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Resin Glycosides. VIII.[1] Four New Glycosidic Acids, Operculinic Acids D, E, F, and G, of the Ether-Soluble Crude Resin Glycosides ("Jalapin") from Rhizoma Jalapae Braziliensis (Roots of *Ipomoea operculata*)

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Four glycosidic acids, operculinic acids D, E, F and G, were isolated from the glycosidic acid fraction afforded by alkaline hydrolysis of the ether-soluble crude resin glycoside ("jalapin") from Rhizoma Jalapae Braziliensis (roots of *Ipomoea operculata*). They were respectively characterized as 11*S*-jalapinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside, 11*S*-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, 11*S*-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside and 11*S*-jalapinolic acid 11-*O*-(2-*O*-methyl-*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, on the bases of chemical and spectroscopic data.

Keywords resin glycoside; jalapin; Rhizoma Jalapae Braziliensis; *Ipomoea operculata*; glycosidic acid; operculinic acid D; operculinic acid E; operculinic acid F; operculinic acid G; 11*S*-jalapinolic acid oligoglycoside

In the preceding paper,[2] we reported the structures of three glycosidic acids, operculinic acids A, B, and C, as well as two organic acids, *n*-decanoic and *n*-dodecanoic acids, which were formed by alkaline hydrolysis of the ether-soluble crude resin glycoside "jalapin" fraction of the roots of *Ipomoea operculata* (Gomes) MART. This paper is concerned with the structure elucidation of operculinic acids D (**1**) and E (**2**) previously obtained, and two new glycosidic acids named operculinic acids F (**3**) and G (**4**).

The fractions 3 and 4 reported previously[2] were each subjected to reversed-phase chromatography over Fuji-gel ODS G-3, and subsequent preparative high-performance liquid chromatography (HPLC) over Nucleosil 5C8 and Kusano CIG Si gel provided the homogeneous methyl esters of operculinic acids F (**3**) and G (**4**).

Operculinic acid D methyl ester (**1′**), a white powder, mp 170—172 °C (dec.), $[\alpha]_D$ −81.7°, $C_{46}H_{82}O_{24}$, negative ion fast atom bombardment mass spectrum (FAB-MS) m/z: 1017 $[M-H]^-$, gave, on acidic hydrolysis, an aglycone and a monosaccharide mixture. Methylation of the former with diazomethane yielded needles, mp 42—44 °C; this product was identified as methyl jalapinolate by gas chromatography (GC) and thin layer chromatography (TLC). The absolute configuration at C_{11} of jalapinolic acid of this plant had been determined as *S* in the preceding paper.[2]

TLC of the monosaccharide mixture revealed the presence of xylose, rhamnose and glucose. In order to determine their absolute configurations, the mixture was derived into trimethylsilyl (TMS) ethers of diastereomeric methyl thiazolidine 4(*R*)-carboxylate and then analyzed by GC according to Hara *et al.*[3] Xylose, rhamnose and glucose were concluded to be in D-, L- and D-form, respectively.

Alkaline hydrolysis of **1′** furnished operculinic acid D (**1**), a white powder, mp 175—177 °C (dec.), $[\alpha]_D$ −88.6°, $C_{45}H_{80}O_{24}$, which exhibited the $[M-H]^-$ ion peak at m/z 1003 along with fragment peaks at m/z 857 $[1003-146$ ($C_6H_{10}O_4$, 6-deoxyhexose unit)$]^-$, 841 $[1003-162$ ($C_6H_{10}O_5$, hexose unit)$]^-$, 695 $[857-162$ and/or $841-146]^-$, 549 $[695-146]^-$, 403 $[549-146]^-$, 271 $[403-132$ ($C_5H_8O_4$, pentose unit), jalapinolic acid-H$]^-$ in the negative FAB-MS (Fig. 1), indicating that **1** is a pentaglycoside composed of 1 mol each of jalapinolic acid, D-glucose and D-xylose, and 3 mol of L-rhamnose. Further, the fragmentation pattern suggested that its sugar moiety forms a branched chain with terminal glucose and rhamnose and that, taking the place of D-fucose in operculinic acids A (**5**) and C (**6**) as well as D-glucose in operculinic acid B (**7**), xylose links directly with the 11-OH of jalapinolic acid.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1′** showed the signals due to one ester methyl (δ 3.62), one terminal methyl (δ 0.90) and 1 eq methylene (2-H_2, δ 2.33) of methyl jalapinolate besides five anomeric proton signals (δ 4.84, 5.19, 5.86, 6.16. 6.28). All the proton signals due to the sugar moieties of **1′** and its peracetate (**8**), a white powder, mp 75—78 °C (dec.), $[\alpha]_D$ −48.0°, were assigned by referring to the ^{1}H–^{1}H shift-correlated 2D-NMR (COSY) and nuclear Overhauser effect 2D-NMR (NOESY) spectra (Table I).

The coupling constants of the anomeric and methine proton signals as well as $J_{C_1-H_1}$ values due to the sugar moiety indicated that all the monosaccharide units are of pyranose type, and further, the mode of glycosidic linkage of rhamnose units is α in $^1C_4$ and those of xylose and glucose units are β in $^4C_1$ conformations.

Comparing the chemical shifts of the signals due to the sugar moieties between **1′** and **8**, the signals of 3-H and 4-H of the xylose unit (Xyl), 2-H and 3-H of the first rhamnose unit (Rha), 2-H of the second rhamnose unit (Rha′), 2-H, 3-H, 4-H and 6-H_2 of the glucose unit (Glc) and 2-H, 3-H and 4-H of the third rhamnose unit (Rha″) were shifted downfield by 0.09—1.53 ppm, while 2-H of Xyl, 4-H of Rha, 3-H and 4-H of Rha′ were shifted upfield by 0.08—0.28 ppm, suggesting that the sugar linkages of **1′** are located at 2-OH of Xyl, 4-OH of Rha, and 3- and 4-OH of Rha′. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) signals due to the sugar moiety of **1′** were assigned by means of ^{1}H–^{13}C heteronuclear shift-correlated 2D-NMR (HETCOR) and then compared with those of methylpyranosides in the literature[4] (Table II). The glycosylation shifts[4] observed at C_2 of Xyl (+2.9 ppm), C_4 of Rha (+8.6 ppm) and C_3 and C_4 of Rha′ (+10.0 and +4.8 ppm) supported the above suggestion.

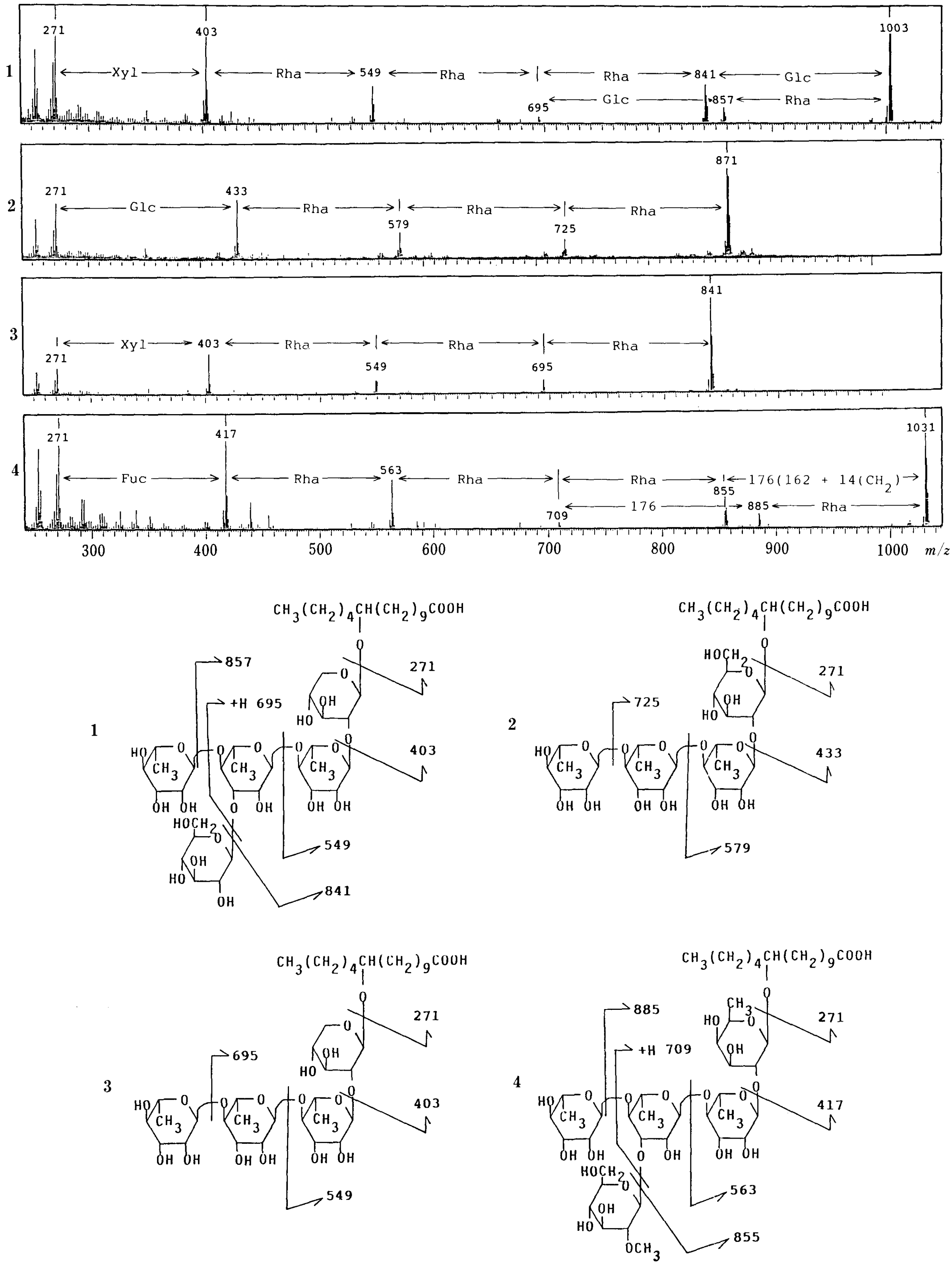


Fig. 1. Negative Ion FAB-MS of **1**, **2**, **3** and **4**

In order to determine the sequence of the sugar moiety, the NOESY spectrum of **1′** was recorded. Four of the cross peaks observed were assigned as those between 1-H of Glc and 3-H of Rha′, 1-H of Rha″ and 4-H of Rha′, 1-H of Rha′ and 4-H of Rha, and 1-H of Rha and 2-H and/or 3-H of Xyl.[5]

Accordingly, the structure of operculinic acid D (**1**) was concluded to be 11*S*-jalapinolic acid 11-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside.

Operculinic acid F methyl ester (**3′**), a white powder, mp 97—101 °C (dec.), $[\alpha]_D$ −93.6°, $C_{40}H_{72}O_{19}$, gave, on alkaline hydrolysis, operculinic acid F (**3**), a white powder,

TABLE I. ^{1}H-NMR Spectral Data for **1′**, **2′**, **3′**, **4′**, **8** and **9** (in Pyridine-d_5, 400 MHz)

	1′	**8**	**2′**	**3′**	**9**	**4′**
Xyl-1	4.84 (d, 7.0)	5.00 (d, 6.4)		4.81 (d, 7.3)	4.98 (d, 7.0)	
2	4.14$^{a)}$	4.06 (dd, 6.4, 8.5)		4.15 (dd, 7.3, 8.0)	4.13 (dd, 7.0, 9.0)	
3	4.14$^{a)}$	5.63 (dd, 8.5, 8.5)		4.12 (dd, 8.0, 8.0)	5.75 (dd, 9.0, 9.0)	
4	4.10$^{a)}$	5.20 (ddd, 5.0, 8.5, 8.5)		4.10 (ddd, 8.0, 9.0, 5.5)	5.25 (ddd, 9.0, 9.0, 6.0)	
5	3.63 (dd, 9.0, 11.0)	3.67 (dd, 8.5, 12.0)		3.61 (dd, 9.0, 11.5)	4.26$^{a)}$	
	4.29 (dd, 5.0, 11.0)	4.27 (dd, 5.0, 12.0)		4.26 (dd, 5.5, 11.5)	3.68 (dd, 9.0, 12.0)	
Glc-1			4.95 (d, 7.0)			
2			4.22$^{a)}$			
3			4.24$^{a)}$			
4			4.13 (dd, 9.0, 9.0)			
5			3.87 (ddd, 3.0, 5.0, 9.0)			
6			4.33 (dd, 5.0, 12.0)			
			4.49 (dd, 3.0, 12.0)			
Fuc-1						4.77 (d, 8.0)
2						4.46 (dd, 8.0, 9.5)
3						4.12 (dd, 9.5, 3.0)
4						3.93$^{a)}$
5						3.78 (q, 6.4)
6						1.51 (d, 6.4)
Rha-1	6.28 (d, 1.2)	5.39 (d, 1.8)	6.35 (d, 1.0)	6.30 (d, 1.0)	5.43 (d, 1.8)	6.20 (s)
2	4.66 (dd, 1.2, 3.0)	5.60 (dd, 1.8, 3.3)	4.64 (dd, 1.0, 3.5)	4.63 (dd, 1.0, 3.5)	5.69 (dd, 1.8, 4.0)	4.64 (br s)
3	4.59 (dd, 3.0, 8.5)	5.68$^{a)}$	4.61 (dd, 3.5, 9.0)	4.58 (dd, 3.5, 9.0)	5.72 (dd, 4.0, 9.0)	4.58 (br d, 9.0)
4	4.24 (dd, 8.5, 8.5)	4.13 (dd, 9.0, 9.0)	4.35 (dd, 9.0, 9.0)	4.33 (dd, 9.0, 9.0)	4.25 (dd, 9.0, 9.0)	4.21 (dd, 9.0, 9.0)
5	4.86 (dq, 8.5, 6.1)	4.65 (dq, 9.0, 6.1)	4.89 (dq, 9.0, 6.0)	4.83 (dq, 9.0, 6.1)	4.83 (dq, 9.0, 6.0)	4.84 (dq, 9.0, 6.1)
6	1.69 (d, 6.1)	1.65 (d, 6.1)	1.69 (d, 6.0)	1.66 (d, 6.1)	1.71 (d, 6.0)	1.58 (d, 6.1)
Rha′-1	5.86 (d, 1.5)	5.31 (d, 2.2)	6.22 (d, 1.0)	6.18 (d, 1.5)	5.42 (d, 1.5)	5.91 (s)
2	5.16 (dd, 1.5, 3.5)	5.48 (dd, 2.2, 3.5)	4.76 (dd, 1.0, 3.5)	4.74 (dd, 1.5, 3.0)	5.73$^{a)}$	5.09 (br s)
3	4.70 (dd, 3.5, 8.5)	4.56 (dd, 3.5, 10.0)	4.55 (dd, 3.5, 9.0)	4.54 (dd, 3.0, 9.0)	5.59 (dd, 3.0, 9.5)	4.61 (br d, 9.0)
4	4.47 (dd, 8.5, 8.5)	4.19 (dd, 10.0, 10.0)	4.42 (dd, 9.0, 9.0)	4.39 (dd, 9.0, 9.0)	4.23 (dd, 9.5, 9.5)	4.49 (dd, 9.0, 9.0)
5	4.40 (dq, 8.5, 6.1)	4.20$^{a)}$	4.36$^{a)}$	4.33$^{a)}$	4.36 (dq, 9.5, 6.0)	4.36 (dq, 9.0, 6.1)
6	1.60 (d, 6.1)	1.57 (d, 5.5)	1.59 (d, 6.0)	1.57 (d, 5.8)	1.62 (d, 6.0)	1.61 (d, 6.1)
Rha″-1	6.16 (d, 1.0)	5.68 (d, 1.5)	6.29 (d, 1.0)	6.25 (d, 1.5)	5.45 (s)	6.00 (s)
2	4.85 (dd, 1.0, 3.0)	5.77 (dd, 1.5, 3.4)	4.80 (dd, 1.0, 3.5)	4.78 (dd, 1.5, 3.0)	5.62$^{a)}$	4.69 (br s)
3	4.41 (dd, 3.0, 8.5)	5.68$^{a)}$	4.45 (dd, 3.5, 9.0)	4.42 (dd, 3.0, 9.0)	5.63 (dd, 3.5, 9.5)	4.39 (br d, 9.5)
4	4.20 (dd, 8.5, 8.5)	5.53 (dd, 10.0, 10.0)	4.25 (dd, 9.0, 9.0)	4.23 (dd, 9.0, 9.0)	5.55 (dd, 9.5, 9.5)	4.25 (dd, 9.5, 9.5)
5	4.29 (dq, 8.5, 6.1)	4.25$^{a)}$	4.34$^{a)}$	4.32$^{a)}$	4.28 (dq, 9.5, 6.0)	4.30 (dq, 9.5, 6.1)
6	1.56 (d, 6.1)	1.33 (d, 6.1)	1.59 (d, 6.0)	1.57 (d, 6.1)	1.37 (d, 6.0)	1.60 (d, 6.1)
Glc′-1	5.19 (d, 7.6)	5.20 (d, 8.0)				5.10 (d, 8.0)
2	3.97 (dd, 7.6, 8.0)	5.32 (dd, 8.0, 9.5)				3.38 (dd, 8.0, 9.0)
3	4.15$^{a)}$	5.68 (dd, 9.5, 9.5)				4.07 (dd, 9.0, 9.0)
4	4.07 (dd, 9.0, 9.0)	5.45 (dd, 9.5, 9.5)				4.06 (dd, 9.0, 9.0)
5	3.92 (ddd, 3.0, 5.0, 9.0)	4.05$^{a)}$				3.84$^{a)}$
6	4.25$^{a)}$	4.48 (dd, 2.1, 12.5)				4.23$^{a)}$
	4.51 (dd, 3.0, 11.0)	4.70 (dd, 3.7, 12.5)				4.48$^{a)}$
Ag- 2	2.33 (t, 7.4)	2.38 (t, 8.0)	2.31 (t, 7.4)	2.31 (t, 7.5)	2.37 (t, 7.2)	2.32 (t, 7.3)
11	3.95$^{a)}$	3.95 (m)	4.00 (m)	3.91 (m)	3.93 (m)	3.98 (m)
16	0.90 (t, 7.3)	0.94 (t, 7.0)	0.92 (t, 7.0)	0.90 (t, 7.5)	0.95 (t, 7.3)	0.92 (t, 6.7)
$COOCH_3$	3.62 (s)	3.64 (s)	3.63 (s)	3.63 (s)	3.63 (s)	3.62 (s)
OCH_3						3.93 (s)
$OCOCH_3$		1.95, 1.98, 2.00			2.00, 2.00, 2.03	
		2.03, 2.04, 2.06			2.05, 2.09, 2.12	
		2.07, 2.11, 2.17			2.16, 2.21, 2.23	
		2.18, 2.22, 2.31				

a) Signals are overlapping. δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses); Xyl, xylopyranosyl; Rha, rhamnopyranosyl; Glc, glucopyranosyl; Ag, aglycone (11*S*-jalapinolic acid). All assignments are based on the ^{1}H–^{1}H COSY and NOESY spectral data.

mp 104—109 °C (dec.), $[\alpha]_D$ −97.9°, $C_{39}H_{70}O_{19}$. The components of the acidic hydrolysate of **3′** were found to be jalapinolic acid, which was identified as the methyl ester, and L-rhamnose and D-xylose in the same manner as for **1′**. The negative ion FAB-MS of **3** revealed the $[M-H]^-$ ion peak at m/z 841 and fragment ion peaks at m/z 695 $[841-146]^-$, 549 $[695-146]^-$, 403 $[549-146]^-$, 271 $[403-132]^-$ (Fig. 1). In addition to the MS data, the fact of coexistence of operculinic acid A (**5**) and its deglucosyl derivative, operculinic acid C (**6**), suggested that the sugar moiety of **3** might be a linear tetrasaccharide lacking the terminal glucose of **1**.

The ^{1}H- and ^{13}C-NMR signals due to the sugar moieties of **3′** and its peracetate (**9**), a white powder, mp 57—61°C (dec.), $[\alpha]_D$ −43.3°, were assigned by referring to the COSY and HETCOR spectra (Tables I and II), indicating that the glycosidic linkages are placed at 2-OH of Xyl, and 4-OH of two rhamnose units (Rha and Rha′). This was confirmed by the fact that three cross peaks between 1-H of Rha and 2-H of Xyl, 1-H of Rha′ and 4-H of Rha, and 1-H of Rha″ and 4-H of Rha′ observed in the NOESY spectrum of **9**.

Therefore, the structure of operculinic acid F (**3**) was

TABLE II. ^{13}C-NMR Spectral Data for **1′**, **2′**, **3′** and **4′** (in Pyridine-d_5, 100 MHz)

	1′	**2′**	**3′**	**4′**	Methyl glycoside α	Methyl glycoside β
Xyl-1	101.7		101.9		101.5	106.0
2	77.5		77.3		73.7	74.6
3	79.5		79.6		75.5	78.1
4	71.5		71.4		71.4	70.9
5	67.1		67.0		63.1	67.0
Glc-1		101.1			101.3	105.5
2		77.1			73.7	74.9
3		79.6			75.3	78.2
4		72.0			72.0	71.4
5		78.0			74.0	78.2
6		62.8			62.7	62.6
Fuc-1				101.2	101.6	105.9
2				75.1	70.0	72.0
3				76.5	71.5	75.2
4				73.4	73.1	72.6
5				71.2	66.9	71.3
6				17.2	17.1	17.2
Rha-1	101.7	101.3	101.6	101.5	102.6	102.6
2	72.6	72.6	72.7	72.6	72.1	72.1
3	72.6	73.1	73.2	72.7	72.7	75.3
4	82.4	80.8	80.7	82.2	73.8	73.7
5	67.8	67.2	67.4	67.5	69.5	73.4
6	18.8	19.1	19.0	18.7	18.6	18.5
Rha′-1	103.5	102.9	102.9	103.1		
2	71.9	73.0	73.1	71.6		
3	82.7	73.4	73.4	82.6		
4	78.6	79.6	79.6	77.9		
5	68.6	68.3	68.4	68.6		
6	19.0	18.8	18.8	19.3		
Rha″-1	103.1	102.9	102.9	102.4		
2	72.6	72.4	72.4	72.5		
3	72.8	72.7	72.7	72.8		
4	73.9	73,9	73.9	73.8		
5	70.4	70.2	70.2	70.4		
6	18.4	18.4	18.4	18.4		
Glc′-1	105.5			104.0		
2	75.1			84.8		
3	78.4			78.2		
4	71.7			71.5		
5	78.4			78.2		
6	62.9			62.6		
Ag- 1	174.1	174.0	174.0	174.1		
11	78.1	78.2	78.3	78.0		
16	14.3	14.4	14.3	14.4		
$COOCH_3$	51.3	51.3	51.3	51.3		
OCH_3				61.2		

δ in ppm from TMS. Xyl, xylopyranosyl; Glc, glucopyranosyl; Fuc, fucopyranosyl; Rha, rhamnopyranosyl; Ag, aglycone. All assignments are based on the HETCOR spectral data.

concluded to be 11*S*-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside, corresponding to deglucosyl operculinic acid D (**1**), as shown in Fig. 2.

Operculinic acid E methyl ester (**2′**), a white powder, mp 99—102 °C (dec.), $[\alpha]_D$ $-65.0°$, $C_{41}H_{74}O_{20}$, liberated, on acidic hydrolysis, jalapinolic acid, which was analyzed as the methyl ester, together with L-rhamnose and D-glucose, which were identified in the same manner as above. The alkaline hydrolysis product of **2′**, operculinic acid E (**2**), a white powder, mp 114—116 °C (dec.), $[\alpha]_D$ $-76.8°$, $C_{40}H_{72}O_{20}$, showed the $[M-H]^-$ ion peak at *m*/*z* 871 and fragment peaks at *m*/*z* 725 $[871-146]^-$, 579 $[725-146]^-$, 433 $[579-146]^-$, 271 $[433-162$, jalapinolic acid-H$]^-$ in the negative ion FAB-MS (Fig. 1), indicating that **2** is a tetraglycoside which consists of 1 mol each of jalapinolic acid and D-glucose, and 3 mol of L-rhamnose, and that the glucose is linked at 11-OH of jalapinolic acid.

Inspection of the ^{1}H- and ^{13}C-NMR spectra (Tables I and II) of **2′** indicated that the glycosidic linkages of **2′** are placed at 2-OH of Glc, 4-OH of Rha, 4-OH of Rha′ and 11-OH of the jalapinolic acid moiety, and that the linkages of the rhamnose units are α in $^1C_4$ conformation and those of the glucose units are *β* in $^4C_1$. The arrangement of sugar linkages was determined from the NOESY spectrum of **2′**, that is, three cross peaks between 1-H of Rha and 2-H of Glc, 1-H of Rha′ and 4-H of Rha, and 1-H of Rha″ and 4-H of Rha′ were seen.

Consequently, the structure of operculinic acid E (**2**) was defined as 11*S*-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2) -*β*-D-glucopyranoside, which corresponds to the deglucosyl derivative of operculinic acid B (**7**), as shown in Fig. 2.

Operculinic acid G methyl ester (**4′**), a white powder, mp 107—113 °C (dec.), $[\alpha]_D$ $-68.2°$, $C_{48}H_{86}O_{24}$, showed an additional methoxy methyl signal (δ 3.93) besides that of ester methyl (δ 3.62) due to the jalapinolic acid moiety, along with signals of five anomeric protons and an equivalent methylene in the ^{1}H-NMR spectrum of **4′**, and the $[M-H]^-$ ion peak at *m*/*z* 1045 in the negative ion FAB-MS. From the data, it was presumed that the structure of **4′** might be a monomethyl ether of operculinic acid A methyl ester (**5′**). Compounds **4′** and **5′** were each methylated to furnish the same permethylate (**10**), a colorless syrup. The products were confirmed to be identical with each other by comparison of their ^{1}H-NMR spectra and hence the presumption was confirmed.

Alkaline hydrolysis of **4′** gave operculinic acid G (**4**), a white powder, mp 110—114 °C (dec.), $[\alpha]_D$ $-62.0°$, which showed the $[M-H]^-$ ion peak at *m*/*z* 1031 and fragment ion peaks at *m*/*z* 885, 855, 709, 563, 417 and 271 in the negative ion FAB-MS (Fig. 1). The peak at *m*/*z* 855 corresponding to $[M-H\text{-}(162(\text{hexose unit})+14(CH_2))]^-$ indicated that the additional methoxy group was located in the terminal glucose unit of **5**. In a comparison of the ^{13}C-NMR data of **4′** and **5′**, the signal due to C_2 of glucose was shifted downfield (9.8 ppm).[4]

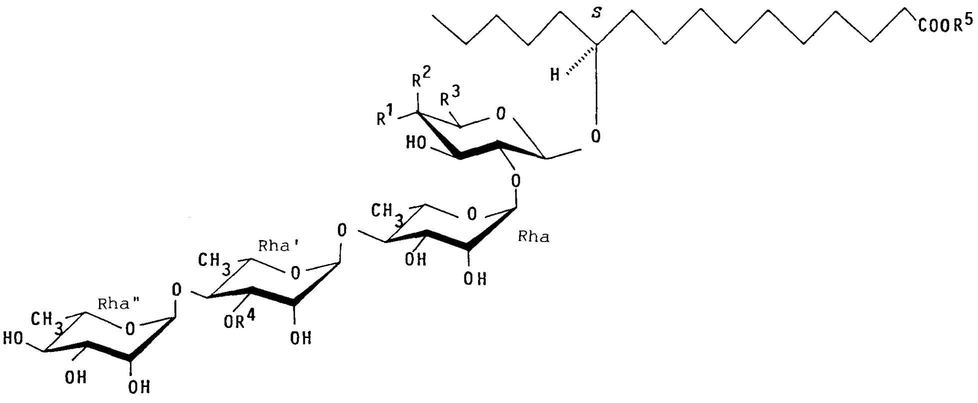
On the basis of all the above data, the structure of **4** is defined as 11*S*-jalapinolic acid 11-*O*-(2-*O*-methyl-*β*-D-glucopyranosyl-(1→3))-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside.

Among the seven glycosidic acids so far isolated from this crude drug, operculinic acids C,[2] E and F correspond to the deglucosyl derivarives of the parent glycosides, perculinic acids A,[2] B[2] and D, while operculinic acid G may be an artifact produced from operculinic acid A during treatment with diazomethane in the separation procedure.[6]

Experimental

The instruments and materials generally used were as cited in the preceding report[2] unless otherwise specified.

Isolation of Methyl Esters of Operculinic acids D (1′), E (2′), F (3′) and G (4′) The fractions 3 (427 mg) and 4 (673 mg) obtained previously[2] from the methyl ester of glycosidic acid fraction were each chromatographed on a Fuji-gel ODS G-3 column (3.6 cm i.d. × 22 cm, 75% MeOH)



1: $R^1 = OH$, $R^2 = R^3 = R^5 = H$, $R^4 = Glc \cdot p$ (operculinic acid D)
1′: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Glc \cdot p$ (Glc′), $R^5 = CH_3$
2: $R^1 = OH$, $R^2 = R^4 = R^5 = H$, $R^3 = CH_2OH$ (operculinic acid E)
2′: $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = CH_2OH$, $R^5 = CH_3$
3: $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = H$ (operculinic acid F)
3′: $R^1 = OH$, $R^2 = R^3 = R^4 = H$. $R^5 = CH_3$
4: $R^1 = R^5 = H$, $R^2 = OH$, $R^3 = CH_3$, $R^4 = Glc \cdot p$ (2-OCH$_3$) (operculinic acid G)
4′: $R^1 = H$, $R^2 = OH$, $R^3 = R^5 = CH_3$, $R^4 = Glc \cdot p$ (2-OCH$_3$) (Glc′)
5: $R^1 = R^5 = H$, $R^2 = OH$, $R^3 = CH_3$, $R^4 = Glc \cdot p$ (operculinic acid A)
6: $R^1 = R^4 = R^5 = H$, $R^2 = OH$, $R^3 = CH_3$ (operculinic acid C)
7: $R^1 = OH$, $R^2 = R^5 = H$, $R^3 = CH_2OH$, $R^4 = Glc \cdot p$ (operculinic acid B)
Glc·*p*: *β*-D-glucopyranosyl, Glc·*p* (2-OCH$_3$): 2-*O*-methyl-*β*-D-glucopyranosyl

Fig. 2

to give fraction (fr.) 9 (34 mg) and fr. 10 (111 mg), and fr. 11 (112 mg) and fr. 12 (519 mg), respectively. Fraction 10 was subjected repeatedly to preparative HPLC on Nucleosil 5C8 (2 cm i.d. × 25 cm, 65% MeOH) and Kusano CIG Si gel (2.2 cm i.d. × 10 cm, $CHCl_3$–MeOH–H_2O, 6 : 4 : 0.3) columns followed by evaporation under reduced pressure to give **3′** (30 mg). HPLC of fr. 12 under similar conditions to those used for fr. 10 furnished **4′** (49 mg).

The procedure for separation of **1′** and **2′** was described in the previous report.[2)]

1′: A white powder (MeOH–H_2O), mp 170—172 °C (dec.), $[\alpha]_D^{23}$ $-81.7°$ ($c = 1.0$, MeOH), IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). Negative FAB-MS *m/z*: 1017 $[(M-H)^-]$. *Anal.* Calcd for $C_{46}H_{82}O_{24}$: C, 54.21; H, 8.11. Found: C, 54.19; H, 7.93. ^{1}H-NMR δ: see Table I. ^{13}C-NMR δ: see Table II, $J_{C_1-H_1}$ (Hz): Rha (170), Rha′ (169), Rha″ (172), Xyl (156), Glc (158).

2′: A white powder (MeOH–H_2O), mp 99—102 °C (dec.), $[\alpha]_D^{18}$ $-65.0°$ ($c = 1.2$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). Negative FAB-MS *m/z*: 885 $[(M-H)^-]$. *Anal.* Calcd for $C_{41}H_{74}O_{20}$: C, 55.52; H, 8.41. Found: C, 55.75; H, 8.32. ^{1}H-NMR δ: see Table I. ^{13}C-NMR δ: see Table II, $J_{C_1-H_1}$ (Hz): Rha (174), Rha′ (171), Rha″ (173), Glc (157).

3′: A white powder, mp 97—101 °C (dec.), $[\alpha]_D^{21}$ $-93.6°$ ($c = 3.6$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). Negative FAB-MS *m/z*: 885 $[(M-H)^-]$. *Anal.* Calcd for $C_{40}H_{72}O_{19}$: C, 56.06; H, 8.47. Found: C, 56.08; H, 8.49. ^{1}H-NMR δ: see Table I. ^{13}C-NMR δ: Table II.

4′: A white powder, mp 107—113 °C (dec.), $[\alpha]_D^{18}$ $-68.2°$ ($c = 4.0$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1725 (C=O). Negative FAB-MS *m/z*: 1045 $[(M-H]^-]$. *Anal.* Calcd for $C_{48}H_{86}O_{24}$: C, 55.05; H, 8.28. Found: C, 55.15; H, 8.34. ^{1}H-NMR δ: see Table I. ^{13}C-NMR δ: see Table II.

Acidic Hydrolysis of 1′, 2′ and 3′ Compounds **1′** (30 mg), **2′** (10 mg) and **3′** (9 mg) were each dissolved in 2 N HCl (2 ml) and the solution was heated at 95 °C for 1 h. The reaction mixture was diluted with H_2O (10 ml) and extracted with ether (3 × 5 ml), and the ether layer was washed with H_2O, dried over $MgSO_4$ and then treated with diazomethane in ether. The mixture was evaporated to give colorless needles (5 mg from **1′**, 2 mg from **2′**, 2 mg from **3′**), mp 42—44 °C. This product was identical with an authentic sample of methyl jalapinolate obtained previously from the root of *I. orizabensis*[7)] by GC (column, 3.2 mm i.d. × 2 m glass column packed with silicone OV-17 (2%); carrier gas, N_2 (1.5 kg/cm^2); column temperature, 190 °C) t_R (min): 7.57 (methyl jalapinolate) and TLC (Silica gel F_{254} (Merck Art 5554), benzene–AcOEt, 4 : 1), *Rf*: 0.70 (methyl jalapinolate). The aqueous layer was neutralized with 3% aqueous KOH and desalted by chromatography over LH-20 (MeOH) to afford a sugar mixture (21 mg from **1′**, (6 mg from **2′**, 6 mg from **3′**), as a syrup, which was subjected to TLC analysis [a) HPTLC plate Silica gel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1; b) TLC (Avicel SF (Funakosi Pharm. Co.), BuOH–pyridine–H_2O (6 : 2 : 3) top layer + pyridine (1)] *Rf*: a) 0.29 (glucose), 0.40 (xylose), 0.54 (rhamnose); b) 0.39 (glucose), 0.55 (xylose), 0.65 (rhamnose) for **1′**; a) 0.29 (glucose), 0.54 (rhamnose); b) 0.39 (glucose), 0.65 (rhamnose) for **2′**; a) 0.40 (xylose), 0.54 (rhamnose); b) 0.55 (xylose), 0.65 (rhamnose) for **3′**.

The monosaccharide mixtures of **1′**, **2′** and **3′** (each 1 mg) were each subjected to GC analysis as the TMS ethers of the methyl thiazolidine 4(*R*)-carboxylate derivatives according to Hara *et al.*[3)] GC (Hitachi G-3000 gas chromatograph equipped with a 30 : 1 splitter and a flame ionizing detector; column, fused silica capillary column Bonded MPS-50 (Quadrex), 0.25 mm i.d. × 50 m, 0.25 μm film thickness; carrier gas, He (30 ml/min); column temperature, 220 °C) t_R (min): 17.21 (D-xylose), 19.51 (L-rhamnose), 27.14 (D-glucose) for **1′**; 19.51 (L-rhamnose), 27.07 (D-glucose) for **2′** and 17.20 (D-xylose), 19.50 (L-rhamnose) for **3′**.

Alkaline Hydrolysis of 1′, 2′, 3′ and 4′ Solutions of **1′** (50 mg), **2′** (22 mg), **3′** (30 mg) and **4′** (20 mg) in 3% aqueous KOH (6 ml) were each heated at 95 °C for 1 h. The reaction mixtures were adjusted to pH 4.0 with 1 N HCl, desalted by using chromatography over MCI gel CHP 20P, and evaporated under reduced pressure to afford **1** (28 mg), **2** (18 mg), **3** (23 mg) and **4** (17 mg), respectively.

1: A white powder, mp 175—177 °C (dec.), $[\alpha]_D^{16}$ $-88.6°$ ($c = 0.7$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1700 (C=O). Negative FAB-MS *m/z*: see Fig. 1. *Anal.* Calcd for $C_{45}H_{80}O_{24}$: C, 53.77; H, 8.02. Found: C, 53.92; H, 8.03.

2: A white powder, mp 114—116 °C (dec.), $[\alpha]_D^{18}$ $-76.8°$ ($c = 1.6$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1700 (C=O). Negative FAB-MS *m/z*: see Fig. 1. *Anal.* Calcd for $C_{40}H_{72}O_{20}$: C, 55.03; H, 8.31. Found: C, 55.06; H, 8.36.

3: A white powder, mp 104—109 °C (dec.), $[\alpha]_D^{32}$ $-97.9°$ ($c = 2.2$, MeOH). IR (KBr) cm^{-1}: 3400 (br, OH), 1700 (C=O). Negative FAB-MS *m/z*: see Fig. 1. *Anal.* Calcd for $C_{39}H_{70}O_{19}$: C, 55.57; H, 8.37. Found: C, 55.59; H. 8.38.

4: A white powder, mp 110—114 °C (dec.), $[\alpha]_D^{24}$ $-62.0°$ ($c = 1.3$, MeOH). IR (KBr) cm^{-1}; 3400 (br, OH), 1700 (C=O). Negative FAB-MS *m/z*: see Fig. 1. *Anal.* Calcd for $C_{47}H_{84}O_{24}$: C, 54.64; H, 8.19. Found: C, 54.66; H, 8.23.

Acetylation of 1′ and 3′ Compounds **1′** (20 mg) and **3′** (10 mg) were each dissolved in Ac_2O–pyridine (1 : 1, 3 ml), and the solution was left to stand at room temperature overnight, then poured into ice-water (50 ml). The precipitate was collected by filtration. **8** (24 mg): a white powder, mp 75—78 °C (dec.), $[\alpha]_D^{20}$ $-48.8°$ ($c = 0.8$, MeOH). IR (KBr) cm^{-1}: 1740 (C=O). ^{1}H-NMR δ: see Table I. **9** (7 mg): a white powder, mp 57—61 °C (dec.), $[\alpha]_D^{27}$ $-43.3°$ ($c=1.0$, MeOH). IR (KBr) cm^{-1}: 1750 (C=O). ^{1}H-NMR δ: see Table I.

Permethylation of 4′ and 5′ A solution of **4′** (5 mg) or **5′** (21 mg) in *N*,*N*-dimethylformamide (DMF) (2 ml) was treated with NaH (50 mg) and CH_3I (2 ml) under stirring. The mixture was stirred at room temperature overnight. The solvent was removed under an N_2 stream and then H_2O (2 ml) was added. The mixture was extracted with Et_2O and the extractive was purified by silica gel chromatography (Merck Art 9385, 2.2 cm i.d. × 15 cm, benzene–acetone, 5 : 1→3 : 1) to furnish **10′** (2 mg from **4′**, 10 mg from **5′**), colorless syrup, ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.90 (3H, t, $J=7.0$ Hz, 16-H_3), 1.36 (3H, d, $J=6.4$ Hz, *sec*-$C\underline{H}_3$), 1.41 (3H, d, $J=6.1$ Hz, *sec*-$C\underline{H}_3$), 1.53 (3H, d, $J=5.8$ Hz, *sec*-$C\underline{H}_3$), 1.56 (3H, d, $J=6.1$ Hz, *sec*-$C\underline{H}_3$), 2.38 (2H, t, $J=7.3$ Hz, 2-H_2 of jalapinolic acid group), 4.70 (1H, d, $J=7.6$ Hz, 1-H of fucose or glucose unit), 4.79 (1H, d, $J=7.9$ Hz, 1-H of fucose or glucose unit), 5.56 (1H, d, $J=1.8$ Hz, 1-H of rhamnose unit), 5.70 (2H, d, $J=1.5$ Hz, 1-H of rhamnose unit × 2), 3.70, 3.69, 3.66, 3.66, 3.64, 3.61, 3.60, 3.58, 3.55, 3.54, 3.53, 3.52, 3.51 (3H, each, s, $OC\underline{H}_3$).

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