Note

Acylated Glycosidic Acid Methyl Esters Generated from the Convolvulin Fraction of Rhizoma Jalapae Braziliensis by Treatment with Indium(III) Chloride in Methanol

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Four hexaglycosides of methyl 3*S*,12*S*-dihydroxyhexadecanoate (1–4) were provided after treatment of the crude convolvulin fraction from Rhizoma Jalapae Braziliensis (the root of *Ipomoea operculata* (Gomes) Mart., Convolvulaceae) with indium(III) chloride in methanol. The structures of 1–4 were elucidated on the basis of spectroscopic and chemical methods. Their sugar moieties were partially acylated with organic acids including (3*S*,9*R*)-3,6:6,9-diepoxydecanoic (exogonic) acid, (*E*)-2-methylbut-2-enoic (tiglic) acid, and isovaleric acid.

Key words resin glycoside; convolvulin; *Ipomoea operculata*; Rhizoma Jalapae Braziliensis; acylated glycosidic acid methyl ester; indium(III) chloride

Rhizoma Jalapae Braziliensis, the root of *Ipomoea operculata* (Gomes) Mart. (Convolvulaceae), is used as a crude laxative drug. Its active ingredients are known to be ether-soluble resin glycoside called jalapin and ether-insoluble resin glycoside called convolvulin.[1,2] In preceding papers, we reported seven glycosidic acids, operculinic acids A–G, and two organic acids, *n*-decanoic acid and *n*-dodecanoic acid, as the alkaline hydrolysis products of the crude jalapin fraction of the crude drug, and isolation and structural elucidation of 18 new genuine jalapins, operculins I–XVIII, from the fraction.[3–8] We also reported a glycosidic acid, operculinic acid H, along with three organic acids such as isovaleric acid, (*E*)-2-methylbut-2-enoic (tiglic) acid, and (3*S*,9*R*)-3,6:6,9-diepoxydecanoic (exogonic) acid, which exists as an equilibrium mixture (*ca.* 1 : 1) at the C-6 spiro center, formed by the alkaline hydrolysis of the crude convolvulin fraction.[9] As part of our studies on the resin glycosides from this crude drug, herein we report the isolation and structural elucidation of four acylated glycosidic acid methyl esters. We could afford these methyl esters by effectively treating the crude convolvulin fraction with indium(III) chloride in methanol (MeOH), a catalyst reagent for the mild methyl esterification of carboxylic acids, as previously reported.[10]

Results and Discussion

Powdered roots of *I. operculata*, which was purchased from Paul Müggenburg Gmbh & Co., a botanical raw materials company in Hannover, Germany, were extracted with MeOH. This extract was suspended in H_2O, and then successively extracted with ether and *n*-butanol (BuOH). The *n*-BuOH-soluble fraction was subjected to MCI gel CHP20 column chromatography to yield a crude convolvulin fraction.[3] Despite numerous trials, our attempt for the isolation of pure resin glycosides from the crude convolvulin fraction has never become successful. This fraction was therefore treated with indium(III) chloride in MeOH, as in the cases of the crude convolvulin fractions from Pharbitis Semen[11] and seeds of *Quamolit pennata*.[12] The treated fraction was successively separated over Sephadex LH-20 and silica gel column chromatography and HPLC on octadecyl silica (ODS), affording four compounds, referred to as IOM-1–IOM-4.

IOM-1 (**1**) was obtained as an amorphous powder and its molecular formula was determined as $C_{73}H_{122}O_{37}$ by high-resolution (HR)-positive-ion FAB-MS at *m*/*z* 1613.7570. Alkaline hydrolysis of **1** afforded an organic acid fraction and a glycosidic acid. The organic acid fraction was subjected to GC to reveal the presence of isovaleric acid, tiglic acid, and exogonic acid. The glycosidic acid was identified as operculinic acid H (**5**) based on its ^{1}H-NMR spectral data.[9] The ^{1}H-NMR spectrum of **1** showed signals due to one each of isovaleroyl residue (Iva), tigloyl residue (Tig), exogonoyl residue (Exg), and methyl ester (**5a**)[9] of **5**. Moreover, the spectrum showed that **1** was a mixture of epimers that differ in the spirocyclic carbon configuration only at C-6 of Exg in a ratio of approximately 1 : 1 (Table 1). We thus envisioned the isolation of these epimers. The HPLC on naphthylethyl group bonded silica (π-nap) of **1** yielded two compounds (**1a**, **b**). However, the ^{1}H-NMR spectra of **1a** and **b** were quite similar to that of **1**, and further each HPLC analysis using the π-nap column of **1a** and **b** afforded two peaks in which the R_t values matched with those of **1a** and **b**, respectively. Because the data show that the interconversion of the epimers of Exg is facile, the structure analyses were carried out using a mixture of the epimers. The facile epimerization at C-6 of exogonic acid was also reported by Lawson *et al.*[13]

Assignments of the ^{1}H- and ^{13}C-NMR signals of **1** were conducted with the aid of the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and ^{1}H–^{1}H total correlation spectroscopy (TOCSY) spectra (Tables 1, 2). Comparing the chemical shifts of the ^{1}H-NMR

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Table 1. ^{1}H-NMR Spectral Data for **1** and **2** (in Pyridine-d_5, 500 MHz)

Position	1	2
Glc-1	4.83 d (7.5)	4.90 d (7.5)
2	4.30[a)]	4.28[a)]
3	4.43[a)]	4.50[a)]
4	3.86[a)]	3.87 dd (9.0, 9.0)
5	3.96[a)]	4.00[a)]
6	4.63 br d (11.0)	4.52[a)]
6	4.05[a)]	4.02[a)]
Glc′-1	5.75 d (8.0)	5.71 d (7.5)
2	4.10[a)]	4.16[a)]
3	3.95[a)]	3.99 dd (8.0, 9.0)
4	3.81 dd (9.0, 9.0)	3.87 dd (9.0, 9.0)
5	3.70 m	3.71 m
6	4.33 dd (2.0, 11.0)	4.32 dd (2.0, 12.0)
6	4.12[a)]	4.15[a)]
Glc″-1	5.11 d (7.5)	5.15 d (8.0)
2	5.52 dd (7.5, 9.5), 5.51 dd (7.5, 9.5)	5.54 dd (8.0, 9.5), 5.54 dd (8.0, 9.5)
3	4.41[a)]	4.44[a)]
4	4.08[a)]	4.12[a)]
5	4.08[a)]	4.14[a)]
6	4.53[a)]	4.52[a)]
6	4.25[a)]	4.27[a)]
Glc‴-1	5.22 d (7.5)	5.49 d (8.0)
2	3.88[a)]	4.06[a)]
3	4.03[a)]	4.16[a)]
4	3.90[a)]	4.04[a)]
5	4.12[a)]	4.02[a)]
6	4.51[a)]	4.48[a)]
6	4.13[a)]	4.19[a)]
Rha-1	5.44 s	5.41 s
2	4.55 d (3.5)	4.52[a)]
3	4.48 dd (3.5, 9.0)	4.48 dd (3.0, 9.0)
4	4.25 dd (9.0, 9.0)	4.22 dd (9.0, 9.0)
5	4.31[a)]	4.30[a)]
6	1.64 d (6.5)	1.64 d (6.5)
Rha′-1	5.94 s	5.90 s
2	6.02 d (3.5), 5.99 d (3.5)	6.01 d (3.5), 6.02 d (3.5)
3	5.20[a)]	4.97[a)]
4	5.91 dd (10.0, 10.0), 5.91 dd (10.0, 10.0)	4.25 dd (9.0, 9.0)
5	5.18[a)]	5.00[a)]
6	1.66 d (6.5)	1.75 d (6.5)
Agl-2	2.75 dd (7.5, 14.5)	2.75 dd (8.0, 15.0)
2	2.71 dd (5.0, 14.5)	2.70 dd (4.0, 15.0)
3	4.44[a)]	4.43[a)]
12	3.86[a)]	3.87[a)]
16	0.90 t (7.5)	0.91 t (7.0)
OCH_3	3.64 s	3.63 s
Exg-2	3.12 dd (6.5, 16.0), 2.89 dd (6.5, 16.0)	3.19 dd (5.5, 15.5), 3.00 dd (5.5, 15.5)
2	2.87 dd (6.5, 16.0), 2.68 dd (6.5, 16.0)	2.90 dd (7.5, 15.5), 2.68 dd (7.5, 15.5)
3	4.71 dddd (6.5, 6.5, 6.5, 6.5), 4.63[a)]	4.74 m, 4.66 m
9	4.30[a)], 4.05[a)]	4.28[a)], 4.04[a)]
10	1.32 d (7.0), 1.13 d (7.0)	1.31 d (6.5), 1.14 d (6.5)
Tig-3	7.43 q (7.0)	
4	1.82 d (7.0)	
5	2.02 s	
Iva-2	2.72[a)]	2.72[a)]
2	2.54 dd (6.5, 16.0), 2.53 dd (6.5, 16.0)	2.51 m
3	2.33[a)]	2.33[a)]
4	1.19 d (6.5)	1.18 d (7.0)
5	1.08 d (6.5)	1.08 d (7.0)

δ in ppm from tetramethyl silane (TMS). Coupling constants (J) in Hz are given in parentheses. *a*) Signals were overlapped with other signals.

signals due to the presence of sugar moieties between **1** and **5a**, significant downfield shifts owing to acylation were observed for the signals because of H-2 of the third glucosyl unit (Glc″), H-2 of the second rhamnosyl unit (Rha′), and H-4 of Rha′ of **1**. From these data, the locations of ester linkages were concluded to be at OH-2 of Glc″, OH-2 of Rha′, and OH-4 of Rha′. The sites of each ester linkage of Iva, Tig, and Exg were corroborated from the HMBC spectrum of **1**, that is, key cross-peaks between H-2 of Glc″ and C-1 of Iva, H-2 of Rha′ and C-1 of Exg, H-4 of Rha′ and C-1 of Tig, and methoxy protons and C-1 of the aglycone moiety (Agl) were observed (Fig. 1). Therefore, Iva, Tig, and Exg were located at OH-2 of Glc′, OH-2 of Rha′, and OH-4 of Rha′, respectively. Consequently, the structure of **1** was defined as methyl 3*S*,12*S*-dihydroxyhexadecanoate 12-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-(2-*O*-(3*S*,9*R*)-exogonoyl, 4-*O*-tigloyl)-*α*-L-rhamnopyranosyl-(1→2)-[*O*-(2-*O*-isovaleroyl)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside, as shown in Fig. 2.

IOM-2 (**2**) was obtained as an amorphous powder and afforded isovaleric acid, exogonic acid, and **5** upon alkaline hydrolysis. Its positive-ion FAB-MS exhibited an $[M+Na]^+$ ion peak at *m*/*z* 1531, 82 mass units (tigloyl residue) less than the corresponding peak of **1**. The molecular formula of **2** was established as $C_{68}H_{116}O_{36}$ by HR-positive-ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1** except for the loss of signals for Tig in **1** (Tables 1, 2). The chemical shifts of the ^{1}H-NMR signals owing to the presence of sugar moieties in **2** and **1** were compared. The signal due to H-4 of Rha′ of **2** showed remarkable upfield shift, whereas the signals of H-2 of Glc″ and H-2 of Rha′ appeared at positions similar to those of **1**. The above data suggest that **2** is a deacyl derivative of **1**, in which Tig attached to C-4 of Rha′ of **1** is cleaved. The sites of each ester linkage of the acyl groups were confirmed using the HMBC spectrum of **2**, with key cross-peaks observed between H-2 of Glc″ and C-1 of Iva and methoxy protons and C-1 of Agl (Fig. 1). Consequently, the structure of **2** was determined as methyl 3*S*,12*S*-dihydroxyhexadecanoate 12-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-(2-*O*-(3*S*,9*R*)-exogonoyl)-*α*-L-rhamnopyranosyl-(1→2)-[*O*-(2-*O*-isovaleloyl)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside, as shown in Fig. 2.

IOM-3 (**3**) was obtained as an amorphous powder and exhibited an $[M+Na]^+$ ion peak at *m*/*z* 1697, 84 mass units (isovaleroyl residue) lager than that of **1**, in the positive-ion FAB-MS. The molecular formula of **3** was determined as $C_{78}H_{130}O_{38}$ based on the HR-positive-ion FAB-MS. The ^{1}H-NMR spectrum of **3** was similar to that of **1**, aside from the appearance of signals because of one more isovaleroyl residue (Iva′). In the ^{1}H-NMR spectrum of **3**, compared with that of **1**, a remarkable downfield shift was observed for the signal due to H-3 of the fourth glucosyl unit (Glc‴), whereas the signals owing to H-2 and H-4 of Rha and H-2 of Glc″ of **3** were observed at chemical shifts similar to those of **1** (Table 3). Moreover, key cross-peaks between H-2 of Glc″ and C-1 of Iva, H-4 of Rha′ and C-1 of Tig, H-3 of Glc‴-3 and C-1 of Iva′, and methoxy protons and C-1 of Agl were detected in the HMBC spectrum of **3**. These data indicate that **3** is a derivative of **1** with Iva′ attached to OH-3 of Glc‴ of **1**.

Table 2. ^{13}C-NMR Spectral Data for **1**–**4** (in Pyridine-d_5, 125 MHz)

Position	1	2	3	4
Glc-1	102.6	102.5	102.7	102.7
2	78.7	79.1	78.7	79.2
3	79.9	79.6	80.0	80.1
4	71.8	72.0	71.8	71.7
5	76.5	76.3	76.6	76.6
6	68.2	68.2	68.3	68.2
Glc′-1	101.1	101.2	101.1	101.4
2	74.8, 74.9	75.5	75.4	75.0
3	86.9	87.1, 87.2	87.0	87.3
4	69.8	69.9	69.8	70.0
5	77.5	77.5	77.5	77.8
6	62.6	62.7	62.5	62.8
Glc″-1	100.3	100.5	100.4	100.5
2	74.5	74.6	74.6	74.6
3	75.3, 75.4	75.3	75.6	75.7
4	72.3	72.4	72.4	72.3
5	78.6	78.7	78.8	78.6
6	62.2	62.3	62.3	62.1
Glc‴-1	105.4	105.9	105.4, 105.3	105.9
2	74.7	75.8	72.8	74.7
3	78.2	78.3	78.6	78.1
4	71.8	71.7, 71.8	69.7	70.9
5	78.2	78.2	78.0	75.1
6	62.9	62.8	62.7	64.1
Rha-1	102.5	102.5	102.6	102.5
2	72.1	72.2	72.3	72.3
3	72.7	72.7	72.6	72.8
4	73.8	74.1	74.0	74.2
5	69.7	69.7	69.9	69.8
6	18.5	18.7	18.4	18.6
Rha′-1	96.1, 96.1	96.4, 96.6	96.5, 96.4	96.5, 96.3
2	73.4, 73.2	73.3, 73.5	73.1	73.3, 73.1
3	74.9, 74.7	78.8	75.4	74.7
4	73.9	73.3	74.2	74.0
5	67.2	69.3	67.2	67.3
6	18.3	18.8	18.7	18.5
Agl-1	172.8	172.9	172.9	172.9
2	43.3	43.4	43.4	43.4
3	68.2	68.3	68.3	68.3
12	81.6	81.3	81.6	81.5
16	14.3	14.4	14.3	14.4
OCH_3	51.2	51.3	51.3	51.3
Exg-1	171.3, 171.0	171.4, 171.0	171.4, 171.1	170.6, 170.4
2	43.2, 41.0	43.2, 41.4	43.2, 41.2	43.3, 41.1
3	75.5, 74.2	75.8, 74.3	75.6, 73.8	75.5, 73.8
6	115.2, 115.0	115.2, 115.1	115.2, 115.1	115.1, 115.0
9	76.0, 74.1	76.1, 74.2	76.1, 74.2	76.0, 74.2
10	23.3, 21.2	23.4, 21.3	23.4, 21.2	23.3, 21.3
Tig-1	167.5		167.6	167.6
2	129.1		129.1	129.2
3	138.2		138.5	138.1
4	14.5		14.5	14.6
5	12.8		12.7	12.9
Iva-1	172.1	172.1	172.1	172.1
2	43.0	43.4	43.1	43.1
3	25.5	25.4	25.3	25.6
4	22.6	22.7	22.6	22.6
5	22.4	22.5	22.5	22.5
Iva′-1			172.6	173.1
2			43.9	43.3
3			25.6	25.9
4			22.5	22.6
5			22.5	22.6

δ in ppm from TMS.

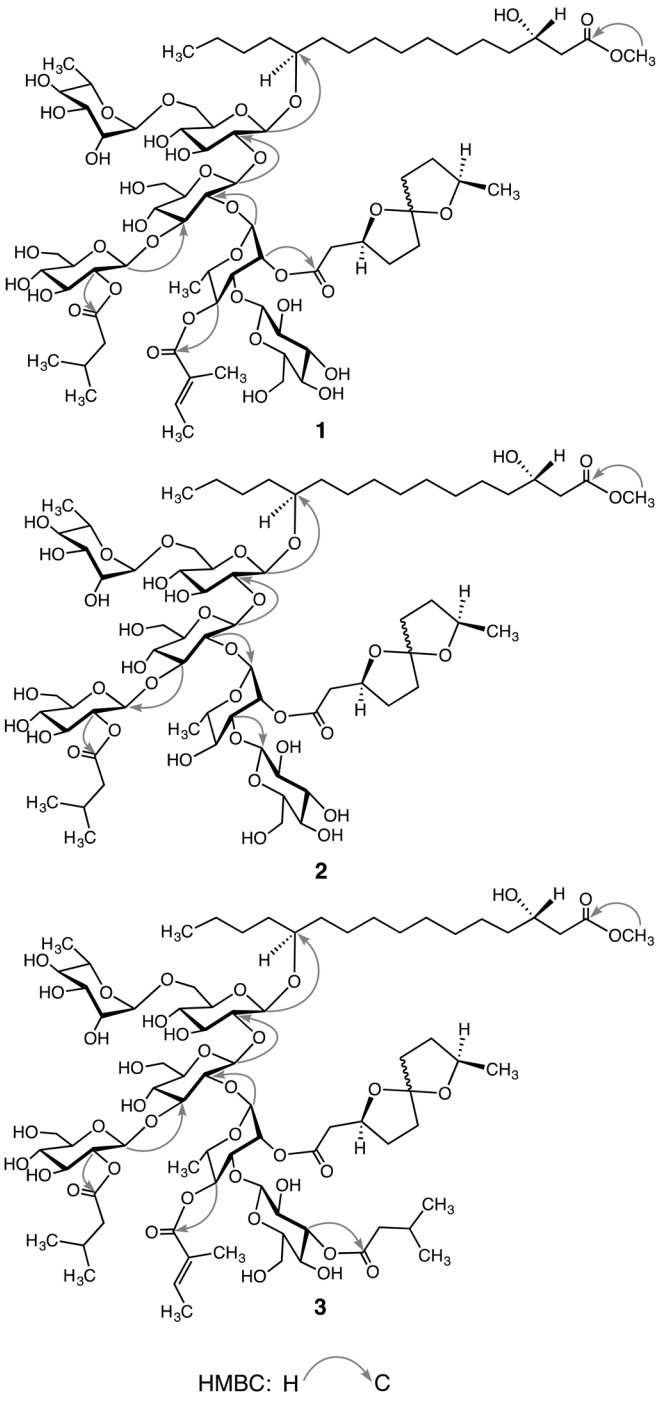

Fig. 1. ^{1}H–^{13}C Long-Range Correlations Observed in the HMBC Spectra of **1**–**3** (in Pyridine-d_5, 500 MHz)

Partial deacylation of **3** was performed in order to determine the sites of ester linkages of the respective organic acids. Compound **3** was refluxed with 3% triethylamine in MeOH for 2 h, and the reaction mixture was separated by HPLC, affording **1**. Consequently, the structure of **3** was identified as methyl 3*S*,12*S*-dihydroxyhexadecanoate 12-*O*-(3-*O*-isovaleloyl)-*β*-D-glucopyranosyl-(1→3)-*O*-(2-*O*-(3*S*,9*R*)-exogonoyl,4-*O*-tigloyl)-*α*-L-rhamnopyranosyl-(1→2)-[*O*-(2-*O*-isovaleroyl)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside, as shown in Fig. 2.

IOM-4 (**4**) was obtained as an amorphous powder. The HR-

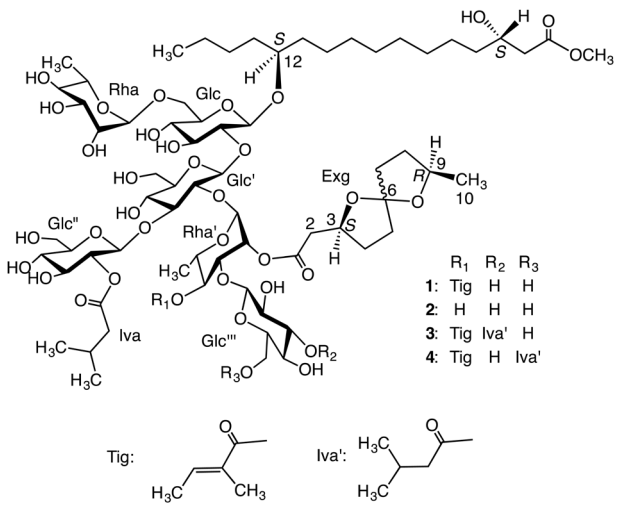

Fig. 2. Structures of **1**–**4**

positive-ion FAB-MS showed that its molecular formula was the same as **3**. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3** and showed signals corresponding to the presence of one each of Tig, Exg, and methoxy group and two isovaleroyl residues (Tables 2, 3). From these data, **4** was deduced to be a positional isomer of **3** concerning ester linkage. The ^{1}H-NMR spectrum of **4** exhibited, in comparison with that of **3**, downfield shifts for the signals assignable to H_2-6 of Glc‴ along with an upfield shift for the signal assignable to H-3 of Glc‴. In contrast, the signals of H-2 and H-4 of Rha and H-2 of Glc″ appeared at positions similar to those in **3**. Further, the treatment of **4** with mild alkali in a similar manner as for **3** afforded **1**. Therefore, **4** was a positional isomer of **3**, in which the isovaleroyl residue of **4** was at the OH-6 of Glc‴ rather than at the OH-3 of Glc‴. Consequently, the structure of **4** was assigned as methyl 3*S*,12*S*-dihydroxyhexadecanoate 12-*O*-(6-*O*-isovaleloyl)-*β*-D-glucopyranosyl-(1→3)-*O*-(2-*O*-(3*S*,9*R*)-exogonoyl,4-*O*-tigloyl)-*α*-L-rhamnopyranosyl-(1→2)-[*O*-(2-*O*-isovaleroyl)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside, as shown in Fig. 2.

The convolvulin from *I. purga* was speculated to be an oligomer of an acylated glycosidic acid.[14] Recently, jalpinoside, which was separated as a minor constituent from the convolvulin fraction of *I. purga*, was reported a macrocyclic bisdesmoside resin glycoside.[15] However, jalapinoside is considered as an acylated glycosidic acid methyl ester monomer. The ^{1}H-NMR spectrum of jalapinoside in the Supporting Information indicates signal due to a methoxy group, which shows a correlation with a carbonyl carbon (C-1) of aglycone moiety in its HMBC spectrum.[15] IOM-1–IOM-4 were all regarded as the methyl ester monomers of the acylated glycosidic acid. Further, negative-ion FAB-MS and HR-negative-ion electrospray ionization-time-of-flight (ESI-TOF)-MS of the crude convolvulin fraction exhibited intense ion peaks at *m*/*z* 1659 and 1659.8010 (calcd for $C_{77}H_{127}O_{38}^{-}$, 1659.8011), corresponding to the values of an $[M–H]^{-}$ ion peak of the demethyl derivatives of **3** and **4**, respectively. In addition, a distinct peak wasn't detected in the region of about *m*/*z* 1700 to 4000 in the negative-ion FAB-MS of the crude convolvulin fraction. Therefore, a part of the crude convolvulin fraction might be a mixture of monomers composed of free carboxylic acid forms

Table 3. ^{1}H-NMR Spectral Data for **3** and **4** (in Pyridine-d_5, 500 MHz)

Position	**3**	**4**
Glc-1	4.81 d (8.0)	4.85 d (7.5)
2	4.29[a]	4.32[a]
3	4.43[a]	4.42[a]
4	3.87[a]	3.91 dd (9.0, 9.0)
5	3.97[a]	4.00[a]
6	4.61 brd (11.0)	4.62 brd (10.5)
6	4.06 dd (7.0, 11.0)	4.08 dd (6.5,10.5)
Glc′-1	5.74 d (8.0)	5.80 d (7.5)
2	4.12 dd (8.0, 9.0)	4.18 dd (7.5, 8.5)
3	3.96 dd (9.0, 9.0)	4.12[a]
4	3.84 dd (9.0, 9.0)	3.89[a]
5	3.68 m	3.79 m
6	4.31[a]	4.36 dd (2.5, 12.0)
6	4.13[a]	4.16[a]
Glc″-1	5.14 d (7.5)	5.18 d (8.0)
2	5.53 dd (7.5, 9.0)	5.55 dd (8.0, 10.0), 5.54 dd (8.0, 10.0)
3	4.41[a]	4.42[a]
4	4.12[a]	4.13[a]
5	4.12[a]	4.13[a]
6	4.53 d (11.0)	4.50[a]
6	4.27[a]	4.28[a]
Glc‴-1	5.16 d (7.5)	5.12 d (7.5)
2	3.87[a]	3.88[a]
3	5.60 dd (9.0, 9.0)	4.01[a]
4	3.98[a]	3.98[a]
5	3.98[a]	4.00[a]
6	4.46[a]	4.88 dd (2.0, 11.5)
6	4.11[a]	4.74[a]
Rha-1	5.44 s	5.44 s
2	4.54 s	4.54 d (3.0)
3	4.48 dd (3.0, 9.0)	4.48 dd (3.0, 9.0)
4	4.25 dd (9.0, 9.0)	4.25 dd (9.0, 9.0)
5	4.31[a]	4.30[a]
6	1.66 d (6.0)	1.64 d (6.5)
Rha′-1	5.94 s	5.99[a]
2	6.07 d (3.0), 6.05 d (3.0)	5.99[a]
3	5.11[a]	5.12[a]
4	5.88 dd (9.5, 9.5), 5.88 dd (9.5, 9.5)	5.89 dd (10.0, 10.0), 5.88 dd (10.0, 10.0)
5	5.20 m	5.16[a]
6	1.66 d (6.0)	1.65 d (7.0)
Agl-2	2.75 dd (7.5, 14.5)	2.75 dd (8.0, 15.0)
2	2.71[a]	2.71 dd (4.5, 15.0)
3	4.43[a]	4.44[a]
12	3.87[a]	3.88[a]
16	0.91 t (7.0)	0.91 t (7.5)
OCH_3	3.63 s	3.63 s
Exg-2	3.12 dd (6.5, 16.0), 2.90 dd (6.5, 16.0)	3.16 dd (4.5, 16.0), 2.93 dd (5.0, 16.0)
2	2.88[a], 2.69[a]	2.90 dd (9.0, 16.0), 2.67 dd (8.5, 16.0)
3	4.73 dddd (6.5, 6.5, 6.5, 6.5), 4.65[a]	4.72 m, 4.70 m
9	4.27[a], 4.01[a]	4.25[a], 4.02[a]
10	1.33 d (6.5), 1.14 d (6.5)	1.27 d (6.5), 1.15 d (6.5)
Tig-3	7.39 q (7.0)	7.42 q (7.0)
4	1.81 d (7.0)	1.82 d (7.0)
5	1.98 s	2.05 brs
Iva-2	2.70[a]	2.74[a]
2	2.53 dd (6.5, 16.0), 2.52 dd (6.5, 16.0)	2.53[a]
3	2.35[a]	2.35[a]
4	1.19 d (7.0)	1.19 d (7.0)
5	1.08 d (7.0)	1.08 d (7.0)
Iva-2	2.20[a]	2.33 d (7.5)
2	2.20[a]	2.33 d (7.5)
3	2.11[a]	2.10 m
4	0.89 d (6.5)	0.94 d (6.5)
5	0.89 d (6.5)	0.94 d (6.5)

δ in ppm from TMS. Coupling constants (*J*) in Hz are given in parentheses. *a*) Signals were overlapped with other signals.

corresponding to **3** and **4**, *etc.*

Experimental

The instruments and materials used were as cited in the preceding report[16] unless otherwise specified. ESI-TOF-MS data was collected using a Q-Exactive Plus (Thermo Fisher Scientific K.K., Kanagawa, Japan) mass spectrometer.

Treatment of the Convolvulin Fraction with Indium(III) Chloride in MeOH and Separation of 1–4 Indium(III) chloride (2.0 g) was added to a solution of the crude covolvulin fraction[9] (4.00 g) from the roots of *I. operculata* in MeOH (160 mL), and the mixture was refluxed for 25 d, while being monitored by TLC. The solvent was removed under reduced pressure, and the residue was subjected to Sephadex LH-20 colum chromatography (CC) eluted with MeOH to give fractions 1 (1189 mg), 2 (2450 mg), and 3 (19 mg). CC of fraction 2 on silica gel eluted with a gradient of mixtures of $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) afforded **1** (56 mg) and fractions 2-1–2-63. Fractions 2-16 (427 mg) and 2-43 (89 mg) were each subjected to HPLC [column, COSMOSIL 5C18-AR-II (Nacalai Tesque, Inc., Kyoto, Japan, 20 mm i.d.×250 mm)] using 85% MeOH for fractions 2-16 and 80% MeOH for fraction 2-43 as eluent to yield **4** (49 mg) and **3** (57 mg) from fraction 2-16 and **2** (13 mg) from fraction 2-43. Compounds **1** (25 mg), **2** (3 mg), **3** (25 mg), and **4** (15 mg) were each subjected to HPLC [column, COSMOSIL π-nap (Nacalai Tesque, Inc., 20 mm i.d.×250 mm)] using 75% MeOH for **2**, 80% MeOH for **1** and **4**, and 85% MeOH for **3** as eluent to yield **1a** (4 mg) and **1b** (7 mg) from **1**, **2a** (1 mg) and **2b** (1 mg) from **2**, **3a** (8 mg) and **3b** (5 mg) from **3**, and **4a** (4 mg) and **4b** (5 mg) from **4**.

The ^{1}H-NMR spectra of **1a**, **2a**, **3a**, and **4a** were superimposable on those of **1b**, **2b**, **3b**, and **4b**, respectively.

IOM-1 (**1**)

Amorphous powder. $[\alpha]_D^{26}$ –19.1° (c=1.0, MeOH). Positive-ion FAB-MS m/z: 1613 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1613.7570 (Calcd for $C_{73}H_{122}O_{37}Na^+$, 1613.7557). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

IOM-2 (**2**)

Amorphous powder. $[\alpha]_D^{19}$ –20.6° (c=1.7, MeOH). Positive-ion FAB-MS m/z: 1531 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1531.7135 (Calcd for $C_{68}H_{116}O_{36}Na^+$, 1531.7139). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

IOM-3 (**3**)

Amorphous powder. $[\alpha]_D^{26}$ –21.3° (c=1.7, MeOH). Positive-ion FAB-MS m/z: 1697 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1697.8157 (Calcd for $C_{78}H_{130}O_{38}Na^+$, 1697.8132). ^{1}H-NMR spectral data: see Table 3. ^{13}C-NMR spectral data: see Table 2.

IOM-4 (**4**)

Amorphous powder. $[\alpha]_D^{26}$ –2.2° (c=1.5, MeOH). Positive-ion FAB-MS m/z: 1697 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1697.8157 (Calcd for $C_{78}H_{130}O_{38}Na^+$, 1697.8132). ^{1}H-NMR spectral data: see Table 3. ^{13}C-NMR spectral data: see Table 2.

Alkaline Hydrolysis of 1 and 2 The alkaline hydrolysis was applied in a slightly modified manner previously reported.[9] Briefly, alkaline hydrolysis (95°C, 1 h) of **1** (15 mg) and **2** (10 mg) with 3% KOH furnished organic acid fraction and glycosidic acid (10 mg from **1**, 5 mg from **2**), respectively. Both glycosidic acids derived from **1** and **2** were identified as **5** based on their ^{1}H-NMR spectra.[9] The organic fraction was analyzed by GC[9] [t_R (min): 5.2 (isovaleric acid) for **1** and **2**, 12.1 (tiglic acid) for **1**]. A part of the organic acid fraction was methylated with diazomethane-ether and then the reaction mixture was analyzed by GC [column, OV-1 (1%), GL Sciences Inc., Tokyo, Japan, 3.2 mm i.d.×2 m glass column; column temperature, 130°C; carrier gas, N_2 1.5 kg/cm^2; t_R (min): 7.6 (methyl exigonate) for **1** and **2**].[9]

Partial Deacylation of 3 and 4 Solutions of **3** (4 mg) and **4** (3 mg) in 3% triethylamine–MeOH (1 mL) were refluxed for 2 h, respectively. After removal of the solvent, reaction mixtures were each subjected to HPLC (column, Inertsil ODS-3, GL Sciences Inc., 4.6 mm i.d.×250 mm) using 85% MeOH to give **1** (2 mg from **3**, 1 mg from **4**), which was identified by comparing of its ^{1}H-NMR spectrum with that of an authentic sample.

HPLC Analyses of 1a and b Each HPLC analysis (column, COSMOSIL π-nap, Nacalai Tesque, Inc., 4.6 mm i.d.×250 mm; solvent, 80% MeOH; flow rate 0.8 mL/min) of **1a** and **b** afforded two peaks in which the R_t values (27.6 min and 30.3 min) matched with those of **1a** and **b**, respectively.

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Conflict of Interest The authors declare no conflict of interest.

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