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Resin Glycosides. IX.[1] Operculins I, II, V, VII and VIII, New Ether-Soluble Resin Glycosides of Rhizoma Jalapae Braziliensis (the Roots of *Ipomoea operculata*)

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Eight ether-soluble resin glycosides (jalapins), operculins I—VIII, were isolated from Rhizoma Jalapae Braziliensis (the roots of *Ipomoea operculata*). Among them, the structures of operculins I, II, V, VII and VIII, which have a common glycosidic acid, operculinic acid A, have been determined on the basis of chemical and spectral data. Similar to the jalapins so far isolated from several Convolvulaceae plants, all of them have intramolecular macrocyclic ester structures. They have *n*-dodecanoic and/or *n*-decanoic acid as a component organic acid in place of hitherto known isobutyric, 2-methylbutyric, tiglic and nilic acids.

Keywords resin glycoside; Rhizoma Jalapae Braziliensis; Convolvulaceae: ether-soluble resin glycoside; macrocyclic ester structure; operculin I; operculin II; operculin V; operculin VII; operculin VIII

In the preceding paper,[1,2] we reported the structures of seven glycosidic acids, operculinic acids A—G, and two organic acids, *n*-decanoic and *n*-dodecanoic acids, as components of the alkaline hydrolysis products of the crude ether-soluble resin glycoside of Rhizoma Jalapae Braziliensis (the roots of *Ipomoea operculata* (Gomes) MART). This paper deals with the isolation of eight new genuine ether-soluble resin glycosides named operculins I—VIII, and the structural elucidation of operculins I, II, V, VII and VIII with a common glycosidic acid, operculinic acid A.

A combination of silica gel chromatography and octadecyl silica (ODS) chromatography of the ether-soluble portion of the crude resin glycoside[2] followed by repeated preparative high performance liquid chromatography (HPLC) over octyl silica and ODS gave operculins I (**1**), II (**2**), III, IV, V (**3**), VI and compound A, which were regarded as being homogeneous on the basis of inspection of the proton nuclear magnetic resonance (^{1}H-NMR) spectra.

Operculin I (**1**), colorless needles, mp 103—111 °C (dec.), $[\alpha]_D$ −24.0°, $C_{70}H_{124}O_{25}$, furnished, on alkaline hydrolysis, a white powder and a glycosidic acid, mp 179—182 °C (dec.). The former was methylated with diazomethane and the product was found to be identical with methyl *n*-dodecanoate by gas chromatography (GC). The latter was identified as operculinic acid A (**6**),[2] (11*S*)-11-hydroxyhexadecanoic acid ((*S*)-jalapinolic acid) 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, by mixed melting point determination and ^{1}H-NMR spectral comparison with an authentic sample.

Compound **1** showed signals due to three carboxy (δ 173.1, 173.5, 173.6) and five anomeric (δ 98.5, 100.0, 103.2, 104.3, 105.4) carbons in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I) suggesting that **1** consists of 1 mol of **6** and 2 mol of *n*-dodecanoic acid.

The negative ion fast atom bombardment-mass spectrum (FAB-MS) of **1** exhibited the $(M-H)^-$ ion peak at *m/z* 1363, indicating all the carboxy groups to be in the form of esters, as in all the ether-soluble resin glycosides, jalapins, so far isolated,[3-5] the carboxy group of the aglycone, jalapinolic acid, of **6** is also intramolecularly linked with a hydroxy group of the sugar moiety to form a macrocyclic ester structure (Fig. 1). This suggestion was supported by the nonequivalent 2-H_2 signals of the jalapinolic acid moiety of **1** observed at δ 2.26 and 2.45 (1H each), whereas **6** exhibited the equivalent signals at δ 2.52 (2H, J=7.6 Hz) in the ^{1}H-NMR spectrum.[2]

In order to clarify the positions of the ester linkages, ^{1}H-NMR signals attributable to the sugar moiety of **1** were assigned on the basis of the ^{1}H–^{1}H shift correlated

TABLE I. ^{13}C-NMR Spectral Data for **1**, **2**, **3**, **4** and **5** (In Pyridine-d_5)

		1	**2**	**3**	**4**	**5**
Fuc	-1	104.3	104.3	101.6	104.3	104.3
	2	80.0	80.0	73.6	80.0	80.0
	3	73.4	73.4	76.5	73.4	73.4
	4	72.9	72.8	73.6	72.9	72.8
	5	70.8	70.8	71.3	70.8	70.8
	6	17.3	17.3	17.2	17.3	17.3
Rha	-1	98.5	98.5	100.1	98.5	98.5
	2	73.6	73.6	70.0	73.7	73.6
	3	69.4	69.4	77.9	69.4	69.4
	4	81.5	81.5	76.8	81.5	81.5
	5	68.9	68.9	68.1	68.9	68.9
	6	19.1	19.1	19.2	19.1	19.1
Rha′	-1	100.0	100.0	99.3	100.0	100.0
	2	73.2	73.2	72.3	73.2	73.2
	3	80.1	80.1	80.2	80.1	80.1
	4	78.6	78.6	78.5	78.6	78.6
	5	68.5	68.5	68.1	68.5	68.5
	6	18.9	18.9	18.7	18.9	18.9
Rha″	-1	103.2	103.2	103.3	103.2	103.2
	2	72.4	72.4	72.3	72.4	72.4
	3	70.3	70.3	70.2	70.3	70.3
	4	75.5	75.5	75.5	75.5	75.5
	5	68.2	68.1	68.0	68.2	68.1
	6	18.0	18.0	18.1	18.0	18.0
Glc	-1	105.4	105.4	104.8	105.4	105.4
	2	75.2	75.2	75.2	75.2	75.1
	3	78.5	78.4	78.4	78.5	78.4
	4	71.5	71.4	70.8	71.5	71.4
	5	78.1	78.1	78.0	78.2	78.1
	6	63.0	62.9	62.6	62.9	62.9
Ag	-11	82.4	82.4	79.5	82.4	82.4
	16	14.3	14.3	14.3	14.3	14.3
C=O		173.6	173.6	174.6	173.6	173.5
		173.5	173.5	173.5	173.5	173.5
		173.1	173.1	173.5	173.1	173.1

δ in ppm from TMS. Fuc, fucopyranosyl; Rha, rhamnopyraosyl; Glc, glucopyranosyl; Ag, aglycone (*S*-jalapinolic acid). All assignments are based on the HETCOR spectral data.

TABLE II. ^{1}H-NMR Spectral Data for **1**, **2**, **3**, **4**, **5** and **6** (in Pyridine-d_5, 400 MHz)

		1	2	3	4	5	6
Fuc	-1	4.69 (d, 7.6)	4.69 (d, 7.5)	4.81 (d, 8.0)	4.69 (d, 7.1)	4.67 (d, 7.5)	4.79 (d, 8.0)
	2	4.12 (dd, 7.6, 9.0)	4.13 (dd, 7.5, 9.0)	4.49 (dd, 8.0, 10.0)	4.13 (dd, 7.1, 9.0)	4.11 (dd, 7.5, 9.5)	4.48 (dd, 8.0, 9.5)
	3	4.01 (dd, 9.0, 4.0)	4.02 (dd, 9.0, 4.0)	4.18 (dd, 10.0, 3.5)	4.03 (dd, 9.0, 4.5)	4.00 (dd, 9.5, 3.5)	4.14 (dd, 9.5, 3.5)
	4	3.94 (d, 4.0)	3.94 (d, 4.0)	3.91 (d, 3.5)	3.96 (d, 4.5)	3.91 (d, 3.5)	3.94 (d, 3.5)
	5	3.72 (q, 6.4)	3.72 (q, 6.4)	3.80 (q, 6.4)	3.73 (q, 6.4)	3.71 (q, 6.1)	3.80 (q, 6.4)
	6	1.49 (d, 6.4)	1.49 (d, 6.4)	1.51 (d, 6.4)	1.49 (d, 6.4)	1.47 (d, 6.1)	1.52 (d, 6.4)
Rha	-1	5.48 (d, 1.2)	5.48 (d, 1.8)	6.31 (d, 1.5)	5.49 (d, 2.0)	5.46 (d, 1.5)	6.21 (d, 1.5)
	2	5.90 (dd, 1.2, 3.1)	5.90 (dd, 1.8, 3.0)	5.22 (dd, 1.5, 2.1)	5.90 (dd, 2.0, 3.0)	5.87 (dd, 1.5, 3.0)	4.67 (dd, 1.5, 3.0)
	3	4.99 (dd, 3.1, 9.2)	4.99 (dd, 3.0, 9.0)	5.64 (dd, 2.1, 10.0)	5.00 (dd, 3.0, 9.0)	4.96 (dd, 3.0, 9.0)	4.61 (dd, 3.0, 9.0)
	4	4.14 (dd, 9.2, 9.2)	4.14 (dd, 9.0, 9.0)	4.65 (dd, 10.0, 10.0)	4.15 (dd, 9.0, 9.0)	4.12 (dd, 9.0, 9.0)	4.21 (dd, 9.0, 9.0)
	5	4.46 (dq, 9.2, 6.1)	4.46 (dq, 9.0, 6.1)	4.98 (dq, 10.0, 6.1)	4.47 (dq, 9.0, 6.1)	4.44 (dq, 9.0, 6.4)	4.86 (dq, 9.0, 6.1)
	6	1.63 (d, 6.1)	1,63 (d, 6.1)	1.58 (d, 6.1)	1.64 (d, 6.1)	1.61 (d, 6.4)	1.61 (d, 6.1)
Rha′	-1	5.87 (d, 1.7)	5.87 (d, 1.5)	5.60 (d, 1.6)	5.88 (d, 1.5)	5.84 (d, 1.5)	5.86 (d, 1.5)
	2	6.29 (dd, 1.7, 3.3)	6.29 (dd, 1.5, 3.2)	5.98 (dd, 1.6, 3.0)	6.30 (dd, 1.5, 3.0)	6.27 (dd, 1.5, 3.0)	5.17 (dd, 1.5, 3.0)
	3	4.75 (dd, 3.3, 9.1)	4.76 (dd, 3.2, 9.0)	4.62 (dd, 3.0, 9.0)	4.76 (dd, 3.0, 9.0)	4.73 (dd, 3.0, 9.5)	4.72 (dd, 3.0, 9.0)
	4	4.33 (dd, 9.1, 9.1)	4.33 (dd, 9.0, 9.0)	4.30 (dd, 9.0, 9.0)	4.34 (dd, 9.0, 9.0)	4.30 (dd, 9.5, 9.5)	4.49 (dd, 9.0, 9.0)
	5	4.38[a]	4.38[a]	4.36[a]	4.37[a]	4.35[a]	4.40 (dq, 9.0, 5.8)
	6	1.65 (d, 5.5)	1.65 (d, 5.8)	1.61 (d, 5.8)	1.66 (d, 5.8)	1.64 (d, 5.8)	1.60 (d, 5.8)
Rha″	-1	6.22 (d, 1.6)	6.22 (d, 1.7)	6.19 (d, 1.5)	6.23 (d, 1.8)	6.19 (d, 1.8)	6.20 (d, 1.5)
	2	4.93 (dd, 1.6, 3.3)	4.94 (dd, 1.7, 3.0)	4.89 (dd, 1.5, 3.0)	4.94 (dd, 1.8, 3.5)	4.91 (dd, 1.8, 3.0)	4.88 (dd, 1.5, 3.0)
	3	4.55 (dd, 3.3, 9.1)	4.55 (dd, 3.0, 9.0)	4.46 (dd, 3.0, 9.1)	4.56 (dd, 3.5, 9.5)	4.52 (dd, 3.0, 9.5)	4.43 (dd, 3.0, 9.0)
	4	5.78 (dd, 9.1, 9.1)	5.78 (dd, 9.0, 9.0)	5.75 (dd, 9.1, 9.1)	5.79 (dd, 9.5, 9.5)	5.75 (dd, 9.5, 9.5)	4.21 (dd, 9.0, 9.0)
	5	4.38[a]	4.38[a]	4.36[a]	4.37[a]	4.35[a]	4.30 (dq, 9.0, 6.1)
	6	1.43 (d, 5.8)	1.43 (d, 6.1)	1.42 (d, 6.1)	1.44 (d, 6.1)	1.42 (d, 6.4)	1.57 (d, 6.1)
Glc	-1	5.05 (d, 7.6)	5.05 (d, 7.5)	5.08 (d, 7.5)	5.06 (d, 7.5)	5.02 (d, 7.5)	5.23 (d, 7.5)
	2	3.95 (dd, 7.6, 9.0)	3.95 (dd, 7.5, 9.0)	3.94 (dd, 7.5, 9.0)	3.97 (dd, 7.5, 9.0)	3.93 (dd, 7.5, 8.5)	3.97 (dd, 7.5, 9.0)
	3	4.03 (dd, 9.0, 9.0)	4.04 (dd, 9.0, 9.0)	4.10 (dd, 9.0, 9.0)	4.04 (dd, 9.0, 9.0)	4.01 (dd, 8.5, 8.5)	4.20 (dd, 9.0, 9.0)
	4	3.93 (dd, 9.0, 9.0)	3.93 (dd, 9.0, 9.0)	4.16 (dd, 9.0, 9.0)	3.94 (dd, 9.0, 9.0)	3.90 (dd, 8.5, 8.5)	4.10 (dd, 9.0, 9.0)
	5	3.74 (ddd, 9.0, 6.0, 2.0)	3.75 (m)	3.88[a]	3.76 (ddd, 9.0, 6.0, 3.0)	3.72[a]	3.99[a]
	6	4.09 (dd, 6.0, 11.5)	4.10 (dd, 6.0, 12.0)	4.36[a]	4.10 (dd, 6.0, 11.5)	4.07 (dd, 5.5, 12.0)	4.27 (dd, 5.0, 11.5)
		4.38 (dd, 2.0, 11.5)	4.38 (dd, 3.0, 12.0)	4.46[a]	4.40 (dd, 3.0, 11.5)	4.37 (dd, 3.0, 12.0)	4.53 (dd, 2.5, 11.5)
Ag	-2	2.26 (ddd, 3.0, 7.5, 13.5)	2.26 (ddd, 4.0, 7.5, 13.5)	2.28 (m)	2.27 (ddd, 4.0, 8.0, 14.0)	2.25 (ddd, 4.5, 7.5, 14.0)	2.52 (t, 7.6)
	2	2.45[a]	2.43 (m)	2.68 (br t, 12.5)	2.45[a]	2.45[a]	
	11	3.83[a]	3.83 (m)	3.90[a]	3.84 (m)	3.82 (m)	3.99[a]
	16	0.87 (t, 7.0)	0.87 (t, 7.0)	0.87 (t, 6.8)	0.87 (t, 7.4)	0.87 (t, 6.7)	0.92 (t, 7.0)
Org	2	2.33 (ddd, 3.5, 7.5, 7.5)	2.32 (ddd, 4.0, 7.5, 7.5)	2.41[a]	2.34 (ddd, 4.0, 7.5, 7.5)	2.32 (ddd, 3.5, 7.5, 7.5)	
		2.47 (ddd, 3.0, 7.5, 7.5)	2.47 (ddd, 2.8, 7.5, 7.5)	2.44[a]	2.47 (ddd, 3.0, 7.5, 7.5)	2.47 (ddd, 3.0, 7.5, 7.5)	
CH_3		0.87 (t, 7.0)	0.85 (t, 7.0)	0.87 (t, 6.8)	0.85 (t, 7.4)	0.85 (t, 7.3)	
		0.87 (t, 7.0)	0.85 (t, 7.0)	0.93 (t, 6.7)	0.87 (t, 7.4)	0.87 (t, 6.7)	

a) Signals are overlapping. δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses); Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Ag, aglycone ((*S*)-jalapinolic acid); Org, *n*-dodecanoyl or *n*-decanoyl. All assignments are based on the ^{1}H–^{1}H COSY and NOESY spectral data.

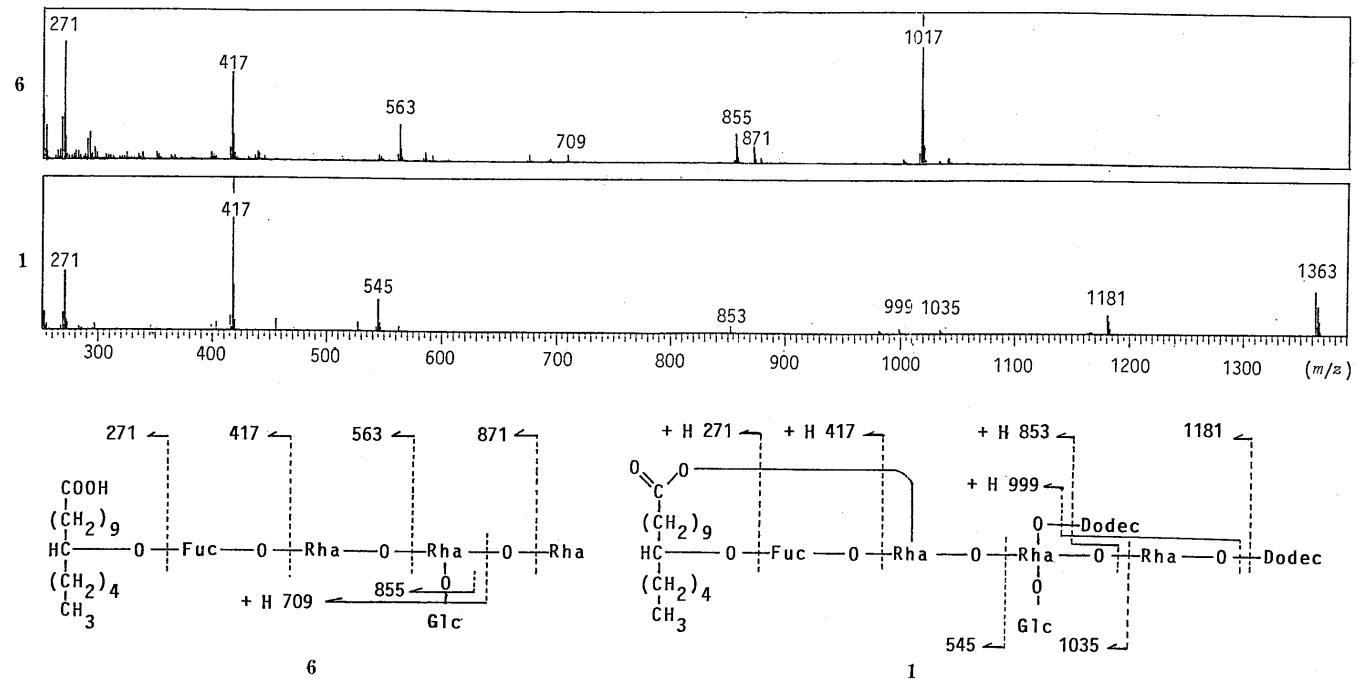

Fig. 1. Negative Ion FAB-MS of **6** and **1**

Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Dodec, *n*-dodecanoyl

2D-NMR (COSY), nuclear Overhauser effect 2D-NMR (NOESY) and ^{1}H–^{13}C heteronuclear shift-correlated 2D-NMR (HETCOR) spectra (Tables I and II).

Comparing the chemical shifts of signals due to the sugar moieties between **1** and **6**, remarkable downfield shifts owing to acylation were seen at 2-H of the first rhamnose (Rha), 2-H of the second rhamnose (Rha′) and 4-H of the third rhamnose (Rha″) by 1.23, 1.12, 1.57 ppm, respectively. Therefore, the ester linkages are concluded to be located at 2-OH of Rha, 2-OH of Rha′ and 4-OH of Rha″.

In the negative FAB-MS of **1** and **6** (Fig. 1), besides the common fragment peaks observed at *m/z* 271 and 417, **1** showed a peak at *m/z* 545 in place of that at *m/z* 563 $[(M-H)-2\times(6-\text{deoxyhexose unit})-\text{hexose unit}]^-$ observed in the spectrum of **6**. The difference of 18 mass units suggested that the ester linkage of jalapinolic acid is placed in Rha, as in the case of orizabins.[3]

To confirm the above suggestion, **1** was converted into the peracetate (**7**), a white powder, mp 64—69 °C (dec.), $[\alpha]_D$ −19.3°. Its electron impact mass spectrum (EI-MS) revealed fragment ion peaks at *m/z* 331, 413, 723 and 1071 which were respectively ascribable to the fragments a, c, d and g shown in Fig. 2.

These data indicated that the ester linkage of jalapinolic acid is located at 2-OH of Rha, and hence the two *n*-dodecanoic acids are attached to 2-OH of Rha′ and 4-OH of Rha″.

Taking the *J* values of the anomeric and methine proton

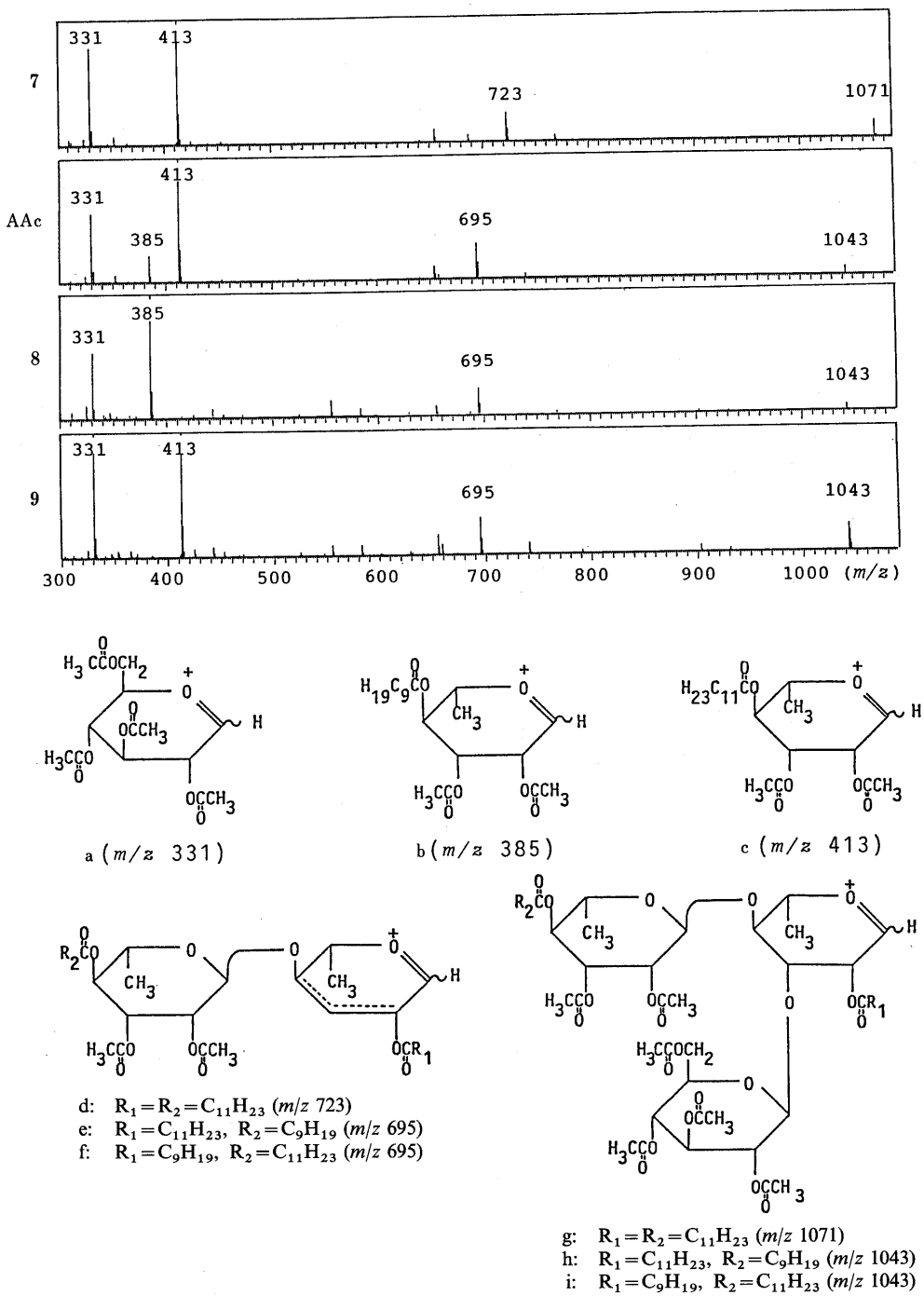

Fig. 2. EI-MS of **7**, AAc, **8** and **9**

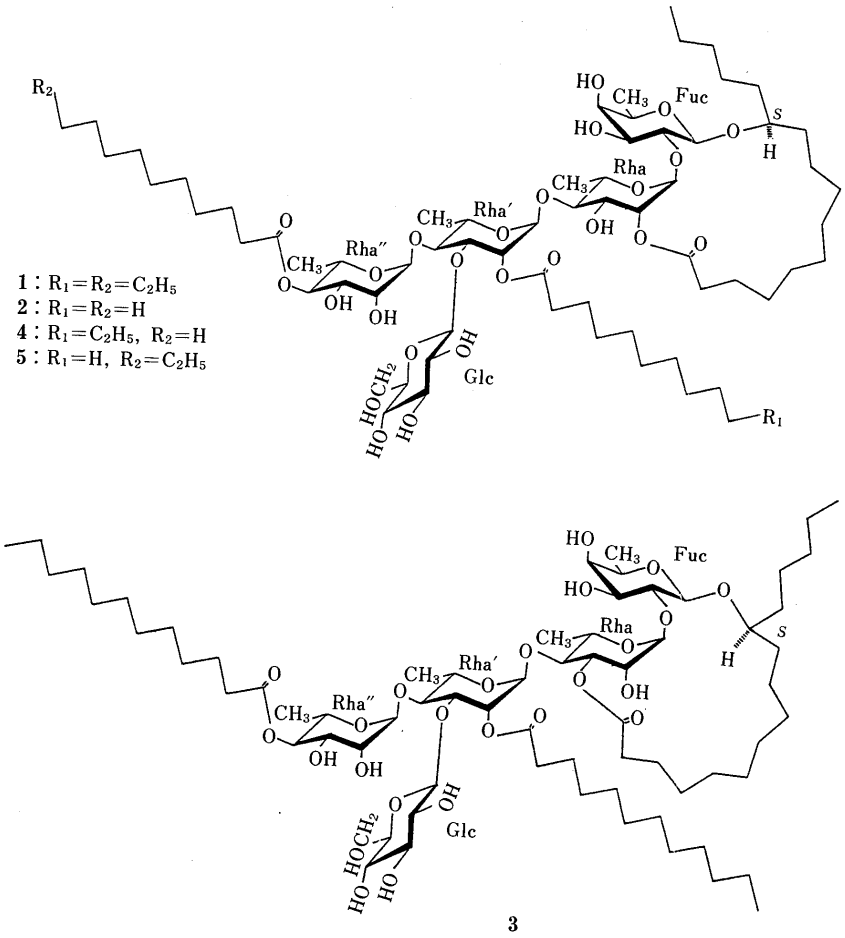

Fig. 3. Structures of **1**—**5**

signals due to the sugar moiety into account, the conformations of the rhamnopyranose units of **1** are concluded to be $^1C_4$ and those of the fucopyranose and glucopyranose units are $^4C_1$ (Table II).

Consequently, the structure of operculin I (**1**) was defined as (*S*)-jalapinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, intramol. 1,2″-ester, as shown in Fig. 3.

Operculin II (**2**), colorless needles, mp 120—128 °C (dec.), $[\alpha]_D$ −23.5°, $C_{66}H_{116}O_{25}$, afforded, on alkaline hydrolysis, *n*-decanoic acid and **6**. Compound **2** exhibited the $(M-H)^-$ ion peak at *m/z* 1307 together with the same fragment peaks as those of **1** at *m/z* 545, 417 and 271 in the negative FAB-MS and three carboxy carbon signals with the same chemical shifts as those of **1** at *δ* 173.1, 173.5 and 173.6 in the ^{13}C-NMR spectrum (Table I). Further, the ^{1}H-NMR spectrum of **2** was quite similar to that of **1** and, in particular, the chemical shifts of the signals due to 2-H of Rha, 2-H of Rha′ and 4-H of Rha″ were identical to those of **1** (Table II).

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

Operculin V (**3**), a white powder, mp 108—111 °C (dec.), $[\alpha]_D$ −53.8°, $C_{70}H_{124}O_{25}$, gave *n*-dodecanoic acid and **6** on alkaline hydrolysis. The negative FAB-MS spectrum of **3** was almost superimposable on that of **1**, where the same $(M-H)^-$ and fragment ion peaks were seen at *m/z* 1363, 545 and 417. Therefore, compound **3** is considered to be a positional isomer of **1** in which the *n*-dodecanoyl groups are located in Rha′ and Rha″, and the intramolecular ester group is placed at one of the hydroxy groups of Rha.

In the ^{1}H-NMR spectrum of **3**, compared with that of **6**, considerable downfield shifts were observed at 3-H of Rha (1.03 ppm), 2-H of Rha′ (0.81 ppm) and 4-H of Rha″ (1.54 ppm) (Table II). Therefore, the ester linkage of jalapinolic acid is located at 3-OH of Rha, and the *n*-dodecanoic acid residues are attached at the same positions as those of **1**.

Consequently, **3** was characterized as (*S*)-jalapinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, intramol, 1,3″-ester, that is, the ester ring of **3** is expanded from 2-OH to 3-OH of Rha of **1**.

Compound A, a white powder, mp 102—110 °C (dec.), $[\alpha]_D$ −25.7°, was hydrolyzed with alkali to afford *n*-dodecanoic and *n*-decanoic acids in the ratio of about 1:1 (GC) along with **6**, and exhibited the $(M-H)^-$ ion peak at *m/z* 1335, which is 28 mass units (C_2H_4) less than that of **1**, together with the same fragment peaks

as those of **1** at *m/z* 545, 417 and 271 in the negative FAB-MS. Furthermore, the ^{1}H-NMR spectrum of A was quite similar to that of **1**. These data suggested that the structure of A is analogous to that of **1**, where one of the *n*-dodecanoic acid residue in **1** is replaced by *n*-decanoic acid.

The EI-MS of the peracetate of A (AAc), a white powder, mp 64—66 °C (dec.), $[\alpha]_D$ −22.5°, showed fragment peaks at *m/z* 331, 385, 413, 695 and 1043 assignable to the fragments a, b, c, e and/or f, and h and/or i shown in Fig. 2. This result suggested that compound A might be a mixture composed of two compounds in which the organic acid groups are mutually interchanged between Rha′ and Rha″.

Preparative recycling HPLC of compound A afforded operculin VII (**4**), a white powder, mp 104—111 °C (dec.), $[\alpha]_D$ −24.3°, and operculin VIII (**5**), a white powder, mp 105—112 °C (dec.), $[\alpha]_D$ −27.0°.

Although the ^{1}H-NMR spectra and negative FAB-MS of **4** and **5** were almost identical with those of compound A, in the EI-MS of the peracetate of **4** (**8**), a white powder, mp 67—71 °C (dec.), $[\alpha]_D$ −21.0°, the fragment peak at *m/z* 413 (c) observed in AAc disappeared, while the peracetate of **5** (**9**), a white powder, mp 65—71 °C (dec.), $[\alpha]_D$ −18.8°, presented no peak at *m/z* 385. Therefore, the *n*-dodecanoic and *n*-decanoic acid groups of **4** are defined to be linked to Rha′ and Rha″, respectively, whereas those of **5** are reversed.

The ^{1}H- and ^{13}C-NMR signals due to the sugar moieties of **4** and **5** were assigned with the aid of the COSY, NOESY and HETCOR methods (Tables I and II). In a comparison of the ^{1}H-signals with those of **6**, pronounced downfield shifts (1.10—1.54 ppm) at 2-H of Rha, 2-H of Rha′ and 4-H of Rha″ were observed for both compounds, **4** and **5** (Table II).

Consequently, the structures of **4** and **5** were respectively concluded to be (*S*)-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[4-*O*-*n*-decanoyl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, intramol. 1,2″-ester and (*S*)-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[4-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, intramol. 1,2″-ester, as shown in Fig. 3.

Like other ether-soluble resin glycosides (jalapins) so far characterized, orizabins,[3] muricatins,[4] merremosides[5] and mammosides,[5] the operculins in this study have intramolecular ester structures, but the component acids, *n*-dodecanoic and *n*-decanoic acids, of the operculins differ from the organic acids (isobutyric, 2-methylbutyric, tiglic and nilic acids) hitherto found in the ether-soluble resin glycosides.

Experimental

General procedures All instruments and materials used were as cited in the preceding report[2] unless otherwise specified.

Isolation of Operculins I (1), II (2), III, IV, V (3), VI, VII (4) and VIII (5) The jalapin fraction (21.08 g) previously obtained from the roots of *I. operculata*[2] was subjected to chromatography over silica gel (Merck Art 7734, 5.5 cm i.d. ×36 cm, $CHCl_3$–MeOH, 14:1→10:1→8:1→7:1→MeOH) to give five fractions, fr. 1 (5.66 g), fr. 2 (9.43 g), fr. 3 (3.39 g), fr. 4 (0.15 g) and fr. 5 (2.08 g). Fraction 1 was chromatographed over silica gel (Merck Art 9385, 3.5 cm i.d. ×25 cm, $CHCl_3$–MeOH, 15:1→10:1→1:1→MeOH) to afford five fractions, fr. 6 (1.70 g), fr. 7 (1.54 g), fr. 8 (0.08 g), fr. 9 (0.52 g) and fr. 10 (1.06 g). Reversed-phase chromatography over Fuji-gel ODS G3 (Fuji Gel Co., 3.6 cm i.d. ×22 cm, 96% MeOH) of fr. 7 furnished fr. 11 (86 mg), fr. 12 (134 mg), fr. 13 (184 mg), fr. 14 (191 mg), fr. 15 (103 mg), fr. 16 (194 mg) and fr. 17 (470 mg). Preparative HPLC on a Hibar RP-8 column (Merck, 2.5 cm i.d. ×25 cm, 94% MeOH) and an Inertsil ODS-2 (Gasukuro Kogyo, 2.0 cm i.d. ×25 cm, MeOH) of fr. 17 yielded fr. 18 (71 mg) and operculin VI (184 mg). Fraction 9 was chromatographed over Fuji-gel ODS G3 (3.6 cm i.d. ×22 cm, 96% MeOH) to give fr. 19 (175 mg) and fr. 20 (295 mg). Successive preparative HPLC of fr. 19 on the Hibar RP-8 (92% MeOH) and the Inertsil ODS-2 (MeOH) columns furnished fr. 21 (36 mg) and **3** (85 mg). Similar preparative HPLC (Hibar RP-8, 97% MeOH and Inertsil ODS-2, MeOH) of fr. 2 (4.70 g) afforded **2** (70 mg), compound A (0.96 g), **1** (1.38 g) and fr. 22 (620 mg). Fraction 3 was chromatographed over silica gel (Merck Art. 9385, 3.6 cm i.d. ×22 cm, $CHCl_3$–MeOH, 8:1→7:1→6:1→MeOH) to give fr. 23 (0.43 g), fr. 24 (0.82 g), fr. 25 (1.90 g) and fr. 26 (0.20 g). Similar chromatography of fr. 24 over silica gel (Merck Art 9385, 2.1 cm i.d. ×27.5 cm, $CHCl_3$–MeOH, 10:1→8:1→6:1→MeOH) afforded fr. 27 (0.25 g), fr. 28 (0.17 g), fr. 29 (0.26 g) and fr. 30 (0.14 g). Fraction 25 and fr. 30 were subjected to HPLC (Hibar RP-8, 97% MeOH) to afford operculin IV (96 mg), compound B (510 mg), operculin III (512 mg) and fr. 31 (510 mg).

1: Colorless needles (MeOH–H_2O), mp 103—111 °C (dec.), $[\alpha]_D^{18}$ −24.0° (*c*=1.0, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1363 ($[M-H]^-$). *Anal.* Calcd for $C_{70}H_{124}O_{25}$: C, 61.56; H, 9.15. Found: C, 61.70; H, 9.16. ^{1}H-NMR δ: see Table II. ^{13}C-NMR δ: see Table I.

2: Colorless needles (MeOH–H_2O), mp 120—128 °C (dec.), $[\alpha]_D^{21}$ −23.5° (*c*=2.8, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1307 $[(M-H)^-]$. *Anal.* Calcd for $C_{66}H_{116}O_{25}$: C, 60.53; H, 8.93. Found: C, 60.75; H, 9.00. ^{1}H-NMR δ: see Table II. ^{13}C-NMR δ: see Table I.

Operculin III: Colorless needles (MeOH–H_2O), mp 115—125 °C (dec.), $[\alpha]_D^{25}$ −25.7° (*c*=2.6, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1379 $[(M-H)^-]$. *Anal.* Calcd for $C_{70}H_{124}O_{26}$: C, 60.85; H, 9.05. Found: C, 60.61; H, 9.12.

Operculin IV: A white powder (MeOH–H_2O), mp 117—120 °C (dec.), $[\alpha]_D^{22}$ −22.2° (*c*=5.4, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1323 $[(M-H)^-]$. *Anal.* Calcd for $C_{66}H_{116}O_{26}$: C, 59.80; H, 8.82. Found: C, 59.98; H, 8.92.

3: A white powder (MeOH–H_2O), mp 108—111 °C (dec.), $[\alpha]_D^{18}$ −53.8° (*c*=2.6, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1363 $[(M-H)^-]$. *Anal.* Calcd for $C_{70}H_{124}O_{25}$: C, 61.56; H, 9.15. Found: C, 61.57; H, 9.16. ^{1}H-NMR δ: see Table II. ^{13}C-NMR δ: see Table I.

Operculin VI: A white powder (MeOH–H_2O), mp 88—91 °C (dec.), $[\alpha]_D^{21}$ −32.3° (*c*=2.1, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1201 $[(M-H)^-]$. *Anal.* Calcd for $C_{64}H_{114}O_{20}\cdot H_2O$: C, 62.93; H, 9.57. Found: C, 62.79; H, 9.40.

Compound A: A white powder (MeOH–H_2O), mp 102—110 °C, $[\alpha]_D^{18}$ −25.7° (*c*=3.0, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1335 $[(M-H)^-]$. *Anal.* Calcd for $C_{68}H_{120}O_{25}$: C, 61.06; H, 9.04. Found: C, 61.17; H, 8.93. Compound A (100 mg) was subjected to recycling HPLC (TSK-gel ODS 120T, Tosoh, 2.15 cm i.d. ×30 cm, MeOH, 6 cycles) to afford **4** (16 mg) and **5** (18 mg).

4: A white powder (MeOH–H_2O), mp 104—111 °C, $[\alpha]_D^{23}$ −24.3° (*c*=1.3, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1335 $[(M-H)^-]$. *Anal.* Calcd for $C_{68}H_{120}O_{25}$: C, 61.06; H, 9.04. Found: C, 61.09; H, 9.04. ^{1}H-NMR δ: see Table II. ^{13}C-NMR δ: see Table I.

5: A white powder (MeOH–H_2O), mp 105—112 °C, $[\alpha]_D^{23}$ −27.0° (*c*=1.0, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (C=O). Negative FAB-MS *m/z*: 1335 $[(M-H)^-]$. *Anal.* Calcd for $C_{68}H_{120}O_{25}$: C, 61.06; H, 9.04. Found: C, 61.07; H, 9.04. ^{1}H-NMR δ: see Table II. ^{13}C-NMR δ: see Table I.

Alkaline hydrolysis of 1, 2, 3 and Compound A Suspensions of **1** (15 mg), **2** (5 mg), **3** (16 mg) and compound A (15 mg) in 3% KOH (2 ml) were each heated at 95 °C for 1 h. The reaction mixture was adjusted to pH 4 with 1 N HCl, then diluted with H_2O (10 ml), and extracted with ether (3×5 ml). The ether layer was washed with H_2O, dried over $MgSO_4$ and treated with diazomethane in ether. After removal of the solvent the residue was subjected to GC (column, Unisole 3000, 3.2 mm i.d. ×2 m glass column; column temperature, 160 °C; carrier gas, N_2 (1.25 kg/cm^{-1}), t_R (min): 8.35 (methyl *n*-dodecanoate) for **1**, 4.17 (methyl *n*-decanoate) for **2**, 8.36 (methyl

n-dodecanoate) for **3**, and 4.18 (methyl *n*-decanoate), 8.37 (methyl *n*-dodecanoate) for compound A.

The aqueous layer was desalted by chromatography over MCI-gel CHP 20P to give a white powder (glycosidic acid) (8 mg from **1** (mp 179—182 °C), 3 mg from **2** (mp 177—179 °C), 8 mg from **3** (mp 171—175 °C), 10 mg from compound A (mp 170—176 °C)). The ^{1}H-NMR spectra of the glycosidic acids were each superimposable on that of an authentic operculinic acid A (**6**).[2)]

Acetylation of 1, 4, 5 and Compound A Solutions of **1** (31 mg), **4** (4 mg), **5** (24 mg) and compound A (10 mg) in Ac_2O–pyridine (1 : 1, 2 ml) were each left to stand at room temperature overnight. The solvent was removed under an N_2 stream to give a white powder, **7** (39 mg from **1**), **8** (5 mg from **4**), **9** (30 mg from **5**), AAc (12 mg from compound A). **7**: mp 64—69 °C (dec.), $[\alpha]_D^{15}$ −19.3° (c=2.9, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). EI-MS m/z: 331, 413, 1071. High resolution EI-MS m/z Calcd for $C_{14}H_{19}O_9$ (a): 331.10287, Found: 331.10234, m/z Calcd for $C_{22}H_{37}O_7$ (c): 413.25390, Found: 413.25431. **8**: mp 67—71 °C (dec.), $[\alpha]_D^{23}$ −21.0° (c=0.4, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). EI-MS m/z: 331, 385, 1043. **9**: mp 65—71 °C (dec.), $[\alpha]_D^{23}$ −18.8° (c=1.9, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). EI-MS m/z: 331, 413, 1043. AAc: mp 64—66 °C (dec), $[\alpha]_D^{15}$ −22.5° (c=0.9, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). EI-MS m/z: 331, 385, 413, 1043.

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References

1) Part VIII. M. Ono, T. Fukunaga, T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.*, **38**, 2650 (1990).
2) M. Ono, T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.*, **37**, 3209 (1989).
3) N. Noda, M. Ono, K. Miyahara, T. Kawasaki and M. Okabe, *Tetrahedron*, **43**, 3889 (1987).
4) N. Noda, H. Kobayashi, K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.*, **36**, 920 (1988); N. Noda, M. Nishi, K. Miyahara and T. Kawasaki, *ibid.*, **36**, 1707 (1988).
5) I. Kitagawa, H. Shibuya, Y. Yokokawa, N. I. Baek, K. Ohashi, M. Yoshikawa, A. Nitta and H. Wiriadinata, *Chem. Pharm. Bull.*, **36**, 1618 (1988); I. Kitagawa, N. I. Baek, K. Ohashi, M. Sakagami, M. Yoshikawa and H. Shibuya, *ibid.*, **37**, 1131 (1989).