Intrapilosins I−VII, Pentasaccharides from the Seeds of *Ipomoea intrapilosa*

Moustapha Bah,† Lilia Chérigo,‡ Alexandre T. Cardoso Taketa,§ Mabel Fragoso-Serrano,‡ Gerald B. Hammond,⊥ and Rogelio Pereda-Miranda*,‡

CEACA, Facultad de Química, Universidad Autónoma de Querétaro, Querétaro, Cerro de Las Campanas, 76010, Querétaro, Mexico, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Mexico, Department of Chemistry, University of Louisville, Louisville, Kentucky 40292, and Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City 04510 DF, Mexico

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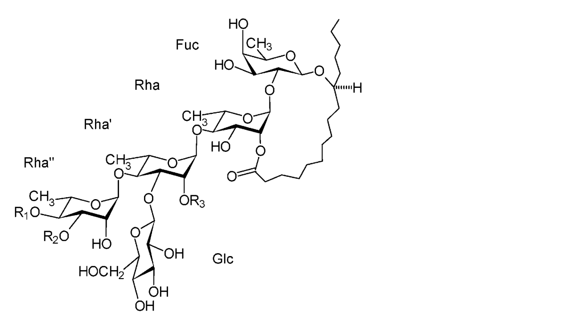
Purification of a $CHCl_3$-soluble extract from seeds of the Mexican medicinal arborescent morning glory, *Ipomoea intrapilosa*, by means of preparative-scale recycling HPLC, yielded seven new resin glycosides, intrapilosins I−VII (**1**−**7**). Their structures were established through the interpretation of their NMR spectroscopic and FABMS data. All pentasaccharides were found to be macrolactones of the known operculinic acid A with different fatty acids esterifying the same positions: C-2 on the second rhamnose unit and C-3 and C-4 on the third rhamnose moiety. The lactonization site of the aglycon could be placed at C-2 of the second saccharide. The fatty acid components of **1**−**7** were identified as (+)-(2*S*)-methylbutanoic, octanoic (caprylic), dodecanoic (lauric), and *trans*-cinnamic. The less common (−)-(2*R*)-methylbutanoic acid was also isolated as one of the saponification-liberated residues from intrapilosin IV (**4**). The presence of the (2*R*)- and (2*S*)-methylbutanoyl enantiomers bonded to the same oligosaccharide core in intrapilosins IV (**4**) and V (**5**) represents an example of diastereoisomerism due to a chiral esterifying moiety in the resin glycoside mixtures of a morning glory species.

The Mexican medicinal plant complex[1] of arborescent morning glories, called vernacularly “cazahuate”,[2] is composed of six related tree-like *Ipomoea* species: *I. arborescens* (Kunth) G. Don,[3] *I. bracteata* Cav., *I. intrapilosa* Rose, *I. murucoides* Roem. & Schult,[4] *I. pauciflora* Martens & Galeotti, and *I. wolcottiana* Rose, and has been used since prehispanic times[5] to treat skin conditions such as itching and rashes.[4,6] *I. intrapilosa* is endemic to the “Sierra Madre Occidental” (Western Sierra Madre) from Southern Sinaloa to Jalisco[7] and grows also in the central volcanic region that includes the states of Michoacán and Morelos. Indeed, in some localities in the State of Morelos, two “cazahuate” species (*I. murucoides* and *I. intrapilosa*) are the dominant plants in the vegetation of the seasonal dry tropical rain forest. In these regions, an infusion of the flowers is used topically to treat rheumatism and ear pain, and the bark is chewed for toothache as well as burned to repel insects. A bark infusion complemented with the wood, leaves, flowers, and seeds of the same plant is also used as an antidote for scorpion and snake bites.[4,6]

This paper presents the results of a study based on the chemical analysis of the resin glycoside mixture obtained from *I. intrapilosa* seeds from which seven new acylated pentasaccharides of jalapinolic acid were isolated and their structures characterized as a step toward understanding the chemical diversity[8] of this inadequately studied group of bioactive principles that represent a potential new treatment for multidrug-resistant bacterial strains.[9]

Results and Discussion

A small portion of the combined two most abundant resin glycoside fractions, obtained from the fractionation of the $CHCl_3$-soluble extract, was submitted to saponification and yielded a water-soluble glycosidic acid and an organic solvent-soluble acidic fraction. The glycosidic acid was identified as operculinic acid A, (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-fucopyranoside, previously obtained from *I. operculata*,[10] *I. leptophylla*,[11] and *I. murucoides*.[4] Evidence for the absolute stereochemistry of the sugars as well as the configuration of the anomeric linkages was published when this oligosaccharide core was first elucidated.[10] HPLC analysis of the acid hydrolysis-liberated monosaccharides led to the identification of rhamnose, fucose, and glucose by coelution experiments with retention time identification using standard samples. Optical activity measurements of the isolated HPLC eluates confirmed that the three monosaccharides were in their naturally occurring form, i.e., the L-series for rhamnose and the D-series for fucose and glucose. The liberated fatty acids were identified by GC-MS as 2-methylbutanoic (mba), *n*-octanoic, *n*-dodecanoic, and *trans*-cinnamic (CA) acids. Individual constituents of the remaining portion of these resin glycoside fractions were separated and purified by the recycling HPLC technique, using a preparative reversed-phase column. These procedures led to the isolation and structural characterization of seven compounds, for which the names intrapilosins I−VII (**1**−**7**) are proposed.



	R_1	R_2	R_3
1	(+)-(2*S*)-methylbutanoyl = (+)-mba	cinnamoyl = CA	(+)-mba
2	*n*-octanoyl = octa	CA	(+)-mba
3	octa	CA	octa
4	(–)-(2*R*)-mba	CA	*n*-dodecanoyl = dodeca
5	(+)-mba	CA	dodeca
6	CA	(+)-mba	dodeca
7	octa	CA	dodeca

Although the interglycosidic linkages have been established already for operculinic acid A,[10] one- and two-dimensional $^1H-^1H$ and $^1H-^{13}C$ NMR spectra were obtained herein for all

* To whom correspondence should be addressed. Tel: +52-55-5622-5288. Fax: +52-55-5622-5329. E-mail: pereda@servidor.unam.mx.
† Universidad Autónoma de Querétaro.
‡ Universidad Nacional Autónoma de México.
§ Universidad Autónoma del Estado de Morelos.
⊥ University of Louisville.

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intrapilosins (**1**−**7**).[8,13] Common features in both ^1H and ^{13}C NMR spectra of the seven compounds are noted in Tables 1 and 2. All ^1H NMR spectra showed significantly downfield shifted signals for H-2 of the second rhamnose unit (rha′), as well as for H-3 and H-4 of the third rhamnose unit (rha″), suggesting esterification at these positions. The multiplets (sometimes splitting as a ddd) centered at δ 2.47 and 2.26 showed cross-peaks in their COSY and TOCSY spectra, revealing the macrocyclic lactone-type structure of compounds **1**−**7** because these signals correspond to the nonequivalent diastereotopic protons of the methylene C-2 of the aglycon (11*S*-hydroxyhexadecanoic acid, jal) when forming a ring.[8,13] The lactonization could be placed at C-2 of the second saccharide (rha) by the observed ^3J correlations (HMBC).[8] In their ^{13}C NMR spectra (Table 2), 2-methylbutanoic acid residues were confirmed in **1**, **2**, and **4**−**6**, due to the observed signals for C-2 at δ 41−42 and the corresponding carbonyls near δ 176. Distinctive *trans*-olefinic (δ 6.61 and 7.86, $J = 16.0$ Hz) and aromatic protons (δ 7.34 and 7.46) confirmed the presence of a *trans*-cinnamoyl group (Table 1). The anomeric configuration in each sugar unit was deduced from a 2D $^1J_{CH}$ NMR experiment.[8] The anomeric signals in the ^{13}C NMR spectra of all intrapilosins (**1**−**7**) showed $^1J_{CH}$ values for fucose (160 Hz) and glucose (164 Hz), supporting their β-anomeric configuration in the $^4C_1$ conformation. The α-configuration was deduced for the L-rhamnopyranosyl unit ($^1J_{CH}$ = 171 Hz). The exact location of the acyl groups on the oligosaccharide core was then determined by the measured $^{2,3}J$ correlations in the HMBC spectra.[8,13] For example, the following interactions were noted for the lowest molecular weight isolated compound, intrapilosin I (**1**, *m*/*z* 1297 [M − H]$^-$): correlations for the carbonyl carbon at δ 166.4 with H-2 (δ 6.61) and H-3 (δ 7.86) of the *trans*-cinnamoyl group, as well as with rha″ H-3 (δ 6.01); C-1 (δ 176.2) of the mba residue with mba H-2 (δ 2.41) and rha′ H-2 (δ 6.34); C-1 (δ 175.9) of the second mba (mba′) with mba′ H-2 (δ 2.49) and rha″ H-4 (δ 6.10); C-1 (δ 173.1) of the aglycon with jal H-2 (δ 2.44 and 2.27) and rha H-2 (δ 5.93). The same experiments were used to locate the *trans*-cinnamoyl group on rha″ C-3 in the rest of the intrapilosins, with the exception of compound **6**, where this residue was found on rha″ C-4 because of the cross-peak observed between signals at δ 6.09 (rha″ H-4) and 166.4 (CA C-1). The nature of the other acid residues was difficult to determine by NMR spectroscopy. However, negative FABMS solved this problem. All compounds displayed the same glycosidic cleavage as previously described for the pescaprein series.[12] Common fragment peaks were observed in all mass spectra, confirming the branched pentasaccharide core, and the resulting diagnostic peaks indicated the position for the esterifying moieties.[8]

For compound **2** (*m*/*z* 1339 [M − H]$^-$; $C_{68}H_{107}O_{26}$), a peak at *m*/*z* 937 [M − H − 130 (C_9H_6O, cinnamoyl) − 126 ($C_8H_{14}O$, octanoyl) − 146 ($C_6H_{10}O_4$, methylpentose)]$^-$ (also seen in **1**; [M − H − C_9H_6O − C_5H_8O (mba) − $C_6H_{10}O_4$,]$^-$) suggested that the mba group is on the rha′ C-2 hydroxyl group and that the additional acid residue on rha″ C-4 must be an octanoate group. Intrapilosin VII (**7**) (*m*/*z* 1437 [$C_{75}H_{121}O_{26}$]$^-$) showed a fragment at *m*/*z* 1035, and the difference of 98 amu (C_7H_{14}) from *m*/*z* 937 observed in **1** and **2** indicated the occurrence of a dodecanoyl group on rha′ C-2. The proton signal for this center (rha′ H-2, δ 6.35) showed a cross-peak with the carbonyl carbon at δ 173.2 in the HMBC spectrum. The observed interaction between rha″ H-4 (δ 6.12) with the carbonyl group at δ 173.5 confirmed the placement of the second fatty acid residue (octanoyl group) at this position of the terminal rhamnose unit. A negative HRFABMS analysis of **3** produced a pseudomolecular ion at *m*/*z* 1381 [M − H]$^-$, which indicated the chemical formula $C_{71}H_{113}O_{26}$. A peak at *m*/*z* 979 [M − H − C_9H_6O − $C_8H_{14}O$ − $C_6H_{10}O_4$]$^-$ suggested that the rha′ unit was esterified by an octanoyl group at C-2 because of the strong deshielding of its geminal proton (δ 5.96). In addition, the fragment at *m*/*z* 1125 [M − C_9H_7O − $C_8H_{14}O$]$^-$ confirmed the presence of a second octanoyl group at rha″ (H-4, δ 6.13).

In compounds **4**−**6**, the same molecular ion peak at *m*/*z* 1395 [M − H]$^-$ indicated the molecular formula $C_{72}H_{115}O_{26}$. The ion at *m*/*z* 1213 [M − H − 182 ($C_{12}H_{22}O$)]$^-$ showed that a dodecanoyl group is present as one of the three esterifying residues. The observed peak at *m*/*z* 1035 [M − H − C_9H_7O − C_5H_8O − $C_6H_{10}O_4$]$^-$ was used to place this residue at rha′ C-2 in all three substances. For compound **6**, the placement of the remaining residues was deduced by the observed HMBC correlations between mba C-1 (δ 175.9) and rha″ H-3 (δ 6.03) and between CA C-1 (δ 166.4) and rha″ H-4 (δ 6.09) (Tables 1 and 2). Compounds **4** and **5** displayed similar FABMS and NMR spectra. HMBC studies were used to locate mba and *trans*-cinnamic acids on the same positions at rha″ C-3 and C-4, respectively. The difference between these two isomers resides in their specific optical rotations and melting points. Therefore, the cause for this diastereoisomerism must be the absolute configuration of the mba group in **4** and **5** since this residue represents the only chiral ester moiety on the oligosaccharide core.

Alkaline hydrolysis of **4** and **5**, followed by esterification with benzyl alcohol of the $CHCl_3$-soluble acids recovered from this procedure[14] together with determination through optical activity of the benzyl 2-methylbutanoates isolated by HPLC, revealed a levorotatory property for the residue present in **4** and a dextrorotation for that present in **5**. The latter value was correlated with (*S*)-(+)-benzyl 2-methylbutanoate, $[\alpha]_D$ +10 (*c* 1.8, $CHCl_3$),[14] prepared from a commercial sample. Therefore, intrapilosin IV (**4**) contains (*R*)-(−)-2-methylbutanoic acid as the chiral esterifying moiety. The rest of the isolated intrapilosins yielded (*S*)-(+)-2-methylbutanoic acid.

The purification of the fraction containing the major compound **5** (in a yield of 85% for the peak area) through recycling HPLC permitted the isolation of **4** (15%). This enantiomeric distribution for both the 2*R* and 2*S* mba enantiomers in intrapilosins IV (**4**) and V (**5**) is similar to that previously reported in some fruits and other foodstuffs, where the major enantiomer represents the *S* configuration.[15] A similar diasteroisomerism has been described in the convolvulaceous resin glycosides for the orizabin series, where the mixtures contained the 2*R*, 3*R* and 2*S*, 3*S* enantiomers of 3-hydroxy-2-methylbutanoic acid.[16]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^1H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted on a Bruker DMX-500 instrument. The NMR techniques were performed according to a previously described methodology.[13] Negative-ion LRFABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX-102A spectrometer. HPLC separations were conducted on a Waters apparatus (Millipore Corp., Waters Chromatography Division, Milford, MA), composed of a 600E multisolvent delivery system equipped with a 996 photodiode array detector. Control of the equipment, data acquisition, processing, and management of the chromatographic information were performed by the Empower 2 software (Waters). GC-MS was performed on a Hewlett-Packard 5890-II instrument coupled to a JEOL SX-102A spectrometer. GC conditions: HP-5MS (5%-phenyl)-methylpolysiloxane column (30 m × 0.25 mm, film thickness 0.25 μm); He, linear velocity 30 cm/s; 50 °C isothermal for 3 min, linear temperature gradient to 300 °C at 20 °C/min; final temperature hold, 10 min. MS conditions: ionization energy, 70 eV; ion source temperature, 280 °C; interface temperature, 300 °C; scan speed, 2 scans s^{-1}; mass range, 33−880 amu.

Plant Material. Seeds of *Ipomoea intrapilosa* were collected in Cuernavaca, Morelos, Mexico, in January and February 1996. Voucher specimens (RP-013 and RP-014) were identified by the botanist Gustavo Soria Rocha, Universidad Autónoma del Estado de Morelos (Mexico), by comparison with an authentic sample collected in Xochitepec,

Table 1. ^{1}H NMR Spectroscopic Data for **1−7** (500 MHz)[a]

proton[b]	**1**	**2**	**3**	**4**	**5**	**6**	**7**
fuc-1	4.73 d (7.5)	4.72 d (7.5)	4.76 d (7.0)	4.75 d (7.5)	4.74 d (7.5)	4.73 d (7.3)	4.75 d (7.0)
2	4.16 dd (9.5, 7.5)	4.16 dd (9.0, 7.5)	4.18 dd (9.5, 7.0)	4.17 dd (9.0, 7.5)	4.17 dd (9.5, 7.5)	4.15 dd (9.5, 7.3)	4.18 dd (9.5, 7.0)
3	4.04−4.10 m*	4.07 dd (9.0, 3.5)	4.11 dd (9.5, 3.0)	4.10 dd (9.0, 3.5)	4.09 dd (9.5, 3.5)	4.0 dd (9.5, 3.4)	4.08 dd (9.5, 3.5)
4	3.96 d (2.5)	3.95 bs	3.98 d (3.0)	3.86 d (3.5)	3.97 d (3.5)	3.95 d (3.4)	3.97 d (3.5)
5	3.74 q (6.2)	3.74 q (6.0)	3.76 q (6.5)	3.77 q (6.5)	3.75 q (6.0)	3.73 q (6.2)	3.75 dq (6.5, 1.0)
6	1.50 d (6.2)	1.50 d (6.0)	1.52 d (6.5)	1.51 d (6.5)	1.51 d (6.0)	1.49 d (6.2)	1.51 d (6.5)
rha-1	5.51 d (1.0)	5.50 d (2.0)	5.53 d (1.0)	5.52 d (2.0)	5.51 d (1.5)	5.51 d (1.0)	5.52 d (1.5)
2	5.93 dd (2.7, 1.0)	5.92 dd (3.2, 2.0)	5.96 dd (2.7, 1.0)	5.94 dd (3.5, 2.0)	5.93 dd (3.5, 1.5)	5.92 dd (2.9, 1.0)	5.94 dd (3.0, 1.5)
3	4.99−5.04 m	4.98−5.02 m	5.05 dd (9.5, 2.7)	5.06−5.01 m	5.01−5.03 m	5.01 m	5.04 dd (9.5, 3.0)
4	4.14 dd (9.5, 9.5)	4.12 dd (9.5, 9.5)	4.18 dd (9.5, 9.5)	4.16 dd (10.0, 9.5)	4.17 dd (9.5, 9.5)	4.15 dd (9.2, 7.3)	4.16 dd (9.5, 9.5)
5	4.50 dq (9.5, 6.0)	4.48 dq (9.5, 6.5)	4.50 dq (9.5, 6.5)	4.51 dq (10.0, 6.0)	4.50 dq (9.5, 6.5)	4.50 dq (9.2, 6.1)	4.50 dq (9.5, 6.0)
6	1.64 d (6.0)	1.64 d (6.5)	1.68 d (6.5)	1.65 d (6.0)	1.65 d (6.5)	1.63 d (6.1)	1.65 d (6.0)
rha′-1	5.78 d (1.0)	5.77 d (1.5)	5.86 d (1.0)	5.86 d (1.5)	5.84 d (2.0)	5.84 d (1.8)	5.85 d (2.0)
2	6.34 dd (3.0, 1.0)	6.33 dd (3.0, 1.5)	6.36 dd (2.5, 1.0)	6.35 dd (1.5, 5.5)	6.34 dd (3.0, 2.0)	6.34 dd (3.0, 1.8)	6.35 dd (3.5, 2.0)
3	4.75 dd (9.0, 3.0)	4.74 dd (3.0, 9.5)	4.82 dd (8.5, 2.5)	4.82 m*	4.81 dd (9.2, 3.0)	4.79 dd (9.0, 3.0)	4.81 dd (8.7, 3.2)
4	4.75 dd (9.5, 9.0)	4.31 dd (9.5, 9.5)	4.40 m*	4.40 m*	4.38 m*	4.37 m*	4.39 m*
5	4.35−4.41 m*	4.37 dq (9.5, 6.0)	4.40 m*	4.40 m*	4.38 m*	4.37 m*	4.39 m*
6	1.66 d (6.0)	1.66 d (6.0)	1.66 d (6.5)	1.67 d (6.0)	1.67 d (6.0)	1.65 d (6.0)	1.67 d (6.0)
rha″-1	6.26 d (1.0)	6.26 d (1.5)	6.33 d (1.0)	6.30 d (2.0)	6.29 d (1.5)	6.29 d (1.0)	6.32 d (1.5)
2	5.26 dd (3.0, 1.0)	5.24 dd (3.0, 1.5)	5.30 dd (3.0, 1.0)	5.29 dd (3.0, 2.0)	5.28 dd (3.0, 1.5)	5.27 dd (2.9, 1.0)	5.28 dd (3.0, 1.5)
3	6.01 dd (10.0. 3.0)	6.00 dd (10.0, 3.0)	6.03 dd (9.5, 3.0)	6.02 dd (10.0, 3.0)	6.01 dd (10.0, 3.0)	6.03 dd (10.0, 2.9)	6.02 dd (10.0, 3.5)
4	6.10 dd (10.0, 10.0)	6.10 dd (10.0, 10.0)	6.13 dd (9.5, 9.5)	6.11 dd (10.0, 10.0)	6.10 dd (10.0, 10.0)	6.09 dd (10.0, 9.0)	6.12 dd (10.0, 9.5)
5	4.51 dq (10.0, 6.0)	4.51 dq (10.0, 6.5)	4.53 dq (9.5, 6.0)	4.50 dq (10.0, 6.0)	4.52 dq (10.0, 6.0)	4.48 dq (9.0, 6.2)	4.52 dq (9.5, 6.0)
6	1.45 d (6.0)	1.48 d (6.5)	1.48 d (6.0)	1.45 d (6.0)	1.45 d (6.0)	1.43 d (6.2)	1.47 d (6.0)
glc-1	5.06 d (8.0)	5.05 d (7.5)	5.13 d (8.0)	5.12 d (8.0)	5.11 d (8.0)	5.10 d (7.7)	5.11 d (7.5)
2	3.92*	3.91 dd (9.0, 7.5)	3.98 m*	3.98 m*	3.97 dd (9.0, 8.0)	3.95 m*	3.98 dd (9.5, 7.5)
3	4.09 m*	4.08 m*	4.11 dd (9.0, 9.0)	4.12 m*	4.09 dd (9.0, 9.0)	4.07 dd (9.0, 9.0)	4.10 dd (9.5, 9.0)
4	3.94 m*	3.91 dd (9.0, 9.0)	3.98 m*	3.98 m*	3.95 dd (9.0, 9.0)	3.95 m*	3.96 dd (9.0, 9.0)
5	3.79 ddd (9.0, 4.4, 2.5)	3.78 ddd (9.0, 6.0, 3.0)	3.82 ddd (9.0, 6.0, 3.0)	3.83 ddd (9.0, 6.0, 3.0)	3.82 ddd (9.0, 6.0, 2.5)	3.81 ddd (9.0, 6.0, 2.5)	3.82 ddd (9.0, 6.0, 3.5)
6	4.06 m*	4.08*	4.11*	4.13 m*	4.09−4.13 m*	4.11 dd (11.0, 2.5)	4.13 dd (12.0, 3.5)
	4.38 m*	4.39 m*	4.44 m*	4.44 m*	4.40−4.45 m	4.42 m*	4.43 dd (11.5, 2.5)
jal-2a	2.27 ddd (14.6, 8.1, 4.0)	2.26 ddd (14.9, 8.1, 4.0)	2.28 m	2.28 ddd (14.6, 7.6, 4.0)	2.27 ddd (14.6, 8.0, 4.0)	2.26 m*	2.28 m*
2b	2.44 m*	2.36 m	2.47 m*	2.47 ddd (14.5, 8.5, 4.0)	2.46 ddd (14.6, 8.5, 4.5)	2.45 m*	2.46 m*
11	3.85 m	3.85 m	3.87 m	3.87 m	3.86 m	3.84 m	3.85 m
16	0.88 t (7.2)	0.88 t (7.0)	0.81 t (7.2)	0.87 t (7.5)	0.87 t (7.0)	0.85 m*	0.87 t (7.0)
mba-2	2.41 tq (7.0, 7.0)	2.42 tq (7.2, 7.2)		2.48 tq (7.0, 6.5)	2.49 tq (7.0, 6.5)	2.48 tq (7.0, 6.8)	
2-Me	1.03 d (7.0)	1.03 d (7.0)		1.16 d (7.0)	1.16 d (7.0)	1.14 d (7.0)	
3-Me	0.79 t (7.5)	0.79 d (7.5)		0.83 d (7.5)	0.83 d (7.5)	0.81 d (7.5)	
mba′-2	2.49 tq (7.0, 7.0)						
2-Me	1.15 d (7.0)						
3-Me	0.83 t (7.5)						
octa-2		2.46 t (7.5)	2.34 m				2.47 t (7.0)
8		0.73 d (7.0, 7.0)	0.89 t (6.8)				0.73 t (7.2)
octa′-2			2.48 t (7.0)				
8			0.74 t (6.8)				
dodeca-2				2.35 t (7.5)	2.34 m	2.33 t (7.3)	2.35 t (7.5)
12				0.88 t (7.0)	0.87 t (6.5)	0.86 m*	0.88 t (7.2)
CA-2	6.61 d (16.0)	6.62 d (16.0)	6.64 d (16.0)	6.61 d (16.0)	6.60 d (16.0)	6.59 d (16.0)	6.63 d (16.0)
3	7.86 d (16.0)	7.87 d (16.0)	7.89 d (16.0)	7.87 d (16.0)	7.86 d (16.0)	7.85 d (16.0)	7.83 d (16.0)
CA Ph-2′	7.34 m*	7.34 m*	7.36 m*	7.34 m*	7.33 m*	7.33 m*	7.35 m*
3′	7.46 m*	7.46 m*	7.47 m*	7.46 m	7.45 m	7.44 m	7.46 m
4′	7.34 m*	7.34 m*	7.36 m	7.34 m*	7.34 m*	7.33 m*	7.35 m*

[a] Data recorded in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b]Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose, jal = 11-hydroxyhexadecanoyl; octa = *n*-octanoyl; dodeca= *n*-dodecanoyl, CA = *trans*-cinnamoyl; Ph = CA aromatic ring.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**7** (125.7 MHz)[a]

carbon[b]	1	2	3	4	5	6	7
fuc-1	104.3	104.3	104.7	104.3	104.3	104.3	104.3
2	80.0	79.9	80.3	79.9	79.9	79.9	79.9
3	73.4	73.3	73.8	73.0	73.3	73.8	73.4
4	72.9	72.9	73.3	72.9	72.9	72.9	72.9
5	70.8	70.8	69.3	70.8	70.8	70.8	70.8
6	17.3	17.3	17.7	17.3	17.3	17.3	17.3
rha-1	98.5	98.5	98.9	98.5	98.5	98.5	98.5
2	73.6	73.6	74.0	73.6	73.6	73.6	73.6
3	69.3	69.3	69.7	69.3	69.3	69.3	69.3
4	82.0	82.0	82.4	82.0	82.0	82.0	82.0
5	68.9	68.9	69.3	68.4	68.9	68.9	68.9
6	19.1	19.1	19.5	19.1	19.1	19.1	19.0
rha′-1	100.3	100.3	100.6	100.3	100.3	100.2	100.3
2	73.2	73.1	73.7	73.4	73.4	73.6	73.3
3	79.9	80.0	80.4	79.9	79.9	79.9	79.9
4	79.1	79.0	79.3	79.0	79.0	79.0	78.9
5	68.4	68.4	68.5	68.2	68.5	68.4	68.5
6	19.0	19.1	19.4	19.0	19.0	19.0	19.1
rha′′-1	103.4	103.3	103.7	103.4	103.4	103.3	103.3
2	69.9	69.9	70.3	69.9	69.9	69.9	69.9
3	73.0	73.1	73.6	73.0	73.0	73.0	73.2
4	71.7	71.9	72.3	71.7	71.7	71.7	71.9
5	68.1	68.1	69.3	68.9	68.1	68.2	68.1
6	17.9	17.9	18.3	17.9	17.9	17.9	17.9
glc-1	105.5	105.5	105.9	105.5	105.5	105.5	105.5
2	75.2	75.2	75.6	75.2	75.2	75.2	75.2
3	78.4	78.4	78.9	78.4	78.4	78.5	78.5
4	71.5	71.5	71.9	71.5	71.5	71.5	71.5
5	77.9	77.9	78.5	78.1	78.1	78.1	78.1
6	62.9	62.9	63.3	62.9	62.9	62.9	62.9
jal-1	173.1	173.1	173.9	173.1	173.1	173.1	173.1
2	34.2	34.2	34.7	34.2	34.2	34.3	34.3
11	82.3	82.3	82.7	82.3	82.3	82.3	82.4
16	14.3	14.3	14.6	14.3	14.3	14.3	14.3
mba-1	176.2	176.2		175.9	175.9	175.9	
2	41.2	41.2		41.6	41.6	41.6	
2-Me	16.6	16.6		16.9	16.9	16.9	
3-Me	11.4	11.4		11.8	11.8	11.8	
mba′-1	175.9						
2	41.6						
2-Me	16.9						
3-Me	11.8						
octa-1		173.1	173.5				173.5
2		34.6	34.9				34.6
8		14.1	14.7				14.1
octa′−1			173.6				
2			35.0				
8			14.5				
dodeca-1				173.5	173.5	173.5	173.2
2				34.6	34.6	34.5	34.6
12				14.3	14.3	14.3	14.3
CA-1	166.4	166.4	166.9	166.4	166.4	166.4	166.5
2	118.6	118.5	118.9	118.6	118.6	118.6	118.6
3	145.3	145.4	145.8	145.3	145.4	145.4	145.4
CA Ph-1′	135.0	134.7	135.1	134.7	134.7	134.7	134.7
2′	129.2	129.2	129.6	129.3	129.3	129.2	129.2
3′	128.5	128.5	129.0	128.5	128.5	128.5	128.6
4′	130.7	130.7	131.1	130.7	130.7	130.8	130.7

[a] Data recorded in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on DEPT, HSQC, and HMBC experiments. [b]Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose, jal = 11-hydroxyhexadecanoyl; octa = *n*-octanoyl; dodeca= *n*-dodecanoyl, CA = *trans*-cinnamoyl; Ph = CA aromatic ring.

Morelos, in February 1990, which is on deposit at the IMSSM Herbarium collection (vouchers 11056 and 11057).

Extraction and Isolation. Dried and milled seeds (389.4 g) were extracted exhaustively by maceration at room temperature with $CHCl_3$ to give, after removal of the solvent, a dark syrup (55.7 g). This extract was subjected to column chromatography. A total of 100 fractions (250 mL each) were collected using a gradient of MeOH in $CHCl_3$ (0:1 to 2:3). The fractions were pooled in 20 main fractions (1−20). Fraction 10 (25 g), eluted with $CHCl_3$−MeOH (9:1) and containing a crude mixture of resin glycosides, was subjected to fractionation by open column chromatography over silica gel (100 g) eluted with the same solvent system, from which 45 secondary fractions (125 mL each) were obtained. Subfractions 10−17 and 18−35 were separately analyzed by reversed-phase C_{18} HPLC using an isocratic elution with CH_3CN−MeOH (2:3). For resolution of subfractions 10−17 (1.760 g), a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm), a flow rate of 9 mL/min, and a detection at 270 nm were used. Peaks with t_R values of 10.62 min (**3**, 35 mg), 11.41 min (**6**, 45 mg), and 16.02 min (**7**, 19 mg) were collected by the technique of heart cutting and independently reinjected in the apparatus operating in the recycle mode to achieve total homogeneity after 15 consecutive cycles. An eluate with a t_R value of 11.81 min was split into two peaks during the recycling process to afford pure compounds **4** (6.0 mg) and **5** (34.2 mg) after 20 consecutive cycles employing the same isocratic elution. For the resolution of subfractions 18−35 (1.373 g), a preparative YMC-pack C_{18} column (Waters; 5 μm, 20 × 250 mm), a flow rate of 5.0 mL/min, and detection at 254 nm were employed. Individual peaks with t_R values of 44.38 and 68.00 min were purified by the recycling technique to afford compounds **1** (22.3 mg) and **2** (32.5 mg).

Intrapilosin I (1): white powder; mp 97−99 °C; $[\alpha]_D$ −3.5 (*c* 0.23, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1297 $[M-H]^-$, 1167 $[M-H-C_9H_6O$ (cinnamoyl)$]^-$, 1083 [1167 − C_5H_8O (α-methylbutyroyl)$]^-$, 937 [1083 − $C_6H_{10}O_4$ (methylpentose)$]^-$, 545 [691 − 146$]^-$, 417 [545 − 128$]^-$, 271 [Jal − H$]^-$; HRFABMS *m/z* 1297.6579 (calcd for $C_{65}H_{101}O_{26}$ requires 1297.6581).

Intrapilosin II (2): white powder; mp 125−127 °C; $[\alpha]_D$ −5.2 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1339 $[M-H]^-$, 1209 $[M-H-C_9H_6O]^-$, 1125 [1209 − $C_5H_8O]^-$, 1083, 937, 545, 417, 271; HRFABMS *m/z* 1339.7053 (calcd for $C_{68}H_{107}O_{26}$ requires 1339.7050).

Intrapilosin III (3): white powder; mp 114−116 °C; $[\alpha]_D$ −40 (*c* 0.15, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1381 $[M-H]^-$, 1251 $[M-H-C_9H_6O]^-$, 1125 [1251 − 126 (octanoyl)], 979 [1125 − $C_6H_{10}O_4$], 853 [979 − $C_8H_{14}O$], 691 [853 − 162 ($C_6H_{10}O_5$)], 545, 417, 271; HRFABMS *m/z* 1381.7519 (calcd for $C_{71}H_{113}O_{26}$ requires 1381.7520)

Intrapilosin IV(4): white powder; mp 98−100 °C; $[\alpha]_D$ −17 (*c* 0.82, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1395 $[M-H]^-$, 1265 $[M-H-C_9H_6O]^-$, 1213 [M − $C_{12}H_{22}O$ (dodecanoyl)], 1083 [1213 − 130], 1035 [1265 − mba − 146], 853 [1035 − 182], 545, 417, 271; HRFABMS *m/z* 1395.7677 (calcd for $C_{72}H_{115}O_{26}$ requires 1395.7676).

Intrapilosin V (5): white powder; mp 95−97 °C; $[\alpha]_D$ −15 (*c* 0.83, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1395 $[M-H]^-$, 1265, 1213, 1083, 1035, 853, 545, 417, 271; HRFABMS *m/z* 1395.7675 (calcd for $C_{72}H_{115}O_{26}$ requires 1395.7676).

Intrapilosin VI (6): white powder; mp 117−119 °C; $[\alpha]_D$ −5 (*c* 0.20, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1395 $[M-H]^-$, 1265, 1213, 1083, 1035, 853, 545, 417, 271; HRFABMS *m/z* 1395.7676 (calcd for $C_{72}H_{115}O_{26}$ requires 1395.7676).

Intrapilosin VII (7): white powder; mp 87−89 °C; $[\alpha]_D$ −14 (*c* 1.53, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1437 $[M-H]^-$, 1307 [1437 − $C_9H_6O]^-$, 1255 [M − H − $C_{12}H_{22}O$], 1181 [1307 − $C_8H_{14}O$], 1125 [1255 − C_9H_6O], 1035 [1181 − $C_6H_{10}O_4$], 999 [1181 − $C_{12}H_{22}O$], 853 [999 − $C_6H_{10}O_4$], 545, 417, 271; HRFABMS *m/z* 1437.8145 (calcd for $C_{75}H_{121}O_{26}$ requires 1437.8146).

Alkaline Hydrolysis of the Resin Glycoside Fraction. A solution of fraction 10 (300 mg), obtained from the column chromatography of the crude extract, in 5% KOH−H_2O (8 mL) was refluxed at 95 °C for 2 h. The reaction mixture was acidified to pH 4.0 and extracted with Et_2O (30 mL). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analyzed by GC-MS with the following peaks detected:[4,12] 2-methylbutanoic acid (t_R 6.8 min): *m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8); *n*-octanoic acid (t_R 10.3 min): *m/z* $[M]^+$ 144 (3), 127 (1), 115 (15), 101 (30), 85 (10), 73 (85), 60 (100), 55 (20), 43 (40), 41 (28), 39 (6); *trans*-cinnamic acid (t_R 16.1 min): *m/z* $[M]^+$ 148 (100), 147 (96), 131 (25), 103 (40), 102 (20), 77 (25), 74 (8), 51 (20), 50 (8), 39 (5), 38 (4); and *n*-dodecanoic acid (t_R 17.5 min): *m/z* $[M]^+$ 200 (15), 183 (2), 171 (18), 157 (40), 143 (10), 129 (48), 115 (20), 101 (15), 85 (33), 73 (100), 60 (80), 57 (30), 55 (47), 43 (44), 41 (30).

The aqueous phase was extracted with *n*-BuOH (30 mL) and concentrated to give a colorless solid (115 mg). The residue (50 mg) was methylated with CH_2N_2 and further acetylated ($Ac_2O-C_5H_5N$, 2:1) to give a residue (64 mg) that was subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 mm). The elution was isocratic with $CH_3CN-MeOH$ (95:5) using a flow rate of 9 mL/min. The eluate with a t_R of 12.5 min was again collected by heart cutting, and the ^1H and ^{13}C NMR data for the isolated product allowed its identification as the peracetylated derivative of operculinic acid A methyl ester: mp 80−82 °C; $[\alpha]_D$ −31 (*c* 1.0, MeOH), which was identified by comparison of NMR data with published values.[4]

Esterification of the Saponification-Liberated Carboxylic Acids. Compound **5** (30 mg) in 5% $KOH-H_2O$ (1 mL) was refluxed at 95 °C for 45 min. The reaction mixture was acidified to pH 3.0 and extracted with CH_2Cl_2 (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. A solution of benzyl alcohol (10.5 mg) in CH_2Cl_2 (1 mL), containing dicyclohexylcarbodiimide (3 mg) and 4-dimethylaminopyridine (1 mg), was added to the mixture of carboxylic acids. The reaction was stirred for 12 h at room temperature and filtered, and the solvent was evaporated. The residue was analyzed by GC-MS: benzyl α-methylbutyrate (t_R 3.64 min) $[M]^+$ 192 (5), 108 (19), 92 (8.0), 91 (100), 77 (10), 65 (15), 57 (16), 39 (12); benzyl *trans*-cinnamoate (t_R 6.20 min) $[M]^+$ 238 (14), 220 (8.6), 194 (10.5), 193 (72), 178 (8), 161 (8), 147 (8), 132, (12), 131 (100), 115 (20), 103 (52), 91 (86), 77 (41), 65 (23), 51 (24), 39 (11); and benzyl dodecanoate (t_R 6.58 min) $[M]^+$ 290 (15), 272 (3), 224 (13), 199 (62), 198 (16), 181 (47), 180 (11), 163 (43), 162 (9), 143 (10), 139 (3), 125 (10), 121 (6), 108 (92), 107 (26), 105 (7), 92 (15), 91 (100), 81 (10), 67 (3), 65 (17), 56 (3), 43 (10), 39 (23). The crude mixture was purified by HPLC on a normal-phase column (μPorasil, 10 μm, 3.9 × 300 mm; Waters) using hexane−EtOAc (99:1, flow rate 0.6 mL/min) to give three peaks: benzyl dodecanoate (t_R 7.92 min), benzyl α-methylbutyrate (t_R 8.43 min), and benzyl cinnamoate (t_R 11.22 min). The physical and spectroscopic constants measured for the eluate with t_R 8.43 min were identical in all aspects to those previously reported[14] for (*S*)-(+)-benzyl α-methylbutyrate: oil, $[\alpha]_{598}$ +9.3, $[\alpha]_{578}$ +9.6, $[\alpha]_{546}$ +10.9, $[\alpha]_{436}$ +17.3, $[\alpha]_{365}$ +26 (*c* 1.0, $CHCl_3$). Treatment of the mixture of carboxylic acids obtained from intrapilosin IV (**4**, 6.0 mg), as described above, yielded the (*R*)-(−)-benzyl α-methylbutyrate: $[\alpha]_{598}$ −9, $[\alpha]_{578}$ −9, $[\alpha]_{546}$ −10.5, $[\alpha]_{436}$ −17, $[\alpha]_{365}$ −25 (*c* 0.8, $CHCl_3$). Saponification of compounds **1**, **2**, and **6** afforded (*S*)-(+)-α-methylbutyric acid: $[\alpha]_D$ +10 (*c* 1.5, $CHCl_3$).

Sugar Analysis. A solution of the crude glycosidic acid (20 mg) obtained from the saponification of the resin glycoside mixture in 4 N HCl (10 mL) was heated at 90 °C for 2 h. The reaction mixture was diluted with H_2O (5 mL) and extracted with Et_2O (30 mL). The aqueous phase was neutralized with 1 N KOH, extracted with *n*-BuOH (30 mL), and concentrated to give a colorless solid. The residue was dissolved in CH_3CN-H_2O (1:1) and directly analyzed by HPLC: Waters standard column for carbohydrate analysis (μBondapak NH_2; 3.9 × 300 mm, 10 μm), using an isocratic elution of CH_3CN-H_2O (85:15), a flow rate of 1 mL/min, and a sample injection of 20 μL (sample concentration: 5 mg/mL). Coelution experiments with standard carbohydrate samples allowed the identification of rhamnose (t_R = 5.9 min), fucose (t_R = 7.7 min), and glucose (10.1 min). Each of these eluates were individually collected, concentrated, and dissolved in H_2O. Optical activity was recorded after stirring the solutions for 2 h at room temperature: L-rhamnose $[\alpha]_{598}$ +8, $[\alpha]_{578}$ +8, $[\alpha]_{546}$ +9, $[\alpha]_{436}$ +15, $[\alpha]_{365}$ +21 (*c* 0.1, H_2O); D-fucose $[\alpha]_{598}$ +81, $[\alpha]_{578}$ +83, $[\alpha]_{546}$ +94, $[\alpha]_{436}$ +155, $[\alpha]_{365}$ +236 (*c* 0.1, H_2O); D-glucose $[\alpha]_{598}$ +50, $[\alpha]_{578}$ +51, $[\alpha]_{546}$ +57, $[\alpha]_{436}$ +97, $[\alpha]_{365}$ +150 (*c* 0.1, H_2O).

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References and Notes

(1) A medicinal plant complex consists of an assemblage of herbal drugs that are taxonomically different at the specific, generic, and/or familial level but that shares a common name, one or more key morphological features, certain organoleptic characteristics, and one therapeutic application. For an example, see: (a) Linares, E.; Bye, R. *J. Ethnopharmacol.* **1987**, *19*, 153−183. (b) Pereda-Miranda, R.; Fragoso-Serrano, M.; Escalante-Sánchez, E.; Hernández-Carlos, B.; Linares, E.; Bye, R. *J. Nat. Prod.* **2006**, *69*, 1460−1466.

(2) The Mexican term "cazahuate" is derived from the Nahuatl ("cuauhzahuatl") words for tree ("quauitl") and mange ("zahuatl") and refers to the uses of this medicinal plant complex to treat itching and rashes ("zahuistle") by rubbing the raw flowers directly on the skin.

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