Orizabins V−VIII, Tetrasaccharide Glycolipids from the Mexican Scammony Root (*Ipomoea orizabensis*)[1]

Beatriz Hernández-Carlos,† Robert Bye,‡ and Rogelio Pereda-Miranda*,†

Departamento de Farmacia, Facultad de Química, and Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, D.F., México

Received February 17, 1999

An extensive investigation of the so-called jalapin resinoid obtained from roots of the Mexican scammony, *Ipomoea orizabensis*, using high field NMR spectroscopy led to the characterization of six glycosides, including the known scammonins I (**1**) and II (**2**) and four new tetrasaccharides of jalapinolic acid, orizabins V−VIII (**3**−**6**). All the isolates (**1**−**6**) were found to be weakly cytotoxic toward human oral epidermoid carcinoma (KB).

Members of the morning glory family (Convolvulaceae) containing cathartic glycoresins are twining herbs, most of them native to tropical regions, that have been prized all over the world since ancient times for their laxative and purgative properties.[2] The genus *Ipomoea* in particular includes a great number of medicinal and economically important species in modern Mexico.[2,3] Varieties of the sweet potato, *I. batatas* (L.) Lam. are appreciated for their nutritious root.[4] Several species of morning glories are also well-known as ornamentals in horticulture and as cover crops in agriculture.[5] The utilization of the hallucinogenic seeds[6] of *I. tricolor* Cav. in divination as well as in religious and curing rituals was very significant in the life of pre-Hispanic Mexico and Central America.[7]

Ipomoea orizabensis (Pelletan) Ledebour ex Steudel is commonly applied to plants known as Mexican scammony[8] or "escamonea"[9] and forms part of the Mexican medicinal plant complex of jalaps,[10] which corresponds to the scammony complex of the Old World.[11] The roots of this plant have also been called "jalapa de Orizaba" (orizaba jalap) in the vernacular and are used as a substitute and adulterant for the roots of *I. jalapa* (L.) Pursh and *I. purga* (Wender.) Hayne, "raíz de Jalapa" (jalap root). These plants are best known and highly esteemed in American and European pharmacopeas for their purgative properties.[2]

Mexican scammony has been considered a less potent purgative than jalap, hence its common name "jalapa ligera" or light jalap. As a replacement for *I. purga*, *I. orizabensis* has likewise been employed as a purgative and anthelmintic. This medicinal plant has also been used to treat abdominal fever, dysentery, epilepsy, hydrocephaly, "manchas" (a spirochete skin infection), meningitis, and tumors.[12]

The cathartic crude drugs are derived from the roots, which are rich in glycoresins (10−18%). The structures of the active ingredients remained poorly known, prior to application of HPLC and high-resolution spectroscopic techniques.[13,14] It has been shown that these resin glycosides consist of complex mixtures of monohydroxy and dihydroxy C_{14} and C_{16} fatty acids glycosidically linked to an oligosaccharide core.[15] The glycolipids, in most of the cases, also contain an intramolecular macrocyclic lactone.[16−19]

As part of a continuing effort to identify bioactive compounds from Mexican medicinal plants,[2] the present study describes the isolation of two known glycosides, scammonins I (**1**) and II (**2**), and four new tetrasaccharides of jalapinolic acid, orizabins V−VIII (**3**−**6**), from the soluble-ether resin (jalapin) of *I. orizabensis.* Several high-resolution NMR techniques,[14] in conjunction with FABMS, were used to characterize a glycosidation sequence in **1**−**6** different from that previously reported for the oligosaccharide core of orizabins I−IV; the first individual glycolipids isolated from the jalapin of the Mexican scammony root.[13]

Results and Discussion

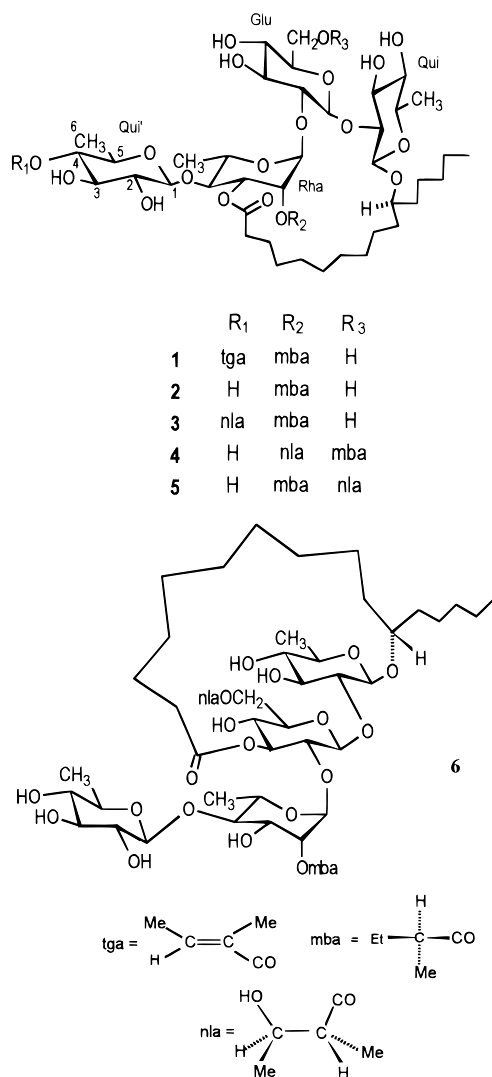
Roots of pulverized *I. orizabensis* were macerated with $CHCl_3$, and the extract was fractionated by column chromatography on Si gel. The major polar fraction rich in resin glycosides, which displayed significant cytotoxic activity (KB: ED_{50} 2.5 μg/mL), was subjected to recycling[20] preparative HPLC using a combination of reversed and normal phases[21] (C_{18} and aminopropyl silica-based bonded phases, respectively). This methodological approach allowed the isolation of glycolipids **1**−**6**.

A small sample of the analyzed glycosidic fraction was saponified to liberate an organic acid fraction together with an H_2O-soluble linear tetraglycoside of (11*S*)-hydroxyhexadecanoic acid. GC−MS analysis of the organic acid fraction, previously treated with CH_2N_2, permitted the identification of its main constituents as (2*S*)-methylbutyric, tiglic, and (3*R*)-hydroxy-(2*R*)-methylbutyric (nilic) acids by comparison with the mass spectra and retention times of the methyl esters of authentic samples. The composition of the oligosaccharide was established by GC−MS analysis[14] of the silylated sugar residues obtained from the acid-catalyzed hydrolysis of the saponification product. The structure of this glycosidic acid was confirmed from its ^{1}H and ^{13}C NMR data as scammonic acid A,[16] (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-quinovopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, previously obtained from *Convolvulus scammonia* L. (scammony root)[16,22] and *I. stans* Cav.[23,24]

The negative ion FABMS of compounds **3**−**6** were almost identical; all four had a pseudo-molecular ion at *m*/*z* 1037 $[M - H]^-$. Therefore, they were isomeric compounds of molecular formula $C_{50}H_{86}O_{22}$. In addition to the common fragmentation peaks[13,14] produced by glycosidic cleavage of the sugar moieties, the observed difference of 100 mass units between **3**−**6** and scammonin II (**2**)[16] corroborated the presence of nilic acid as an additional ester group on the oligosaccharide core of the new orizabins.

* To whom correspondence should be addressed. Tel.: (525) 622-5288. Fax: (525) 622-5329. E-mail: pereda@servidor.unam.mx.
† Departamento de Farmacia, Facultad de Química.
‡ Jardín Botánico, Instituto de Biología.

10.1021/np9900627 CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/27/1999



Patterns of substitution on individual saccharide units in **3**–**6** were established by 2D homonuclear NMR techniques (COSY and TOCSY).[14] From the ^{1}H NMR spectra (Table 1), esterified positions on the tetrasaccharide cores were easily identified through strong deshielding effects relative to the nonesterified equivalents on **2**. All four new compounds showed the following common features: (a) signals attributable to the nonequivalent protons of the methylene group at C-2 in the aglycon moiety, which confirmed their macrocyclic lactone-type[13,14] structure; (b) signals for two short-chain fatty acid residues esterifying the oligosaccharide core: H-2 of these moieties was used as a diagnostic resonance centered at δ 2.3–2.5 (1H, tq) for the methylbutyroyl groups and at δ 2.8–2.9 (1H, dq, $J_{2,3}$ = 7 Hz) for the *threo*-isomer[25] of the niloyl residues; (c) the hydroxyl group on position C-2 of the rhamnose unit was obviously acylated as in model compound **2** (δ_H ca. 6.2; δ_C 69–72); (d) the site of lactonization at C-3 of rhamnose for **3**–**5** was established by the significant downfield shift observed for its geminal proton ($\Delta\delta$ ca. 1.8) in contrast to the same resonance in **6** (δ 4.62), where the acylation was observed for the methylene proton on C-3 of glucose (δ 5.71). These postulations were verified by HMBC analysis[14,17] and the usual downfield shift due to acylation of the carbon bearing the ester group (Table 2).

The most shielded carbonyl resonance was assigned to the lactone functionality by the ^2J-coupling with each of the methylene protons δ_H ca. 2.3–2.8 on the adjacent C-2 position of the aglycon. In **3**–**5**, the site of lactonization was placed at C-3 of rhamnose by the observed ^3J-coupling between the carbonyl carbon of the aglycon (δ 173) and its geminal proton (δ ca. 6.4) in the pyranose unit. For **6**, the position of lactonization was verified by the observed connectivities between the carbonyl group of the aglycon (δ 172) and H-3 of glucose. Therefore, compound **6** is unique, among the reported derivatives of scammonic acid A,[22–24] in having the site of lactonization at C-3 of the second sugar unit rather than at C-3 of the third saccharide moiety. By a similar analysis, the $^{2,3}J_{CH}$ interactions were used to differentiate between the carbonyl resonances for the residues of nilic and methylbutyric acids esterifying the oligosaccharide core. It was possible in all cases to establish the links between a specific carbonyl ester group with their corresponding vicinal methyne resonance ($^2J_{CH}$) and the pyranose ring proton at the site of esterification ($^3J_{CH}$). For example, a methylbutyroyl group could be placed at C-2 in the rhamnose unit of **3**, **5**, and **6**. For compound **4**, the $^2J_{CH}$ coupling between the carbonyl resonance at δ 175.6 with the quintet-like proton at δ 2.7 were used to identify the nilate residue and its location at C-2 of the rhamnose by the $^3J_{CH}$ coupling with the signal at δ 6.14. Therefore, the remaining esterified position represented the location of the additional ester linkage, that is, C-4 of terminal quinovose in **3** and C-6 of glucose in **4**–**6**.

Finally, the cytotoxicity of isolates **1**–**6** was evaluated using four human cancer cultured cell lines. All compounds exhibited weak activity against oral epidermoid carcinoma (KB; ED_{50} 7–10 μg/mL) and were inactive (ED_{50} > 15 μg/mL) against colon carcinoma, squamous cell cervix carcinoma, and ovarian cancer cell lines. Similar KB selectivity was previously observed for the structurally related tetrasaccharides isolated from *I. stans*.[24] However, the cytotoxicity displayed for **1**–**6** was one order of magnitude lower than the values reported (ED_{50} < 2 μg/mL) for the isolates from *I. stans*. In the latter, the macrocyclic structures are composed of the same basic oligosaccharide of **2**, but with three, rather than one or two, short-chain fatty acids.[23,24] This variation in activity, relating to the degree of esterification of the oligosaccharide cores,[2] emphazises the value of additional structure–activity investigations of the so-called resin glycosides of convolvulaceous plants.

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. Negative ion LRFABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX102A spectrometer. ^{1}H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted either on a Bruker AMX-500 or a Varian XL-500 instruments. The experimental procedures, including preparative HPLC instrumentation and NMR techniques, were performed according to previously described methodology, unless stated otherwise.[14,26]

Plant Material. Roots of *Ipomoea orizabensis* were obtained from northwestern Veracruz near the state of Hidalgo in February 1990. The voucher specimen (R. Bye 17761) was deposited in the Ethnobotanical Collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Isolation of Compounds 1–6. The dried roots (5.0 kg) were powdered and defatted by maceration at room temperature with hexane. The residual material was extracted exhaustively with $CHCl_3$ to give, after removal of the solvent, a dark brown syrup (743.7 g). The crude resinous extract (300 g; KB: ED_{50} 7.7 μg/mL) was subjected to column chromatography over Si gel (1.5 kg) in a gravity column using a gradient

Table 1. ^{1}H NMR Spectral Data of Compounds **3**−**6** (500 MHz)[a]

proton[b]	3	4	5	6
Qui-1	4.98 d (7.5)	4.93 d (7.7)	5.02 d (8.0)	4.73 d (8.0)
2	4.29 dd (9.0, 7.5)	4.24 dd (9.0,7.7)	4.32 dd (9.0, 8.0)	4.39 dd (9.0, 8.0)
3	4.10 dd (9.0, 9.0)	4.11 dd (9.0, 9.0)	4.18 dd (9.0, 9.0)	4.26 dd (9.0, 9.0)
4	3.55 dd (9.0, 9.0)	3.54 dd (9.0, 9.0)	3.62 dd (9.0, 9.0)	3.55 dd (9.0, 9.0)
5	3.67−3.80*	3.63−3.70*	3.68−3.78*	3.60 m
6	1.58 d (6.5)	1.58 d (6.0)	1.58 d (6.0)	1.52 d (5.5)
Glu-1	5.92 d (7.5)	5.84 d (7.4)	5.91 d (7.0)	5.85 d (7.5)
2	4.24 dd (9.0, 7.5)	4.12 dd (9.0, 7.4)	4.18 dd (9.0, 7.0)	4.10 dd (9.0, 7.5)
3	4.30 dd (9.0, 9.0)	4.16 dd (9.0, 9.0)	4.24 dd (9.0, 9.0)	5.71 dd (9.0, 9.0)
4	4.08 dd (9.0, 8.5)	3.83 dd (10.0, 9.0)	3.92 dd (9.0, 9.0)	4.17 dd (9.5, 9.0)
5	3.87ddd (8.5, 6.5, 3.0)	3.92 dd (10.0, 6.8, 2.0)	3.96−4.1*	3.93 ddd (9.5, 6.0, 2.5)
6	4.42 dd (11.5, 3.0)	4.59 dd (12.0, 6.8)	4.98−5.03*	4.80 dd (11.7, 2.5)
6	4.24 dd (11.5, 6.5)	4.85−4.93*	4.60 dd (11.5, 6.5)	4.71−4.76*
Rha-1	6.34*	6.19 d (1.0)	6.24 d (1.5)	5.45 d (1.5)
2	6.27 dd (3.0, 1.5)	6.14 dd (3.0, 1.0)	6.18 dd (3.0, 1.5)	5.70 dd (3.5, 1.5)
3	6.41 dd (9.5, 3.0)	6.33 dd (10.0, 3.0)	6.40 dd (10.0, 3.0)	4.62 dd (9.5, 3.5)
4	4.29 dd (9.5, 9.5)	4.29 dd (10.0, 9.8)	4.36 dd (10.0, 9.5)	4.22 dd (9.5, 9.5)
5	4.80 dq (9.5, 6.5)	4.77 dq (9.8, 6.0)	4.85 dq (9.5, 6.5)	4.71−4.76*
6	1.59 d (6.5)	1.57 d (6.0)	1.64 d (6.5)	1.88 d (6.5)
Qui′-1	4.99 d (7.5)	4.94 d (7.8)	5.0 d (8.0)	5.34 d (8.0)
2	3.86 dd (8.0, 7.5)	3.80 dd (8.9, 7.8)	3.87 dd (9.0, 8.0)	3.96 dd (9.0, 8.0)
3	4.12 dd (9.0, 8.0)	4.01 dd (8.9, 8.7)	4.08 dd (9.0, 8.5)	4.05 dd (9.0, 9.0)
4	5.30 dd (9.0, 9.0)	3.62 dd (9.0, 8.7)	3.71 dd (9.0, 8.5)	3.65 dd (9.0, 9.0)
5	3.67−3.8 m	3.63−3.70*	3.68−3.78*	3.66 m
6	1.42 d (6.0)	1.51*	1.60 d (6.0)	1.55 d (5.5)
Jal-2a	2.82 ddd (15.0, 7.5, 3.0)	2.71 ddd (15.0, 8.0, 3.0)	2.77 ddd (15.0, 8.5, 3.0)	2.80−2.86*
2b	2.58 ddd (15.0, 10.5, 2.5)	2.48 ddd (15.0, 10.0, 3.0)	2.55 ddd (15.0, 9.5, 2.5)	2.36 ddd (17.8, 10.0, 2.0)
11	3.98−3.94*	3.85−3.88 m	4.18−4.22*	3.82 m
16	0.851 t (7.0)	0.89 t (7.0)	0.87 t (7.0)	0.82 t (7.0)
mba-2	2.34−2.39 m	2.30 tq (7.0, 7.0)	2.37 tq (7.0, 7.0)	2.50 tq (7.0, 7.0)
2-Me	1.16 d (7.5)	1.07 d (7.0)	1.15 d (7.0)	1.18 d (7.5)
3-Me	0.99 t (7.5)	0.79 t (7.0)	0.97 t (7.5)	0.88 t (7.5)
nla-2	2.89 dq (7.0, 7.0)	2.77 dq (7.0, 7.0)	2.84 dq (7.0, 6.5)	2.79 dq (7.0, 7.0)
3	4.37 dq (7.0, 7.0)	4.28−4.32*	4.30−4.40*	4.33 dq (7.0, 7.0)
2-Me	1.34 d (7.0)	1.22 d (7.0)	1.31 d (7.0)	1.27 d (7.5)
3-Me	1.44 d (6.5)	1.29 d (6.0)	1.36 d (6.0)	1.34 d (6.0)

[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, m = multiplet, q = quartet. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b] Abbreviations: Qui = quinovose; Glu = glucose; Rha = rhamnose; Jal = 11-hydroxyhexadecanoyl; mba= 2-methylbutanoyl; nla = 3-hydroxy-2-methylbutanoyl.

of MeOH in $CHCl_3$. A total of 330 fractions (150 mL each) was collected and combined to give several pools containing complex mixtures of resin glycosides. The most polar pool, fractions 274−320 (850 mg; KB: ED_{50} 2.5 μg/mL), was subjected to preparative HPLC on an ISCO C_{18} column (250 × 10 mm, 10 μm). The elution was isocratic with CH_3CN−H_2O (22:3) using a flow rate of 8.0 mL/min. Eluates (subfractions 1−4) across the peaks with t_R of 8 min (50.2 mg), 11 min (53.1 mg), 14 min (29.4 mg), and 17 min (195.2 mg) were collected by the technique of heart-cutting,[27] and independently reinjected on a Waters aminopropylmethylsilyl amorphous Si gel column (150 × 19 mm; μBondapak, 10 μm).[14] The preparative HPLC system was then operated in the recycle mode[20] to further separate and guarantee maximal purity of the major constituents in each subfraction. Elution was conducted isocratically with CH_3CN−H_2O (92:8; flow rate = 4 mL/min) and complete separation of all components to homogeneity was achieved after eight to ten consecutive cycles using the same aminopropyl column. Scammonins I (**1**; t_R 16 min, 15 mg) and II (**2**; t_R 36 min, 15.2 mg) were isolated from 50 mg of the trailing and leading eluted subfractions (4 and 1), respectively. The third peak contained compound **3** (t_R 20 min, 12.0 mg). Glycolipids **4** (t_R 22.1 min, 7.7 mg), **5** (t_R 22.2 min, 21.3 mg), and **6** (t_R 30 min, 11.8 mg) were isolated from the second eluate.

Scammonin I (1): obtained as a white amorphous powder; mp 130−134 °C (lit. 132−141 °C dec); HRFABMS [M − H]$^-$ 1019.5422 (calcd for $C_{50}H_{83}O_{21}$, 1019.5426); identified by comparison of spectral data with published values.[16]

Scammonin II (2): obtained as a white amorphous powder; mp 156−160 °C; HRFABMS [M − H]$^-$ 937.5005 (calcd for $C_{45}H_{77}O_{20}$, 937.5008); identified by comparison of spectral data with published values.[16]

Orizabin V (3): obtained as a white amorphous powder; mp 128−130 °C; $[\alpha]_D$ −33° (c 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS m/z 1037 [M − H]$^-$, 993 [M − H − C_2H_4O]$^-$, 937 [M − H − $C_5H_8O_2$]$^-$, 909 [993 − C_5H_8O]$^-$, 891 [909 − H_2O]$^-$, 835 [937 − C_5H_8O − H_2O], 617, 561, 417, 271; HRFABMS m/z 1037.5525 [M − H]$^-$ (calcd for $C_{50}H_{85}O_{22}$ requires 1037.5533).

Orizabin VI (4): obtained as a white amorphous powder; mp 128−133 °C; $[\alpha]_D$ −38.6° (c 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS m/z 1037 [M − H]$^-$, 1023 [M − CH_3]$^-$, 993 [M − H − C_2H_4O]$^-$, 937 [M − H − $C_5H_8O_2$]$^-$, 909 [993 − C_5H_8O]$^-$, 891 [909 − H_2O]$^-$, 887, 789, 661, 617, 561, 417, 271; HRFABMS m/z 1023.5365 [M − CH_3]$^-$ (calcd for $C_{49}H_{83}O_{22}$ requires 1023.5376).

Orizabin VII (5): obtained as a white amorphous powder; mp 127−134 °C; $[\alpha]_D$ −29° (c 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS m/z 1037 [M − H]$^-$, 1023 [M − CH_3]$^-$, 937 [M − H − $C_5H_8O_2$]$^-$, 891 [909 − H_2O]$^-$, 789, 679, 661, 617, 561, 417, 271; HRFABMS m/z 1023.5353 [M − CH_3]$^-$ (calcd for $C_{49}H_{83}O_{22}$ requires 1023.5376).

Orizabin VIII (6): obtained as a white amorphous powder; mp 110−115 °C, $[\alpha]_D$ −21.3° (c 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS m/z 1037 [M − H]$^-$, 993 [M − H − C_2H_4O]$^-$, 937 [M − H − $C_5H_8O_2$]$^-$, 891, 661, 617, 561, 417, 271; HRFABMS m/z 1037.5547 [M − H]$^-$ (calcd for $C_{50}H_{85}O_{22}$ requires 1037.5533).

Alkaline Hydrolysis of the Resin Glycoside Fraction. A solution of the resin glycoside fractions 274−320 (500 mg) in 5% KOH−H_2O (10 mL) was refluxed at 95 °C for 1 h. The reaction mixture was acidified to pH 4.0 and extracted with $CHCl_3$ (20 mL). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was treated with CH_2N_2 and directly

Table 2. ^{13}C NMR Spectral Data of Compounds **3**–**6** (125.7 MHz)[a]

carbon[b]	3	4	5	6
Qui-1	101.2	100.9	100.9	102.3
2	77.3	79.0	78.6	76.5
3	78.3	78.6	78.9	78.8
4	77.2	77.4	77.3	77.0
5	72.5	73.1	72.9	72.5
6	18.5	18.7	18.6	18.4
Glu-1	101.7	101.2	100.9	99.8
2	79.3	77.4	79.3	80.5
3	79.2	77.4	77.3	78.9
4	72.6	72.4	72.4	70.2
5	77.5	75.0	74.9	73.9
6	63.2	64.9	64.6	64.2
Rha-1	96.2	96.6	96.4	99.2
2	69.5	70.0	70.0	72.9
3	71.2	71.5	71.5	69.7
4	79.0	78.8	78.4	84.3
5	68.7	69.0	68.8	68.0
6	18.5	18.7	18.6	18.6
Qui′-1	105.2	105.5	105.3	105.9
2	75.0	75.2	75.1	76.1
3	75.4	78.2	78.1	78.0
4	76.6	76.8	76.6	76.5
5	70.3	72.6	72.5	73.1
6	18.0	18.7	18.6	18.4
Jal-1	173.3	173.6	173.4	172.3
2	34.5	34.6	34.5	34.3
11	79.3	79.4	79.3	80.3
16	14.3	14.5	14.3	14.2
mba-1	175.3	175.8	175.4	176.0
2	41.1	41.2	41.1	41.2
2-Me	17.0	17.2	17.0	17.0
3-Me	11.8	11.9	11.7	11.6
nla-1	174.9	175.6	175.4	175.3
2	48.6	48.7	48.8	48.6
3	69.5	69.2	69.1	69.1
2-Me	13.2	13.6	13.3	13.2
3-Me	21.0	21.1	20.9	20.8

[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: Qui = quinovose; Glu = glucose; Rha = rhamnose; Jal = 11-hydroxyhexadecanoyl; mba = 2-methylbutanoyl; nla = 3-hydroxy-2-methylbutanoyl; tga = 2(*E*)-methyl-2-butenoyl.

analyzed by GC–MS[28] (30 m × 0.25 mm DB-1 column; He, 2 mL/min; 120–280 °C, Δ 4 °C/min) with two major peaks detected that were identified as methyl 2-methylbutyrate: t_R 15.5 min; m/z [M]$^+$ 116 (0.3), [M − CH_3]$^+$ 101 (18), 88 (100), 85 (20), 59 (29), 57 (80) and methyl 3-hydroxy-2-methylbutyrate: t_R 21.3 min; m/z [M]$^+$ 132 (0.4), [M − CH_3]$^+$ 117 (8), 101 (20), 88 (100), 59 (18), 57 (80); an additional minor peak was identified as methyl tiglate: t_R 18.0 min; m/z [M]$^+$ 114 (2), [M − CH_3]$^+$ 99 (15), 83 (25), 59 (8), 57 (100). The preparation and identification from **3**–**6** of methyl (2*S*)-methylbutyrate ([α]$_D$ +15°; *c* 4.0, MeOH) and methyl (3*R*)-hydroxy-(2*R*)-methylbutyrate[29] ([α]$_D$ −27°; *c* 5.0, MeOH) were performed according to previously reported procedures.[13]

The aqueous phase was extracted with *n*-BuOH (20 mL). Then the organic layer was concentrated to give a colorless solid (319.8 mg). The physical and spectroscopic constants registered for this derivative were identical in all aspects to those previously reported[16,22] for scammonic acid A: mp 154–157 °C; [α]$_D$ −51.1° (MeOH, *c* 1.0); negative FABMS m/z 871 [M − H]$^-$, 725 [M − H − 146 (methylpentose unit)]$^-$, 579 [725−146 (methylpentose unit)]$^-$, 417 [579−162 (hexose unit)]$^-$, and 271 [417−146 (methylpentose unit); jalapinolic acid − H]$^-$.

Sugar Analysis. Scammonic acid A (100 mg) in 4 N HCl (20 mL) was heated at 90 °C for 2 h. The reaction mixture was extracted with Et_2O. The organic layer was evaporated to dryness and treated with an excess of CH_2N_2. The purification of the aglycon was carried out by normal-phase HPLC (21.1 × 250 mm column) using *n*-hexane–$CHCl_3$–Me_2CO to give 23.5 mg of methyl (11*S*)-hydroxyhexadecanoate (jalapinolic acid methyl ester):[13] t_R 16.4 min; mp 42–44 °C; [α]$_D$ +7.27° (*c* 0.2, $CHCl_3$); ^{13}C NMR: 174.3, 72.0, 51.4, 37.5, 37.4, 34.1, 31.9, 29.6, 29.5, 29.4, 29.2, 29.1, 25.6, 25.3, 24.9, 22.6, 14.1. The aqueous phase was neutralized with 1 M KOH and lyophilized to give a colorless powder. The residue was dissolved in dry pyridine and treated with Sigma Sil-A at 70 °C for 30 min. GC–MS analysis[28] (120–280 °C, Δ 4 °C/min) allowed the identification of the following TMS-sugars by coelution with authentic samples: tetra-TMS-rhamnose, t_R = 14.9 and 16.6 min; tetra-TMS-quinovose, t_R = 17.9 and 19.5; penta-TMS-glucose, t_R = 21.9 and 24.2.

Cytotoxicity Assay. Squamous cell cervix carcinoma (SQC-1 UISO), nasopharyngeal carcinoma (KB), ovarian cancer (OVCAR), and colon cancer (HCT-15) cell lines were maintained in RMPI 1640 (10×) medium supplemented with 10% fetal bovine serum. All cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at log phase of their growth cycle were treated in triplicate at various concentrations of the glycolipids (0.16–20 μg/mL), and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by the sulforhodamine B method.[30] Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}).[26] Ellipticine was included as a positive drug control: ED_{50} 0.24 (HCT-15); 0.41 (SQC-1 UISO); 0.58 (OVCAR); 0.33 (KB).

Acknowledgment. This reseach was supported by grants from Dirección General de Asuntos del Personal Académico (IN205197) and Dirección General de Estudios de Posgrado, UNAM (PADEP: 5301; PAEP-207341 and 207005). Financial support from Consejo Nacional de Ciencia y Tecnología (400313-5-25118-N) is also acknowledged. B. H.-C. is grateful to CONACyT for a graduate student scholarship. The sequence of chemical degradations used for characterization of the oligosacharide core of the resin glycosides was performed by Francisco Sosa Vázquez as part of his B. Sc. thesis. We wish to thank Isabel Chávez (Instituto de Química, UNAM) and Atilano Gutiérrez (Universidad Autónoma Metropolitana) for recording the highfield NMR spectra. Also, thanks are due to "Unidad de Servicios de Apoyo a la Investigación" (Facultad de Química, UNAM), in special to Graciela Chávez, Marisela Gutiérrez, Oscar Yáñez, and Georgina Duarte. Francisco Basurto assisted in the study of herbarium specimens. We thank Dr. Mario Sousa, curator, and his staff at the National Herbarium (MEXU).

References and Notes

(1) Part VII in the series "Bioactive Natural Products from Traditionally Used Mexican Plants"; taken in part from the Ph.D. dissertation of B. Hernández-Carlos.
(2) Pereda-Miranda, R. In *Phytochemistry of Medicinal Plants*; Arnason, J. T., Mata, R., Romeo, J. T., Eds. Plenum Press: New York, 1995; pp 83–112.
(3) McDonald, A. *An. Inst. Biol. Universidad Nac. Autón. México, Ser. Bot.* **1991**, *62*, 65–82.
(4) It is thought that all edible varieties of *Ipomoea batatas* were derived from *I. tiliacea* (Willd.) Choisy, as a result of selection by the native inhabitants of Mesoamerica.
(5) Mata, R.; Pereda-Miranda, R.; Lotina-Hennsen, B. In *Secondary Metabolites from Mexican Plants: Chemistry and Biological Properties*; Pandalai, S. G., Ed.; Research Signpost: Trivandrum, India, 1996; pp 59–68.
(6) Schultes, R. E.; Hofmann, A. *Plants of the Gods. Their Sacred, Healing and Hallucinogenic Powers*; Healing Arts Press: Rochester, 1992; pp 158–163.
(7) The narcotic power of the morning glory seeds, due to the high content of ergot-type indole alkaloids, remained hidden by the Chinantec and Mazatec Indians in the hinterlands of Oaxaca, in southern Mexico, until the present century.
(8) *Ipomoea orizabensis* is placed taxonomically in the Series *Tyrianthinae* (House) Austin of the Section *Pharbitis* (Choisy) Griseb. of the subgenus *Ipomoea* along with *I. stans*. This species includes among its synonyms *I. tyrianthina* Lindley, *I. longepedunculata* (Mart., & Gal.) Hemsley, and *Convolvulus orizabensis* Pelletan.
(9) This tuberous rooted, perennial morning glory vine grows in secondary vegetation in the dry tropical forests, grasslands, and conifer–oak forests of the mountains between 1000 and 2500 m above sea level from northeastern Mexico to northern Guatemala.

(10) The roots of the jalaps were introduced into Europe as early as 1565, and since then they have represented an important item of commerce between the New and the Old World. At the beginning of the 20th century, the jalap medicinal plant complex included "jalapa hembra" or "oficinal" (*I. purga*), "jalapa macho" (*I. orizabensis*) and "jalapa de Tampico" (*I. simulans* Hanbury). The substitution of *I. purga* with other jalaps has resulted in the confusing application of such terms as "jalapa falsa" or false jalap.
(11) Gerard, J. *The Herbal or General History of Plants*; Dover: New York, 1975; pp 866−869.
(12) Martínez, M. *Las Plantas Medicinales de México*; Ediciones Botas: Mexico, 1990; pp 276−279.
(13) Noda, N.; Ono, M.; Miyahara, K.; Kawasaki, T.; Okabe, M. *Tetrahedron* **1987**, *43*, 3889−3902.
(14) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1996**, *52*, 13063−13080.
(15) The sugars are frequently esterified by long chain fatty acids (*n*-decanoic and *n*-dodecanoic) or by lower molecular-weight acids (including tiglic, isobutyric, 2-methylbutyric, and nilic acids).
(16) Noda, N.; Kogetsu, H.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1990**, *11*, 3565−3569.
(17) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007−9022.
(18) MacLeod, J. K.; Ward, A.; Oelrichs, P. B. *J. Nat. Prod.* **1997**, *60*, 467−471.
(19) Du, X.-M.; Kohinata, K.; Kawasaki, T.; Guo, Y.-T.; Miyahara, K. *Phytochemistry* **1998**, *48*, 843−850.
(20) Kubo, I.; Nakatsu, T. *LC−GC* **1990**, *8*, 933−939.
(21) Neue, U. D. *HPLC Columns. Theory, Technology and Practice*; Wiley-VCH: New York, 1997; pp 164−216.
(22) Kogetsu, H.; Noda, N.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1991**, *30*, 957−963.
(23) Enriquez, R. G.; Leon, I.; Perez, F.; Walls, F.; Carpenter, K. A.; Puzzuoli, F. V.; Reynolds, W. F. *Can. J. Chem.* **1992**, *70*, 1000−1008.
(24) Reynolds, W. F.; Yu, M.; Enriquez, R. G.; Gonzalez, H.; Leon, I.; Magos, G.; Villareal, M. L. *J. Nat. Prod.* **1995**, *58*, 1730−1734.
(25) Massiot, G.; Chen, X.-F.; Lavaud, C.; Le Men-Olivier, L.; Delaude, C.; Viari, A.; Vigny, P.; Duval, J. *Phytochemistry* **1992**, *31*, 3571−3576.
(26) Fragoso-Serrano, M.; González-Chimeo, E.; Pereda-Miranda, R. *J. Nat. Prod.* **1999**, *62*, 45−50.
(27) Hostettmann, K.; Hostettmann, M.; Marston, A. *Preparative Chromatography Techniques. Applications in Natural Product Isolation*; Springer-Verlag: Berlin, 1986; pp 37−40.
(28) Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Wickramaratne, D. B. M.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1993**, *56*, 571−582.
(29) Tai, A.; Imaida, M. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1114−1117.
(30) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Boskesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

NP9900627