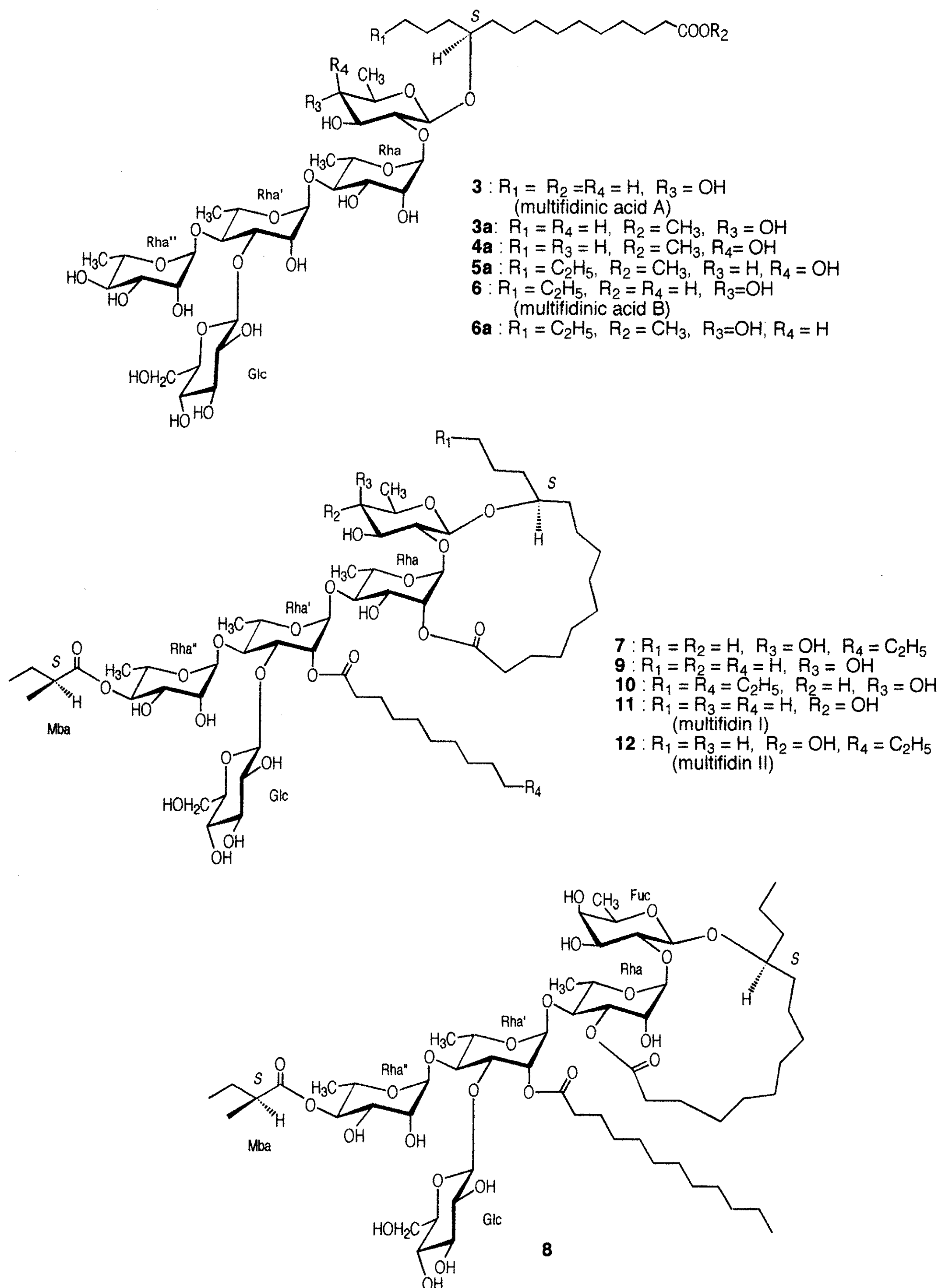

Fig. 1. Structures of **3**—**12**

L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-quinovopyranoside.

Compound **6a**, on alkaline hydrolysis, provided a new glycosidic acid named multifidinic acid B (**6**), white powder, mp 184—190 °C, $[\alpha]_D$ −90°. On acidic hydrolysis, **6a** gave the methyl ester of jalapinolate (**2**), glucose, quinovose and rhamnose. Negative ion FAB-MS of **6** showed the quasi molecular ion peak at *m/z* 1017 $[M-H]^-$ and fragment ion peaks at *m/z* 871, 855, 563 and 417, all of which were 14 mass units larger than those of **3a**. These facts suggested that **6** differs only in the aglycone from **3**, *i.e.*, convolvulinolic acid in **3** is replaced by jalapinolic acid. The ^{1}H- and ^{13}C-NMR spectra of **6a** were almost superimposable on thosle of **3a** except for the signals due to the aglycones (Table 1).

Accordingly, the structure of multifidinic acid B (**6**) was concluded to be (11*S*)-jalapinolic 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-quinovopyranoside.

The ether-soluble resin glycoside fraction was subjected to repetitive column chromatography on silica gel and preparative HPLC to afford six genuine resin glycosides (**7**—**12**). Among them, **7**, **8**, **9** and **10** were identified as

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **3**, **6**, **12** and **13** (in Pyridine-d_5)

	3a		**6a**		**11**		**12**	
	^{1}H$^{a)}$	^{13}C$^{b)}$	^{1}H$^{a)}$	^{13}C$^{b)}$	^{1}H$^{a)}$	^{13}C$^{b)}$	^{1}H$^{a)}$	^{13}C$^{c)}$
Qui-1	4.85, d (7.3)	100.7	4.88, d (7.7)	100.8	4.76, d (7.8)	103.9	4.75, d (7.3)	103.9
2	*ca.* 4.16	79.2	4.19, dd (7.7, 9.0)	79.2	3.84, dd (7.8, 8.9)	82.0	3.82, dd (7.3, 8.9)	81.9
3	4.13, dd (9.3, 9.3)	77.6	4.14, dd (9.0, 9.0)	77.6	4.03, dd (8.9, 9.0)	76.2	4.01, dd (8.9, 8.9)	76.2
4	*ca.* 3.62	77.2	*ca.* 3.62	77.2	3.56, dd (9.0, 9.0)	76.9	3.54, dd (8.9, 8.9)	76.9
5	3.70, dq (9.3, 6.0)	72.6	3.72, dq (9.0, 6.0)	72.7	3.65, dq (9.0, 6.0)	72.5	3.63, dq (8.9, 6.0)	72.4
6	1.57, d (6.0)	18.5	1.60, d (6.0)	18.6	1.54, d (6.0)	18.5	1.52, d (1.8)	18.5
Rha-1	6.28, d (1.6)	101.6	6.31, d (1.2)	101.6	5.57, d (1.8)	98.3	5.56, d (1.8)	98.3
2	4.65, dd (1.6, 3.6)	72.6	4.66, dd (1.2, 3.2)	72.7	5.98, dd (1.8, 3.2)	73.6	5.97, dd (1.8, 3.2)	73.6
3	4.59, dd (3.6, 9.5)	72.6	4/61, dd (3.2, 9.5)	72.7	5.02, dd (3.2, 9.5)	69.4	4.99, dd (3.2, 9.5)	694
4	4.23, dd (9.5, 9.5)	82.3	5.25, dd (9.5, 9.5)	82.3	4.16, dd (9.5, 9.5)	81.2	4.13, dd (9.5, 9.5)	81.3
5	*ca.* 4.87	67.8	*ca.* 4.90	67.8	4.43, dq (9.5, 6.0)	69.0	4.41, dq (9.5, 6.0)	68.9
6	1.67, d (6.1)	18.8	1.71, d (6.5)	18.9	1.60, d (6.0)	19.1	1.58, d (6.0)	19.1
Rha′-1	5.86, d (2.0)	103.5	5.88, d (2.0)	103.6	5.88, d (1.5)	99.9	5.86, d (1.8)	99.9
2	5.16, dd (2.0, 2.8)	72.0	5.17, dd (2.0, 3.2)	72.0	6.29, dd (2.0, 3.2)	73.1	6.27, dd (1.8, 3.2)	73.1
3	4.71, dd (2.8, 9.0)	82.8	4.73, dd (3.2, 9.2)	82.8	4.76, dd (3.2, 9.0)	80.1	4.74, dd (3.2, 8.9)	80.1
4	4.48, dd (9.0, 9.0)	78.6	4.50, dd (9.2, 9.2)	78.6	4.33, dd (9.0, 9.0)	78.6	4.31, dd (8.9, 8.9)	78.6
5	4.39, dq (9.0, 6.0)	68.6	*ca.* 4.41	68.6	4.39, dd (9.0, 5.5)	68.5	*ca.* 4.33	68.5
6	1.59, d (6.0)	18.9	1.61, d (6.0)	19.0	1.65, d (5.5)	18.9	1.64, d (5.6)	18.9
Rha″-1	6.18, s	103.1	6.20, d (1.2)	103.1	6.21, d (2.0)	103.3	6.19, d (1.5)	103.2
2	*ca.* 4.86	72.6	*ca.* 4.88	72.7	4.92, dd (2.0, 3.2)	72.5	4.90, dd (1.5, 3.2)	72.4
3	4.41, dd (3.4, 9.1)	72.8	*ca.* 4.42	72.9	4.51, dd (3.2, 9.2)	70.3	4.48, dd (3.2, 9.5)	70.3
4	4.21, dd (9.1, 9.1)	73.9	4.21, dd (9.1, 9.1)	74.0	5.73, dd (9.2, 9.2)	75.2	5.71, dd (9.5, 9.5)	75.2
5	4.29, dq (9.1, 6.1)	70.5	4.31, dq (9.1, 6.1)	70.5	4.37, dd (9.2, 6.0)	68.2	*ca.* 4.34	68.2
6	1.57, d (6.1)	18.5	1.58, dq (6.1)	18.4	1.40, d (6.0)	18.0	1.39, d (6.0)	18.0
Glc-1	5.20, d (7.7)	105.6	5.23, d (7.7)	105.6	5.05, d (7.6)	105.4	5.03, d (7.7)	105.4
2	*ca.* 3.97	75.1	3.97, dd (7.7, 9.0)	75.1	3.99, dd (7.6, 9.0)	75.2	3.93, dd (7.7, 8.8)	75.1
3	*ca.* 4.14	78.5	4.17, dd (9.0, 9.0)	78.5	4.02, dd (9.0, 9.0)	78.5	4.00, dd (8.8, 8.8)	78.4
4	4.08, dd (9.1, 9.1)	71.7	4.09, dd (9.0, 9.0)	71.7	3.92, dd (9.0, 9.0)	71.4	3.90, dd (8.8, 8.8)	71.3
5	3.92, ddd (2.6, 5.8, 9.1)	78.5	3.95, ddd (2.4, 5.2, 9.0)	78.5	3.73, ddd (9.0, 6.0, 2.5)	78.2	3.71, ddd (8.8, 6.0, 3.2)	78.1
6	4.24, dd (5.8, 11.9)	62.9	*ca.* 4.25	62.9	4.08, dd (6.0, 12.0)	62.9	4.05, dd (6.0, 11.9)	62.9
	4.51, dd(2.6, 11.9)		4.35, dd (2.4, 12.1)		4.38, d (2.5, 12.0)		*ca.* 4.35	
Conv-1		174.1				173.4		173.4
2	2.32, t (7.5)	34.2			2.45, ddd (4.0, 8.5, 14.5)	34.4	2.45, ddd (4.0, 8.5, 14.5)	34.3
					2.28, ddd (3.6, 8.5, 14.5)		2.28, ddd (3.6, 8.0, 14.5)	
11	*ca.* 3.97	77.6			*ca.* 3.82, m	82.2	*ca.* 3.81, m	82.1
14	1.00, t (7.1)	14.5			0.92, t (7.3)	14.6	0.91, t (7.3)	14.6
OCH_3	3.63, s	51.3						
Jla-1				174.1				
2			2.33, t (7.5)	34.2				
11			*ca.* 3.99	78.0				
16			0.92, t (7.1)	14.4				
OCH_3			3.62, s	51.3				
Mba-1						176.3		176.3
2					2.52, ddq (7.0, 7.0, 7.0)	41.6	2.51, ddq (7.0, 7.0, 7.0)	41.5
4					0.94, t (7.0)	11.8	0.93, t (7.0)	11.8
5					1.20, d (7.0)	17.0	1.20, d (7.0)	17.0
Deca-1						173.7		
2					2.34, dt (7.5, 4.3)	34.6		
10					0.85, t (7.0)	14.3		
Dodeca-1								173.7
2							2.33, m	34.5
12							0.87, t (7.3)	14.3

δ in ppm from TMS. Coupling constants (J) in Hz are given in parentheses. Qui, quinovopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Conv, *S*-convolvulinolic acid unit; Jla, *S*-jalapinolic acid unit; Mba, 2*S*-methylbutyric acid; Deca, *n*-decanoyl; Dodeca, *n*-dodecanoyl. Assignments are based on the H–H COSY, C–H COSY, NOESY and HETCOR spectra. *a*) 600 MHz, *b*) 100 MHz, *c*) 150 MHz.

quamoclins I, II, III and IV, previously isolated from the seed of *Q. pinnata*[5] respectively, as judged by ^{1}H- and ^{13}C-NMR spectral comparisons.

Compound **11**, named multifidin I, powder, mp 123—130 °C, $[\alpha]_D$ −31.5°, was subjected to alkaline hydrolysis followed by esterification with ethereal diazomethane to afford methyl 2-methylbutyrate, *n*-decanoate and the methyl ester of multifidinic acid A (**3a**). Further, the $[M-H]^-$ ion at m/z 1209 observed in the negative ion FAB-MS indicated that **11** is composed of one mol each of (2*S*)-2-methylbutyric acid, *n*-decanoic acid and **3a**, and that three ester groups exist in the molecule; hence the carboxyl group of the aglycone in **11** combines with one of the hydroxyl groups in the sugar moiety to form a macrocyclic ester structure, as in already-known jalapins. Negative ion FAB-MS of **11** exhibited fragment peaks at

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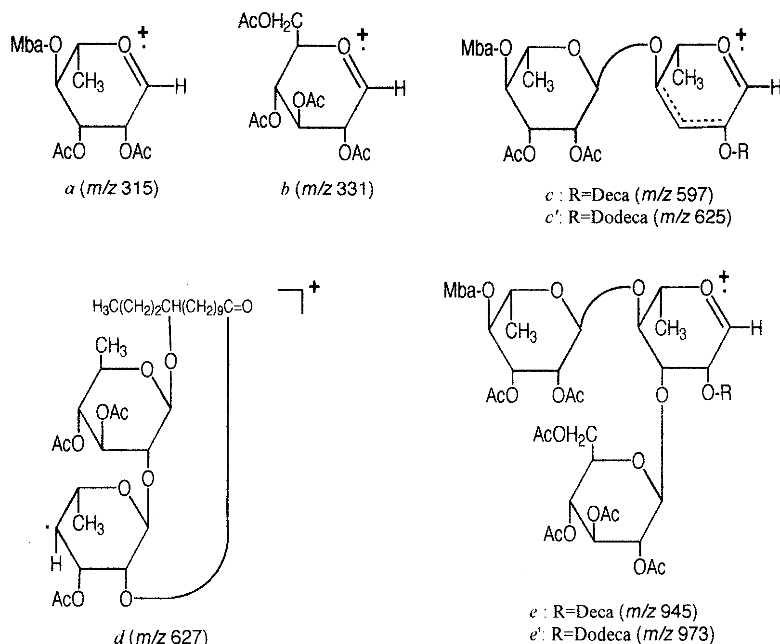

Fig. 2. Mass Fragment Ions of **13** and **14**

Ac, acetyl; Mba, 2-methylbutyryl; Deca, *n*-decanoyl; Dodeca, *n*-dodecanoyl.

m/*z* 1055 [1209 − 154 (decanoic acid unit)]$^-$, 517 [1055 − 146 − 2 − 162 − 84 (2-methylbutyric acid unit)]$^-$, 389 [517 − 128 (146 − 18)]$^-$ and 243 [389 − 146]$^-$. The last three peaks indicate that the macrocyclic ester linkage might be in the second sugar (Rha).[13)]

The ^{1}H-NMR spectrum of **11** showed the presence of a cyclic ester group as judged from the nonequivalent 2-H_2 signals of the aglycone[11)] at δ 2.30 (1H, ddd), 2.46 (1H, ddd), while those of **3a** were equivalent (δ 2.32, 2H, t). Furthermore, the positions of ester groups were clarified by the remarkable downfield shifts observed at 2-H of Rha, and 2-H of Rha′ and 4-H of Rha″ by 1.33, 1.13 and 1.52 ppm, respectively. The position of each acyl group were identified by carbon signal assignment based on the C–H COSY spectrum (Table I) and subsequent measurement of the hetero nuclear multiple bond connectivity (HMBC) spectrum, which showed correlations between 1-C of aglycone and 2-H of Rha, 1-C of *n*-decanoic acid and 2-H of Rha′, 1-C of (2*S*)-2-methylbutyric acid and 4-H of Rha″. These results were supported by the fragment peaks *a*—*e* in the EI-MS of the peracetate (**13**) of **11**, as shown in Fig. 2.[14)]

Consequently, the structure of multifidin I (**11**) was established to be (11*S*)-convolvulinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[4-*O*-(2*S*)-2-methylbutyryl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2″-ester (Fig. 1).

Compound **12**, named multifidin II, white powder, mp 127—130 °C, $[\alpha]_D$ −20.0°, was subjected to basic hydrolysis to yield 2-methylbutyric acid, *n*-dodecanoic acid and multifidinic acid A (**3**). In the negative ion FAB-MS, **12** exhibited the quasi molecular ion at *m*/*z* 1237 [M−H]$^-$, and a fragment ion at *m*/*z* 535, 28 mass units greater than those of **11**, accompanied by fragments at *m*/*z* 389 and 243 that are common to those of **11**. These data suggested that **12** has the same structure as that of **11** except for a difference in the acyl group (*n*-decanoic acid *vs*. *n*-dodecanoic acid). This suggestion was confirmed by the ^{1}H- and ^{13}C-NMR spectral data, which were assigned as in the case of **11** (Table I), and the electron impact (EI)-MS of the peracetate (**14**) of **12**, which revealed fragment ions ascribable to *a*, *b*, *c*′, *d* and *e*′ (Fig. 2).

The structure of multifidin I (**12**) was therefore established to be (11*S*)-convolvulinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[4-*O*-(2*S*)-2-methylbutyryl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1, 2″-ester (Fig. 1).

Among six jalapins obtained in this study, compounds **7**—**10** were identified as quamoclins I—IV, isolated previously from the seeds of *Q. pinnata*.[5)] Although the other two new jalapins, multifidin I and II, have substantially similar structure to quamoclin III and I, respectively, their component glycosidic acid, multifidinic acid A, has D-quinovose in place of D-fucose as the first sugar. Multifidins I and II could not be detected in the jalapin fraction from seeds of *Q. pinnata* by HPLC analysis. Thus, it seems that the metabolic system for multifidins I and II arose from *Q. coccinea*.

Experimental

Melting points were determined on a Yanaco MP-S3 and are uncorrected. Optical rotations were measured on a JASCO DIP-140. ^{1}H- and ^{13}C-NMR were recorded on JEOL JNM GX-400 and GE OMEGA-600 spectrometers at 26 °C with tetramethylsilane as an internal standard. The signal patterns are described as follows (s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; dq, double quartet; ddd, double double doublet). Mass spectra (MS) were taken on a JEOL JMS-DX-300/JMA-3500 system. The negative ion FAB-MS were obtained by use of Xe as a collision gas and triethanolamine as a matrix, and EI-MS with an accelerating voltage 30 eV. GC analyses were performed on a Shimadzu GC-8A or Hitachi G-3000 equipped with a flame ionization detector. HPLC was carried out on a JASCO TWINCLE and 880-PU pump, equipped with a Shodex RI SE-11 differential refractometer or a

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JASCO 875-UV detector. TLC analyses were performed on Alufolien Kieselgel 60 F254 (Merck #5554) and HPTLC-Fertigplatten RP-18 F254 (Merck #13724) and visualized by heating the plate after spraying with 10% H_2SO_4–MeOH.

Extraction and Separation of Ether-Soluble and Insoluble Resin Glycoside Fractions The powdered seeds (698 g) of *Q. multifida* were extracted with MeOH (3.7 l) for 7 d at room temperature. The extract was evaporated *in vacuo* to give a brown solid (85.6 g), which was partitioned between H_2O (150 ml) and *n*-BuOH (250 ml × 3). Both phases were reduced to give the *n*-BuOH-soluble (80.8 g) and H_2O-soluble (16.9 g) fractions. The former was defatted with *n*-hexane (200 ml × 2) to give a yellow powder (42.8 g). The latter was subjected to DIAION HP 20 column chromatography (46 × 110 mm 70% MeOH→MeOH→acetone) to afford fr. 1 (12.9 g), fr. 2 (20.6 g) and fr. 3 (0.7 g). Fraction 2 was chromatographed on a Sephadex LH-20 column (56 × 390 mm) to afford fr. 4 (0.4 mg), fr. 5 (pale yellow powder, ether-insoluble resin glycoside fraction 11.5 g), fr. 6 (pale yellow powder, ether-soluble resin glycoside fraction 3.0 g) and fr. 7 (1.5 g).

Alkaline Hydrolysis of Ether-Soluble Resin Glycoside Fraction Fraction 6 (500 mg) was added to 1 M KOH (1 ml) and heated at 95 °C for 1 h. The mixture was adjusted to pH 3 with 1 M HCl then extracted with Et_2O (5 ml × 5). The Et_2O layer was evaporated to furnish an organic acid fraction (100 mg). The H_2O layer was desalted over a DIAION HP-20 column (28 × 200 mm, washed with excess H_2O then eluted with acetone) to give a glycosidic acid fraction (231 mg). An aliquot of the organic acid fraction was subjected to GC analysis (condition 1: glass column, 3.2 mm × 2 m, packed with 5% Unisole 30T; N_2, 1.0 kg/cm^2; isothermal 100 °C) t_R: 9.72 min (2-methylbutyric acid). A part of the fraction was treated with CH_2N_2–Et_2O and the product was examined by GC (condition 2: glass column, 3.2 mm × 2 m, packed with 3% Unisole 3000; N_2, 1.25 kg/cm^2; isothermal 160 °C) t_R: 4.60 min (methyl *n*-decanoate) and 9.15 min (methyl *n*-dodecanoate).

Determination of the Absolute Configuration of 2-Methylbutyric Acid A mixture of organic acid fraction (95 mg), benzene (10 ml) and thionyl chloride (40 mg) was refluxed for 15 min, then (1*S*)-1-phenylethylamine (100 mg) was added. The mixture was refluxed for 30 min, then partitioned between H_2O (10 ml) and AcOEt (20 ml). The AcOEt layer was washed with 0.1 M HCl (10 ml), 5% $NaHCO_3$ (50 ml) and H_2O (10 ml), successively. The EtOAc was evaporated, and the residue was subjected to HPLC (condition 3: column, Chemcosorb 5 Si (6 × 250 mm; solvent, *n*-hexane–AcOEt (4 : 1); flow rate, 2.0 ml/min; detected at 254 nm) to give (1*S*)-*N*-1-phenylethyl-(2*S*)-2-methylbutyrylamide (9 mg). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.59 (1H, br s, NH), 5.15 (1H, dq, J=6.9, 6.9 Hz, NCH), 2.08 (1H, m, 2-H), 1.64 (1H, m, 3-H_a), 1.49 (3H, d, J=6.9 Hz, $NHCHCH_3$), 1.42 (1H, m, 3-H_b), 1.14 (3H, d, J=6.9 Hz, 5-H_3), 0.86 (3H, t, J=7.4 Hz, 4-H_3). HPLC (condition 3) t_R: 13.00 min (*cf.*: (1*S*)-*N*-1-phenylethyl-(2*R*)-2-methylbutyrylamide, t_R: 10.85 min).

Acidic Hydrolysis of Glycosidic Acid Fraction A solution of glycosidic acid fraction (231 mg) in 1 M HCl (1 ml) was heated at 95 °C for 30 min. After cooling, the mixture was extracted with Et_2O (5 ml × 3). The Et_2O layer was evaporated to give a hydroxyfatty acid fraction (67 mg). This was treated with CH_2N_2–Et_2O and the product was analyzed by GC (condition 4: glass column, 3.2 mm × 3 m, packed with 2% silicone OV-17; N_2, 1.5 kg/cm^2 (at 200 °C); column temperature, 200→240 °C (5 °C/min)) t_R: 4.71 min (methyl convolvulinolate) and 8.41 min (methyl jalapinolate). The product was subjected to low-pressure HPLC (column: Kusano CIG Si, 22 × 100 mm; solvent, *n*-hexane–AcOEt, 8 : 1) to afford **1** (17 mg), white powder, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.4 (C(1)), 71.7 (C(11)), 51.5 (–O$\underline{C}H_3$), 39.7, 37.5, 34.1, 29.7, 29.5, 29.4, 29.2, 29.1, 25.0, 25.0, 18.8, 14.1 (C(14)), and ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: 174.0 (C(1)), 70.6 (C(11)), 51.3 (–O$\underline{C}H_3$), 40.7, 38.6, 34.1, 30.2, 30.0, 29.7, 29.6, 29.4, 26.4, 25.3, 19.5 and 14.6 (C(14)), and **2** (4 mg), white powder, ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.4 (C(1)), 72.0 (C(11)), 51.4 (–O$\underline{C}H_3$), 37.5 (×2), 34.1, 31.9, 29.7, 29.5, 29.4, 29.2, 29.1, 25.6, 25.3, 24.9, 22.7, 14.1 (C(16)) and ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: 174.0 (C(1)), 71.0 (C(11)), 51.3 (–O$\underline{C}H_3$), 38.6, 38.5, 34.2, 32.4, 30.2, 30.0, 29.8, 29.6, 29.4, 26.4, 26.1, 25.3, 23.1, 14.3 (C(16)).

The H_2O layer was neutralized with 1 M KOH and the mixture was evaporated *in vacuo*. The residue was applied to a Sephadex LH-20 column (32 × 390 mm) to give a monosaccharide fraction (87 mg) which was subjected to TLC (condition 6: plate, Avicel SF (Funakoshi Pharm. Co.); solvent, *n*-BuOH–pyridine–H_2O [6 : 2 : 3 (upper layer) + pyridine 1) *Rf*: 0.34 (glucose), 0.45 (fucose), 0.55 (rhamnose and/or quinovose). The fraction was derived to the trimethylsilyl ethers of the thiazolidine-(4*R*)-4-carboxyl derivative by Hara's method[9] and analyzed by GC (column, GL Sciences fused silica capillary column Bonded MPS-50 (Quadrex), 0.25 mm × 50 m, film thickness 0.25 μm; carrier gas, He, 1.36 kg/cm^2; isothermal at 220 °C) t_R: 24.25 min (D-glucose deriv.), 17.63 min (L-rhamnose deriv.), 17.05 min (D-quinovose deriv.) and 18.20 min (D-fucose deriv.).

Determination of Absolute Configurations of 1 and 2 Carbon tetrachloride (3 drops) and (+)-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) chloride[8] (15 mg) were added to a solution of **1** (2 mg) and or **2** (2 mg) in pyridine (0.3 ml), and the mixture was left to stand at room temperature overnight. The mixture was evaporated under an N_2 stream and the residue was purified by silica gel chromatography [Merck #9385, 21 × 120 mm, *n*-hexane→*n*-hexane–AcOEt, 15 : 1→10 : 1→5 : 1] to furnish the (+)-MTPA ester of **1** (3 mg, colorless oil), ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.918 (3H, t, J=7.2 Hz, C14-H_3), 2.299 (2H, t, J=7.2 Hz, C2-H_2), 3.558 (3H, q, J=1.2 Hz, –OC$\underline{H}_3$), 3.665 (3H, s, COOC$\underline{H}_3$), 5.096 (1H, m, C11-H)), or the (+)-MTPA ester of **2** (3 mg, colorless oil), ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.876 (3H, t, J=7.2 Hz, C16-H_3), 2.302 (2H, t, J=7.8 Hz, C2-H_2), 3.561 (3H, q, J=0.3 Hz, –OC$\underline{H}_3$), 3.667 (3H, s, COOC$\underline{H}_3$), 5.085 (1H, m, C11-H). The ^{1}H-NMR spectrum in $CDCl_3$ was identical with that of an authentic sample of the (+)-MTPA ester of methyl (11*S*)-convolvulinolate or the (+)-MTPA ester of methyl (11*S*)-jalapinolate.[8]

Isolation of 3a—6a A glycosidic acid fraction (200 mg) in MeOH (3 ml) was treated with CH_2N_2–Et_2O. The product was chromatographed on silica gel 60 [Merck #9385, 21 × 120 mm, $CHCl_3$–MeOH–H_2O (10 : 2 : 1→8 : 2 : 0.2→7 : 3 : 0.5→6 : 4 : 1)] to afford fr. 8 (30 mg) and fr. 9 (150 mg). The latter was subjected to preparative HPLC (column, Inertsil PREP-ODS, 20 × 250 mm; solvent, 75% MeOH) to give fr. 10 (13 mg), and **3a** (30 mg).

3a, white powder, mp 180—182 °C, $[\alpha]_D^{21}$ −86.4° (c=0.8, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1720 (C=O). Negative ion FAB-MS *m/z* (rel. int. %): 1003 (100) [M−H]$^-$, 857 (15), 841 (5), 549 (14), 403 (7). ^{1}H-NMR and ^{13}C-NMR (pyridine-d_5) δ: see Table 1. J_{C1-H1} (pyridine-d_5) (Hz): Qui (158.9), Glc (159.0), Rha (173.0), Rha′ (169.9), Rha″ (172.9). **4a** (45 mg), white powder, mp 162—168°C, $[\alpha]_D^{25}$ −81.4° (c=3.6, MeOH), fr. 11 (15 mg). **5a** (10 mg), white powder, mp 171—175°C, $[\alpha]_D^{23}$ −87.1° (c=0.8, MeOH). **6a** (8 mg), white powder, mp 171—175°C, $[\alpha]_D^{24}$ −78.8° (c=0.6, MeOH), ^{1}H-NMR and ^{13}C-NMR (pyridine-d_5) δ: see Table 1.

^{1}H- and ^{13}C-NMR spectra of **4a** and **5a** were superimposable on those of quamoclinic acid A methyl ester[5] and operculinic acid A methyl ester,[10] respectively.

Alkaline Hydrolysis of 3a Compound **3a** (15 mg) was added to 3% Na_2CO_3 (3 ml) and the mixture was heated at 90°C for 2 h. After cooling, the mixture was acidified to pH 1, then desalted by Waters Sep-Pak ODS to afford **3**, white powder, mp 187—191°C, $[\alpha]_D^{19}$ −91° (c=1.9, MeOH), IR (KBr) cm^{-1}: 3400 (OH), 1720 (C=O). *Anal.* Calcd for $C_{44}H_{78}O_{24}$: C, 53.32; H, 7.93. Found: C, 53.06; H, 8.01.

Alkaline Hydrolysis of 6a Compound **6a** (6 mg) in 1 M KOH (1 ml) was heated at 95 °C for 1 h. After cooling, the mixture was acidified to pH 3 with 1 M HCl and subjected to DIAION HP-20 column chromatography (22 × 150 mm, H_2O→acetone) to furnish **6** (4 mg), white powder, mp 184—190 °C, $[\alpha]_D^{23}$ −90.3° (c=0.4, MeOH), negative ion FAB-MS *m/z* (rel. int. %); 1017 (44) [M−H]$^-$, 871 (3) [1017−146 (deoxyhexose unit)]$^-$, 855 (6) [1017−162 (hexose unit)]$^-$, 563 (4) [855−146×2]$^-$, 417 (8) [563−146]$^-$. *Anal.* Calcd for $C_{46}H_{82}O_{24}$·$3H_2O$: C, 51.90; H, 8.24. Found: C, 51.78; H, 8.22.

Acidic Hydrolysis of 3 Compound **3** (10 mg) in 1 M H_2SO_4 (2 ml) was heated at 90°C for 2 h. The mixture was extracted with Et_2O and the extract afforded a solid (3 mg). This was treated with CH_2N_2 then crystallized from EtOAc–*n*-hexane to furnish colorless plates (2 mg), mp 29.5—30.5°C, FD-MS *m/z*: 259 [M+H]$^+$, 215 [M−C_3H_7]$^+$, 73 [$C_3H_7CH(OH)$]$^+$. It was identified as methyl convolvulinolate by direct comparison with an authentic sample. The lower layer was neutralized with $Ba(OH)_2$ and the precipitate was filtered off. The filtrate was reduced to give a syrup (7 mg). The syrup was analyzed in the same way as described for the jalapin fraction, giving D-glucose, L-rhamnose and D-quinovose in the ratio of about 1 : 3 : 1.

Acidic Hydrolysis of 6 Compound **6** (4 mg) was hydrolyzed in the same manner as in the case of glycosidic acid fraction to furnish a solid (0.9 mg) and a syrup (1.1 mg). The former was treated with CH_2N_2 and the product was identified as methyl jalapinolate by GC (condition 4). The latter was analyzed by TLC (condition 6), and glucose and rhamnose

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and/or quinovose were identified.

Isolation of 7—12 The ether-soluble fraction (1.5 g) was chromatographed on a silica gel column [Merck #7734, 36×220 mm, $CHCl_3$–MeOH–H_2O (14:2:0.1→MeOH)] to furnish fr. 11 (35 mg), fr. 12 (212 mg), fr. 13 (417 mg), fr. 14 (563 mg), fr. 15 (96 mg), fr. 16 (59 mg) and fr. 17 (32 mg). Fraction 13 was subjected to preparative HPLC (condition 9: column, Inertsil PREP-ODS 20×250 mm; solvent, MeOH) to give fr. 18 (85 mg), fr. 19 (80 mg), fr. 20 (8 mg), fr. 21 (84 mg) and fr. 22 (63 mg). Similar HPLC (92% MeOH) of fr. 21 gave fr. 23 (13 mg), **8** (13 mg), fr. 24 (16 mg), fr. 25 (14 mg) and fr. 26 (12 mg). HPLC (condition 9) of fr. 14 afforded fr. 27 (32 mg), fr. 28 (75 mg), fr. 29 (300 mg), fr. 30 (45 mg) and fr. 31 (29 mg). HPLC (98% MeOH) of fr. 28 gave **9** (21 mg), **11** (14 mg), fr. 32 (10 mg) and fr. 33 (3 mg). HPLC (condition 9) of fr. 29 afforded **7** (134 mg), **12** (76 mg) and fr. 34 (17 mg). HPLC (condition 9) of fr. 30 gave **10** (14 mg) and fr. 35 (13 mg).

7: White powder, mp 125—130°C, $[\alpha]_D^{23}$ −23° (c=2.2, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1237 (97) $[M-H]^-$, 1055 (37) $[1237-182$ (dodecanoic acid unit)$]^-$, 517 (57) $[1055-146\times2-162-84$ (2-methylbutyric acid unit)$]^-$, 389 (100) $[517-128\ (146-18)]^-$, 243 $[389-146$, convolvulinolic acid$-H]^-$.

8: White powder, mp 110—113°C, $[\alpha]_D^{24}$ −58° (c=1.3, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1237 (100) $[M-H]^-$, 1055 (35) $[1237-182]^-$, 517 (43) $[1055-146\times2-162-84]^-$, 389 (100) $[517-128]^-$, 243 (45) $[389-146]^-$.

9: White powder, mp 128—132°C, $[\alpha]_D^{25}$ −26° (c=1.7, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1209 (96) $[M-H]^-$, 1055 (40) $[1237-154$ (decanoic acid unit)$]^-$, 517 (72) $[1055-146\times2-162-84]^-$, 389 (100) $[517-128]^-$, 243 (100) $[389-146]^-$.

10: White powder, mp 110—115°C, $[\alpha]_D^{25}$ −21° (c=1.5, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1265 (63) $[M-H]^-$, 1083 (25) $[1237-182]^-$, 545 (23) $[1083-146\times2-162-84]^-$, 417 (100) $[545-128]^-$, 271 (26) $[417-146$, jalapinolic acid$-H]^-$.

11: White powder, mp 123—130°C, $[\alpha]_D^{21}$ −30° (c=0.9, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1209 (100) $[M-H]^-$, 1055 (43) $[1237-154]^-$, 517 (70) $[1055-146\times2-162-84$ (methylbutyric acid)$]^-$, 389 (100) $[517-128]^-$, 243 (55) $[389-146]^-$. ^{1}H-NMR and ^{13}C-NMR (pyridine-d_5) δ: see Table 1. *Anal.* Calcd for $C_{59}H_{102}O_{25}$: C, 58.50; H, 8.49. Found: C, 58.33; H, 8.46.

12: White powder, mp 127—130 °C, $[\alpha]_D^{23}$ −20° (c=5.2, MeOH). Negative ion FAB-MS m/z (rel. int. %): 1237 (100) $[M-H]^-$, 1055 (38) $[1237-182]^-$, 517 (76) $[1055-146\times2-162-84]^-$, 389 (100) $[517-128]^-$, 243 (75) $[389-146]^-$. ^{1}H-NMR and ^{13}C-NMR (pyridine-d_5) δ: see Table 1. *Anal.* Calcd for $C_{61}H_{106}O_{25}\cdot H_2O$: C, 58.26; H, 8.66. Found: C, 58.00; H, 8.68. Among **7**—**12**, compounds **7**, **8**, **9** and **10** were identified with quamoclin I, II, III and IV,[5] respectively, by comparisons of their ^{1}H-NMR and ^{13}C-NMR spectra with those of authentic samples.

Alkaline Hydrolysis of 11 Compound **11** (18 mg) in 3% Na_2CO_3 (MeOH–H_2O, 1:1, 5 ml) was refluxed for 1 h. After cooling, the mixture was acidified to pH 2 with 1 M HCl, then extracted with Et_2O (20 ml×3). The Et_2O layer was dried over Mg_2SO_4 then evaporated to give an oil (4 mg). This was analyzed by GC (condition 10: column, Unisole 30T (5%) glass column, 4 mm i.d.×2 m; carrier gas N_2, 1.5 kg/cm^2; isothermal 120°C) and 2-methylbutyric acid was detected (t_R: 5.40 min). An aliquot of the oil was methylated with CH_2N_2–Et_2O. The product was subjected to GC analysis under the same condition as above, t_R, 4.85 min (methyl n-decanoate, 4.85 min).

The aqueous phase was subjected to a Sep-Pak ODS column (H_2O→MeOH) and the MeOH eluate was evaporated to dryness under reduced pressure. The residue was dissolved in 1% H_2SO_4 in dry MeOH (5 ml), then left to stand at room temperature for 2 h. The reaction mixture was diluted with H_2O (10 ml) then worked up in the same manner as above to afford a white powder (14 mg), mp 175—180°C, $[\alpha]_D^{21}$ −86.4° (c=0.8, MeOH). Negative ion FAB-MS m/z: 1003 $[M-H]^-$. Its ^{13}C-NMR spectrum was superimposable on that of **3a**.

Alkaline Hydrolysis of 12 Compound **12** (10 mg) was hydrolyzed in the same manner as described for the ether-soluble resin glycoside fraction to yield a white powder (7 mg), mp 186—190 °C. Negative ion FAB-MS m/z (rel. int. %): 989 (100) $[M-H]^-$, 843 (13) $[989-146]^-$, 827 (22) $[989-162]^-$, 681 (5) $[827-146]^-$, 535 (19) $[681-146]^-$, 389 (40) $[535-146]^-$, 243 (22) $[389-146]^-$, and an organic acid fraction (2 mg). It was identified as multifidinic acid A (**3**) by comparison of the ^{1}H-NMR spectrum with that of an authentic sample. GC (condition 1) of the organic acid fraction showed the presence of 2-methylbutyric acid, and after methylation with CH_2N_2–Et_2O, methyl n-dodecanoate was detected by GC analysis (condition 2).

Acetylation of 11 Compound **11** (0.8 mg) was dissolved in a mixture of pyridine (0.05 ml) and Ac_2O (0.10 ml) and left to stand at room temperature overnight. The mixture was evaporated under an N_2 stream and the residue was dissolved in Et_2O (0.1 ml), then n-hexane was added. The precipitates were collected to afford a colorless solid, **13** (1 mg), mp 76—80 °C, IR (KBr) cm^{-1}: no OH absorption. EI-MS m/z (rel. int. %): 945 (6), 627 (9), 597 (20), 331 (33), 315 (100).

Acetylation of 12 Compound **12** (5 mg) was added to a mixture of pyridine (0.3 ml) and Ac_2O (0.3 ml) and the whole was left to stand at room temperature for 3 h. The mixture was worked up as above to furnish **14** (6 mg), white powder, mp 73—80°C. EI-MS m/z (rel. int. %): 973 (3), 627 (6), 625 (15), 331 (30), 315 (100). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.19, 2.12, 2.08, 2.04, 2.03, 2.00, 1.98, 1.98, 1.94 (each 3H, all s, $OCOC\underline{H}_3$).

Acknowledgments The authors wish to thank Dr. Naoki Noda of this University for helpful discussions and also Dr. Masatoshi Nishi and Mr. Shoji Yamaguchi of the Faculty of Pharmaceutical Sciences, Setsunan University for measurements of NMR and mass spectra.

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