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Resin Glycosides from *Ipomoea pes-caprae*[†]

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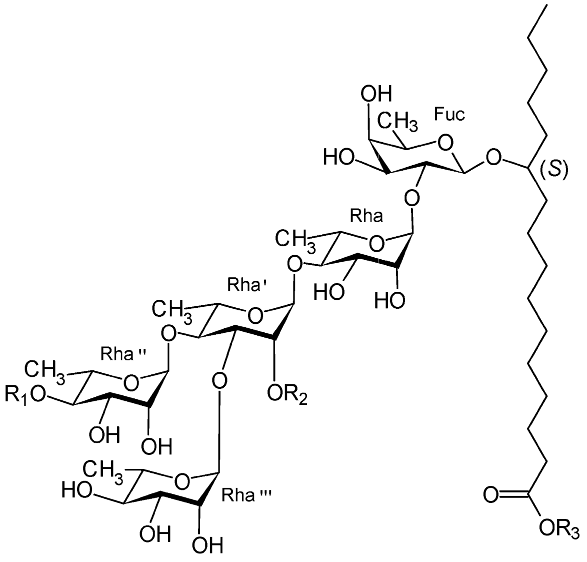
Ipomoea pes-caprae (beach morning-glory; "riñonina" for the herbal drug in Mexico) is prescribed by traditional healers to moderate "heat" in an infected kidney. The hexane-soluble extract from the aerial parts of this medicinal plant, through preparative-scale recycling HPLC, yielded six new lipophilic oligosaccharides of jalapinolic acid: pescaproside B (**1**) and pescapreins V−IX (**2**−**6**). The previously known pescaproside A (**7**), pescapreins I−IV (**8**−**11**), and stoloniferin III (**12**) were also identified in the analyzed material by means of HPLC comparison with authentic samples. The glycosidic acid structure for all pentasaccharides was confirmed as simonic acid B. Pescaproside B (**1**), an acylated glycosidic acid methyl ester, is structurally related to pescaprein III (**10**). Pescapreins V (**2**) and VI (**3**) are diasteroisomeric tetraglycosidic lactones of operculinic acid C. Both of these compounds contain (2*S*)-methylbutyric and *n*-dodecanoic acids as their esterifying residues. Pescapreins VII (**4**) and IX (**6**) are pentasaccharides that contain an *n*-decanoyl group as their esterifying fatty acid residue instead of the *n*-dodecanoyl found in pescapreins I (**8**) and IV (**11**). Pescaprein VIII (**5**) represents an isomer of pescaprein II (**9**) containing an *n*-dodecanoyl unit as the esterifying residue at position C-4 of the third rhamnose moiety and a 2-methylpropanoyl at C-2 of the second rhamnose. High-field NMR spectroscopy and FAB mass spectrometry were used to characterize all new isolated compounds.

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae)[1] is used in infusions for urinary or kidney complaints, hypertension, and scrofula and in decoctions to treat functional digestive disorders, internal pain, colic, lumbago, arthritis, rheumatism, dysentery, and inflammatory conditions.[2] This herbal drug is called "riñonina"[3] in Mexico, reflecting the belief of traditional healers that it moderates the "heat" in an infected kidney.[4] The dried whole plant is found readily in herbal markets and stores throughout the country.

Our previous research has been focused on the resin glycosides of the Convolvulaceae family, whose structural diversity holds considerable potential for medicinal usage.[5,6] This paper describes the isolation and characterization of six new oligosaccharides from the hexane-soluble resin obtained from "riñonina": pescaproside B (**1**) and pescapreins V−IX (**2**−**6**). Several NMR techniques and FABMS were used to characterize the structure of these complex glycolipids. The known compounds pescaproside A (**7**), pescapreins I−IV (**8**−**11**), and stoloniferin III (**12**) were also identified by HPLC.[7]

Hexane-soluble extracts of the crude drug "riñonina", purchased from a herbal store located in downtown Mexico City, were compared by C_{18} reversed-phase HPLC (CH_3CN−MeOH, 9:1) with reference solutions of authentic samples.[7] This analysis allowed for the detection of resin glycosides **8**−**12** (t_R 11−17 min) as well as for a mixture of highly lipophilic compounds (t_R 32−45 min). The total extract was fractionated by column chromatography on silica gel. The major fractions, rich in these low-polarity resin glycosides, were purified further by passing through activated charcoal to eliminate pigmented residues. These processes were followed by recycling preparative-scale HPLC,[8] resulting in the isolation of six new glycolipids (**1**−**6**).



	R_1	R_2	R_3
1	(2*S*)-methylbutanoyl = mba	*n*-dodecanoyl	CH_3
7	H	*n*-dodecanoyl	CH_3
13	H	H	CH_3

Results and Discussion

Pescaproside B (**1**) gave a quasimolecular ion at *m*/*z* 1281 $[M - H]^-$ ($C_{64}H_{113}O_{25}$) in the negative-ion FABMS; the observed difference of 84 mass units (C_5H_8O) between this natural product and the previously reported pescaproside A (**7**; *m*/*z* 1197; $[M - H]^-$) corroborated the presence of α-methylbutyric acid as an additional ester group on the oligosaccharide core. From the ^{1}H NMR spectrum (Table 1), the esterified position at C-4 of the third rhamnose moiety (Rha″-1, *δ* 5.53) was identified through the strong deshielding effect (*δ*Δ 1.3) relative to the nonesterified equivalent[7] on compound **7**. The triplet-like signal for the methylene protons at C-2 (*δ* 2.3) and the methyl ester group (*δ* 3.6) of the aglycon confirmed its acyclic structure. HPLC analysis of several hexane-soluble crude extracts prepared from commercial samples of this herbal drug confirmed the presence of compounds **1** and **7** as natural constituents. This type of non-macrocyclic resin glycoside has been identified in only two other plant species apart from that used in

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[†] This work was taken in part from the Ph.D. thesis of C. Escobedo-Martínez.

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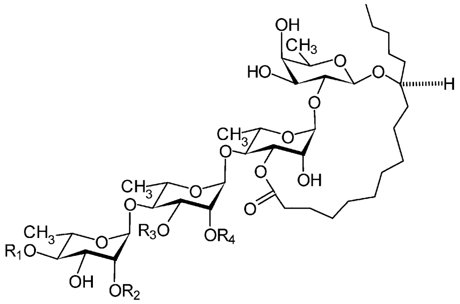

rha = α-L-rhamnopyranosyl

	R_1	R_2	R_3	R_4
2	mba	H	H	*n*-dodecanoyl
3	H	*n*-dodecanoyl	mba	H
4	H	H	rha	*n*-decanoyl
5	*n*-dodecanoyl	H	rha	2-methylpropanoyl = iba
6	*n*-hexanoyl	H	rha	*n*-decanoyl
8	H	H	rha	*n*-dodecanoyl
9	iba	H	rha	*n*-dodecanoyl
10	mba	H	rha	*n*-dodecanoyl
11	*n*-hexanoyl	H	rha	*n*-dodecanoyl
12	mba	H	rha	*n*-decanoyl

the present investigation.[7] The first of these yielded cuscutic acids A−D isolated from the seeds of *Cuscuta chinensis*, a member of the Convolvulaceae family.[9] The second yielded crypthophilic acids A−C from the aerial parts of *Scrophularia crypthophila*, a species taxonomically unrelated to Convolvulaceae but belonging instead to the Scrophulariaceae.[10]

Negative-ion FAB mass spectra generated by pescapreins V (**2**) and VI (**3**) were found to be very similar, with a pseudomolecular $[M - H]^-$ ion at *m*/*z* 1103, and therefore these constituents represented diastereoisomeric tetrasaccharides of molecular formula $C_{57}H_{100}O_{20}$. The ^1H NMR spectrum of **2** showed signals of four anomeric protons and, when compared with the values previously reported for operculinic acid C,[11] exhibited the acylation shifts for H-3 of Rha, H-2 of Rha′, and H-4 of Rha″. HMBC studies[5,12] using long-range heteronuclear coupling correlation ($^{2,3}J_{CH}$) provided evidence for the location of each ester substituent at the oligosaccharide core. Thus, the site of lactonization by the aglycon (δ_{C-1} 174.9) could be placed at C-3 of the second saccharide (Rha) at C-3 (δ_H 5.59); a methylbutyroyl group (δ_{C-1} 176.4) was located at C-4 of Rha″ (δ_H 5.82); and the additional acyl substituent (*n*-dodecanoic acid; δ_{C-1} 173.2) exhibited a $^3J_{CH}$ coupling with the H-2 signal of Rha′ (δ_H 5.78).

From the ^1H NMR spectrum of pescaprein VI (**3**), the three acylation sites were identified at H-3 of Rha, H-3 of Rha′, and H-2 of Rha″. However, a significant signal overlap in the proton region δ 5.6−5.8 hampered $^3J_{CH}$ complete assigments for these signals. Careful analysis of the negative FABMS generated by both tetrasaccharides **2** and **3** confirmed the substitution pattern proposed for each glycolipid. The initial loss of the esterifying group at the terminal saccharide unit (Rha″) afforded a peak at *m*/*z* 921 in pescaprein VI (**3**) representing $[M - H - C_{12}H_{22}O]^-$, while the same elimination afforded a peak at *m*/*z* 1019 $[M - H - C_5H_8O]^-$ in pescaprein V (**2**). The loss of the terminal deoxyhexose afforded the peak at *m*/*z* 873 [1019 − 146 (methylpentose unit)]$^-$ in **2** and at *m*/*z* 775 [921 − 146 (methylpentose unit)]$^-$ in **3**. Thus, these peaks suggested that an *n*-dodecanoyl residue should be placed at C-2 of Rha″ in compound **3**. The pattern for the nonequivalent protons at C-2 of the aglycon in the ^1H NMR spectrum (Table 1) of pescapreins V (**2**) and VI (**3**) showed a multiplet in the form of a broad triplet-like signal (ddd) for the deshielding resonance (δ 2.71 for **2** and δ 2.29 for **3**) and a clear doublet of doublet of doublets for the shielded proton (δ 2.23 for **2** and δ 2.14 for **3**). This pattern was similar to that previously observed in the pentasaccharides **8**−**11**, which are macrocyclic lactones with the esterification by the aglycon at C-3 of Rha.[7] Therefore, the remaining esterified position represented the location for the additional ester linkage (2*S*-methylbutyric acid), that is, C-4 of Rha″ and C-3 of Rha′, in pescapreins V (**2**) and VI (**3**), respectively.

Pescaprein VII (**4**) afforded a $[M - H]^-$ peak at *m*/*z* 1137 ($C_{56}H_{97}O_{23}$) in contrast to the ion at *m*/*z* 1165 for its homologue **8** (pescaprein I), indicating a difference of two methylene groups between these compounds. Pescaprein VIII (**5**; $[M - H]^-$ ion at *m*/*z* 1235, $C_{62}H_{107}O_{24}$) is an isomer of pescaprein II (**9**). Pescaprein IX (**6**) showed also the $[M - H]^-$ ion at *m*/*z* 1235 ($C_{62}H_{107}O_{24}$) and a difference of 28 amu in relation to pescaprein IV (**11**) and confirmed the presence of an *n*-decanoyl group as its acylating residue. All compounds displayed the same fragmentation pattern previously described for the pescaprein series,[7] and the resulting diagnostic peaks were useful to confirm the position for the esterifying moieties; for example, the ion at *m*/*z* 907 $[M - H - C_{12}H_{22}O - C_6H_{10}O_4]^-$ indicated that the *n*-dodecanoyl residue is located at C-4 of the third rhamnose moiety (Rha″), and therefore the isobutyroyl residue should be placed at C-2 of the second rhamnose (Rha′).

Pescapreins VII−IX (**4**−**6**) showed similar ^1H and ^{13}C NMR spectra (Tables 1 and 2) and displayed diagnostic signals for the pescaprein series,[7] thus confirming their pentasaccharide core as simonic acid B, which has also been identified previously in the resin glycosides of *I. batatas*,[13] *I. stolonifera*,[14] and *I. murucoides*.[15] The features registered were (a) the site of lactonization at C-3 (δ_C 77.9) of the second saccharide (Rha) established by the significant downfield shift observed for its geminal proton (δ 5.60); (b) a triplet-like signal for the methylene group at C-2 (δ_H 2.35) for the fatty acid esterifying the hydroxyl group on position C-2 of the third saccharide unit (Rha′: δ_H 5.79; δ_C 73); and (c) signals attributable to the nonequivalent protons of the methylene group at C-2 in the aglycon moiety that confirmed their macrocyclic lactone-type[7] structure: δ_{H2a} 2.91−2.94 (broad triplet-like doublet of doublet of doublets) and δ_{H2b} 2.24−2.25 (doublet of doublet of doublets). Furthermore, compound **5** showed a diagnostic signal at δ 2.63 (1H, septet) for one isobutyroyl residue that esterified position C-2 at Rha′ (δ_C 73). Compound **6** also showed the characteristic triplet-like signal for the H-2 methylene equivalent protons of the hexanoyl group at δ 2.38. From the TOCSY experiment, edited ^1H NMR subspectra for each individual monosaccharide moiety were obtained for all oligosaccharides and permitted the assignment of their resonances (Table 1). Homonuclear spin-decoupling experiments were carried out to verify coupling constants. The anomeric configuration in each sugar unit was deduced from a 2D $^1J_{CH}$ NMR experiments. For D-sugars in the $^4C_1$ conformation, the α-anomeric configuration (β-equatorial C−H bond) has a $^1J_{CH}$ value of 170 Hz, which is 10 Hz higher than that (ca. 160 Hz) for the β-anomer (α-axial C−H bond). From the anomeric signals in the ^{13}C NMR spectra of the glycosidic acids (i.e., simonic acid B and operculinic acid C), $^1J_{CH}$ values for fucose (159 Hz) supported its found β-anomeric configuration. The α-configuration was deduced for the rhamnose units ($^1J_{CH} = 170-172$ Hz).[5] All monosaccharides were in their naturally occurring form (D-fucose and L-rhamnose), as confirmed by optical rotation measurements of the acid-liberated individual monosaccharides.

The results of the present investigation suggest that convolvulaceous plants may have the ability to synthesize an array of amphipatic oligosaccharides from such simple building blocks as sugars and fatty acids. The highly elaborated chemical diversity of this plant family, as this study illustrates, may confer selective biological properties for which these species have long been used in traditional agriculture and medicinal practices.[5]

Experimental Section

General Experimental Procedures. All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. ^1H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted

Table 1. ^{1}H NMR Spectroscopic Data for **1**−**6** (500 MHz)[a]

proton[b]	**1**	**2**	**3**	**4**	**5**	**6**
fuc-1	4.80−4.83*	4.79 d (8.0)	4.79 d (7.5)	4.80 d (7.9)	4.81 d (7.9)	4.79 d (7.8)
2	4.52 dd (9.5, 8.0)	4.53 dd (9.5, 8.0)	4.54 dd (9.5, 7.5)	4.52 dd (9.6, 7.9)	4.52 dd (9.5, 7.9)	4.50 dd (9.4, 7.8)
3	4.16 dd (9.5, 3.5)	4.19 dd (9.5, 3.5)	4.20 dd (9.5, 3.5)	4.18 dd (9.6, 3.4)	4.20 dd (9.5, 3.3)	4.20 dd (9.4, 3.4)
4	3.94 d (3.5)	3.92 d (3.5)	3.93 d (3.5)	3.91 d (3.4)	3.91 d (3.3)	3.90 d (3.4)
5	3.80 q (6.5)	3.83 q (6.5)	3.83 q (6.5)	3.81 q (6.4)	3.82 q (6.5)	3.80 q (6.4)
6	1.53 d (6.5)	1.53 d (6.5)	1.52 d (6.5)	1.51 d (6.4)	1.51 d (6.5)	1.50 d (6.4)
rha-1	6.28 d (1.0)	6.38 bs	6.4 d (1.5)	6.33 d (1.6)	6.33 d (1.3)	6.32 d (1.6)
2	4.64 dd (3.0, 1.0)	5.29 bs	5.26 dd (2.3, 1.5)	5.30 dd (2.3, 1.6)	5.31 dd (2.8, 1.3)	5.29 d (2.8, 1.6)
3	4.33 dd (9.5, 3.0)	5.60 dd (9.5, 2.5)	5.68 dd (9.8, 2.3)	5.61 dd (9.8, 2.3)	5.61 dd (9.6, 2.8)	5.60 dd (9.8, 2.8)
4	4.31 dd (9.5, 9.5)	4.59 dd (9.5, 9.5)	4.70 dd (9.8, 9.8)	4.65 dd (9.8, 9.8)	4.63 dd (9.6, 9.6)	4.62 dd (9.8, 9.8)
5	4.91 dq (9.0, 6.0)	5.02 dq (9.5, 6.5)	5.08 dq (9.8, 6.1)	5.00 dq (9.8, 6.4)	5.01 dq (9.6, 6.3)	5.00 dq (9.8, 6.2)
6	1.62 d (6.0)	1.60 d (6.5)	1.55 bs	1.55 d (6.4)	1.57 d (6.3)	1.56 d (6.2)
rha′-1	6.11 d (2.0)	5.56 d (1.5)	5.90 d (1.0)	5.66 d (1.7)	5.66 d (1.7)	5.64 d (1.3)
2	6.04 dd (3.5, 2.0)	5.78 dd (2.8, 1.5)	4.75 bs	5.82 dd (3.4, 1.7)	5.82 dd (3.0, 1.7)	5.81 dd (2.4, 1.3)
3	4.67 dd (9.5, 3.5)	4.59 dd (9.5, 2.8)	5.70 dd (9.5, 3.0)	4.50 dd (9.1, 3.4)	4.51 dd (9.5, 3.0)	4.49 dd (9.3, 2.4)
4	4.30 dd (9.5, 9.5)	4.25 dd (9.5, 9.5)	4.57 dd (9.5, 9.5)	4.27−4.34*	4.26 (9.5, 9.5)	4.25−4.32*
5	4.35 dq (9.5, 6.0)	4.36 dq (9.5, 6.0)	4.34 dq (9.5, 6.3)	4.27−4.34*	4.33 dq (9.5, 6.1)	4.25−4.32*
6	1.73 d (6.0)	1.67 d (6.0)	1.54 d (6.3)	1.57 d (6. 0)	1.59 d (6.1)	1.58 d (6.3)
rha″-1	5.92 bs	6.15 d (1.5)	5.50 d (1.5)	5.92 bs	5.91 bs	5.90 bs
2	4.64 dd (3.5, 1.5)	4.77 dd (3.0, 1.5)	5.71 dd (3.0, 1.5)	4.65 dd (3.3, 1.4)	4.62 dd (3.3, 2.0)	4.62 bs
3	4.42 dd (9.8, 3.0)	4.18 dd (9.5, 3.0)	4.48 dd (9.3, 3.0)	4.36 dd (9.2, 3.3)	4.42 dd (9.8, 3.3)	4.36 dd (9.8, 3.4)
4	5.53 dd (9.8, 9.8)	5.82 dd (9.5, 9.5)	4.13 dd (9.3, 9.3)	4.26 dd (9.2, 9.2)	5.76 dd (9.8, 9.8)	5.79 dd (9.8, 9.8)
5	4.37 dq (9.8, 6.0)	4.41 dq (9.5, 6.5)	4.29 dq (9.3, 6.3)	4.27−4.34*	4.33 dq (9.3, 6.3)	4.34 dq (9.6, 6.2)
6	1.40 d (6.0)	1.44 d (6.5)	1.63 d (6.3)	1.65 d (6.1)	1.37 d (6.3)	1.39 d (6.3)
rha‴-1	5.62 bs			5.58 d (1.5)	5.56 bs	5.55 d (1.1)
2	4.80−4.83 bs*			4.52 dd (3.3, 1.5)	4.78 bs	4.77 bs
3	4.48 dd (9.3, 3.3)			4.53 dd (9.3, 3.3)	4.52 dd (9.5, 3.2)	4.51 dd (9.3, 3.3)
4	4.23 dd (9.3, 9.3)			4.24 dd (9.3, 9.3)	4.24 dd (9.5, 9.5)	4.25−4.32*
5	4.27 dq (9.3, 6.0)			4.27−4.34*	4.29 dq (9.5, 6.2)	4.25−4.32*
6	1.58 d (6.5)			1.71 d (6.1)	1.70 d (6.2)	1.69 d (6.1)
jal-2a	2.33 t (7.5)	2.23 ddd (14.0, 7.0, 3.0)	2.14 ddd (14.5, 7.0, 2.5)	2.24 ddd (15.7, 7.7, 2.1)	2.25 ddd (14.0, 6.5, 2.6)	2.24 m
2b		2.71 ddd[c] (14.0, 12.0, 1.7)	2.29 ddd[c] (14.5, 12.5, 2.0)	2.91 ddd[c] (15.7, 12.5, 1.8)	2.94 ddd[c] (14.0, 11.6, 1.7)	2.91 ddd[c] (13.1, 12.5, 1.8)
11	4.01 m	3.87 m	3.91 m	3.86 m	3.87 m	3.85 m
16	0.94 t (7.0)	1.2 t (7.0)	0.96 t (7.3)	0.94 t (7.0)	0.95 t (6.9)	0.98 t (7.1)
iba-2					2.63 sept (7.0)	
3					1.19 d (7.0)	
3′					1.16 d (7.0)	
mba-2	2.49 tq (7.0, 6.5)	2.53 tq (7.0, 6.5)	2.51 tq (7.0, 6.5)			
2-Me	1.20 d (7.0)	1.22 d (7.0)	1.20 d (7.0)			
3-Me	0.93 t (7.5)	0.96 t (7.5)	0.91 t (7.5)			
hexa-2						2.38 t (7.3)
6						0.93 t (7.1)
deca-2				2.35 t (7.4)		2.35 t (7.5)
10				0.86 t (7.2)		0.77 t (7.2)
dodeca-2	2.32 t (7.5)	2.34 ddd (15.5, 8.5, 8.5) 2.25 ddd (15.5, 8.0, 8.0)	2.42 ddd (14.0, 6.5, 6.5) 2.77 ddd (14.0, 6.5, 6.0)		2.36 t (7.3)	
12	0.87 t (7.0)	0.87 t (7.0)	0.86 t (7.1)		0.87 t (7.1)	

[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, bs = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet, sept = septet. All assignments are based on ^{1}H-^{1}H COSY and TOCSY experiments. [b]Abbreviations: fuc = fucose; rha = rhamnose; jal = 11-hydroxyhexadecanoyl; iba = 2-methylpropanoyl; mba = 2-methylbutanoyl; hexa = hexanoyl; deca = decanoyl; dodeca = dodecanoyl. [c]Broad triplet-like signal.

on a Bruker DMX-500 instrument. The NMR techniques were performed according to previously described methodology.[12] Negative-ion LRFABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX-102A spectrometer. The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600E multisolvent delivery system equipped with a refractive index detector (Waters 410). 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 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. The herbal drug "riñonina" was purchased in June 2004 at the herbal store "La Magnolia" in Mexico City. A small sample (50 g) was archived at the Departamento de Farmacia, Facultad de Química, UNAM. Macroscopic anatomical features enabled the drug to be identified by one of the authors (R.P.-M.) as *Ipomoea pes-caprae* through comparison with a voucher specimen collected on the Mexican Pacific coast (R. Bye 17707)[7] and deposited in the Ethnobotanical Collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation. The whole plant (1 kg) was powdered and extracted exhaustively by maceration at room temperature with hexane to give, after removal of the solvent, a dark green syrup (41.8 g). This crude extract was subjected to column chromatography over silica gel (650 g) using a gradient of $CHCl_3$ in hexane followed by $CHCl_3$−Me_2CO (7:3). A total of 85 fractions (250 mL each) were collected and combined in 12 fractions (I−XII), which were partially purified by passing through activated charcoal to eliminate pigmented residues. Each fraction was analyzed by HPLC on a Symmetry C_{18} column (Waters; 5 μm, 4.6 × 250 mm) with an isocratic elution of

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**6** (125.7 MHz)[a]

carbon[b]	**1**	**2**	**3**	**4**	**5**	**6**
fuc-1	101.2	101.5	101.7	101.6	101.6	101.6
2	74.8	73.2	73.0	73.4	73.4	73.4
3	76.7	76.8	76.9	76.6	76.6	76.7
4	73.5	73.5	73.6	73.5	73.6	73.6
5	71.3	71.3	71.2	71.2	71.3	70.8
6	17.2	17.2	17.2	17.2	17.2	17.2
rha-1	101.3	100.3	100.3	100.3	100.3	100.3
2	72.9	69.6	69.1	69.8	69.8	69.8
3	80.6	77.9	79.0	77.9	77.8	77.9
4	79.7	70.9	75.7	77.5	78.0	79.2
5	66.9	67.7	67.5	67.9	67.9	68.3
6	18.9	19.0	19.3	19.2	19.2	19.2
rha′-1	99.2	100.7	102.3	99.1	99.2	99.2
2	72.6	74.3	70.3	73.0	73.0	73.0
3	79.8	79.2	75.7	79.8	80.3	79.0
4	79.6	80.8	77.9	78.6	79.2	79.6
5	68.2	68.5	69.1	68.5	68.1	68.0
6	18.7	18.7	18.5	18.6	18.7	18.8
rha″-1	103.7	103.8	100.6	103.7	103.9	103.7
2	72.7	72.3	73.6	72.5	72.6	72.7
3	70.2	70.2	70.1	70.7	70.2	69.8
4	74.8	75.1	73.6	74.0	74.9	75.1
5	68.1	68.0	70.7	70.7	68.3	68.1
6	17.9	18.0	18.3	18.3	17.8	17.9
rha‴-1	104.9			104.4	104.3	104.4
2	72.6			72.6	72.7	72.6
3	72.7			72.5	72.5	71.3
4	73.7			73.5	73.8	73.8
5	73.7			70.7	70.8	70.2
6	19.1			18.8	18.8	18.8
jal-1	174.0	174.9	174.4	174.9	174.9	174.9
2	34.5	34.2	34.6	33.7	33.7	33.7
11	77.8	79.3	79.8	79.4	79.4	79.3
16	14.5	14.6	14.6	14.4	14.3	14.5
iba-1					176.7	
2					34.5	
3					19.3	
3′					19.1	
mba-1	176.3	176.4	176.1			
2	41.6	41.6	41.4			
2-Me	11.8	11.8	11.7			
3-Me	17.0	17.0	16.8			
hexa-1						173.0
2						34.4
6						14.3
deca-1				173.0		173.5
2				34.4		34.7
12				14.3		14.3
dodeca-1	172.9	173.2	173.0		172.9	
2	34.4	34.4	34.3		34.4	
12	114.3	14.3	14.3		14.4	

[a] Data recorded in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HMQC and HMBC experiments. [b]Abbreviations: fuc = fucose; rha = rhamnose; jal = 11-hydroxyhexadecanoyl; iba = 2-methylpropanoyl; mba = 2-methylbutanoyl; hexa = hexanoyl; deca = decanoyl; dodeca = dodecanoyl.

CH_3CN−MeOH (9:1), a flow rate of 0.7 mL/min, and a sample injection of 10 μL (1 mg/mL). This analysis allowed the comparison with reference solutions of the previously reported resin glycosides from this species,[7] confirming the detection of the following compounds: stoloniferin III (**12**, t_R 11.8 min), pescaprein II (**9**, t_R 13.5 min), pescaprein III (**10**, t_R 16.2 min), pescaprein IV (**11**, t_R 17.5 min), pescaproside A (**7**, t_R 37.0 min), and pescaprein I (**8**, t_R 39.5 min) in fractions VII−IX (4.6 g). The eluate across the peaks with t_R of 12.9 min (13 mg) was collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode.[8] These techniques afforded pure compound **6** (3 mg) after 20 consecutive cycles employing the same isocratic elution. The eluate with t_R 15.1 (12.8 mg) represented a complex mixture of related oligosaccharides, which was further recycled (35 consecutive times) to afford pure compound **5** (2 mg). The crude fractions IV and V were combined (740 mg) and subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 nm), using a flow rate of 9 mL/min (sample injection: 500 μL). The elution was isocratic with CH_3CN−MeOH (9:1) using a flow rate of 9 mL/min. Eluates across the peaks with t_R of 32.91 (peak I), 38.44 (peak II), and 44.65 (peak III) were collected by the technique of heart cutting and independently reinjected to be recycled in order to achieve total homogeneity after 20 consecutive cycles employing the same isocratic elution for peaks I and II. The solvent system used for recycling peak III was CH_3CN−MeOH (7:3). These techniques afforded pure compounds **4** (6.4 mg) and **12** (15.0 mg) from peak I; **2** (1.0 mg) and **3** (0.8 mg) from peak II; and **1** (12.5 mg), **7** (23.3 mg), and **10** (7.6 mg) from peak III.

Pescaproside B (1): white powder; mp 87−88 °C; $[\alpha]_D$ −35 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 1281.7572 $[M - H]^-$ (calcd for $C_{64}H_{113}O_{25}$ requires 1281.7570).

Pescaprein V (2): white powder; mp 140−142 °C; $[\alpha]_D$ −57 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1103 $[M - H]^-$, 1019 $[M - H - C_5H_8O]^-$, 921 $[M - H - C_{12}H_{22}O]^-$, 873 $[1019 - C_6H_{10}O_4]^-$, 691, 545, 417, 271; HRFABMS *m/z* 1103.6730 $[M - H]^-$ (calcd for $C_{57}H_{99}O_{20}$ requires 1103.6729).

Pescaprein VI (3): white powder; mp 136−138 °C; $[\alpha]_D$ −17 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1103 $[M - H]^-$, 921 $[M - H - C_{12}H_{22}O]^-$, 837, 775 $[921 - C_6H_{10}O_4]^-$, 691, 545, 417, 271; HRFABMS *m/z* 1103.6728 $[M - H]^-$ (calcd for $C_{57}H_{99}O_{20}$ requires 1103.6729).

Pescaprein VII (4): white powder; mp 134−136 °C; $[\alpha]_D$ −72 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1137 $[M - H]^-$, 983 $[M - H - C_{10}H_{18}O]^-$, 837 $[983 - C_6H_{10}O_4]^-$, 545, 417, 271; HRFABMS *m/z* 1137.6421 $[M - H]^-$ (calcd for $C_{56}H_{97}O_{23}$ requires 1137.6420).

Pescaprein VIII (5): white powder; mp 110−112 °C; $[\alpha]_D$ −17 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1235 $[M - H]^-$, 1089 $[M - H - C_6H_{10}O_4]^-$, 1053 $[M - H - C_{12}H_{22}O]^-$, 907 $[1053 - C_6H_{10}O_4]^-$, 837, 545, 417, 271; HRFABMS *m/z* 1235.7154 $[M - H]^-$ (calcd for $C_{62}H_{107}O_{24}$ requires 1235.7152).

Pescaprein IX (6): white powder; mp 123−124 °C; $[\alpha]_D$ −53 (*c* 0.1 MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1235 $[M - H]^-$, 1137 $[M - H - C_6H_{10}O]^-$, 1081 $[M - H - C_{10}H_{18}O]^-$, 991 $[1137 - C_6H_{10}O_4]^-$, 837, 545, 417, 271; HRFABMS *m/z* 1235.7153 $[M - H]^-$ (calcd for $C_{62}H_{107}O_{24}$ requires 1235.7152).

Alkaline Hydrolysis of Resin Glycoside Mixture. A solution of the crude fractions IV and V (80 mg) in 5% KOH−H_2O (5 mL) was refluxed at 95 °C for 3 h. The reaction mixture was acidified to pH 4.0 and extracted with $CHCl_3$ (30 mL). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The aqueous phase was extracted with *n*-BuOH (20 mL) and concentrated to dryness. The residue from the organic phase was directly analyzed by GC-MS: 2-methylpropanoic acid (t_R 4.12 min): *m/z* $[M]^+$ 88 (10), 73 (27), 60 (3), 55 (5), 45 (7), 43 (100), 41 (40), 39 (10), 29 (6), 27 (24); 2-methylbutanoic acid (t_R 7.2 min): *m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8); *n*-hexanoic acid (t_R 9.5 min): *m/z* $[M]^+$ 116 (2), 99 (3), 87 (21), 73 (63), 60 (100), 41 (16), 39 (7); *n*-decanoic acid (t_R 14.6 min): *m/z* $[M]^+$ 172 (2), 155 (3), 143 (12), 129 (62), 115 (15), 112 (12), 87 (20), 73 (100), 60 (90), 57 (40), 55 (45), 43 (30), 41 (35), 39 (6); and *n*-dodecanoic acid (t_R 17.8 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 residue extracted (35 mg) from the aqueous phase was subjected to preparative HPLC on a Waters μBondapak NH_2 column (7.8 × 300 mm; 10 μm). The elution was isocratic with CH_3CN−H_2O (4:1), using a flow rate of 3 mL/min, and a sample injection of 500 μL (35 mg/mL). This procedure yielded operculinic acid C[11] (5.2 mg, t_R = 8.7 min) and simonic acid B[13] (18.6 mg, t_R = 14.6 min), which were identified by comparison of physical constants and NMR data with published values.

Sugar Analysis. A solution of fractions IV and V (15 mg) 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 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 (17:3), 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 L-rhamnose (t_R = 7.2 min) and D-fucose (t_R = 9.8 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).

Alkaline Hydrolysis of Pescaproside B (1). A solution of compound **1** (10 mg) was submitted to an alkaline hydrolysis following the same procedures described above. The organic layer residue from extraction was directly analyzed by GC-MS, and two peaks were detected: 2-methylbutanoic acid (t_R 7.2 min) [*m*/*z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8)] and *n*-dodecanoic acid (t_R 17.8 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 preparation and identification of 4-bromophenyacyl (2*S*)-2-methylbutyrate [40−42 °C; $[\alpha]_D$ +18 (*c* 1.0, MeOH)] from pescaproside B (**1**) were performed according to previously reported procedures.[8] The aqueous phase-soluble residue was methylated with CH_2N_2 to yield 3.5 mg of a white powder. The physical and spectroscopic constants (^1H and ^{13}C NMR) registered for this derivative were identical in all aspects to those previously reported[13] for simonic acid B methyl ester: mp 113−115 °C; $[\alpha]_D$ −82.5 (*c* 1.0, MeOH); HRFABMS *m*/*z* 1015.5322 $[M - H]^-$ (calcd for $C_{47}H_{83}O_{23}$ requires 1015.5325).

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References and Notes

(1) *Ipomoea pes-caprae*, an herbaceous pantropical trailing vine, colonizes sand dunes and is called railroad vine, bay hops, and beach morning-glory in Florida and Georgia (bay winters in Bahamas) and “bejuco de playa” in Cuba, the Antilles, and the Caribbean coasts of Mexico and Central and South America.

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(3) “Riñonina”, coming from the Spanish word “riñón”, meaning kidney, is the Mexican name for this herbal tea used for the treatment of “mal de orín” (bladder and kidney ailments).

(4) In Mexico, traditional healers classify illnesses and herbal remedies as “hot” or “cold”. A hot−cold imbalance must be redressed by the ingestion of contrary elements. For the hot−cold dichotomy, see: (a) López Austin, A. *The Human Body and Ideology. Concepts of the Ancient Nahuas*; University of Utah Press: Salt Lake City, 1988; pp 270−282. (b) Ortiz de Montellano, B. R. *Aztec Medicine, Health, and Nutrition*; Rutgers University Press: New Brunswick, NJ, 1990; pp 213−235.

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