Characterization of Lipophilic Pentasaccharides from Beach Morning Glory (*Ipomoea pes-caprae*)†

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The hexane-soluble extract from the aerial parts of the herbal drug *Ipomoea pes-caprae* (beach morning-glory), through preparative-scale recycling HPLC, yielded six lipophilic glycosides, namely, five new pentasaccharides of jalapinolic acid, pescaproside A (**1**) and pescapreins I−IV (**2**−**5**), as well as the known stoloniferin III (**6**). Saponification of the crude resin glycoside mixture yielded simonic acid B (**7**) as the glycosidic acid component, whereas the esterifying residues of the natural oligosaccharides comprised five fatty acids: 2-methylpropanoic, (2*S*)-methylbutyric, *n*-hexanoic, *n*-decanoic, and *n*-dodecanoic acids. Pescaproside A (**1**), an acylated glycosidic acid methyl ester, is related structurally to the product obtained from the macrolactone hydrolysis of pescapreins I−IV (**2**−**5**). All the isolated compounds (**1**−**6**), characterized through high-field NMR spectroscopy, were found to be weakly cytotoxic to a small panel of cancer cell lines.

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae), a herbaceous pantropical trailing vine, commonly called railroad vine, bay hops, and beach morning-glory, routinely colonizes sand dunes.[1] Its goat footprint-like leaf shape is the descriptive anatomical feature naming the species. Pinkish lavender funnel-shaped flowers ($2^1/_2$ to 3 in. wide) bloom throughout summer and fall. This plant is an important pioneering species along tropical coastlines, spreading profusely to form large mats that prevent erosion. It is occasionally cultivated for the economic benefits of stabilizing sand dunes against damaging storms.[1,2] In North America, it is found from the coast of Georgia, Florida, and west through the Gulf of Mexico to the northern Yucatan Peninsula as well as on the Mexican Pacific coast up to lower California.[3] It competes for space and light with another coastal morning-glory species, *I. stolonifera*, which has white flowers and is usually found in the more protected inland dunes.

In addition to its ecological importance, railroad vine has been used in folk medicine since time immemorial. For example, the Australian aborigines still apply the heated leaves directly to wounds, skin infections, and inflamed sores, as well as to stings from poisonous fish, manta-ray, and insects. Decoctions of the plant have a worldwide use in medicinal baths to treat fatigue, strain, arthritis, and rheumatism. The infusions are used to treat hypertension and scrofula. The roots are employed for their diuretic and mild laxative actions.[4] Published reports have shown that the resin glycosides present in the convolvulaceous roots are the responsible principles for their purgative properties.[5]

"Riñonina", coming from the Spanish word "riñón" meaning kidney, is the Mexican name for this herbal drug, which is used both in infusions for kidney ailments and in decoctions to treat functional digestive disorders, colic, internal and external pain, dysentery, and inflamatory conditions.[6,7] In Mexico City, this drug is easily found in the numerous herbal markets and health food stores.

This vine is protected from most insects and grazing mammals because of its high content of indole alkaloids,[8] e.g., ergotamine. Several pharmacological evaluations have demonstrated the effectiveness of the crude extracts for neutralization of jellyfish venom contact dermatitis,[9] inhibition of prostaglandin synthesis,[10] and as hypoglycemic[11] and analgesic agents.[12] Bioactivity-guided fractionation of a lipophilic extract resulted in the isolation of an acyclic diterpene (*E*-phytol) and a partially degraded sesquiterpene (*β*-damascenone) as the antispasmodic principles.[13] Clinical trials in Thailand have resulted in certification of a tincture as an antiinflammatory treatment.[14]

Previous to this investigation, the only known study of the lipophilic resin glycosides of *I. pes-caprae* was conducted in India from a locally collected sample, which elucidated the structure of its major oligosaccharide core as 11-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-fucopyranoside-(1→2)-*O*-α-L-rhamnopyranosyl using a degradation approach.[15]

Over the past decade, our laboratory has used the documented history of traditional Mexican medicine to investigate the chemistry of morning glories in a continuing effort to elucidate the structural diversity of the convolvulaceous resin glycosides.[5] This paper describes the isolation of six lipopentasaccharides (**1**−**6**) from the hexane-soluble resin obtained from *I. pes-caprae*. A complete knowledge of the HPLC resin glycoside profile of this medicinal plant is necessary since pure samples are needed as chromatographic standards to assess quality of the herbal drug. The standardization of analytical HPLC procedures, easily scaled up for preparative purposes, was an important prerequisite for isolation. Several NMR techniquies and FABMS were used to characterize the glycosidation sequence and the location of the *O*-acyl groups in these complex glycolipids.

Results and Discussion

The herbal drug "riñonina" (*I. pes-caprae*) was purchased as dried whole plants from a health food store located in downtown Mexico City. A macroscopic examination was used to identify this material by comparing it with an

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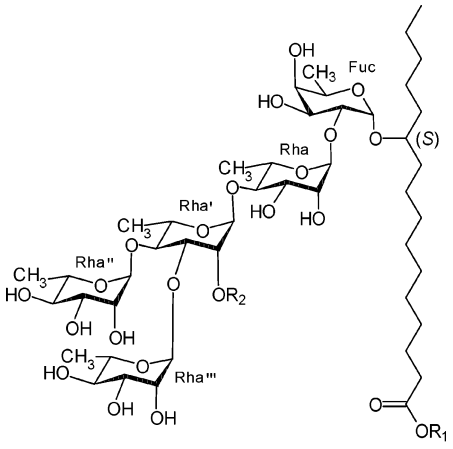
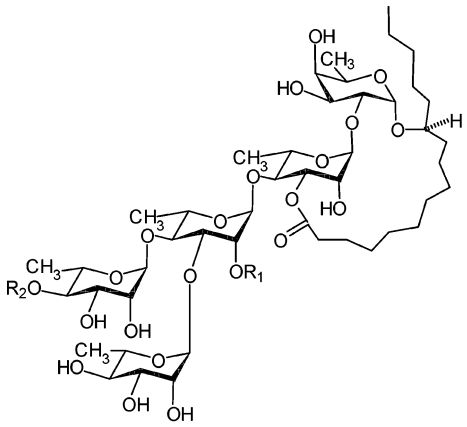
authentic dried sample collected on the Mexican Pacific coast. This evaluation determined that the drug was in an entirely pure condition, containing no appreciable quantities of foreign matter. Hexane extracts of the crude drug were prepared and compared by TLC and HPLC with a reference solution from the authentic plant material sample. This analysis confirmed the same lipophilic resin glycoside content in both tested samples. Then, the crude herbal drug was pulverized and macerated with hexane, and the extract was fractionated by column chromatography on silica gel. The major fraction, rich in resin glycosides, was further separated by first using C_{18} reversed-phase column chromatography followed by recycling preparative-scale HPLC.[16] This methodological approach allowed for the isolation of glycolipids **1**−**6**.



	R_1	R_2
1	CH_3	*n*-dodecanoyl
7	H	H
8	CH_3	H

A small sample of the crude resin was saponified to liberate an organic solvent-soluble acid fraction together with an H_2O-soluble pentasaccharide of jalapinolic acid. The structure of this glycosidic acid was confirmed as simonic acid B (**7**), previously obtained from *I. batatas*[17] and *I. stolonifera*.[18] The physical properties and NMR data of its derivative **8** matched, within experimental error, those values reported for simonic acid B methyl ester.[17] On the basis of the GC analysis of the base-hydrolyzed products recovered from the organic fraction, five residues, 2-methylpropanoic (isobutyric), (2*S*)-methylbutyric, *n*-hexanoic (caproic), *n*-decanoic (capric), and *n*-dodecanoic (lauric) acids, were identified by comparison of their mass spectra and retention times with those of authentic samples.

Pescaproside A (**1**), an amorphous powder, gave a quasimolecular ion at *m/z* 1197 $[M - H]^-$ ($C_{59}H_{105}O_{24}$) in the negative-ion FABMS; the observed difference of 182 mass units ($C_{12}H_{22}O$) between this natural product and simonic acid B methyl ester (**8**; *m/z* 1015; $[M - H]^-$) corroborated the presence of dodecanoic acid as an ester group on the oligosaccharide core. From the ^1H NMR spectra (Table 1), the esterified position at C-2 of the second saccharide moiety (δ 6.0) was easily identified through the strong deshielding effect ($\delta\Delta$ 1.4) relative to the nonesterified equivalent on **8**.[17] 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. Accordingly, compound **1** was characterized as methyl (11*S*)-hydroxyhexadecanoate 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-dodecanoyl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside. This component represents the third example of a naturally acylated glycosidic acid related to the macrocyclic ester structures of the convolvulaceous resin glycosides. Previously, two acylated trisaccharides named cus-1 and cus-2 were isolated from the traditional Chinese medicine *Cuscuta chinensis*.[19]



	R_1	R_2
2	*n*-dodecanoyl	H
3	*n*-dodecanoyl	2-methylpropanoyl
4	*n*-dodecanoyl	(2*S*)-methylbutanoyl
5	*n*-dodecanoyl	*n*-hexanoyl
6	*n*-decanoyl	(2*S*)-methylbutanoyl

Negative-ion FABMS of compounds **2**−**5** were obtained. As reported,[20] this technique was successful in providing intense pseudomolecular $[M - H]^-$ ions for the analysis of this type of glycolipids. Pescaprein I afforded a peak at *m/z* 1165, indicating a molecular formula of $C_{58}H_{102}O_{23}$ for natural product **2**. For pescaprein II (**3**), the mass spectra revealed a $[M - H]^-$ ion at *m/z* 1235 ($C_{62}H_{107}O_{24}$) in contrast to the ion detected at *m/z* 1249 ($C_{63}H_{109}O_{24}$) for its homologue **4** (pescaprein III), indicating a difference of one methylene group between both compounds. Pescaprein IV (**5**) showed the $[M - H]^-$ ion at *m/z* 1263 ($C_{64}H_{111}O_{24}$). The initial loss of one of the esterifying groups afforded a peak at *m/z* 1165 representing $[M - C_4H_6O]^-$, $[M - C_5H_8O]^-$, or $[M - C_6H_{10}O]^-$ (Figure 1; fragment a) in prescapreins II−IV (**3**−**5**), in addition to the peak $[M - C_{12}H_{22}O]^-$ at *m/z* 983, 1067, and 1081 corresponding to the $[M - 182]^-$ ion (Figure 1; fragment b) in pescapreins I (**2**), III (**4**), and IV (**5**), respectively. All spectra displayed the common fragment peaks (Figure 1) produced by glycosidic cleavage of each sugar moiety at *m/z* 1019, 837, 545, and 417, which were similar to those reported for the stoloniferin series,[18] confirming the branched pentasaccharide core of prescapreins as that previously described for simonic acid B (**7**).

All four pescapreins (**2**−**5**) showed the following common features in the ^1H and ^{13}C NMR spectra (Tables 1 and 2): (a) signals attributable to the nonequivalent protons of the methylene group at C-2 in the aglycon moiety confirmed their macrocyclic lactone-type[20] structure; (b) the site of lactonization at C-3 (δ_C 77) of the second saccharide (Rha) was established by the significant downfield shift observed for its geminal proton (δ 5.6) in contrast to the same resonance in compounds **1** and **8** (δ 4.6); (c) a triplet-like signal for the methylene group at C-2 (δ_H 2.3) for a dodecanyl group esterifying the hydroxyl group on position C-2 of the third saccharide unit (Rha′: δ_H 5.8; δ_C 72−73); (d) compounds **3**−**5** showed signals for one short-chain fatty acid residue esterifying position C-4 at Rha″ (δ_C 73); H-2

Table 1. ^{1}H NMR Data of Compounds **1**−**5** (500 MHz)[a]

proton[b]	1	2	3	4	5
fuc-1	4.79 d (7.8)	4.79 d (7.8)	4.81 d (7.6)	4.81 d (7.9)	4.78 d (7.9)
2	4.52 dd (9.1, 7.8)	4.49−4.52*	4.52 dd (9.5, 7.6)	4.53 dd (9.5, 7.9)	4.50 dd (9.5, 7.9)
3	4.14 dd (9.1, 2.7)	4.18 dd (9.5, 3.1)	4.19 dd (9.5, 3.4)	4.19 dd (9.5, 3.4)	4.16 dd (9.5, 3.3)
4	3.93 d (2.7)	3.90 bs	3.91 d (3.4)	3.91 d (3.4)	3.89 d (3.3)
5	3.78 q (6.4)	3.80 q (6.4)	3.82 q (6.4)	3.81 q (6.4)	3.79 q (6.4)
6	1.51 d (6.4)	1.50 d (6.4)	1.52 d (6.4)	1.51 d (6.4)	1.49 d (6.4)
rha-1	6.26 d (1.6)	6.33 bs	6.32 d (1.5)	6.35 d (1.4)	6.32 d (1.0)
2	4.64 dd (2.7, 1.6)	5.30 bs	5.30 dd (2.8, 1.5)	5.32 dd (2.6, 1.4)	5.29 dd (2.5, 1.0)
3	4.48 dd (9.4, 2.7)	5.60 dd (9.7, 2.5)	5.61 dd (9.8, 2.8)	5.60 dd (9.8, 2.6)	5.58 dd (10.1, 2.5)
4	4.31 dd (9.4, 9.4)	4.64 dd (9.7, 9.7)	4.63 dd (9.8, 9.8)	4.63 dd (9.8, 9.8)	4.61 dd (10.1, 10.1)
5	4.88 dq (9.4, 6.3)	4.98 dq (9.3, 6.2)	5.00 dq (9.8, 6.7)	5.00 dq (9.8, 6.2)	4.98 dq (10.1, 6.2)
6	1.59 d (6.3)	1.57 d (6.2)	1.58 d (6.7)	1.57 d (6.2)	1.55 d (6.2)
rha′−1	5.94 d (1.0)	5.65 d (1.0)	5.65 d (1.5)	5.66 d (1.5)	5.63 d (1.5)
2	6.02 dd (2.2, 1.0)	5.81 dd (3.7, 1.0)	5.82 dd (3.3, 1.5)	5.82 dd (3.2, 1.5)	5.80 dd (2.0, 1.5)
3	4.67 dd (9.1, 2.2)	4.49−4.52*	4.52 dd (8.7, 3.3)	4.52 dd (9.5, 3.2)	4.50 dd (9.4, 2.0)
4	4.33−4.35*	4.31 dd (8.5, 8.5)	4.25 dd (8.7, 8.7)	4.23 dd (9.4, 9.4)	4.20 dd (9.4, 9.4)
5	4.32 dq (9.1, 6.0)	4.29 dq (8.5, 5.9)	4.32 dq (8.7, 6.4)	4.32 dq (9.4, 6.0)	4.31 dq (9.4, 6.0)
6	1.63 d (6.0)	1.63 d (5.9)	1.60 d (6.4)	1.59 d (6.0)	1.57 d (6.0)
rha″-1	6.12 d (1.6)	5.92 bs	5.89 bs	5.92 bs	5.90 bs
2	4.65 dd (3.2, 1.6)	4.64*	4.62 dd (3.3, 1.3)	4.62 dd (3.4, 1.3)	4.61 dd (3.2, 1.0)
3	4.37 dd (9.5, 3.2)	4.36 dd (9.5, 3.1)	4.42 dd (9.8, 3.3)	4.41 dd (9.7, 3.4)	4.41 dd (9.7, 3.2)
4	4.24 dd (9.5, 9.5)	4.25 dd (9.5, 9.5)	5.76 dd (9.8, 9.8)	5.78 dd (9.7, 9.7)	5.78 dd (9.7, 9.7)
5	4.33−4.35*	4.27−4.33*	4.33 dq (9.8, 6.1)	4.35 dq (9.7, 6.3)	4.33 dq (9.7, 6.2)
6	1.52 d (6.2)	1.54 d (6.14)	1.38 d (6.1)	1.38 d (6.3)	1.38 d (6.2)
rha‴-1	5.74 bs	5.58 bs	5.56 bs	5.56 d (1.0)	5.55 bs
2	4.85 dd (3.5, 1.0)	4.82 dd (2.9, 1.3)	4.78 dd (3.0, 1.3)	4.79 dd (3.7, 1.0)	4.76 dd (3.0, 1.0)
3	4.49 dd (9.5, 3.5)	4.49−4.52*	4.51 dd (9.5, 3.0)	4.53 dd (9.1, 3.7)	4.50 dd (9.2, 3.0)
4	4.26 dd (9.5, 9.5)	4.27−4.33*	4.21 dd (9.5, 9.5)	4.26 dd (9.1, 9.1)	4.24 dd (9.2, 9.2)
5	4.33−4.35*	4.27−4.33*	4.27 dq (9.5, 5.8)	4.28 dq (9.1, 6.1)	4.27 dq (9.2, 6.0)
6	1.73 d (6.2)	1.71 d (6.0)	1.71 d (5.8)	1.70 d (6.1)	1.68 d (6.0)
jal-2	2.31 t (7.52)	2.25 ddd (15.3, 6.1, 2.0)	2.28 m	2.25 ddd (15.3, 7.1, 3.1)	2.24 m
		2.91 t (12.1)	2.93 t (12.5)	2.93 t (11.7)	2.91 t (12.7)
11	3.97 t (5.8)	3.84 m	3.87 m	3.86 m	3.89 m
16	0.85 t (7.2)	0.85 t (7.5)	0.86 t (7.0)	0.85 t (6.8)	0.83 t (6.7)
iba-2			2.64 sept (7.0)		
3			1.17 d (7.0)		
3′			1.20 d (7.0)		
mba-2				2.48 tq (7.0, 6.7)	
2-Me				1.19 d (7.2)	
3-Me				0.92 t (7.4)	
hexa-2					2.38 m
6					0.75 t (7.1)
dodeca-2	2.32 t (7.5)	2.33 t (7.4)	2.38 t (7.3)	2.35 t (7.2)	2.35 t (7.5)
12	0.91 t (6.8)	0.92 t (7.1)	0.95 t (7.0)	0.93 t (6.8)	0.91 t (6.8)

[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; mba = 2-methylbutanoyl; iba= 2-methylpropanoyl; hexa = hexanoyl; deca = decanoyl; dodeca= dodecanoyl.

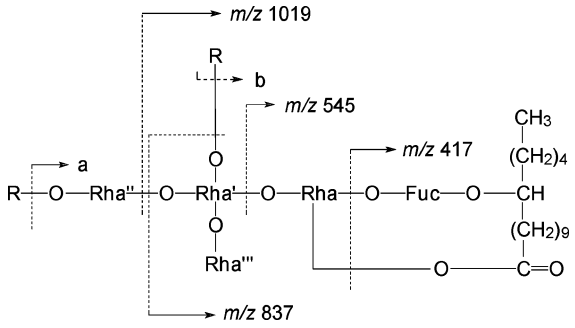


Figure 1. Negative-ion FABMS fragmentation pattern of resin glycosides **2**−**5**.

of these moieties was used as a diagnostic resonance centered at δ 2.64 (1H, septet) for the isobutyroyl group in pescaprein II (**3**), at δ 2.5 (1H, tq) for the methylbutyroyl group in **4** (pescaprein III), and at δ 2.4 (2H, t) for the methylene equivalent protons of the hexanoyl group in pescaprein IV (**5**). In all cases it was possible by HMBC analysis[5,20] to establish the links between a specific carbonyl ester group with their corresponding vicinal proton resonance ($^2J_{CH}$) and the pyranose ring proton at the site of esterification ($^3J_{CH}$). For example, the carbonyl resonance of the lactone functionality (δ 174) was assigned by the ^2J-coupling with each of the methylene protons at δ_H ca. 2.3 (1H, ddd) and 2.9 (1H, t) on the adjacent C-2 position of the aglycon. The site of lactonization was placed at C-3 of Rha by the observed ^3J-coupling between this carbonyl carbon and its geminal proton (δ 5.6). The dodecanoyl residue was identified through the observed $^2J_{CH}$ coupling between the carbonyl resonance at δ 172 with the triplet-like signal for the vicinal methylene protons (δ 2.3, 2H) and its location at C-2 of Rha′ by the $^3J_{CH}$ coupling with the signal at δ 5.8. Therefore, the remaining esterified position represented the location of the additional ester linkage in pescapreins II−IV (**3**−**5**), that is, C-4 of Rha″.

The cytotoxic potential of isolates **1**−**6** was evaluated against four human cancer cell lines. All compounds exhibited weak cytotoxicity against nasopharyngeal, colon, squamous cell cervical, and ovarian carcinomas (ED_{50} 5−20 μg/mL). Although the level of potency was similar to that displayed by other lipophilic resin glycosides, e.g., the orizabins (tetrasaccharides from the Mexican scammony

Table 2. ^{13}C NMR Data of Compounds **1**−**5** (125.7 MHz)[a]

carbon[b]	**1**	**2**	**3**	**4**	**5**
fuc-1	101.2	101.6	101.2	101.6	101.2
2	72.7	73.4	73.0	73.4	73.0
3	76.6	76.6	76.3	76.7	76.3
4	73.5	73.6	73.2	73.6	73.2
5	71.2	71.2	70.9	71.3	70.9
6	17.2	17.2	16.8	17.2	16.8
rha-1	101.3	100.5	99.9	100.3	99.9
2	72.9	69.8	69.4	69.8	69.4
3	74.9	77.9	77.4	77.8	77.5
4	80.1	77.5	77.6	78.1	77.6
5	66.9	67.9	67.5	67.9	67.5
6	18.8	18.6	18.8	19.2	18.8
rha′-1	103.6	99.4	98.8	99.2	98.8
2	73.5	73.0	72.6	73.0	72.6
3	80.1	80.4	79.9	80.3	79.9
4	78.9	78.5	78.8	79.2	78.7
5	68.4	68.5	67.9	68.4	67.9
6	18.3	18.3	18.4	18.8	18.4
rha″-1	98.6	103.2	103.3	104.3	103.2
2	72.6	72.6	72.3	72.7	72.3
3	72.7	72.5	69.8	70.2	69.8
4	73.7	73.8	74.4	74.8	74.7
5	70.6	70.7	67.7	68.2	67.7
6	19.1	19.1	17.5	17.9	17.5
rha‴−1	104.8	104.4	103.9	103.7	104.0
2	72.6	72.6	72.3	72.6	72.2
3	72.7	72.5	72.2	72.6	72.1
4	73.6	73.6	73.4	73.7	73.4
5	70.5	70.7	70.4	70.8	70.4
6	18.6	18.8	18.4	18.8	18.4
jal-1	174.0	174.9	174.5	174.9	174.5
2	34.2	33.7	33.3	33.7	33.3
11	77.9	79.4	79.0	79.4	79.0
16	14.3	14.3	13.9	14.3	13.9
iba-1			175.9		
2			41.2		
3			11.4		
3′			16.6		
mba-1				176.4	
2				41.6	
2-Me				17.0	
3-Me				11.8	
hexa-1					173.1
2					34.0
6					13.6
dodeca-1	172.9	172.9	172.6	173.0	172.6
2	34.4	34.4	34.0	34.4	34.2
12	14.4	14.4	14.0	14.4	14.0

[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.

root),[16] it was lower than that observed with the more amphiphilic glycolipids (e.g., tricolorin A).[5] Detailed structure−activity studies are needed to explain the observed differences in the cytotoxic potential of the convolvulaceous resin glycosides. It is hypothesized that the cytotoxic properties of the above-mentioned compounds could reside in their ability to perturb cell membranes (through a possible ion flux disturbance), reflecting surface interactions with target cells. These resin glycosides have a peculiar organization in aqueous solution in the form of micelles or molecular aggregates similar to those formed by tricolorin A in the crystalline state.[21] The hydrophobic pentasaccharides discussed in this paper constitute an example of the subtle solubility balance in low-polarity organic solvents (i.e., hexane, ether, and chloroform) resulting from the acylation of the oligosaccharide core by long-chain fatty acids.

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 on a Bruker AMX-500 instrument. The NMR techniques were performed according to previously described methodology.[20] Negative-ion LR-FABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX102A spectrometer. Cytotoxicity was conducted at UNAM (Facultad de Química) using cultured KB (nasopharyngeal carcinoma), SQC-1 UISO (squamous cell cervical carcinoma), HCT-15 (colon cancer), and OVCAR (ovarian adenocarcinoma) cells, according to previously described protocols.[22] Vinblastine was included as a positive drug control: ED_{50} (μg/mL) 0.01 (KB); 0.05 (SQC-1 UISO); 0.02 (HCT-15); 0.06 (OVCAR).

Plant Material. The herbal drug "riñonina" was purchased in March 2002 at the health food store "Las Plantas Medicinales de América, S.A. de C.V." in Mexico City. A small sample (150 g) was taken for preliminary examination and 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 an authentic sample collected by Dr. Robert Bye in dunes along an upper beach in Las Salinas, Chamela Bay, Jalisco, Mexico, in November 1989. A voucher specimen (R. Bye 17707) was deposited in the Ethnobotanical Collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation. The whole plant (4 kg) was powdered and extracted by maceration at room temperature with hexane to give, after removal of the solvent, a dark green syrup (40.6 g). The extract was compared by TLC (silica gel 60 F_{254} aluminum sheets; $CHCl_3$−MeOH, 4:1) with a reference solution of an authentic *I. pes-caprae* collection, which confirmed the detection of a similar lipophilic resin glycoside mixture (R_f 0.45) in both samples. The crude extract prepared from the herbal drug was subjected to column chromatography over silica gel (250 g) using a gradient of $CHCl_3$ in hexane followed by $CHCl_3$−Me_2CO (7:3). A total of 80 fractions (150 mL each) were collected and combined to give several pools containing mixtures of resin glycosides. The most lipophilic fractions (2−11), eluted with hexane−$CHCl_3$ (3:1), were subjected to reversed-phase C_{18} column chromatography (50 g), eluting with MeOH (400 mL), to eliminate a pigmented oily residue. The process was monitored by TLC, and 20 fractions (20 mL each) were collected and combined (subfractions 5−11), yielding a mixture of lipophilic pentasaccharides (3.19 g).

Recycling HPLC Separation. 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). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 32 software program (Waters). The analytical HPLC separations were done on a Symmetry C_{18} column (Waters; 5 μm, 4.6 × 250 mm) with an isocratic elution of CH_3CN−MeOH (9:1), a flow rate of 0.7 mL/min, and a sample injection of 10 μL (1 mg/mL). The crude fraction 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 (9:1) using a flow rate of 9 mL/min. Eluates across the peaks with t_R of 11.8 min (peak I), 13.5 min (peak II), 16.2 min (peak III), 17.5 min (peak IV), and 39.6 min (peak V) were collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode[16] to achieve total homogeneity after 10−20 consecutive cycles employing the same isocratic elution. These techniques afforded pure compound **3** (7.6 mg) from peak II; **4** (53.2 mg) from peak III; **5** (8 mg) from peak IV; and stoloniferin III (**6**) (15.8 mg) from peak I. An isocratic elution

with CH_3CN−MeOH (65:35) was used for the resolution of peak V to afford pure compounds **1** (t_R 9.2 min; 3 mg) and **2** (t_R 10.2 min; 3.8 mg).

Pescaproside A (1): amorphous white powder; mp 89−91 °C; $[\alpha]_D$ −40° (*c* 0.1, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 1197.6990 $[M - H]^-$ (calcd for $C_{59}H_{105}O_{24}$ requires 1197.6995).

Pescaprein I (2): amorphous white powder; mp 131−133 °C; $[\alpha]_D$ −65° (*c* 0.06, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1165 $[M - H]^-$, 983 $[M - H - C_{12}H_{22}O]^-$, 837, 545, 417, 271; HRFABMS *m/z* 1165.6730 $[M - H]^-$ (calcd for $C_{58}H_{101}O_{23}$ requires 1165.6733).

Pescaprein II (3): amorphous white powder; mp 120−123 °C; $[\alpha]_D$ −81° (*c* 0.19, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1235 $[M - H]^-$, 1165 $[M - H - C_4H_6O]^-$, 1019, 837, 545, 417, 271; HRFABMS *m/z* 1235.7147 $[M - H]^-$ (calcd for $C_{62}H_{107}O_{24}$ requires 1235.7152).

Pescaprein III (4): amorphous white powder; mp 121−123 °C; $[\alpha]_D$ −70° (*c* 0.43, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1249 $[M - H]^-$, 1165 $[M - H - C_5H_8O]^-$, 1067 $[M - H - C_{12}H_{22}O]^-$, 1019, 837, 545, 417, 271; HRFABMS *m/z* 1249.7224 $[M - H]^-$ (calcd for $C_{63}H_{109}O_{24}$ requires 1249.7309).

Pescaprein IV (5): amorphous white powder; mp 120−122 °C; $[\alpha]_D$ −60° (*c* 0.81, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1263 $[M - H]^-$, 1165 $[M - H - C_6H_{10}O]^-$, 1081 $[M - H - C_{12}H_{22}O]^-$, 1019, 837, 545, 417, 271; HRFABMS *m/z* 1263.7499 $[M - H]^-$ (calcd for $C_{64}H_{111}O_{24}$ requires 1263.7465).

Stoloniferin III (6): amorphous white powder; mp 120−123 °C; $[\alpha]_D$ −64° (*c* 0.43, MeOH); HRFABMS *m/z* 1221.7049 $[M - H]^-$ (calcd for $C_{61}H_{105}O_{24}$ requires 1221.6996); identified by comparison of NMR data with published values.[18]

Alkaline Hydrolysis of the Resin Glycoside Fraction. A solution of the resin glycoside fraction (200 mg) in 5% KOH−H_2O (5 mL) was refluxed at 95 °C for 2 h. The reaction mixture was acidified to pH 4.0 and extrated 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 residue was directly analyzed by GC-MS: HP-5MS (5%-phenyl)-methylpolysiloxane column (30 m × 0.31 mm, film thickness 2 μm); He, 2 mL/min; 50 °C isothermal for 3 min, linear gradient to 300 °C at 20 °C/min; with five peaks detected. These were 2-methylpropanoic acid (t_R 3.0 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-methylbutyric acid (t_R 6.9 min): *m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8); *n*-hexanoic acid (t_R 8.5 min): *m/z* $[M]^+$ 116 (2), 99 (3), 87 (21), 73 (63), 60 (100), 41 (16), 39 (7); *n*-decanoic acid (t_R 14.7 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 17.5 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 preparation and identification of 4-bromophenacyl (2*S*)-2-methylbutyrate [mp 40−42 °C; $[\alpha]_D$ +18.6 (*c* 1.0, MeOH)] from pescaprein II (**3**) were performed according to previously reported procedures.[16]

The aqueous phase was extracted with *n*-BuOH (30 mL) and concentrated to give a colorless solid (85 mg). The residue was methylated with CH_2N_2 to give compound **8**. The physical and spectroscopic constants (^1H and ^{13}C NMR) registered for this derivative were identical in all aspects to those previously reported[17] for simonic acid B methyl ester: white powder; 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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