Components of Ether-Insoluble Resin Glycoside (Rhamnoconvolvulin) from Rhizoma Jalapae Braziliensis

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Alkaline hydrolysis of the ether-insoluble resin glycoside (convolvulin) fraction of the roots of *Ipomoea operculata* (Gomes) Mart. (Convolvulaceae) afforded a glycosidic acid named operculinic acid H along with isovaleric, tiglic, and exogonic (3,6:6,9-diepoxydecanoic) acids. Operculinic acid H was characterized to be 3*S*,12*S*-dihydroxyhexadecanoic acid 12-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-*O*-β-D-glucopyranoside on the basis of spectroscopic data and chemical evidence. The absolute configuration of exogonic acid determined from the α-methoxy-α-trifluoromethylphenylacetic acid esters of the hydrogenolysis products revealed that exogonic acid exists as a mixture (*ca.* 1 : 1) of two epimers, (3*S*,6*S*,9*R*)- and (3*S*,6*R*,9*R*)-diepoxydecanoic acids.

Key words resin glycoside; *Ipomoea operculata*; Rhizoma Jalapae Braziliensis; operculinic acid H; 3*S*,12*S*-dihydroxyhexadecanoic acid; exogonic acid

Rhizoma Jalapae Braziliensis, the root of *Ipomoea operculata* (Gomes) Mart. (Convolvulaceae), is known to be a substitute for Jalapae Tuber (Vera Curz Jalap, *I. purga*), and it is used as a laxative crude drug. It contains both ether-soluble ("jalapin") and ether-insoluble ("convolvulin") resin glycosides as the active ingredients.[1)] In our previous papers,[2—4)] we have reported the isolation and structural elucidation of seven glycosidic acids named operculinic acids A—G, which were obtained along with two organic acids—*n*-decanoic acid and *n*-dodecanoic acid—upon alkaline hydrolysis of the jalapin fraction. Further, we isolated eighteen genuine jalapins, named operculins I—XVIII.[5—7)]

Studies conducted on the convolvulin component of this crude laxative drug were inconclusive because of difficulties encountered in distinguishing between different source materials, especially that from Jalapae Tuber. Graf *et al.*[8)] carried out alkaline hydrolysis of convolvulin (named rhamnoconvolvulin, M.W. *ca.* 31000) and reported that it contained nine organic acids—acetic, propionic, *n*-pentanoic, 2-methylbutanoic, 3-methylbutanoic, 2,2-dimethylpropanoic, tiglic, 4-oxooctanoic,[9)] and exogonic (3,6:6,9-diepoxydecanoic)[10,11)] acids—as well as a glycosidic acid (rhamnoconvolvulinic acid). Votocek and Valentin[12)] proposed that rhamnoconvolvulinic acid ($C_{52}H_{92}O_{32}\cdot 7H_2O$) was possibly a hexaglycoside in which two trisaccharides that constituted with 2 mol glucose and 1 mol rhamnose bind to the OH groups of dihydroxyhexadecanoic acid, which was characterized to be 3,12-dihydroxyhexadecanoic acid by Votocek and Prelog.[13)] Later, Wagner and Kazmaier[14)] isolated a major glycosidic acid named operculinic acid from rhamnoconvolvulin and characterized it as 3,12-dihydroxyhexadecanoic acid 12-*O*-α-D-glucopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→6)]-*O*-α-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranoside. In this paper, we discuss the re-examination of the chemical composition of rhamnoconvolvulin.

Powdered roots of *I. operculata* were extracted with MeOH, and the extract was partitioned between ether and H_2O. Then, the H_2O-soluble fraction was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction was chromatographed over MCI gel CHP20 to afford a convolvulin fraction. Alkaline hydrolysis of the fraction with 5% *aq*. KOH afforded organic acid and glycosidic acid fractions. Then, organic acid was esterified with *p*-bromophenacyl bromide, and the product was subjected to chromatographic separation to afford *p*-bromophenacyl isovalerate (**1**), *p*-bromophenacyl tiglate (**2**), and two compounds **3** and **4** (Fig. 1). The field desorption mass spectrum (FD-MS) of **3** showed an $[M]^+$ ion peak at *m/z* 396, and the ^{1}H-NMR spectrum of **3** showed signals due to two oxygenated methine protons, one secondary methyl group, and one nonequivalent methylene group. The ^{13}C-NMR spectrum of **3** showed ten carbon signals, including signals due to one carbonyl carbon, one acetal carbon, and two oxygenated methine carbons, besides signals due to the *p*-bromophenacyl group. These NMR signals were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{13}C heteronuclear shift-correlated 2D-NMR (HETCOR) spectra. From the above mentioned data, **3** was identified as the *p*-bromophenacyl ester of the exogonic acid reported by Graf and Dahlke.[11)] The ^{1}H- and ^{13}C-NMR spec-

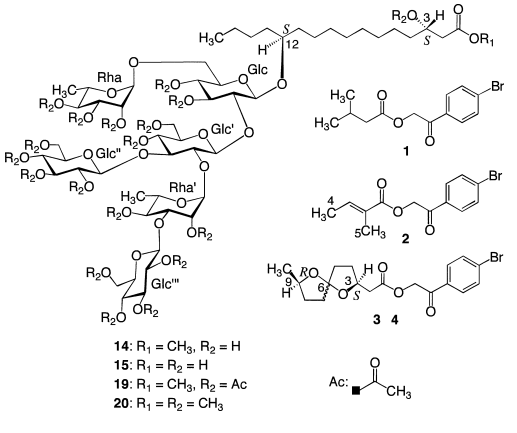

Fig. 1. Structures of **1**—**4**, **14**, **15**, **19**, and **20**

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Table 1. ^{1}H-NMR Spectral Data for **8**—**13** (in $CDCl_3$, 600 MHz)

	8	**9**	**10**
2a	2.627 dd (4.4, 15.7)	2.599 dd (4.6, 16.1)	2.410 dd (6.6, 14.9)
2b	2.706 dd (8.3, 15.7)	2.661 dd (8.2, 16.1)	2.559 dd (6.6, 14.9)
3	5.532 dddd (4.4, 5.4, 6.6, 8.3)	5.543 dddd (4.6, 5.2, 7.0, 8.2)	4.201 dddd (6.6, 6.6, 6.6, 6.6)
6	3.736 dddd (5.4, 6.6, 6.6, 6.6)	3.806 dddd (5.3, 6.9, 7.1, 7.7)	3.774 dddd (6.6, 6.6, 6.6, 6.6)
9	3.907 ddq (6.0, 7.1, 6.0)	3.934 ddq (5.2, 7.5, 6.2)	5.183 ddq (6.0, 7.2, 6.5)
10	1.178 d (6.0)	1.212 d (6.2)	1.341 d (6.5)
$COOCH_3$	3.656 s	3.583 s	3.677 s
OCH_3	3.538 q (0.8)	3.530 q (0.9)	3.560 q (1.2)

	11	**12**	**13**
2a	2.454 dd (6.6, 15.6)	2.584 dd (4.6, 16.1)	2.565 dd (5.0, 16.1)
2b	2.595 dd (6.6, 15.6)	2.684 dd (8.1, 16.1)	2.645 dd (8.1, 16.1)
3	4.236 dddd (6.6, 6.6, 6.6, 6.6)	5.445 m	5.466 m
6	3.839 dddd (6.6, 6.6, 6.6, 6.6)		
9	5.170 ddq (4.8, 7.2, 6.2)	5.117 ddq (4.8, 7.8, 6.5)	5.121 ddq (6.2, 6.6, 6.2)
10	1.262 d (6.2)	1.322 d (6.5)	1.250 d (6.2)
$COOCH_3$	3.683 s	3.665 s	3.591 s
OCH_3	3.544 q (1.3)	3.534 q (1.2)	3.512 q (1.3)
OCH_3		3.560 q (1.2)	3.532 q (0.9)

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses).

tra of **4** were very similar to those of **3**. Further, on refluxing in $CHCl_3$ for 8 h, **3** was transformed to **4** to afford an equilibrium mixture in which the ratio of **3** : **4** was *ca.* 1 : 1. Consequently, it was suggested that **3** and **4** are isomers and that interconversion between them occurred because of inversion at the C-6 spiro center.

In order to determine the configurations at C-3 and C-9 of exogonic acid, we performed the following experiments. The methyl ester of exogonic acid was hydrogenated and then purified by HPLC to afford three compounds, **5**, **6**, and **7**. The ^{1}H-NMR spectra of both **5** and **6** showed signal due to one of each of the following groups: a methoxyl group, secondary methyl group, and a nonequivalent methylene group; the spectra also showed signals due to three oxygenated methine protons. Further, the ^{13}C-NMR spectra of both **5** and **6** showed eleven signals, including signals due to one carbonyl carbon, one methoxyl carbon, and three oxygenated methine carbons. On the basis of these data, it was proposed that **5** and **6** were either methyl 3-hydroxy-6,9-epoxydecanoate or methyl 9-hydroxy-3,6-epoxydecanoate, respectively. The ^{1}H-NMR spectra of both (−)- and (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)[15] esters (**8** and **9**, respectively) of **5** showed, when compared with that of **5**, the remarkable downfield shift of the signal due to H-3 and additional signals due to 1 mol of the MTPA residue, thereby indicating that **5** is methyl 3-hydroxy-6,9-epoxydecanoate (Table 1). Comparison of the ^{1}H-NMR spectrum of **8** with that of **9** revealed significant difference in chemical shift [$\Delta\delta$ (δ**8**—δ**9**)]: +0.073 ($CO_2C\underline{H}_3$), +0.028 (Ha-2), +0.045 (Hb-2), −0.070 (H-6), −0.027 (H-9), and −0.034 (H_3-10) ppm (Fig. 2). Therefore, the configuration at C-3 of **5** was determined to be *S*. In contrast, the ^{1}H-NMR spectra of (−)- and (+)-MTPA esters (**10** and **11**, respectively) of **6** showed that **6** is methyl 9-hydroxy-3,6-epoxydecanoate, and further, the $\Delta\delta$ (δ**10**—δ**11**) values indicated that the configuration at C-9 in **6** is *R*. The ^{1}H-NMR spectrum of **7** showed signals assignable to one methoxyl group, one nonequivalent methylene group, two oxygenated methine protons, and one secondary methyl group. Further, the ^{13}C-NMR spectrum of **7** showed eleven signals, including signals due to one carbonyl carbon, one methoxyl carbon, and two oxygenated methine carbons. Thus, **7** was determined to be methyl 3,9-dihydroxydecanoate. The spectra of both the (−)-MTPA ester (**12**) and the (+)-MTPA ester (**13**) of **7** showed signals due to 2 mol of MTPA residues, and a comparison of their ^{1}H-NMR spectra revealed the following $\Delta\delta$ (δ**12**—δ**13**) values: +0.074 ($CO_2C\underline{H}_3$), +0.019 (Ha-2), +0.039 (Hb-2), and +0.072 (H_3-10) ppm. Therefore, exogonic acid was identified to be (3*S*,9*R*)-3,6:6,9-diepoxydecanoic acid, and it was found that this acid existed as a mixture of epimers that differ in the spiro-cyclic carbon configuration only at C-6. These results were consistent with those of the enantioselective syntheses performed by Lawson *et al.*[16]

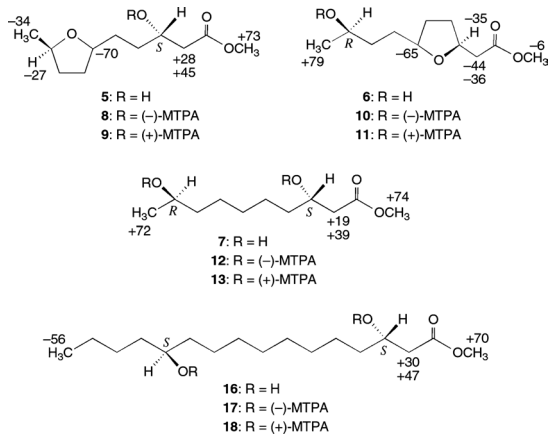

Fig. 2. Structures of **5**—**13** and **16**—**18**, and Values of ^{1}H-NMR Chemical Shift Difference (ppm) [$\Delta\delta$: δ(−)-MTPA–δ(+)-MTPA Ester ($\times 10^{-3}$)] for MTPA Esters (in $CDCl_3$, 600 MHz)

The glycosidic acid fraction was treated with diazomethane and subsequently subjected to chromatography to

Table 2. ^{1}H-NMR Spectral Data for **17** and **18** (in $CDCl_3$, 400 MHz)

	17	**18**
2a	2.601 dd (4.9, 15.9)	2.571 dd (4.9, 15.9)
2b	2.694 dd (8.0, 15.9)	2.647 dd (7.9, 15.9)
3	5.469 ddt (4.9, 8.0, 6.5)	5.470 ddt (4.9, 7.9, 6.3)
12	5.084 tt (5.3, 6.5)	5.082 tt (5.2, 6.7)
16	0.827 t (7.1)	0.883 t (6.9)
$COOCH_3$	3.659 s	3.589 s
OCH_3	3.539 q (0.9)	3.525 q (1.2)
OCH_3	3.557 q (1.2)	3.555 q (1.2)

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses).

isolate the methyl ester (**14**), alkaline hydrolysis of which afforded a glycosidic acid (**15**), which was named operculinic acid H. Acidic hydrolysis of **14** afforded an aglycone and a mixture of monosaccharides. Methylation of the aglycone with diazomethane yielded a methyl ester of a hydroxyfatty acid (**16**). The ^{1}H-NMR spectrum of **16** showed signals due to two oxygenated methine protons, one ester methyl group, and one primary methyl group. The FD-MS of **16** showed an $[M+H]^+$ ion peak at *m/z* 303 and fragment ion peaks at *m/z* 245 $[M-CH_3(CH_2)_2]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$, and 87 $[CH_3(CH_2)_3CHOH]^+$. These data were consistent with those estimated for methyl 3,12-dihydroxyhexadecanoate reported by Votocek and Prelog.[13] Compound **16** was completely acylated using (−)- and (+)-MTPA chloride to afford **17** and **18**, respectively. From a comparison of the ^{1}H-NMR spectra of **17** and **18**, the $\Delta\delta$ (δ**17**—δ**18**) values were found to be +0.070, +0.047, +0.030, and −0.056 ppm for signals due to the methoxyl group, Ha-2, Hb-2, and H_3-16, respectively (Table 2, Fig. 2). This suggests that the configurations at both C-3 and C-12 are *S*,[17] which is in agreement with the results obtained by Jakob and Gerlach.[18] On the other hand, HPLC separation of the monosaccharide fraction yielded D-glucose and L-rhamnose. Further, the negative FAB-MS spectrum of **15** showed an $[M-H]^-$ ion peak at *m/z* 1227 along with fragment ion peaks at *m/z* 1065 [1227−162 ($C_6H_{10}O_5$, hexose unit)]$^-$, 919 [1065−146 ($C_6H_{10}O_4$, 6-deoxyhexose unit)]$^-$, 757 [919−146]$^-$, and 595 [903−162]$^-$. These data indicated that **15** is composed of 1 mol of 3*S*,12*S*-dihydroxyhexadecanoic acid, 2 mol of L-rhamnose, and 4 mol of D-glucose. This composition is consistent with that of operuclinic acid reported by Wagner *et al.*[14]

The ^{1}H-NMR spectrum of **14** showed signals due to six anomeric protons and two secondary methyl groups, and these signals were assignable to the H_3-6 of the rhamnose units; this spectrum also showed signals due to one nonequivalent methylene group, one primary methyl group, and one methoxyl group ascribable to the aglycone moiety (Ag). The proton signals due to the sugar moiety of **14** were assigned by referring to the COSY and nuclear Overhauser and exchange spectroscopy (NOESY) spectra (Table 3). The coupling constants of the signals due to the anomeric and methine protons as well as the $^1J_{C-H}$ values due to the anomeric carbons indicated that all the monosaccharide units were of the pyranose type and that the mode of glycosidic linkage in glucose units was β in $^4C_1$ conformation and that in rhamnose units was α in $^1C_4$ conformation.

The ^{13}C-NMR signals due to the sugar moiety of **14** were assigned by HETCOR and then compared with those of

Table 3. ^{1}H-NMR Spectral Data for **14** and **19** (in Pyridine-d_5, 600 MHz)

	14	**19**
Glc-1	4.93 d (7.7)	4.80 d (7.3)
2	4.36 dd (7.7, 9.2)	4.23 dd (7.3, 9.5)
3	4.55 dd (9.2, 9.2)	5.71 dd (9.5, 9.5)
4	3.87 dd (9.2, 9.2)	5.29 dd (9.5, 9.5)
5	*ca.* 4.01	*ca.* 4.03
6	*ca.* 4.03	3.87 dd (6.7, 12.2)
	4.56 dd (4.3, 11.0)	*ca.* 4.03
Glc′-1	5.79 d (7.7)	5.00 d (7.6)
2	4.22 dd (7.7, 9.2)	4.08 dd (7.6, 9.5)
3	*ca.* 3.93	4.50 dd (9.5, 9.5)
4	*ca.* 3.93	5.25 dd (9.5, 9.5)
5	3.75 ddd (2.0, 6.1, 9.2)	*ca.* 4.05
6	4.14 dd (6.1, 11.6)	4.34 dd (2.5, 12.8)
	4.34 dd (2.0, 11.6)	4.58 dd (4.6, 12.8)
Glc″-1	4.93 d (7.8)	*ca.* 5.20
2	3.99 dd (7.8, 9.2)	*ca.* 5.20
3	4.21 dd (9.2, 9.2)	*ca.* 5.77
4	4.07 dd (9.2, 9.2)	5.38 dd (10.0, 10.0)
5	4.02 dd (2.2, 6.1, 9.2)	*ca.* 4.17
6	4.21 dd (6.1, 11.6)	4.14 dd (2.2, 13.5)
	4.53 dd (2.2, 11.6)	*ca.* 4.64
Glc‴-1	5.40 d (7.7)	*ca.* 5.40
2	4.07 dd (7.7, 9.4)	*ca.* 5.40
3	4.15 dd (9.4, 9.4)	*ca.* 5.67
4	4.04 dd (9.4, 9.4)	5.50 dd (9.5, 9.5)
5	3.94 ddd (2.4, 6.1, 9.4)	4.20 ddd (2.5, 4.0, 9.5)
6	4.12 dd (6.1, 12.2)	4.42 dd (4.0, 12.5)
	4.42 dd (2.4, 12.2)	4.60 dd (2.5, 12.5)
Rha-1	5.43 d (1.4)	5.16 s
2	4.55 dd (1.4, 3.1)	5.70 d (3.4)
3	4.49 dd (3.1, 9.6)	5.64 dd (3.4, 10.0)
4	4.24 dd (9.6, 9.6)	5.49 dd (10.0, 10.0)
5	4.31 dq (9.6, 6.1)	4.12 dq (10.0, 6.4)
6	1.62 d (6.1)	1.32 d (6.4)
Rha′-1	6.31 d (1.4)	5.70 s
2	5.10 dd (1.4, 3.1)	5.85 d (4.0)
3	4.83 dd (3.1, 9.0)	4.74 dd (4.0, 9.8)
4	4.49 dd (9.0, 9.0)	5.51 dd (9.8, 9.8)
5	5.08 dq (9.0, 6.2)	*ca.* 4.68
6	1.76 d (6.2)	1.53 d (6.1)
Ag-2	2.68 dd (4.5, 14.5)	2.73 dd (5.8, 15.8)
	2.73 dd (7.9, 14.5)	2.77 dd (7.0, 15.8)
3	*ca.* 4.41	*ca.* 5.50
12	*ca.* 3.89	*ca.* 3.83
16	0.89 t (7.3)	0.97 t (7.3)
OCH_3	3.63 s	3.62 s

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl; Rha, rhamnopyranosyl; Ag, aglycone moiety.

methyl pyranosides in the literature[19] (Table 4). Glycosylation shifts[20,21] were observed at C-2 and C-6 of the first glucose residue (Glc) (+4.2 and +5.6 ppm), C-2 and C-3 of the second glucose residue (Glc′) (+2.5 and +11.4 ppm), C-3 of the second rhamnose residue (Rha′) (+10.4 ppm), and C-12 of Ag (+10.4 ppm). Moreover, comparison of the chemical shifts of the signals due to **14** with those of the signals due to its peracetate (**19**) revealed that the signals due to H-2 and H_2-6 of Glc, H-2 of Glc′, H-3 of Rha′, and H-12 of Ag exhibited no acylation shift (Table 3). These data suggested that the sugar linkages in **14** were formed at OH-2 and OH-6 of Glc, OH-2 and OH-3 of Glc′, OH-3 of Rha′, and OH-12 of Ag. The NOESY spectrum of **19** showed five cross peaks, which were identified as those between H-1 of Glc and H-12 of Ag, H-1 of Glc′ and H-2 of Glc, H-1 of Rha and H-5 and/or H-6 of Glc, H-1 and/or H-2 of Glc″ and H-3 of Glc′,

Table 4. ^{13}C-NMR Spectral Data for **14** and **21**—**25** (in Pyridine-d_5)

	14	**21**	**22**	**23**	**24**	**25**	Methyl glycoside[19]
Glc-1	102.6	103.8	101.8	101.9	102.6	102.4	105.5
2	79.1	75.3	83.6	83.3	79.2	79.7	74.9
3	79.4	78.6	78.2	78.2	79.3	79.3	78.3
4	72.1	72.0	71.6	71.6	72.1	72.0	71.6
5	76.5	76.9	78.0	76.5	76.4	77.8	78.3
6	68.3	68.7	62.8	68.3	68.4	62.8	62.7
Glc′-1	101.8		105.4	105.2	101.8	101.9	
2	77.4		75.5	75.3	77.8	77.3	
3	89.7		87.9	88.0	89.3	89.5	
4	70.3		69.9	69.9	70.1	70.4	
5	77.5		78.0	77.9	77.4	77.2	
6	62.9		62.6	62.6	62.9	63.1	
Glc″-1	104.7		105.7	105.6	104.5	104.6	
2	74.9		75.6	75.5	74.9	74.9	
3	78.5		78.2	78.2	78.6	78.5	
4	71.5		71.6	71.6	71.5	71.9	
5	78.6		78.6	78.5	78.6	78.5	
6	62.4		62.5	62.6	62.4	62.4	
Glc‴-1	106.1					106.1	
2	75.9					75.9	
3	78.2					78.2	
4	71.1					71.5	
5	78.2					78.1	
6	62.8					62.9	
Rha-1	102.5	102.6		102.4	102.5		102.6
2	72.3	72.3		72.2	72.3		72.1
3	72.7	72.8		72.7	72.7		72.7
4	74.1	74.0		74.0	74.0		73.8
5	69.8	69.7		69.7	69.7		69.5
6	18.7	18.7		18.6	18.7		18.6
Rha′-1	102.0				102.1	101.9	
2	71.7				72.3	71.7	
3	83.1				72.6	83.1	
4	73.2				74.3	73.2	
5	69.3				69.6	69.4	
6	19.0				19.0	18.9	
Ag-1	172.8	172.8	172.9	172.8	172.9	172.8	
2	43.4	43.4	43.4	43.4	43.4	43.4	
3	68.3	68.2	68.2	68.2	68.2	68.3	
12	81.3	79.5	79.7	80.2	81.1	80.8	
16	14.4	14.4	14.3	14.3	14.4	14.2	
OCH_3	51.3	51.3	51.3	51.3	51.3	51.2	

δ in ppm from TMS. **14** at 125 MHz and **21**—**25** at 100 MHz. Glc, glucopyranosyl; Rha, rhamnopyranosyl; Ag, aglycone moiety.

and H-1 and/or H-2 of Glc‴ and H-3 of Rha′. Moreover, the high-resolution electron impact ionization mass spectrum (HI-EI-MS) of the permethylate (**20**) of **14** showed fragment ion peaks at *m*/*z* 299.2590, 393.2127, 565.2848, and 801.4121, which were assigned to the fragment ions denoted by *a*, *b*, *c,* and *d*, respectively (Fig. 3). The structure of **15** determined on the basis of these facts is shown in Fig. 1.

Despite the large difference between the structures of the sugar moieties in **15** and Wagner's operuclinic acid,[14] both compounds were regarded to be identical. This is because they were most glycosidic acid of convolvulin fraction and their physical properties (mp 156—163 °C, $[\alpha]_D^{26}$ −49.1° for **15**; mp 182—185 °C, $[\alpha]_D^{24}$ −48.6° for operuclinic acid[14]) were similar. To confirm the structure of **15** in further detail, partial methanolysis of **14** was attempted. Compound **14** was refluxed with 0.3% HCl–MeOH for 100 min to obtain five hydrolysates **21**—**25**. The structures (Fig. 4) of **21**—**25** were determined on the basis of their COSY, NOESY, and HETCOR spectra (Tables 4, 5). The structures of **21**—**25** validated the structure of **15** shown in Fig. 1. Therefore, the structure of operculinic acid H (**15**) was characterized to be 3*S*,12*S*-dihydroxyhexadecanoic acid 12-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside.

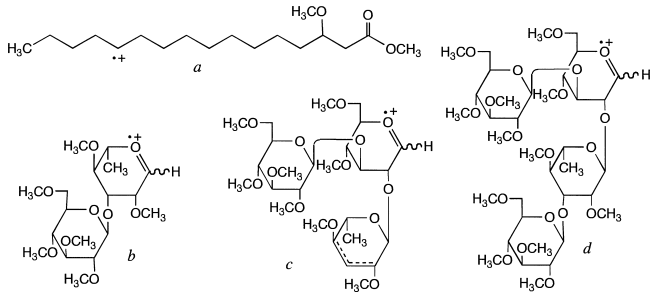

Fig. 3. Fragment Ions Observed in the EI-MS Spectrum of **20**

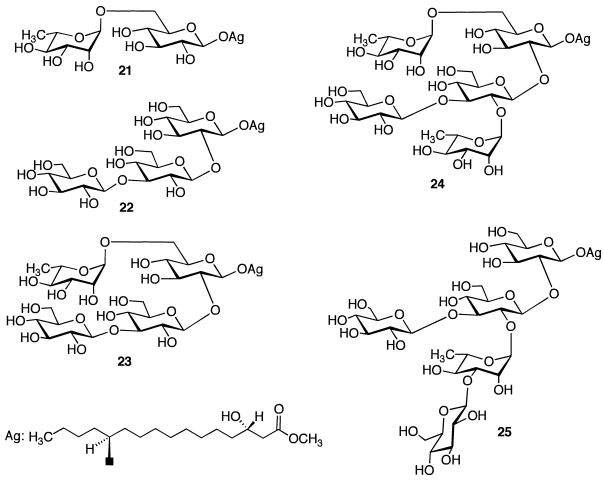

Fig. 4. Structures of the Products (**21**—**25**) Obtained by Partial Methanolysis of **14**

In this study, it was found that the components of rhamnoconvolvulin are isovaleric, tiglic, and exogonic acids as well as **15**. Further, the absolute configuration of exogonic acid was determined by using the MTPA esters of its hydrogenolysis products, and exogonic acid was confirmed to exist as a mixture of two epimers, (3*S*,6*S*,9*R*)- and (3*S*,6*R*,9*R*)-diepoxydecanoic acids. Moreover, the proposed arrangement of monosaccharides in the sugar moiety of Wagner's operculinic acid should be revised as shown in Fig. 1. Compound **15** has a branched-chain hexasaccharide moiety bound to the OH-12 of aglycone 3*S*,12*S*-dihydroxyhexadecanoic acid, and as in the case of pharbitic acids,[22] OH-3 remains free. On the basis of its behavior towards alkali and acid, rhamnoconvolvulin is presumed to be a complex glycoside composed of a number of repeating units which are operculinic acid H (**15**) partially acylated by isovaleric, tiglic, and exogonic acids at the sugar moiety.

Experimental

The instruments and materials used were as cited in the preceding report[3,23] unless otherwise specified.

Preparation of Convolvulin Fraction The *n*-BuOH fraction (15.84 g) obtained previously[3] from the roots of *I. operculata* was subjected to MCI

Table 5. ^{1}H-NMR Spectral Data for **21**—**25** (in Pyridine-d_5, 400 MHz)

	21	**22**	**23**	**24**	**25**
Glc-1	4.88 d (8.0)	4.94 d (7.5)	4.85 d (7.6)	4.93 d (7.3)	4.97 d (7.6)
2	4.00 dd (8.0, 9.0)	4.13 dd (7.5, 8.5)	4.07 dd (7.6, 8.5)	4.33 dd (7.3, 9.5)	4.28 dd (7.6, 9.0)
3	4.22 dd (9.0, 9.0)	4.29 dd (8.5, 8.5)	4.21 dd (8.5, 8.5)	4.48 dd (9.5, 9.5)	4.52 dd (9.0, 9.0)
4	4.00 dd (9.0, 9.0)	4.16 dd (8.5, 8.5)	*ca.* 3.91	3.88 dd (9.5, 9.5)	4.07 dd (9.0, 9.0)
5	4.07 ddd (1.0, 6.7, 9.0)	3.87 ddd (3.0, 5.5, 8.5)	*ca.* 3.91	*ca.* 3.97	*ca.* 3.89
6	4.14 dd (6.7, 11.0)	4.32 dd (5.5, 11.5)	4.05 dd (5.0, 11.5)	*ca.* 4.05	4.27 dd (5.0, 11.5)
	4.68 dd (1.0, 11.0)	4.48 dd (3.0, 11.5)	4.56 d (11.5)	4.59 dd (1.0, 11.0)	*ca.* 4.40
Glc′-1		5.31 d (7.5)	5.27 d (7.6)	5.80 d (7.3)	5.71 d (7.6)
2		4.09 dd (7.5, 8.5)	4.06 dd (7.6, 8.5)	*ca.* 4.25	*ca.* 4.20
3		4.20 dd (8.5, 8.5)	4.13 dd (8.5, 8.5)	4.07 dd (9.0, 9.0)	*ca.* 3.94
4		4.15 dd (8.5, 8.5)	4.09 dd (8.5, 8.5)	*ca.* 4.03	*ca.* 3.94
5		3.85 ddd (3.5, 5.5, 8.5)	3.83 ddd (3.0, 6.0, 8.5)	3.79 m	3.72 m
6		4.30 dd (5.5, 11.5)	4.25 dd (6.0, 11.5)	4.18 dd (5.5, 11.0)	*ca.* 4.13
		4.42 dd (3.5, 11.5)	*ca.* 4.38	*ca.* 4.34	4.32 dd (2.0, 11.8)
Glc″-1		5.25 d (7.5)	5.20 d (7.9)	5.00 d (7.6)	4.92 d (7.9)
2		4.04 dd (7.5, 8.5)	4.00 dd (7.9, 8.5)	4.00 dd (7.6, 8.5)	3.97 dd (7.9, 8.5)
3		4.21 dd (8.5, 8.5)	4.15 dd (8.5, 8.5)	4.14 dd (8.5, 8.5)	*ca.* 4.13
4		4.15 dd (8.5, 8.5)	4.10 dd (8.5, 8.5)	4.10 dd (8.5, 8.5)	4.02 dd (8.5, 8.5)
5		4.00 ddd (2.0, 6.0, 8.5)	3.95 ddd (2.6, 6.2, 8.5)	*ca.* 4.00	*ca.* 3.98
6		4.28 dd (6.0, 11.5)	4.24 dd (6.2, 11.5)	*ca.* 4.25	*ca.* 4.20
		4.51 dd (2.0, 11.5)	4.47 dd (2.6, 11.5)	*ca.* 4.53	4.50 dd (2.7, 12.0)
Glc‴-1					5.39 d (7.6)
2					4.04 dd (7.6, 9.0)
3					4.15 dd (9.0, 9.0)
4					4.04 dd (9.0, 9.0)
5					*ca.* 3.95
6					4.11 dd (4.8, 11.0)
					ca. 4.40
Rha-1	5.51 d (1.5)		5.41 d (1.5)	5.44 d (1.0)	
2	4.60 dd (1.5, 3.4)		4.51 dd (1.5, 3.0)	4.54 dd (1.0, 3.0)	
3	4.52 dd (3.4, 9.5)		4.45 dd (3.0, 8.5)	4.49 dd (3.0, 9.0)	
4	4.25 dd (9.5, 9.5)		4.19 dd (8.5, 8.5)	4.23 dd (9.0, 9.0)	
5	4.35 dq (9.5, 6.1)		4.28 dq (8.5, 5.8)	*ca.* 4.33	
6	1.64 d (6.1)		1.61 d (5.8)	1.63 d (6.1)	
Rha′-1				6.30 d (1.5)	6.28 s
2				4.81 dd (1.5, 3.5)	5.03 d (3.5)
3				4.61 dd (3.5, 9.0)	4.79 dd (3.5, 9.5)
4				4.26 dd (9.0, 9.0)	4.45 dd (9.5, 9.5)
5				*ca.* 4.96	5.01 dq (9.5, 6.1)
6				1.78 d (6.4)	1.73 d (6.1)
Ag-2	2.68 dd (4.6, 14.7)	2.69 dd (5.0, 15.0)	2.67 dd (5.0, 14.5)	2.68 dd (5.0, 15.0)	2.67 dd (5.0, 14.5)
	2.75 dd (8.2, 14.7)	2.75 dd (8.0, 15.0)	2.73 dd (8.0, 14.5)	2.73 dd (8.5, 15.0)	2.72 dd (8.5, 14.5)
3	4.42 ddt (4.6, 8.2, 7.0)	*ca.* 4.41	*ca.* 4.39	4.41 m	*ca.* 4.39
12	3.98 m	3.93 tt (5.5, 5.5)	*ca.* 3.89	3.93 m	*ca.* 3.92
16	0.93 t (7.3)	0.86 t (7.3)	0.92 t (7.2)	0.91 t (7.3)	0.84 t (7.0)
OCH_3	3.64 s	3.64 s	3.64 s	3.62 s	3.62 s

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl; Rha, rhamnopyranosyl; Ag, aglycone moiety.

gel CHP20 column chromatography (cc) (70% MeOH→95% MeOH→acetone) to afford fr. 1 (4.60 g), fr. 2 (7.34 g, brown powder, mp 148—155 °C, convolvulin fraction), and fr. 3 (0.20 g).

Alkaline Hydrolysis of Convolvulin Fraction Fraction 2 (7.10 g) in 5% KOH (100 ml) was heated at 95 °C for 3 h. After cooling, the pH of the reaction mixture was adjusted to 4 with 1 M HCl, and the mixture was shaken with ether (30 ml×3). The ether layer was washed with H_2O (50 ml), dried over $MgSO_4$, and evaporated under reduced pressure to afford an oil (988 mg, organic acid fraction). The H_2O layer was loaded onto an MCI gel CHP20 column and eluted first with H_2O and then with acetone. The acetone eluate was evaporated to dryness to afford a yellow powder (5.8 g, glycosidic acid fraction).

A small portion of the organic acid fraction was analyzed by GC [column: 3.2 mm i.d.×2.0 m glass column packed with Unisole 30T (10%); carrier gas: N_2 (2.0 kg/cm^2); column temperature: 120 °C] and was found to contain isovaleric acid (t_R 5.1 min) and tiglic acid (t_R 2.0 min).

Identification of Organic Acids The organic acid fraction (980 mg) in dry acetone (50 ml) was neutralized with triethylamine. Then, *p*-bromophenacylbromide (600 mg) was added; the mixture was left to stand at room temperature for 5 h and then concentrated under reduced pressure. The residue was fractionated between H_2O (40 ml) and ether (20 ml). Chromatographic separation of the ether-soluble fraction on a silica gel column [*n*-hexane–ethyl acetate (AcOEt) (2 : 1)] afforded fr. 4 (730 mg) and fr. 5 (192 mg). Fraction 4 was subjected to silica gel cc [*n*-hexane–AcOEt (19 : 1)] to obtain fr. 6 (448 mg) and fr. 7 (282 mg). HPLC separation of fr. 7 on a Kusano CIG Si column [22 mm i.d.×100 mm; solvent: *n*-hexane–AcOEt (60 : 1)] afforded **1** (140 mg) and **2** (202 mg). HPLC separation of fr. 5 on a Kusano CIG Si column [22 mm i.d.×300 mm; solvent: *n*-hexane–AcOEt (5 : 2)] afforded **3** (109 mg) and **4** (62 mg). Compounds **3** and **4** were refluxed separately in $CHCl_3$ for 8 h, and then the solvent was evaporated. The HPLC chromatogram [column: Kusano CIG Si, 22 mm i.d.×100 mm; solvent: *n*-hexane–AcOEt (5 : 1); flow rate: 3.0 ml/min] of both the residues showed two peaks in the ratio of *ca*. 1 : 1, and the t_R values of the two residues, 61 and 68 min, were equal to those of **3** and **4**, respectively.

1: Colorless needles (*n*-hexane–AcOEt), mp 67—68 °C. ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 1.02 (6H, d, J=6.4 Hz, H_3-4, 4′), 2.18 (1H, tqq, J=7.0, 6.4, 6.4 Hz, H-3), 2.36 (2H, d, J=7.0 Hz, H_2-2), 5.28 (2H, s, O–CH_2–CO), 7.63 (2H, ddd, J=1.7, 1.7, 8.6 Hz, arom. H), 7.77 (2H, ddd, J=1.7, 1.7, 8.6 Hz, arom. H).

2: Colorless needles (*n*-hexane–AcOEt), mp 68—69 °C. ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 1.84 (3H, dd, J=0.9, 7.3 Hz, H_3-4), 1.90 (3H, dd, J=0.9, 1.5 Hz, H_3-5), 5.33 (2H, s, OCH_2CO), 7.02 (1H, dq, J=7.3, 1.5 Hz, H-3), 7.63 (2H, ddd, J=1.5, 1.5, 8.3 Hz, arom. H), 7.79 (2H, ddd, J=1.5, 1.5, 8.3 Hz, arom. H).

3: Colorless oil. FD-MS *m/z* (%): 399 (59) $[M+3]^+$, 398 (95) $[M+2]^+$, 397 (67) $[M+1]^+$, 396 (100) $[M]^+$. ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 1.21 (3H, d, J=6.1 Hz, H_3-10), 1.44 (1H, m, Ha-8), 1.70 (1H, m, Ha-4), *ca.* 2.04 (2H, H_2-5), *ca.* 2.04 (2H, H_2-7), 2.10 (1H, m, Hb-8), 2.31 (1H, m, Hb-4), 2.62 (1H, dd, J=7.3, 15.3 Hz, Ha-2), 2.79 (1H, dd, J=6.1, 15.3 Hz, Hb-2), 4.19 (1H, ddq, J=6.0, 8.0, 6.1 Hz, H-9), 4.52 (1H, dddd, J=6.1, 7.0, 7.0, 7.3 Hz, H-3), 5.26 (1H, d, J=16.5 Hz, OCHa), 5.31 (1H, d, J=16.5 Hz, OCHb), 7.63 (2H, ddd, J=2.5, 2.5, 9.0 Hz, arom. H), 7.77 (2H, ddd, J=2.5, 2.5, 9.0 Hz, arom. H). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 21.1 (C-10), 30.2 (C-4), 32.1 (C-8), 35.1 (C-5 or C-7), 35.5 (C-5 or C-7), 40.3 (C-2), 65.8 (OCH_2), 74.0 (C-3), 74.3 (C-9), 115.1 (C-6), 129.1 (arom. C), 129.3 (arom. C), 132.2 (arom. C), 133.0 (arom. C), 170.4 (C-1), 191.2 ($OCH_2\underline{C}O$).

4: Colorless oil. FD-MS *m/z* (%): 399 (79) $[M+3]^+$, 398 (88) $[M+2]^+$, 397 (100) $[M+1]^+$, 396 (60) $[M]^+$. ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 1.29 (3H, d, J=6.3 Hz, H_3-10), 1.73 (1H, m, Ha-8), 1.90 (1H, m, Ha-4), 2.00 (1H, m, Hb-8), *ca.* 2.05 (2H, H_2-5), *ca.* 2.05 (2H, H_2-7), 2.22 (1H, m, Hb-4), 2.74 (1H, dd, J=6.7, 15.6 Hz, Ha-2), 2.94 (1H, dd, J=7.0, 15.6 Hz, Hb-2), 4.13 (1H, ddq, J=6.0, 8.5, 6.3 Hz, H-9), 4.48 (1H, dddd, J=6.7, 7.0, 7.0, 7.0 Hz, H-3), 5.26 (1H, d, J=16.0 Hz, OCHa), 5.31 (1H, d, J=16.0 Hz, OCHb), 7.63 (2H, ddd, J=1.5, 1.5, 8.5 Hz, arom. H), 7.77 (2H, ddd, J=1.5, 1.5, 8.5 Hz, arom. H). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 22.9 (C-10), 30.6 (C-4), 32.5 (C-8), 35.6 (C-5 or C-7), 36.0 (C-5 or C-7), 42.2 (C-2), 65.6 (OCH_2), 75.3 (C-3), 76.1 (C-9), 114.8 (C-6), 129.0 (arom. C), 129.2 (arom. C), 132.1 (arom. C), 133.0 (arom. C), 170.7 (1-C), 191.2 ($OCH_2\underline{C}O$).

Hydrogenolysis of Methyl Exogonate The organic acid fraction (1.75 g) was methylated using a diazomethane-ether solution. The solvent was removed under reduced pressure, and the residue was chromatographed over silica gel [*n*-hexane–AcOEt (10 : 1→7 : 1→5 : 1)] to afford a colorless oil (methyl exogonate, 310 mg). PtO_2 (50 mg) was added to a solution of the oil in acetic acid (5 ml), and the mixture was stirred in a hydrogen atmosphere (pressure: 1 atm) for 1 h. After filtration, the filtrate was concentrated under reduced pressure, and the residue was subjected to HPLC [column: Kusano CIG Si, 22 mm i.d.×300 mm; solvent: *n*-hexane–AcOEt (2 : 1)] to afford **5** (52 mg), **6** (3 mg), and **7** (2 mg).

5: Colorless oil, ^{1}H-NMR (in $CDCl_3$, 600 MHz) δ: 1.23 (3H, d, J=6.1 Hz, H_3-10), 2.46 (1H, dd, J=7.8, 15.7 Hz, Ha-2), 2.49 (1H, dd, J=4.0, 15.7 Hz, Hb-2), 3.70 (3H, s, $COOCH_3$), 3.83 (1H, dddd, J=4.8, 5.0, 7.8, 7.8 Hz, H-6), 3.97 (1H, ddq, J=6.1, 7.3, 6.1 Hz, H-9), 4.05 (1H, dddd, J=4.0, 4.0, 7.2, 7.8 Hz, H-3). ^{13}C-NMR (in $CDCl_3$, 150 MHz) δ: 21.4, 31.4, 32.7, 32.8, 33.9, 41.7, 51.7 ($COO\underline{C}H_3$), 68.3 (C-O), 75.6 (C-O), 79.5 (C-O), 173.1 ($\underline{C}OOCH_3$).

6: Colorless oil, ^{1}H-NMR (in $CDCl_3$, 600 MHz) δ: 1.18 (3H, d, J=6.0 Hz, H_3-10), 2.48 (1H, dd, J=6.6, 15.3 Hz, Ha-2), 2.63 (1H, dd, J=6.6, 15.3 Hz, Hb-2), 3.69 (3H, s, $COOCH_3$), 3.79 (1H, ddq, J=3.6, 4.6, 6.0 Hz, H-9), 3.87 (1H, dddd, J=4.8, 6.6, 6.6, 6.6 Hz, H-6), 4.26 (1H, dddd, J=6.6, 6.6, 6.6, 6.6 Hz, H-3). ^{13}C-NMR (in $CDCl_3$, 150 MHz) δ: 23.6, 31.0, 31.1, 33.0, 36.5, 40.9, 51.7 ($COO\underline{C}H_3$), 68.2 (C-O), 75.5 (C-O), 80.1 (C-O), 171.6 ($\underline{C}OOCH_3$).

7: Colorless oil, ^{1}H-NMR (in $CDCl_3$, 600 MHz) δ: 1.19 (3H, d, J=6.2 Hz, H_3-10), 2.42 (1H, dd, J=3.2, 16.5 Hz, Ha-2), 2.51 (1H, dd, J=8.9, 16.5 Hz, Hb-2), 3.72 (3H, s, $COOCH_3$), 3.79 (1H, ddq, J=4.8, 6.0, 6.2 Hz, H-9), 4.00 (1H, dddd, J=3.2, 5.0, 6.1, 8.9 Hz, H-3). ^{13}C-NMR (in $CDCl_3$, 150 MHz) δ: 23.6, 25.4, 25.6, 29.5, 36.5, 39.2, 41.1, 51.7 ($COO\underline{C}H_3$), 68.0 (C-O), 78.1 (C-O), 173.5 ($\underline{C}OOCH_3$).

Preparation of (−)- and (+)-MTPA Esters, 8—13 Freshly prepared (−)-MTPA chloride (30 mg) was added to individual solutions of hydrogenated compounds (**5**—**7**, 1—3 mg) in a mixture of pyridine (2 ml) and CCl_4 (5 drops); the resulting mixture was left to stand at room temperature overnight. After removing the solvent under a N_2 stream, the residue was purified by chromatography on a silica gel column [*n*-hexane–AcOEt (20 : 1→10 : 1→8 : 1→5 : 1→2 : 1→0 : 1)] to afford (−)-MTPA esters (**8**, **10**, **12**). Further, (+)-MTPA esters (**9**, **11**, **13**, 2—7 mg) were obtained from individual solutions of **5**, **6**, and **7**, respectively, by using (+)-MTPA chloride (30 mg) and following the same procedure as described above.

8: Colorless oil, ^{1}H-NMR δ: see Table 1.

9: Colorless oil, ^{1}H-NMR δ: see Table 1.

10: Colorless oil, ^{1}H-NMR δ: see Table 1.

11: Colorless oil, ^{1}H-NMR δ: see Table 1.

12: Colorless oil, ^{1}H-NMR δ: see Table 1.

13: Colorless oil, ^{1}H-NMR δ: see Table 1.

Isolation of Operculinic Acid H Methyl Ester (14) The glycosidic acid fraction (5.87 g) in MeOH (100 ml) was methylated using a diazomethane–ether solution. The methylated product was chromatographed on a silica gel column [$CHCl_3$–MeOH–H_2O (6 : 4 : 1)] and subsequently purified by HPLC (column: Nucleosil 5C8, 20 mm i.d.×250 mm; solvent: 70% MeOH) to afford **14** (4.10 g).

14: White powder, mp 142—145 °C, $[\alpha]_D^{19}$ −33.8° (c=1.0, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). Negative FAB-MS *m/z*: 1241 $[M-H]^-$. ^{1}H- and ^{13}C-NMR δ: see Tables 3 and 4. $^1J_{C-H}$: Glc (156 Hz), Glc′ (164 Hz), Glc″ (159 Hz), Glc‴ (158 Hz), Rha (168 Hz), Rha′ (174 Hz). *Anal.* Calcd for $C_{53}H_{94}O_{32}$: C, 51.20; H, 7.62. Found: C, 51.13; H, 7.63.

Alkaline Hydrolysis of 14 A solution of **14** (30 mg) in 3% KOH (6 ml) was heated at 95 °C for 1 h. The pH of the reaction mixture was adjusted to 4 with 1 M HCl, and the mixture was desalted through an MCI gel CHP20 column (H_2O→acetone) to afford **15** (26 mg).

15: White powder, mp 156—163 °C, $[\alpha]_D^{26}$ −49.1° (c=2.5, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1710 (C=O). Negative FAB-MS *m/z*: 1227 $[M-H]^-$, 1065 $[1227-162]^-$, 919 $[1065-146]^-$, 757 $[919-162]^-$, 595 (26) $[757-162]^-$. *Anal.* Calcd for $C_{52}H_{92}O_{32}$: C, 50.81; H, 7.54. Found: C, 50.81; H, 7.54.

Acidic Hydrolysis of 14 A solution of **14** (200 mg) in 2 M H_2SO_4 (3 ml) was heated at 95 °C for 2 h. The reaction mixture was diluted with H_2O (10 ml) and extracted with ether (10 ml×3). The ether layer was dried over $MgSO_4$ and then treated with a solution of diazomethane–ether. The reaction mixture was evaporated, and the residue was crystallized from *n*-hexane–AcOEt to yield **16** (23 mg). The H_2O layer was neutralized with 4% KOH and then desalted on a Sephadex LH-20 column (MeOH) to afford a sugar mixture, which was subjected to HPLC (column: Nucleosil 5NH2, 10 mm i.d.×300 mm; solvent: 80% CH_3CN) to afford L-rhamnose (28 mg) [syrup, $[\alpha]_D^{24}$ +10.1° (c=1.7, H_2O)] and D-glucose (25 mg) [syrup, $[\alpha]_D^{24}$ +49.8° (c=1.6, H_2O)].

16: Colorless needles (*n*-hexane–AcOEt), mp 79—80 °C, $[\alpha]_D^{21}$ +12.5° (c=3.5, $CHCl_3$). FD-MS *m/z* (%): 303 (100) $[M+H]^+$, 285 (20) $[303-H_2O]^+$, 245 (14) $[M-CH_3(CH_2)_3]^+$, 216 (14) $[303-CH_3(CH_2)_3CH(OH)]^+$, 103 (44) $[CH(OH)CH_2COOCH_3]^+$, 87 (27) $[CH_3(CH_2)_3CH(OH)]^+$. ^{1}H-NMR (in pyridine-d_5, 400 MHz) δ: 0.90 (3H, t, J=7.0 Hz, H_3-16), 2.68 (1H, dd, J=4.8, 15.0 Hz, Hb-2), 2.75 (1H, dd, J=8.0, 15.0 Hz, Ha-2), 3.64 (3H, s, $COOCH_3$), 3.82 (1H, m, H-12), 4.42 (1H, m, H-3). ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 0.91 (3H, t, J=7.0 Hz, H_3-16), 2.41 (1H, dd, J=8.9, 16.2 Hz, Hb-2), 2.51 (1H, dd, J=3.3, 16.2 Hz, Ha-2), 3.58 (1H, m, H-12), 3.71 (3H, s, $COOCH_3$), 4.00 (1H, m, H-3). ^{13}C-NMR (in pyridine-d_5, 100 MHz) δ: 14.4 (C-16), 23.2, 26.2, 26.4, 28.6, 30.0 (×2), 30.0, 30.2, 38.1, 38.2, 38.5, 43.5, 51.3 (OCH_3), 68.2 (C-3), 70.9 (C-12), 172.8 (C-1). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 14.1 (C-16), 22.8, 25.5, 25.6, 27.9, 29.5 (×2), 29.5, 29.7, 36.5, 37.2, 37.5, 41.2, 51.7 ($COO\underline{C}H_3$), 68.0 (C-3), 72.0 (C-12), 173.5 (C-1).

Preparation of (−)-MTPA Ester (17) and (+)-MTPA Ester (18) of 16 Compound **16** (8 mg or 5 mg) was treated with (−)-MTPA chloride (30 mg) or (+)-MTPA chloride (15 mg) in a mixture of dry pyridine (2 ml) and CCl_4 (5 drops), and the reaction mixture was left to stand overnight at room temperature. After removal of the solvent, the residue was purified by silica gel cc [benzene–AcOEt (10 : 1→8 : 1→5 : 1→0 : 1)] to yield the corresponding ester.

17: Colorless oil, ^{1}H-NMR δ: see Table 2.

18: Colorless oil, ^{1}H-NMR δ: see Table 2.

Acetylation of 14 Compound **14** (30 mg) in Ac_2O–pyridine (1 : 1, 3 ml) was left to stand at room temperature overnight. The solvent was removed under a N_2 stream to afford **19**.

19: White powder, mp 83—87 °C, $[\alpha]_D^{26}$ −21.3° (c=6.6, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). ^{1}H-NMR δ: see Table 3.

Permethylation of 14 NaH (50 mg) and CH_3I (2 ml) were added to a solution of **14** (14 mg) in DMF (2 ml) under stirring. The mixture was stirred overnight at room temperature. The solvent was removed under a N_2 stream and then H_2O (2 ml) was added. The mixture was extracted with ether (1 ml×4). The extract was purified by silica gel cc [benzene–acetone (5 : 1, 3 : 1)] to afford **20** (8 mg).

20: Colorless syrup, ^{1}H-NMR (in pyridine-d_5, 400 MHz) δ: 1.00 (3H, t, J=7.0 Hz, H_3-16 of Ag), 1.46 (3H, d, J=6.1 Hz, H_3-6 of rhamnose), 1.62 (3H, d, J=6.1 Hz, H_3-6 of rhamnose), 2.59 (1H, dd, J=5.5, 15.3 Hz, Ha-2 of Ag), 2.71 (1H, dd, J=7.2, 15.3 Hz, Hb-2 of Ag), 3.37, 3.43, 3.45, 3.52, 3.54, 3.55, 3.55, 3.56, 3.57, 3.60, 3.66, 3.67, 3.67, 3.68, 3.72, 3.72, 3.86, 3.97 (each 3H, s, OCH_3), 4.67 (1H, d, J=7.6 Hz, H-1 of glucose), 4.82 (1H, d, J=7.9 Hz, H-1 of glucose), 4.88 (1H, d, J=7.5 Hz, H-1 of glucose), 5.16 (1H, d, J=7.6 Hz, H-1 of glucose), 5.21 (1H, d, J=1.8 Hz, H-1 of rham-

nose), 5.64 (1H, d, J=1.5 Hz, H-1 of rhamnose). EI-MS m/z: 299, 393, 565, 801. HR-EI-MS m/z: 299.2590 [Calcd for $C_{18}H_{35}O_3$ (*a*): 299.2586]; 393.2127 [Calcd for $C_{18}H_{33}O_9$ (*b*): 393.2124]; 565.2848 [Calcd for $C_{26}H_{45}O_{13}$ (*c*): 565.2860]; 801.4121 [Calcd for $C_{36}H_{65}O_{19}$ (*d*): 801.4119], see Fig. 3.

Partial Methanolysis of 14 Compound **14** (531 mg) was dissolved in 0.3% HCl–MeOH (3 ml), and the solution was refluxed for 100 min. After cooling, the reaction mixture was neutralized with triethylamine, the solvent was removed under reduced pressure, and the residue was successively chromatographed on a Sephadex LH-20 column (MeOH) and a Fuji-gel ODS G3 column (60% MeOH, 70% MeOH) to afford fr. 8 (170 mg) and fr. 9 (320 mg). Fraction 9 was subjected to HPLC (column: Inertsil ODS, 20 mm i.d.×250 mm; solvent: 70% MeOH) to give fr. 10 (37 mg), fr. 11 (35 mg), fr. 12 (4 mg), fr. 13 (121 mg), fr. 14 (10 mg), fr. 15 (42 mg), fr. 16 (10 mg), and fr. 17 (38 mg). Fraction 10 was chromatographed on a silica gel column [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2→7 : 3 : 0.5→0 : 0 : 1)] to afford **23** (30 mg). Fractions 11, 13, 15, and 17 were chromatographed individually on a silica gel column under conditions similar to those used for the chromatographic purification of fr. 10 to afford the following compounds: **21** (15 mg) from fr. 11, **14** (81 mg) from fr. 13, **22** (11 mg) and **24** (14 mg) from fr. 15, and **25** (17 mg) from fr. 17.

21: Colorless needles (MeOH–H_2O), mp 118—121 °C, $[\alpha]_D^{21}$ −41.9° (c=2.5, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1720 (C=O). ^{1}H- and ^{13}C-NMR δ: see Tables 4 and 5.

22: White powder, mp 102—111 °C, $[\alpha]_D^{21}$ −17.3° (c=1.8, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). ^{1}H- and ^{13}C-NMR δ: see Tables 4 and 5.

23: White powder, mp 110—115 °C, $[\alpha]_D^{21}$ −24.0° (c=4.5, MeOH). IR (KBr) cm^{-1}: 3400 (br OH), 1725 (C=O). ^{1}H- and ^{13}C-NMR δ: see Tables 4 and 5.

24: White powder, mp 118—126 °C, $[\alpha]_D^{21}$ −48.5° (c=1.9, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). ^{1}H- and ^{13}C-NMR δ: see Tables 4 and 5.

25: White powder, mp 126—132 °C, $[\alpha]_D^{21}$ −29.7° (c=2.5, MeOH). IR (KBr) cm^{-1}: 3400 (br OH), 1725 (C=O). ^{1}H- and ^{13}C-NMR δ: see Tables 4 and 5.

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