Acylated Glycosides of Hydroxy Fatty Acid Methyl Esters Generated from the Crude Resin Glycoside (Pharbitin) of Seeds of *Pharbitis nil* by Treatment with Indium(III) Chloride in Methanol

Masateru Ono,*[,†] Ayako Takigawa,[†] Tomoko Mineno,[‡] Hitoshi Yoshimitsu,[§] Toshihiro Nohara,[§] Tsuyoshi Ikeda,[⊥] Emiko Fukuda-Teramachi,[∥] Naoki Noda,[∥] and Kazumoto Miyahara[∥]

School of Agriculture, Tokai University, 5435 Minamiaso, Aso, Kumamoto 869-1404, Japan, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui, Takasaki, Gunma 370-0033, Japan, Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan, and Faculty of Pharmaceutical Sciences, Setsunan University; 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan

Received July 19, 2010

Treatment of the crude ether-insoluble resin glycoside (convolvulin) from seeds of *Pharbitis nil* (Pharbitis Semen), called pharbitin, with indium(III) chloride in methanol provided seven oligoglycosides of hydroxy fatty acid methyl esters partially acylated by 2-methyl-3-hydroxybutyric (nilic) and 2*S*-methylbutyric acids. Their structures were elucidated on the basis of NMR and MS data and chemical conversions.

The so-called resin glycosides are well known as purgatives and are characteristic ingredients of crude drugs such as Mexican Scammony Root, Orizaba Root, Jalapae Tuber, and Rhizoma Jalapae Braziliensis. They are commonly found in plants of the Convolvulaceae. Chemical investigations on these resin glycosides were initiated in the middle of 19th century, and they can be roughly divided into an ether-soluble resin glycoside called jalapin and an ether-insoluble one called convolvulin.[1] In 1987, some of the present authors succeeded in the isolation and structural elucidation of four jalapins.[2] Almost all jalapins hitherto isolated and characterized had common intramolecular macrocyclic ester structures composed of an oligoglycoside of a hydroxy fatty acid partially acylated by some organic acids at the sugar moiety (acylated glycosidic acid); only six examples were ester-type dimers.[3–5] On the other hand, convolvulin is regarded as an oligomer of a variety of acylated glycosidic acids.[3,6] However, no pure convolvulin has thus far been isolated, and chemical studies have been confined only to characterization of the component organic acids and glycosidic acids afforded by alkaline hydrolysis of mixtures of convolvulins.[7–11]

Pharbitis Semen, the seed of *Pharbitis nil* Choisy (Convolvulaceae), is used as a purgative crude drug, and its resin glycoside is a typical convolvulin. Asahina and co-workers[12–14] reported that alkaline hydrolysis of the crude glycoside named pharbitin gave (+)-2-methylbutyric acid, tiglic acid, nilic acid (2-methyl-3-hydroxybutyric acid), and a glycosidic acid named pharbitic acid, which was composed of ipurolic acid (3,11-dihydroxytetradecanoic acid), D-glucose, and L-rhamnose. In 1970, Okabe and co-workers[15–18] isolated two glycosidic acids, named pharbitic acids C (**1**) and D (**2**), along with valeric, tiglic, nilic, and (+)-2-methylbutyric acids, as components of the alkaline hydrolysis products of pharbitin. In preceding papers,[10,19,20] we reported corrected structures of **1** and **2**, the characterization of a glycosidic acid named pharbitic acid B (**3**), and the absolute configurations of 2-methylbutyric and nilic acids obtained from alkaline hydrolysis of pharbitin.

As part of our continuing study on pharbitin, the present paper deals with the isolation and structural elucidation of seven acylated glycosidic acid methyl esters that were generated by treatment of pharbitin with indium(III) chloride in methanol (MeOH).

Results and Discussion

Despite numerous attempts, isolation of the pure resin glycosides from pharbitin could not be achieved. In consideration of previous results, it was evident that the component resin glycosides of pharbitin possessed at least one carboxyl group. Hence, pharbitin was treated with indium(III) chloride in MeOH, which was reported to be a catalyst for mild methyl esterification of carboxylic acids by Mineno and Kansui,[21] and the treated pharbitin exhibited a number of separate spots by TLC on silica gel. This treated pharbitin was successively subjected to Diaion HP20, Sephadex LH-20, silica gel column chromatography, and HPLC on octadecyl silica, to afford seven compounds, temporarily referred to as PM-1 (**4**)−PM-7 (**10**).

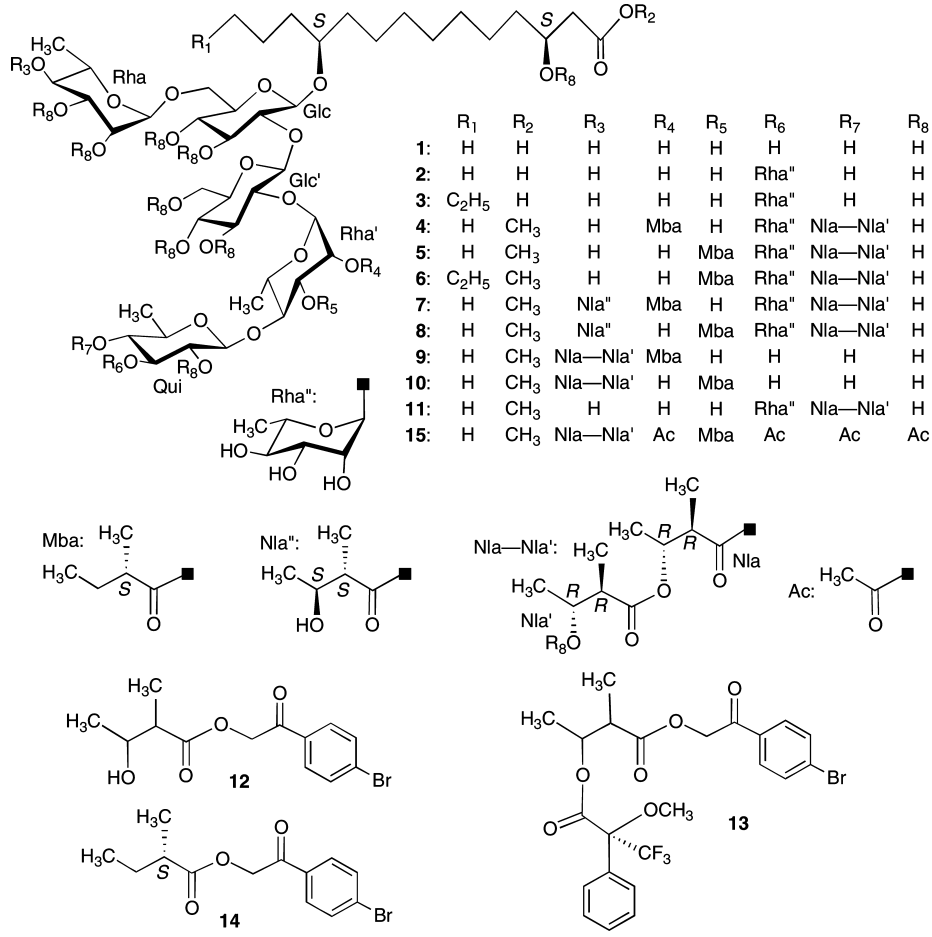
PM-1 (**4**) was obtained as an amorphous powder and exhibited an $[M - H]^-$ ion peak at *m*/*z* 1465 in the negative FABMS. HR-positive FABMS indicated the molecular formula of **4** to be $C_{66}H_{114}O_{35}$. The ^{1}H NMR spectrum of **4** showed signals due to one 2-methylbutyryl unit, two nilyl units, one methoxy group, one primary methyl group, two nonequivalent methylene protons adjacent to a carbonyl group, six anomeric protons, and four secondary methyl groups assignable to 6-deoxyhexosyl units (Table 1). The ^{13}C NMR spectrum of **4** yielded signals due to 66 carbons, including six anomeric carbons and four ester carbonyl carbons (Table 2). These ^{1}H and ^{13}C NMR signals due to the sugar moiety of **4** were assigned on the basis of ^{1}H−^{1}H COSY, HMQC, and TOCSY spectra and indicated that **4** was composed of two nilic acid moieties, one 2-methylbutyric acid moiety, and a methyl ester of **2**. Comparing the chemical shifts of the ^{1}H NMR signals due to the sugar moieties between **2** and **4**,[19] the signals due to H-4 of the quinovosyl unit (Qui) and H-2 of the second rhamnosyl unit (Rha′) showed remarkable downfield shifts of 1.58 and 1.22 ppm, respectively, due to acylation. In addition, the signal due to H-3 of the first nilyl unit (Nla) was shifted downfield by ca. 1.14 ppm when compared to the signal due to the second nilyl unit (Nla′) in **4**. These data indicated the ester linkages to be located at OH-4 of Qui, OH-2 of Rha′, and OH-3 of Nla.

To determine the sites of ester linkages of the respective organic acids, partial deacylation of **4** and HMBC experiments were conducted. Compound **4** was refluxed with 28% aqueous NH_3−MeOH (1:4) for 1 h, and the products were separated by

* To whom correspondence should be addressed. Phone: +81-9676-7-3947. Fax: +81-9676-7-3960. E-mail: mono@agri.u-tokai.ac.jp.
† Tokai University.
‡ Takasaki University of Health and Welfare.
§ Sojo University.
⊥ Kumamoto University.
∥ Setsunan University.

10.1021/np1004888
Published on Web 10/12/2010

Chart 1



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1:	H	H	H	H	H	H	H	H
2:	H	H	H	H	H	Rha"	H	H
3:	C_2H_5	H	H	H	H	Rha"	H	H
4:	H	CH_3	H	Mba	H	Rha"	Nla—Nla'	H
5:	H	CH_3	H	H	Mba	Rha"	Nla—Nla'	H
6:	C_2H_5	CH_3	H	H	Mba	Rha"	Nla—Nla'	H
7:	H	CH_3	Nla"	Mba	H	Rha"	Nla—Nla'	H
8:	H	CH_3	Nla"	H	Mba	Rha"	Nla—Nla'	H
9:	H	CH_3	Nla—Nla'	Mba	H	H	H	H
10:	H	CH_3	Nla—Nla'	H	Mba	H	H	H
11:	H	CH_3	H	H	H	Rha"	Nla—Nla'	H
15:	H	CH_3	Nla—Nla'	Ac	Mba	Ac	Ac	Ac

HPLC to give **5** and **11** together with recovered **4**. The negative FABMS of **11** showed an $[M - H]^-$ ion peak at *m*/*z* 1381, which was 84 mass units (2-methylbutyryl unit) smaller than that of **4**. The ^{1}H NMR spectrum of **11** exhibited signals due to one methoxy group and two nilyl units and two deshielded signals attributable to H-4 of Qui and H-3 of Nla (Table 1). However, signals due to a 2-methylbutyryl unit (Mba) were not observed. In addition, the HMBC spectrum of **4** showed cross-peaks between the methoxy protons and C-1 of the aglycone moiety (Agl); H-2 of Rha′ and C-1 of Mba; H-4 of Qui and C-1 of Nla; and H-3 of Nla and C-1 of Nla′. These data suggested that Mba, Nla, and Nla′ were attached at OH-2 of Rha′, OH-4 of Qui, and OH-3 of Nla, respectively.

On alkaline hydrolysis, **11** furnished an organic acid and a glycosidic acid. The former was converted into the *p*-bromophenacyl ester and then purified by HPLC to give *p*-bromophenacyl nilate (**12**). The ^{1}H NMR spectrum of (−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester[22] (**13**) of **12** indicated that the absolute configuration of **12** was 2*R*,3*R*.[10] The latter was identified as **2** by comparison of the ^{1}H NMR spectrum with that of an authentic sample.[19] The configuration of the 2-mehylbutyric acid component of pharbitin had been previously determined to be *S*.[19] Consequently, the structures of **4** and **11** were defined as methyl 3*S*,11*S*-dihydroxytetradecanoate 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[4-*O*-2*R*-methyl-3*R*-*O*-(2*R*-methyl-3*R*-hydroxybutyryl)butyryl]-β-D-quinovopyranosyl-(1→4)-*O*-(2-*O*-2*S*-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside and its deacyl derivative, in which Mba of **4** was cleaved.

PM-2 (**5**) was obtained as an amorphous powder, and its negative FABMS indicated the same $[M - H]^-$ ion peak as that of **4** at *m*/*z* 1465. The ^{1}H NMR spectrum of **5**, which was imposable on that of **4**, indicated signals due to one Mba, two nilyl units, one methoxy group, and six anomeric protons (Table 1). When compared with the ^{1}H NMR signals of **4**, a downfield shift of ca. 1.27 ppm for the signal due to H-3 of Rha′ along with an upfield shift of 1.23 ppm for the signal assignable to H-2 of Rha′ were observed, whereas H-4 of Qui and H-3 of Nla resonated at positions similar to those of **4**. Further, reflux of **5** in 70% MeOH produced **4**. Accordingly, **5** was concluded to be a positional isomer of **4**, in which Mba of **5** was at OH-3 of Rha′ rather than at OH-2 of Rha′.

PM-3 (**6**) was obtained as an amorphous powder, and its negative FABMS exhibited an $[M - H]^-$ ion peak at *m*/*z* 1493, which was 28 mass units (C_2H_4) larger than that of **5**. On alkaline hydrolysis, **6** furnished nilic acid, 2-methylbyryric acid, and **3**.[19] Further, the ^{1}H and ^{13}C NMR spectra of **6** were quite similar to those of **5**, except for an upfield shift (0.09 ppm) of the signal due to the primary methyl group assignable to Agl in the ^{1}H NMR spectrum of **6** and the observation of two additional signals due to methylene carbons in the ^{13}C NMR spectrum of **6** (Tables 1 and 2). Consequently, **6** was concluded to be a homologue of **5** in which methyl 3*S*,11*S*-ipurorate of the aglycone was replaced by methyl 3*S*,11*S*-dihydroxyhexadecanoate.

PM-4 (**7**) and PM-5 (**8**) were obtained as amorphous powders. Both compounds exhibited an $[M - H]^-$ ion peak at *m*/*z* 1565, which was 100 mass units (nilyl unit) larger than those of **4** and **5** in the negative FABMS. The ^{1}H NMR spectra of **7** and **8** were analogous to those of **4** and **5**, respectively, apart from the appearance of signals due to one more nilyl unit (Table 3). The ^{13}C NMR spectra of **7** and **8** exhibited signals due to five ester carbonyl carbons and six anomeric carbons, respectively (Table 2). Further, **8** was converted to **7** by reflux in 70% MeOH, which suggested that **7** and **8** were, like **4** and **5**, positional isomers. Alkaline hydrolysis of **7** afforded organic acids and a glycosidic acid. The former was converted into *p*-bromophenacyl ester and

Table 1. ^{1}H NMR Spectroscopic Data (Pyridine-d_5) for Compounds **4**−**6** and **11**[a]

position	**4**[b]	**11**[c]	**5**[b]	**6**[b]
Glc-1	4.91 d (8.0)[d]	4.89 d (7.5)	4.88 d (7.0)	4.90 d (7.0)
2	ca. 4.33	4.33 dd (7.5, 9.0)	4.19 dd (7.0, 8.5)	4.20 dd (7.0, 9.0)
3	ca. 4.52	4.50 dd (9.0, 9.0)	4.63 dd (8.5, 8.5)	4.63 dd (9.0, 9.0)
4	ca. 3.94	3.91 dd (9.0, 9.0)	ca. 3.88	3.89 dd(9.0, 9.0)
5	ca. 3.99	3.99 ddd (1.5, 6.0, 9.0)	ca. 3.97	3.98 ddd (2.0, 6.0, 9.0)
6	4.11 dd (6.0, 11.0)	4.07 dd (6.0, 11.0)	4.11 dd (6.0, 11.0)	4.12 dd (6.0, 11.5)
6	4.54 dd (1.0, 11.0)	4.53 dd (1.5, 11.0)	ca. 4.50	4.50 dd (2.0, 11.5)
Glc′-1	5.90 d (7.0)	5.88 d (7.5)	5.72 d (7.0)	5.72 d (7.5)
2	ca. 4.22	4.23 dd (7.5, 8.5)	4.27 dd (7.0, 9.0)	4.27 dd (7.5, 9.0)
3	4.20 dd (9.0, 9.0)	4.19 dd (8.5, 8.5)	4.23 dd (9.0, 9.0)	ca. 4.22
4	4.08 dd (9.0, 9.0)	4.06 dd (8.5, 8.5)	4.04 dd (9.0, 9.0)	4.05 dd (9.0, 9.0)
5	ca. 3.84	3.85 ddd (3.0, 6.0, 8.5)	ca. 3.85	3.85 ddd (3.0, 6.0, 9.0)
6	ca. 4.31	4.26 dd (6.0, 11.0)	4.28 dd (6.0, 11.0)	4.28 dd (6.0, 11.5)
6	ca. 4.44	4.42 dd (3.0, 11.0)	ca. 4.46	4.46 dd (3.0, 11.5)
Rha-1	5.46 d (1.0)	5.44 d (1.0)	5.45 d (1.0)	5.46 d (1.0)
2	ca. 4.46	ca. 4.48	4.43 dd (1.0, 3.0)	4.43 dd (1.0, 3.0)
3	ca. 4.46	ca. 4.49	ca. 4.50	4.49 dd (3.0, 9.0)
4	ca. 4.22	4.23 dd (9.0, 9.0)	4.22 dd (9.0, 9.0)	ca. 4.22
5	ca. 4.33	ca. 4.34	4.39 dq (9.0, 6.0)	ca. 4.38
6	1.66 d (6.0)	1.64 d (6.0)	1.67 d (6.0)	1.67 d (6.0)
Rha′-1	6.24 d (1.0)	6.29 d (1.0)	6.36 d (2.0)	6.35 d (1.5)
2	5.91 br s	4.66 dd (1.0, 3.0)	4.68 dd (2.0, 3.0)	4.68 dd (1.5, 3.0)
3	ca. 4.98	4.76 dd (3.0, 9.0)	6.25 dd (3.0, 9.5)	6.25 dd (3.0, 9.5)
4	ca. 4.29	4.37 dd (9.0, 9.0)	4.57 dd (9.5, 9.5)	4.56 dd (9.5, 9.5)
5	5.10 dq (9.5, 6.0)	5.02 dq (9.0, 6.0)	5.23 dq (9.5, 6.5)	5.22 dq (9.5, 6.0)
6	1.89 d (6.0)	1.83 d (6.0)	1.83 d (6.5)	1.83 d (6.0)
Rha″-1	6.05 d (1.0)	6.09 d (1.0)	5.98 d (1.5)	5.98 d (1.0)
2	4.74 dd (1.0, 3.5)	4.72 dd (1.0, 3.0)	4.75 dd (1.5, 3.0)	4.74 dd (1.0, 3.0)
3	ca. 4.48	4.46 dd (3.0, 9.0)	ca. 4.50	4.49 dd (3.0, 9.0)
4	ca. 4.22	4.25 dd (9.0, 9.0)	4.22 dd (9.0, 9.0)	ca. 4.22
5	ca. 4.33	ca. 4.34	4.35 dq (9.0, 6.5)	4.36 dq (9.0, 6.0)
6	1.70 d (6.0)	1.71 d (6.0)	1.69 d (6.5)	1.69 d (6.0)
Qui-1	5.30 d (8.0)	5.15 d (8.0)	4.90 d (7.0)	4.90 d (8.0)
2	ca. 3.98	3.93 dd (8.0, 9.0)	ca. 3.88	3.87 dd (8.0, 9.0)
3	ca. 4.22	4.21 dd (9.0, 9.0)	4.17 dd (9.5, 9.5)	4.16 dd (9.0, 9.0)
4	5.14 dd (9.5, 9.5)	5.11 dd (9.0, 9.0)	5.10 dd (9.5, 9.5)	5.10 dd (9.0, 9.0)
5	3.58 dq (9.5, 6.0)	3.47 dq (9.0, 6.0)	3.56 dq (9.5, 6.0)	3.56 dq (9.0, 6.0)
6	1.32 d (6.0)	1.25 d (6.0)	1.27 d (6.0)	1.27 d (6.0)
Agl-2	2.71 dd (5.0, 15.0)	2.69 dd (5.5, 15.0)	2.70 dd (5.0, 15.0)	2.69 dd (5.5, 15.0)
2	2.75 dd (8.0, 15.0)	2.73 dd (7.5, 15.0)	2.74 dd (8.0, 15.0)	2.73 dd (8.0, 15.0)
3	ca. 4.44	ca. 4.42	ca. 4.43	ca. 4.43
11	ca. 3.94	ca. 3.91	ca. 3.96	ca. 3.95
14	0.97 t (7.0)	0.95 t (7.0)	0.95 t (7.5)	
16				0.86 t (7.0)
OCH_3	3.63 s	3.62 s	3.63 s	3.63 s
Nla-2	3.18 dq (7.0, 7.0)	3.17 dq (7.0, 7.0)	3.13 dq (7.0, 7.0)	3.13 dq (7.0, 7.0)
3	5.56 dq (7.0, 7.0)	5.54 dq (7.0, 7.0)	5.52 dq (7.0, 7.0)	5.52 dq (7.0, 7.0)
4	1.40 d (7.0)	1.39 d (7.0)	1.38 d (7.0)	1.38 d (7.0)
5	1.36 d (7.0)	1.34 d (7.0)	1.34 d (7.0)	1.34 d (7.0)
Nla′-2	2.99 dq (7.0, 7.0)	2.97 dq (7.0, 7.0)	3.03 dq (7.0, 7.0)	3.02 dq (7.0, 7.0)
3	ca. 4.42	ca. 4.43	ca. 4.48	ca. 4.48
4	1.39 d (7.0)	1.37 d (7.0, 7.0)	1.40 d (7.0)	1.40 d (7.0)
5	1.26 d (7.0)	1.24 d (7.0)	1.27 d (7.0)	1.27 d (7.0)
Mba-2	2.49 ddq (7.0, 7.0, 7.0)		2.51 ddq (7.0, 7.0, 7.0)	2.51 ddq (7.0, 7.0, 7.0)
3	ca. 1.48		ca. 1.42	ca. 1.41
3	ca. 1.78		ca. 1.84	ca. 1.83
4	0.89 dd (7.0, 7.0)		0.85 dd (7.5, 7.5)	0.85 dd (7.5, 7.5)
5	1.14 d (7.0)		1.19 d (7.0)	1.20 d (7.0)

[a] Chemical shifts (δ) are in ppm relative to TMS. [b] Values are recorded at 400 MHz. [c] Values are recorded at 600 MHz. [d] Coupling constants (J) in Hz are given in parentheses.

separated by HPLC to give *p*-bromophenacyl 2-methylbutyrate (**14**) and **12′**. The ^{1}H NMR spectrum of the (−)-MTPA ester (**13′**) of **12′** suggested that **12′** was a mixture of *p*-bromophenacyl 2*R*,3*R*-nilate and its enantiomer in the ratio of 2:1.[10] The later was identified as **2** by comparison of the ^{1}H NMR data with those of an authentic sample.[19] Thus, **7** and **8** were composed of one 2*S*-methylbutyric acid, one 2*S*,3*S*-nilic acid, one methyl ester of **2**, and two 2*R*,3*R*-nilic acid moieties. The ^{1}H NMR signals of **7** and **8** were assigned on the basis of the 2D-NMR techniques. Comparing the chemical shifts of the signals between **4** and **7** and between **5** and **8**, remarkable downfield shifts (ca. 1.59 ppm in **7**; 1.57 ppm in **8**) were observed, assignable to H-4 of the first rhamnose (Rha) in **7** and **8**. On the other hand, the signals owing to H-2 and H-3 of Rha′, H-4 of Qui, and H-3 of Nla of **7** and **8** were observed at quite similar chemical shifts to those of **4** and **5**, respectively. In addition, the HMBC spectrum of **8** indicated correlations between methoxy protons and C-1 of Agl; H-3 of Rha′ and C-1 of Mba; H-4 of Qui and C-1 of Nla; H-3 of Nla and C-1 of Nla′; and H-4 of Rha and C-1 of the third nilyl unit (Nla″). From these data, **7** and **8** were assumed to be derivatives of **4** and **5**, in which a 2*S*,3*S*-nilic acid was attached to OH-4 of Rha of **4** and **5**, respectively. The ester linkage of 2*S*,3*S*-nilic acid was confirmed by the following evidence. Treatment of **8** with mild alkali in a similar manner to that done for **4** gave **4**, **5**, and **11**. Since the sites of ester linkages

Table 2. ^{13}C NMR Spectroscopic Data (Pyridine-d_5) for Compounds **4**−**11**[a]

position	**4**[b]	**5**[c]	**6**[c]	**7**[c]	**8**[d]	**9**[d]	**10**[c]	**11**[d]	position	**4**[b]	**5**[c]	**6**[c]	**7**[c]	**8**[d]	**9**[d]	**10**[c]	**11**[d]
Glc-1	102.7	102.7	102.8	102.7	102.7	102.7	102.7	102.8	Agl-1	172.9	172.9	173.0	172.9	172.9	172.9	172.9	173.0
2	79.5	80.1	80.0	79.7	80.0	79.5	80.3	79.5	2	43.4	43.4	43.4	43.4	43.4	43.4	43.4	43.5
3	79.2	78.6	78.7	79.2	78.7	79.2	78.6	79.3	3	68.2	68.3	68.3	68.2	68.3	68.3	68.2	68.3
4	71.8	71.4	71.4	71.6	71.2	71.9	71.5	71.9	11	81.0	80.9	81.3	80.9	80.8	81.0	80.6	81.2
5	76.3	76.4	76.5	76.0	75.9	76.0	75.9	76.4	14	14.5	14.5		14.6	14.6	14.6	14.5	14.6
6	67.9	67.9	68.0	68.0	68.0	68.2	68.1	68.1	16			14.4					
Glc′-1	101.6	102.4	102.4	101.7	102.5	101.6	102.5	101.9	OCH_3	51.2	51.2	51.3	51.3	51.3	51.3	51.3	51.4
2	78.2	76.9	76.9	78.6	77.3	78.3	76.5	78.9	CH_2	19.0	19.1	23.0	19.1	19.1	19.0	19.1	19.1
3	78.9	79.5	79.5	78.9	79.3	78.9	79.5	79.3	CH_2	25.2	24.9	25.0	25.2	24.9	25.3	24.8	25.3
4	72.4	72.6	72.5	72.5	72.5	72.5	72.6	72.4	CH_2	26.2	26.2	25.6	26.2	26.2	26.2	26.2	26.3
5	77.8	77.6	77.8	77.7	77.7	77.7	77.6	77.9	CH_2	30.1	30.1	26.3	30.1	30.1	30.1	30.1	30.2
6	63.0	63.3	63.3	63.1	63.3	63.1	63.4	63.1	CH_2	30.2	30.2	30.2	30.2	30.2	30.2	30.2	30.3
Rha-1	102.3	102.4	102.4	102.2	102.2	102.3	102.2	102.4	CH_2	30.5	30.6	30.3	30.6	30.6	30.6	30.6	30.6
2	72.4	72.3	72.3	71.9	71.9	72.3	72.3	72.3	CH_2	34.9	35.0	30.6	35.0	34.9	35.0	34.9	35.0
3	72.6	72.6	72.6	70.6	70.4	70.3	70.3	72.7	CH_2	37.6	37.6	32.3	37.7	37.6	37.6	37.6	37.7
4	73.9	74.1	74.1	75.5	75.5	75.7	75.7	74.1	CH_2	38.1	38.1	34.8	38.2	38.1	38.1	38.1	38.2
5	69.6	69.8	69.6	67.0	67.0	67.0	67.0	69.7	CH_2			35.4					
6	18.7	18.8	18.8	18.2	18.3	18.3	18.4	18.8	CH_2			38.2					
Rha′-1	98.4	100.6	100.6	98.5	100.8	98.5	100.5	101.8	Nla-1	172.7	172.8	172.8	172.7	172.7	173.6	173.6	172.8
2	73.3	70.5	70.5	73.3	70.5	73.2	70.6	72.1	2	44.3	44.4	44.4	44.3	44.3	45.0	45.0	44.4
3	69.9	74.7	74.7	69.9	74.7	70.1	74.9	72.6	3	70.9	70.9	70.9	70.9	70.9	71.3	71.3	71.0
4	83.0	77.9	77.9	83.4	78.0	84.2	77.6	83.8	4	16.8	17.0	17.1	16.8	16.9	16.7	16.7	17.0
5	67.4	67.9	67.9	67.5	67.9	67.7	68.0	67.8	5	12.7	13.1	13.2	12.7	12.9	12.8	12.8	12.9
6	18.7	18.7	18.7	18.7	18.7	18.8	18.8	18.8	Nla′-1	174.7	174.6	174.6	174.8	174.7	174.7	174.6	174.8
Rha″-1	102.4	102.7	102.8	102.3	102.3			102.5	2	48.0	47.8	47.7	48.2	48.0	48.5	48.5	48.1
2	72.4	72.6	72.6	72.4	72.5			72.6	3	68.7	68.6	68.5	68.8	68.7	68.8	68.8	68.7
3	72.2	72.2	72.1	72.3	72.3			72.3	4	19.9	19.5	19.4	20.2	20.0	20.8	20.8	20.0
4	74.0	74.0	74.0	73.9	74.0			73.9	5	12.2	11.7	11.5	12.4	12.1	13.1	13.1	12.2
5	69.9	69.6	69.8	70.0	69.9			70.0	Nla″-1				175.5	175.5			
6	18.9	19.1	19.1	18.9	18.9			19.0	2				48.5	48.4			
Qui-1	104.6	103.8	103.8	104.8	103.8	105.7	104.3	105.0	3				69.7	69.7			
2	76.4	75.7	75.7	76.5	75.5	76.2	75.2	76.7	4				21.1	21.0			
3	79.7	80.2	80.3	79.6	80.0	78.0	77.7	79.8	5				13.3	13.3			
4	74.8	74.8	74.7	74.8	74.8	76.6	76.7	74.8	Mba-1	176.1	176.8	176.8	176.1	176.8	176.1	176.8	
5	70.4	70.2	70.2	70.4	70.3	73.1	72.9	70.5	2	41.3	41.8	41.8	41.3	41.8	41.3	41.7	
6	18.2	18.1	18.1	18.2	18.2	18.6	18.5	18.3	3	27.0	26.7	26.7	27.0	26.7	27.0	26.6	
									4	11.6	11.8	11.8	11.6	11.8	11.6	11.8	
									5	16.7	16.6	16.6	16.8	16.6	16.8	16.4	

[a] Chemical shifts (δ) are in ppm relative to TMS. [b] Values are recorded at 125 MHz. [c] Values are recorded at 100 MHz. [d] Values are recorded at 150 MHz.

of the two 2*R*,3*R*-nilic acids present in **4**, **5**, and **11** were located at OH-4 of Qui and OH-3 of Nla, the 2*S*,3*S*-nilyl unit should be attached to OH-4 of Rha. Consequently, the structures of **7** and **8** were defined as methyl 3*S*,11*S*-dihydroxytetradecanoate 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[4-*O*-2*R*-methyl-3*R*-*O*-(2*R*-methyl-3*R*-hydroxybutyryl)butyryl]-*β*-D-quinovopyranosyl-(1→4)-*O*-(2-*O*-2*S*-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-(4-*O*-2*S*-methyl-3*S*-hydroxybutyryl)-α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside and the positional isomer of **7**, in which Mba at OH-2 of Rha′ was transferred to OH-3 of the same sugar unit, respectively.

PM-6 (**9**) and PM-7 (**10**) were obtained as amorphous powders, and their negative FABMS indicated an $[M - H]^-$ ion peak at *m*/*z* 1319. Both compounds showed signals due to one Mba, two nilyl units, two nonequivalent methylene protons assignable to H_2-2 of the aglycone, one methoxy group, and five anomeric protons in the ^{1}H NMR spectra and exhibited signals due to four ester carbonyl carbons and five anomeric carbons in the ^{13}C NMR spectra (Tables 2 and 4). Further, reflux of **10** in 70% MeOH generated **9**. These data indicated that **9** and **10** were composed of one 2-methylbutyric acid, two nilic acid, and one methyl ester of **1** moieties and further that **9** and **10** were positional isomers involving the Mba group. On alkaline hydrolysis, **9** gave nilic acid, 2-methylbutyric acid, and **1**. The absolute configuration of nilic acid in **9** was defined as 2*R*,3*R* in the same manner as **4**. The ^{1}H NMR spectrum of **9** showed two remarkable downfield shifts of signals due to H-4 (1.47 ppm) of Rha and H-2 (1.19 ppm) of Rha′, when compared to that of **1**,[19] along with a deshielded signal assignable to H-3 of Nla. On the other hand, comparison of the ^{1}H NMR spectrum of **10** with that of **9** indicated an upfield shift (ca. 1.21 ppm) of the H-2 of Rha′ and a downfield shift (1.37 ppm) of the signal due to H-3 of Rha′, whereas the signals due to H-4 of Rha and H-3 of Nla in the ^{1}H NMR spectrum of **10** were observed at chemical shifts similar to those of **9**. In addition, the HIEIMS of the peracetate of **10** (**15**) revealed fragment ion peaks at *m*/*z* 273.0966, 473.2010, and 545.2236, which were assigned to the fragment ions of the 2,3,4-*O*-triacetylquinovopyranosyl unit, the [4-*O*-2-methyl-3-*O*-(2-methyl-3-acetoxybutyryl)butyryl, 2,3-*O*-diacetyl]rhamnopyranosyl unit, and the 2,3,4-*O*-triacetylquinovopyranosyl-(1→4)-*O*-(2-*O*-acetyl-3-*O*-2-methylbutyryl)rhamnopyranosyl unit, respectively. Moreover, the HMBC spectrum of **9** showed three key cross-peaks, which were assigned as those between H-4 of Rha and C-1 of Nla; H-3 of Nla and C-1 of Nla′; and methoxy protons and C-1 of Agl. Consequently, **9** and **10** were characterized as methyl 3*S*,11*S*-dihydroxytetradecanoate 11-*O*-*β*-D-quinovopyranosyl-(1→4)-*O*-(2-*O*-2*S*-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-{[4-*O*-2*R*-methyl-3*R*-*O*-(2*R*-methyl-3*R*-hydroxybutyryl)butyryl]-*O*-α-L-rhamnopyranosyl-(1→6)}-*β*-D-glucopyranoside and the positional isomer of **9**, in which the Mba at OH-2 of Rha′ was migrated to OH-3 of the same sugar unit, respectively.

Mannich and Schumann[6] presumed that convolvulin from *I. purga* was an oligomer partially acylated by some organic acids at the sugar moiety. However, PM-1−PM-7 were all monomers of methyl esters of glycosidic acid, several esterfied organic acids at the sugar moiety. Further, negative FABMS of pharbitin exhibited ion peaks at *m*/*z* 1551, 1451, and 1305, which corresponded to the values of $[M - H]^-$ ion peaks of the demethyl derivatives of **7**, **4**, and **9**, respectively. However, no intense ion peaks were observed

Table 3. ^{1}H NMR Spectroscopic Data (Pyridine-d_5) for Compounds **7** and **8**[a]

position	**7**[b]	**8**[c]
Glc-1	4.90 d (8.0)[d]	4.86 d (7.5)
2	ca. 4.28	ca. 4.17
3	4.45 dd (9.5, 9.5)	4.59 dd (8.5, 8.5)
4	ca. 3.94	ca. 3.92
5	ca. 3.95	ca. 3.91
6	4.08 dd (5.0, 11.0)	4.10 dd (4.5, 11.0)
6	4.43 dd (3.0, 11.0)	ca. 4.42
Glc′-1	5.83 d (7.0)	5.70 d (7.5)
2	ca. 4.19	ca. 4.23
3	ca. 4.17	ca. 4.21
4	4.07 dd (9.0, 9.0)	4.03 dd (9.5, 9.5)
5	3.82 ddd (3.5, 5.5, 9.0)	ca. 3.85
6	ca. 4.27	4.26 dd (5.0, 11.5)
6	ca. 4.40	4.45 dd (2.5, 11.5)
Rha-1	5.39 d (1.0)	5.40 s
2	ca. 4.40	ca. 4.34
3	4.50 dd (3.5, 10.0)	4.50 dd (3.0, 9.5)
4	5.81 dd (10.0, 10.0)	5.79 dd (9.5, 9.5)
5	4.34 dq (10.0, 6.0)	ca. 4.38
6	1.49 d (6.0)	1.54 d (6.0)
Rha′-1	6.18 d (1.5)	6.31 d (1.5)
2	5.90 dd (1.5, 3.5)	4.69 dd (1.5, 3.0)
3	4.95 dd (3.5, 9.0)	6.21 dd (3.0, 9.5)
4	4.25 dd (9.0, 9.0)	4.56 dd (9.5, 9.5)
5	5.04 dq (9.0, 6.0)	5.18 dq (9.5, 6.0)
6	1.87 d (6.0)	1.82 d (6.0)
Rha″-1	6.03 d(1.0)	5.99 d (1.5)
2	4.68 dd (1.0, 3.0)	4.71 dd (1.5, 3.0)
3	ca. 4.42	4.46 dd (3.0, 9.0)
4	ca. 4.20	4.20 dd (9.0, 9.0)
5	ca. 4.32	ca. 4.34
6	1.68 d (6.0)	1.69 d (6.0)
Qui-1	5.27 d(8.0)	4.92 d(8.0)
2	ca. 3.96	ca. 3.86
3	4.23 dd (9.0, 9.0)	4.18 dd (9.5, 9.5)
4	5.14 dd (9.0, 9.0)	5.13 dd (9.5, 9.5)
5	3.61 dq (9.0, 6.0)	3.59 dq (9.5, 6.0)
6	1.32 d (6.0)	1.29 d (6.0)
Agl-2	2.70 dd (5.5, 15.0)	2.70 dd (5.0, 14.5)
2	2.74 dd (7.5, 15.0)	2.73 dd (8.0, 14.5)
3	ca. 4.41	ca. 4.42
11	ca. 3.93	ca. 3.92
14	0.99 t (7.0)	0.99 t (7.0)
OCH_3	3.63 s	3.63 s
Nla-2	3.20 dq (7.0, 7.0)	3.16 dq (7.0, 7.0)
3	5.58 dq (7.0, 7.0)	5.54 dq (7.0, 7.0)
4	1.41 d (7.0)	1.40 d (7.0)
5	1.37 d (7.0)	1.36 d (7.0)
Nla′-2	2.94 dq (7.0, 7.0)	2.96 dq (7.0, 7.0)
3	ca. 4.40	ca. 4.42
4	1.37 d (7.0)	1.38 d (7.0)
5	1.26 d (7.0)	1.27 d (7.0)
Nla″-2	2.86 dq (7.0, 7.0)	2.86 dq (7.0, 7.0)
3	ca. 4.31	ca. 4.30
4	1.41 d (7.0)	1.40 d (7.0)
5	1.30 d (7.0)	1.30 d (7.0)
Mba-2	2.50 ddq (7.0, 7.0, 7.0)	2.52 ddq (7.0, 7.0, 7.0)
3	ca. 1.51	ca. 1.44
3	1.78 ddq (7.0, 13.0, 7.0)	ca. 1.84
4	0.90 dd (7.0, 7.0)	0.86 dd (7.5, 7.5)
5	1.15 d (7.0)	1.21 d (7.0)

[a] Chemical shifts (δ) are in ppm relative to TMS. [b] Values are recorded at 400 MHz. [c] Values are recorded at 600 MHz. [d] Coupling constants (J) in Hz are given in parentheses.

in the m/z 1580 to 3500 region. Therefore, pharbitin appears to be a mixture of monomers composed of free carboxylic acid forms corresponding to **4**−**10**, etc.

Although the isolation of a pure form of convolvulin from pharbitin could not be achieved, the detailed structure of convolvulin is reported here for the first time. Further, it should be noted that **7** and **8** had 2*R*,3*R*-nilic acid and its enantiomer as the component organic acids in each molecule. The indium(III) chloride-catalyzed methyl esterification of the carboxylic acids is considered to be useful for the investigation of convolvulins.

Table 4. ^{1}H NMR Spectroscopic Data (Pyridine-d_5) for Compounds **9**, **10**, and **15**[a]

position	**9**[b]	**10**[b]	**15**[c,d]
Glc-1	4.89 d (7.5)[e]	4.85 d (7.5)	4.88 d (8.0)
2	4.29 dd (7.5, 9.0)	4.14 dd (7.5, 9.0)	4.18 dd (8.0,9.5)
3	4.50 dd (9.0, 9.0)	ca. 4.70	5.63 dd(9.5, 9.5)
4	3.88 dd (9.0, 9.0)	3.91 dd (9.0, 9.0)	5.30 dd (9.5, 9.5)
5	3.97 ddd (1.5, 6.5, 9.0)	3.96 ddd (1.5, 7.0, 9.0)	4.00 ddd (2.0, 6.0, 9.5)
6	4.03 dd (6.5, 11.0)	4.05 dd (7.0, 11.0)	ca. 3.87
6	ca. 4.42	4.36 dd (1.5. 11.0)	4.06 dd (2.0, 11.0)
Glc′-1	5.84 d (7.5)	5.70 d (7.5)	5.12 d (8.0)
2	4.20 dd (7.5, 9.5)	ca. 4.29	4.11 dd (8.0, 9.0)
3	4.18 dd (9.5, 9.5)	4.24 dd (9.5, 9.5)	5.76 dd (9.0, 9.0)
4	ca. 4.05	4.05 dd (9.5, 9.5)	5.35 dd (9.0, 9.0)
5	3.82 ddd (3.0, 6.5, 9.5)	ca. 3.85	4.14 ddd (2.5, 4.5, 9.0)
6	4.24 dd (6.5, 11.5)	ca. 4.29	4.36 dd (2.5, 12.0)
6	ca. 4.42	ca. 4.47	4.63 dd (4.5, 12.0)
Rha-1	5.36 d (1.0)	5.33 s	5.22 d (1.5)
2	4.45 dd (1.0, 3.5)	ca. 4.47	5.79 dd (1.5, 3.5)
3	4.49 dd (3.5, 9.5)	4.52 dd (3.0, 9.5)	5.73 dd (3.5, 10.0)
4	5.73 dd (9.5, 9.5)	5.76 dd (9.5, 9.5)	ca. 5.54
5	ca. 4.28	ca. 4.30	4.24 dq (10.0, 5.5)
6	1.43 d (6.5)	1.46 d (6.0)	1.45 d (5.5)
Rha′-1	6.19 d (1.5)	6.41 d (1.5)	5.37 d (2.0)
2	5.91 dd (1.5, 3.5)	ca. 4.70	5.63 dd (2.0, 3.0)
3	4.97 dd (3.5, 9.5)	6.34 dd (3.0, 10.0)	5.76 dd (3.0, 9.5)
4	ca. 4.29	4.67 dd (10.0, 10.0)	4.31 dd (9.5, 9.5)
5	5.07 dq (9.5, 6.0)	5.29 dq (10.0, 6.5)	4.59 dq (9.5, 6.0)
6	1.93 d(6.0)	1.90 d (6.5)	1.79 d (6.0)
Qui-1	5.32[f]	4.98 d (8.0)	5.31 d (8.0)
2	ca. 4.04	3.93 dd (8.0, 9.0)	5.45 dd (8.0, 9.5)
3	ca. 4.05	4.00 dd (9.0, 9.0)	5.58 dd (9.5, 9.5)
4	ca. 3.66	3.71 dd (9.0, 9.0)	5.12 dd (9.5, 9.5)
5	ca. 3.69	3.64 dq (9.0, 7.0)	3.78 dq (9.5, 5.5)
6	1.59 d (6.0)	1.56 d (7.0)	1.23 d (5.5)
Agl-2	2.70 dd (5.0, 15.0)	2.71 dd (5.0, 15.0)	2.76 dd (6.0, 16.0)
2	2.73 dd (8.0, 15.0)	2.75 dd (8.0, 15.0)	2.80 dd (7.0, 16.0)
3	ca. 4.40	4.43 m	ca. 5.54
11	ca. 3.89	ca. 3.85	ca. 3.87
14	1.00 t (7.0)	0.98 t (7.5)	1.06 t (6.5)
OCH_3	3.63 s	3.64 s	3.65 s
Nla-2	3.03 dq (6.5, 7.0)	3.02 dq (7.0, 7.0)	2.81 dq (7.0, 7.0)
3	5.53 dq (6.5, 6.5)	5.53 dq (7.0, 7.0)	ca. 5.33
4	1.35 d (6.5)	1.35 d (7.0)	1.25 d (6.5)
5	1.28 d (7.0)	1.28 d (7.0)	1.18 d (7.0)
Nla′-2	2.78 dq (6.5, 7.0)	2.78 dq (7.0, 7.0)	2.89 dq (7.0, 7.0)
3	4.31 dq (6.5, 6.5)	4.32 dq (7.0, 7.0)	5.39 dq (7.0, 6.5)
4	1.33 d (6.5)	1.33 d (7.0)	1.28 d (6.5)
5	1.25 d (7.0)	1.25 d (7.0)	1.20 d (7.0)
Mba-2	2.49 ddq (7.0, 7.0, 7.0)	2.53 ddq (7.0, 7.0, 7.0)	2.57 ddq (7.0, 7.0, 7.0)
3	ca. 1.48	ca. 1.48	1.58 m
3	ca. 1.75	ca. 1.75	1.88 m
4	0.88 dd (7.5, 7.5)	0.81 dd (7.5, 7.5)	0.98 dd (7.0, 7.0)
5	1.13 d (7.0)	1.20 d (7.0)	1.31 d (7.0)

[a] Chemical shifts (δ) are in ppm relative to TMS. [b] Values are recorded at 600 MHz. [c] Values are recorded at 400 MHz. [d] δ 2.01 (s), 2.01 (s), 2.03 (s), 2.03 (s), 2.05 (s), 2.05 (s), 2.07 (s), 2.08 (s), 2.15 (s), 2.16 (s), 2.20 (s), 2.24 (s), 2.30 (s) ($COCH_3$). [e] Coupling constants (J) in Hz are given in parentheses. [f] Signal is deformed by virtual coupling.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO DIP-140 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded by using a JEOL JNM GSX-400, a JEOL alpha 500, and a GE Omega 600 spectrometer, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. MS data were collected using a JEOL JMS DX-300, a JEOL JMS-HX110, and a JEOL JMS-700T spectrometer. Analytical GC was carried out with a Shimadzu gas chromatograph GC-8A with a flame ionizing detector. Column chromatography (CC) was carried out over Diaion HP20 (Mitsubishi Chemical Industries), Sephadex LH-20 (Pharmacia Fine Chemicals), and silica gel 60 (Merck, Art 9385). HPLC separation was performed on a Shimadzu LC-10AS micropump with a Shimadzu RID-10A RI-detector or Shimadzu SPD-10A UV-detector (Shimadzu, Kyoto).

Plant Material. The seeds of *Pharbitis nil* Choisy were kindly provided by Kanebo, Ltd., a commercial supplier of herbs in Tokyo, Japan, and were identified by one of the authors (K.M.). A voucher

specimen has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Treatment of Pharbitin with Indium(III) Chloride in MeOH and Isolation of 4−10. Pharbitin (6235 mg) previously obtained[19] from seeds of *Pharbitis nil* was dissolved in MeOH (600 mL), and indium(III) chloride (1.5 g) was added to the solution at room temperature. The mixture was heated at reflux for 6 days, while being monitored by TLC. The concentrated reaction mixture was chromatographed on a Diaion HP20 column, eluted with H_2O and MeOH. The MeOH eluate (6078 mg) was subjected to Sephadex LH-20CC eluted with MeOH to give fractions 1 (1082 mg), 2 (1609 mg), 3 (2231 mg), and 4 (341 mg). CC of fraction 2 on silica gel eluted with a gradient of mixtures of $CHCl_3$−MeOH−H_2O (14:2:0.1, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0) afforded fractions 2.1 (29 mg), 2.2 (24 mg), 2.3 (329 mg), 2.4 (105 mg), 2.5 (319 mg), 2.6 (232 mg), 2.7 (197 mg), 2.8 (209 mg), 2.9 (42 mg), 2.10 (38 mg), and 2.11 (74 mg). Fractions 2.4, 2.5, and 2.6 were each subjected to HPLC (column 1: COSMOSIL 5C18 AR-II, Nacalai Tesque Inc., 20 mm i.d. × 250 mm) using 80% MeOH for fractions 2.4 and 2.5 and 85% MeOH for fraction 2.6 to give **8** (22 mg) from fraction 2.4, **5** (23 mg), **9** (27 mg), and **7** (18 mg) from fraction 2.5, and **4** (34 mg) and **6** (13 mg) from fraction 2.6. Fraction 3 was chromatographed on a silica gel column using a gradient of mixtures of $CHCl_3$−MeOH−H_2O (14:2:0.1, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0) to give fractions 3.1 (29 mg), 3.2 (88 mg), 3.3 (90 mg), 3.4 (95 mg), 3.5 (347 mg), 3.6 (168 mg), 3.7 (122 mg), 3.8 (370 mg), 3.9 (123 mg), 3.10 (98 mg), 3.11 (320 mg), 3.12 (157 mg), and 3.13 (72 mg). HPLC (column 1) of fractions 3.2, 3.4, 3.5, 3.7, and 3.8, using 80% MeOH as eluent, afforded **10** (15 mg) from fraction 3.2, **8** (13 mg) from fraction 3.4, **8** (24 mg) from fraction 3.5, **7** (24 mg) from fraction 3.7, and **4** (28 mg) from fraction 3.8.

PC-1 (4): amorphous powder; $[\alpha]^{28}_D$ −56.7 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS (negative mode) *m/z* 1465 $[M - H]^-$; HRFABMS (positive mode) *m/z* 1489.6975 (calcd for $C_{66}H_{114}O_{35}Na$, 1489.7038); *anal*. C 54.22, H 7.84%, calcd for $C_{66}H_{114}O_{35}$, C, 54.01, H 7.83%.

PC-2 (5): amorphous powder; $[\alpha]^{23}_D$ −51.7 (*c* 0.8, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS (negative mode) *m/z* 1465 $[M - H]^-$; *anal*. C 54.06, H 7.89%, calcd for $C_{66}H_{114}O_{35}$, C 54.01, H 7.83%.

PC-3 (6): amorphous powder; $[\alpha]^{23}_D$ −47.7 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS (negative mode) *m/z* 1493 $[M - H]^-$; *anal*. C 54.78, H 8.03%, calcd for $C_{68}H_{118}O_{35}$, C 54.61, H 7.95%.

PC-4 (7): amorphous powder; $[\alpha]^{27}_D$ −46.6 (*c* 1.8, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1730 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 2 and 3; FABMS (negative mode) *m/z* 1565 $[M - H]^-$; *anal*. C 54.35; H 7.98%, calcd for $C_{71}H_{122}O_{37}$, C 54.40, H 7.84%.

PC-5 (8): amorphous powder; $[\alpha]^{22}_D$ −51.8 (*c* 2.4, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 2 and 3; FABMS (negative mode) *m/z* 1565 $[M - H]^-$; *anal*. C 54.29, H 7.87%, calcd for $C_{71}H_{122}O_{37}$, C 54.40, H 7.84%.

PC-6 (9): amorphous powder; $[\alpha]^{28}_D$ −40.0 (*c* 4.3, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH); 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 2 and 4; FABMS (negative mode) *m/z* 1319 $[M - H]^-$; *anal*. C 54.58, H 7.98%, calcd for $C_{60}H_{104}O_{31}$, C 54.54, H 7.93%.

PC-7 (10): amorphous powder; $[\alpha]^{22}_D$ −48.7 (*c* 2.2, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 2 and 4; FABMS (negative mode) *m/z* 1319 $[M - H]^-$; *anal*. C 54.44, H 8.00%, calcd for $C_{60}H_{104}O_{31}$, C 54.54, H 7.93%.

Partial Deacylation of 4 and 8. Solutions of **4** (18 mg) in 28% aqueous NH_3−MeOH (1:4) (1 mL) and **8** (28 mg) in 28% aqueous NH_3−MeOH (1:8) (2 mL) were refluxed for 1 and 1.5 h, respectively. The reaction mixtures were neutralized with 1 M HCl. After removal of the solvent, the residues were desalted by Sephadex LH-20 CC using MeOH as eluent and subjected to preparative HPLC on a Nucleosil 5C8 column (Chemco Scientific Co., Ltd., 20 mm i.d. × 250 mm) using 75% MeOH as eluent to give **11** (3 mg), **5** (7 mg), and **4** (3 mg) from the reaction mixture of **4** and **11** (11 mg), **5** (5 mg), and **4** (5 mg) from the reaction mixture of **8**. The generated compounds, except for **11**, from **4** and **8** were each identified on the basis of ^1H NMR spectroscopic data (pyridine-d_5, 400 MHz).

Compound 11: amorphous powder; $[\alpha]^{28}_D$ −59.8 (*c* 4.3, MeOH); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1725 cm^{-1} (C=O); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS (negative mode) *m/z* 1381 $[M - H]^-$; *anal*. C 52.81, H 7.74%, calcd for $C_{61}H_{106}O_{34}$, C 52.96, H, 7.72%.

Alkaline Hydrolysis of 7, 9, and 11. Suspensions of **7** (25 mg), **9** (25 mg), and **11** (10 mg) in 1 M KOH (1 mL) were each heated at 95 °C for 1 h. After cooling, the pH of the reaction mixture was adjusted to pH 3 with 1 M HCl, then diluted with H_2O (5 mL), and extracted with AcOEt (10 mL, 5 mL × 8). The AcOEt layer was dried over $MgSO_4$ and solvent removed to give a residue (organic acid fraction, 6 mg from **7**, 5 mg from **9**, 1 mg from **11**). The organic acid fraction in dry acetone (5 mL) was neutralized with triethylamine. Then, *p*-bromophenacyl bromide (20 mg) was added and the mixture was left to stand at room temperature overnight and then concentrated under reduced pressure. The residue was partitioned between H_2O (5 mL) and ether (5 mL × 7). The ether layer was washed with H_2O (5 mL) and dried over $MgSO_4$ to give the ether fraction, which was subjected to HPLC (column, Kusano CIG prepacked Si-gel, Kusano Kagakukiki Co., 22 mm i.d. × 100 mm) using hexane−AcOEt (2:1) as eluent to give **12** (2 mg) from **11**, **14** (1 mg) and **12′** (6 mg) from **7**, and **14** (2 mg) and **12″** (5 mg) from **9**. The retention times (t_R) of **12**, **12′**, and **12″** on HPLC (column, COSMOSIL 5SL-II, 4.6 mm i.d. × 250 mm; flow rate, 0.8 mL/min; detector, Shimadzu SPD-10A UV−vis detector) were each identical to that of *p*-bromphenacyl nilate [t_R 11.3 min; solvent, hexane−AcOEt (2:1)], and that of **14** was identical to that of *p*-bromphenacyl 2-methylbutyrate [t_R 7.4 min; solvent, hexane−AcOEt (9:1)].[19]

The aqueous layer was desalted by chromatography over a MCI gel CHP20 column (Mitsubishi Chemical Industries) using H_2O and acetone as eluent to give a white powder (glycosidic acid) (15 mg from **7**, 16 mg from **9**, and 7 mg from **11**). The ^1H NMR spectra (pyridine-d_5, 400 MHz) of glycosidic acids of **7** and **11** were both superimposable on that of **2**, and that of **9** was quite similar to that of **1**.[19]

Preparation of (−)-MTPA Esters of 12, 12′, and 12″. Compounds **12** (2 mg), **12′** (2 mg), and **12″** (2 mg) were each dissolved in pyridine (0.5 mL) and CCl_4 (5 drops). Then, (−)-MTPACl[22] (14 mg) was added and the mixture was left to stand at room temperature overnight. The solvent was removed under a N_2 stream, and the residue was purified by silica gel CC, using hexane−AcOEt (10:1, 5:1), to give an oil [**13** (1 mg) from **12**, **13′** (2 mg) from **12′**, **13″** (2 mg) from **12″**]. The ^1H NMR spectrum of **13″** was superimposable on that of **13**.

Compound 13: ^1H NMR ($CDCl_3$, 600 MHz) δ 1.241 (3H, d, J = 7.5 Hz, H_3-5), 1.435 (3H, d, J = 6.0 Hz, H_3-4), 2.928 (1H, dq, J = 7.0, 7.5 Hz, H-2), 3.555 (3H, s, OCH_3), 5.056 (1H, d, J = 16.0 Hz, COOCH_2CO), 5.123 (1H, d, J = 16.0 Hz, COOCH_2CO), 5.459 (1H, dq, J = 7.0, 6.0 Hz, H-3).

Compound 13′: ^1H NMR ($CDCl_3$, 600 MHz) δ 1.241 (3H, d, J = 7.5 Hz, H_3-5 of *R*,*R*-form), 1.304 (3/2H, d, J = 7.5 Hz, H_3-5 of *S*,*S*-form), 1.358 (3/2H, d, J = 6.5 Hz, H_3-4 of *S*,*S*-form), 1.435 (3H, d, J = 6.5 Hz, H_3-4 of *R*,*R*-form), 2.929 (1H, dq, J = 7.5, 7.5 Hz, H-2 of *R*,*R*-form), 2.960 (1/2H, dq, J = 7.5, 7.5 Hz, H-2 of *S*,*S*-form), 3.512 (3/2H, d, J = 1.0 Hz, OCH_3 of *S*,*S*-form), 3.555 (3H, d, J = 1.0 Hz, OCH_3 of *R*,*R*-form), 5.056 (1H, d, J = 16.0 Hz, COOCH_2CO of *R*,*R*-form), 5.123 (1H, d, J = 16.0 Hz, COOCH_2CO of *R*,*R*-form), 5.180 (1/2H, d, J = 16.0 Hz, COOCH_2CO of *S*,*S*-form), 5.252 (1/2H, d, J = 16.0 Hz, COOCH_2CO of *S*,*S*-form), 5.460 (3/2H, dq, J = 7.5, 6.5 Hz, H-3 of *R*,*R*- and *S*,*S*-form).

Alkaline Hydrolysis of 6. A suspension of **6** (7 mg) in 5% KOH (1 mL) was heated at 95 °C for 1 h. The reaction mixture was adjusted to pH 4 with 1 M HCl, diluted with H_2O (10 mL), and extracted with ether (3 × 3 mL). The ether layer was washed with H_2O (5 mL), dried over $MgSO_4$, and evaporated under reduced pressure to afford a residue (1 mg, organic acid fraction). A part of the residue was subjected to GC [column, Unisole 30T (10%), Gasukuro Kogyo Inc., 3.2 mm i.d. × 2 m glass column; column temperature, 120 °C; carrier gas, N_2 2 kg/cm^2] and was found to contain 2-methylbutyric acid (t_R 5.1 min). An aliquot of the organic acid fraction was methylated with ethereal diazomethane and analyzed by GC [column, Unisole 30T (10%), 3.2 mm i.d. × 2 m glass column; column temperature, 70 °C; carrier gas, N_2 2 kg/cm^2], and methyl nilate (t_R 21.4 min) was detected.[19]

The aqueous layer was desalted by chromatography over a MCI gel CHP20 column, using H_2O and acetone as eluent, to give a white powder (glycosidic acid, 5 mg). The glycosidic acid was identified as **3** by comparison of ^1H NMR spectroscopic data (in pyridine-d_5, 400 MHz) with those of an authentic sample.[19]

Reflux of 5, 8, and 10 in 70% MeOH. Compounds **5** (2 mg), **8** (2 mg), and **10** (2 mg) were each refluxed in 70% MeOH (2 mL) for 4 h.

The mixture derived from **5**, **8**, and **10** exhibited the presence of **4** (t_R 15.16 min) and **5** (t_R 8.04 min) in the approximate ratio of 5:7, **7** (t_R 21.76 min) and **8** (t_R 10.55 min) in the approximate ratio of 1:1, and **9** (t_R 9.00 min) and **10** (t_R 5.86 min) in the approximate ratio of 1:1, respectively, on HPLC analysis (column, COSMOSIL 5C18 AR-II, 4.6 mm i.d. × 250 mm; solvent, 80% MeOH; flow rate, 0.8 mL/min; detector, JASCO RI-2031 Plus RI-detector).

Acetylation of 10. A solution of **10** (10 mg) in Ac_2O−pyridine (1:1, 1 mL) was left to stand at room temperature overnight. The solvent was removed under an N_2 stream to give **15** (14 mg).

Compound 15: amorphous powder; $[\alpha]^{27}_D$ −20.1 (*c* 1.8, MeOH); ^{1}H NMR, see Table 4; EIMS *m*/*z* 833, 545, 473, 273; HREIMS *m*/*z* 545.2236 (calcd for $C_{25}H_{37}O_{13}$, 545.2234), 473.2010 (calcd for $C_{22}H_{33}O_{11}$, 473.2022), 273.0966 (calcd for $C_{12}H_{17}O_7$, 273.0974).

Acknowledgment. We express our appreciation to Dr. M. Nishi of Setsunann University for measurements of NMR spectra, mass spectra, and elemental analyses, and Mr. T. Iriguchi of Kumamoto University for measurement of the positive HR-FABMS.

Supporting Information Available: Figures of NMR spectra of **1**−**11** and **15** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Shellard, E. J. *Plant Med.* **1961**, *9*, 102–116.
(2) Noda, N.; Ono, M.; Miyahara, K.; Kawasaki, T. *Tetrahedron* **1987**, *43*, 1389–3902.
(3) Noda, N.; Tsuji, K.; Kawasaki, T.; Miyahara, K.; Nakazono, H.; Yang, C.-R. *Chem. Pharm. Bull.* **1995**, *43*, 1061–1063.
(4) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *27*, 9007–9022.
(5) Escalante-Sánchez, E.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 1029–1034.
(6) Mannich, C.; Schumann, P. *Arch. Pharm.* **1938**, *276*, 211–226.
(7) MacLeod, J. K.; Ward, A. *J. Nat. Prod.* **1997**, *60*, 467–471.
(8) Pereda-Miranda, R.; Fragoso-Serrano, M.; Escalante-Sánchez, E.; Hernández-Carlos, B.; Linares, E.; Bye, R. *J. Nat. Prod.* **2006**, *69*, 1460–1466.
(9) Ono, M.; Nishioka, H.; Fukushima, T.; Kunimatsu, H.; Mine, A.; Kubo, H.; Miyahara, K. *Chem. Pharm. Bull.* **2009**, *57*, 262–268.
(10) Ono, M.; Takagi-Taki, Y.; Honda-Yamada, F.; Noda, N.; Miyahara, K. *Chem. Pharm. Bull.* **2010**, *58*, 666–672.
(11) Ono, M.; Imao, M.; Miyahara, K. *Chem. Pharm. Bull.* **2010**, *58*, 1232–1235.
(12) Asahina, Y.; Terada, S. *Yakugaku Zasshi* **1919**, *452*, 821–836.
(13) Asahina, Y.; Shimizu, T. *Yakugaku Zasshi* **1922**, *479*, 1–18.
(14) Asahina, Y.; Nakanishi, Y. *Yakugaku Zasshi* **1925**, *520*, 515–520.
(15) Okabe, H.; Kawasaki, T. *Tetrahedron Lett.* **1970**, *11*, 3123–3126.
(16) Kawasaki, T.; Okabe, H.; Nakatsuka, I. *Chem. Pharm. Bull.* **1971**, *19*, 1144–1149.
(17) Okabe, H.; Koshito, N.; Tanaka, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1971**, *19*, 2394–2403.
(18) Okabe, H.; Kawasaki, T. *Chem. Pharm. Bull.* **1972**, *20*, 514–520.
(19) Ono, M.; Naoki, N.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1990**, *38*, 1892–1897.
(20) Ono, M.; Yamada, F.; Noda, N.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1993**, *41*, 1023–1026.
(21) Mineno, T.; Kansui, H. *Chem. Pharm. Bull.* **2006**, *54*, 918–919.
(22) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–518.

NP1004888