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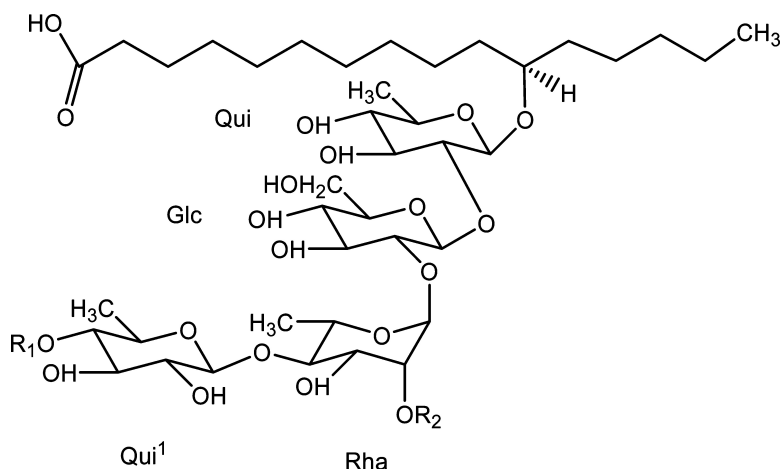
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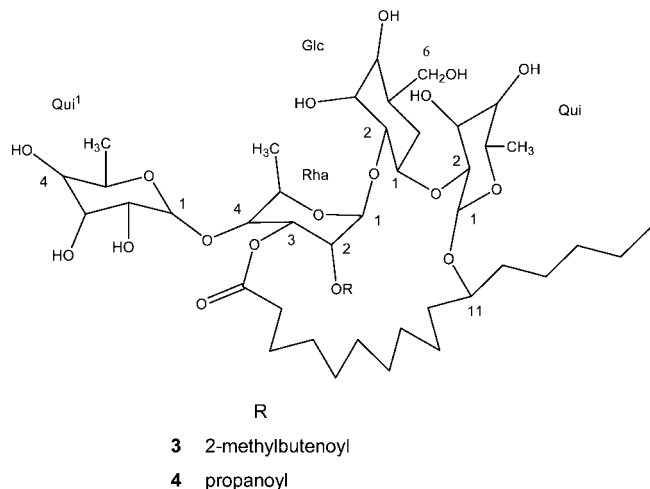
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Tyrianthinic Acids from *Ipomoea tyrianthina* and Their Antimycobacterial Activity, Cytotoxicity, and Effects on the Central Nervous System[#]

Ismael León-Rivera,*,[†] Gumersindo Mirón-López,[†] Gloria María Molina-Salinas,[‡] Maribel Herrera-Ruiz,[§] Samuel Estrada-Soto,[⊥] María del Carmen Gutiérrez,[||] Daniel Alonso-Cortes,[§] Gabriel Navarrete-Vázquez,[⊥] María Yolanda Ríos,[†] and Salvador Said-Fernández[‡]

Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa 62209 Cuernavaca, Morelos, México, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, San Luis Potosí y Dos de Abril, Col. Independencia 64720 Monterrey, Nuevo León, México, Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, Argentina 1 Col. Centro Xochitepec, Morelos, México, Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa 62209 Cuernavaca, Morelos, México, and Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa 62209 Cuernavaca, Morelos, México

Four new partially acylated tetrasaccharides of 11-hydroxyhexadecanoic acid (**1**–**4**) were isolated from a methanolic extract of *Ipomoea tyrianthina*. The structures of these compounds were elucidated by spectroscopic and chemical methods. The resin glycoside composition of *I. tyrianthina* varied with the location of growth in Mexico. Compounds **1**–**4** showed antimycobacterial activity, were cytotoxic against the KB cell line, and, in a mouse model, exhibited potentiation of hypnosis induced by pentobarbital, protected against seizures induced by pentylenetetrazole, and released GABA and glutamic acid.

Ipomoea tyrianthina Lindley (syn. *Ipomoea orizabensis* Pelletan, Lebed. ex Steud., Convolvulaceae) is a twining herb with large roots that has been used mainly as a purgative since prehispanic times. The chemical components of the glycoresin obtained from the root of this plant have been characterized as tetrasaccharides of jalapinolic acid.[1-3] It has been shown that the growing location affects the chemical composition of the glycoresin in *Ipomoea* species.[3]

Tuberculosis (TB) is still a major cause of death all over the world. There were an estimated 8.8 million new TB cases in 2005, and 1.6 million people died, including 195 000 patients infected with HIV/AIDS. When considered along with the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB), the magnitude of the problem becomes clear, as it will become inevitably even more difficult to treat TB in developing countries in particular.[4] The need for new drugs to extend the variety of TB drug options is urgent. New chemical entities with novel mechanisms of action should possess activity against MDR-TB. There are some reports of Mexican plants with ethnomedicinal use against respiratory infections or related conditions that have been shown to be sources of antitubercular compounds.[5-8] The antimycobacterial activities of glycolipids have been reported by Barnes et al., using a crude extract of *Ipomoea leptophylla*[9] and of some macrolactone tetrasaccharide glycolipids isolated from *Ipomoea tricolor* by Rivero-Cruz et al.[10] More recently, crypthophilic acids A–C were examined in vitro as potential antimycobacterial substances.[11]

The roots of *I. orizabensis* have been also used to treat epilepsy.[12] In a previous report, we have described the antidepressant and protective effects against pentylenetetrazole-induced seizures by

tetrasaccharide glycolipids purified from a dichloromethane extract of *I. tyrianthina*.[3] Similar effects on the central nervous system (CNS) have been reported by Herrera-Ruiz et al., as a result of the administration of an ethyl acetate extract from the roots of *Ipomoea stans*.[13] The molecular structures of compounds **1**–**4** are very similar to those of the glycolipids isolated from *I. tyrianthina*[3] and *I. stans*[13] that have exhibited activity on the CNS of mice. The above-mentioned data suggested the possibility that tyrianthinic acids (**1** and **2**) are able to induce effects on the CNS of mice.

	R_1	R_2
1	nil	mba
2	tig	mba

mba = 2-methylbutanoyl, nil = 2-methyl-3-hydroxybutanoyl, tig = 2-methylbutenoyl

In a continuing investigation on secondary metabolites with biological activity from *Ipomoea* species, we have studied the resin glycosidic content of the methanolic extract from the roots of *I. tyrianthina* collected in three different regions of Mexico. We report herein on the isolation and characterization of four new partially acylated tetrasaccharide resin glycosides (compounds **1**–**4**) from the methanolic extract of *I. tyrianthina*, as well as their antimycobacterial activity, cytotoxicity in four cell lines, and effects on the CNS.

Results and Discussion

The roots of *I. tyrianthina* were dried, pulverized, defatted with hexane, and macerated succesively in dichloromethane and metha-

3   2-methylbutanoyl
4   propanoyl

nol. The methanolic extract was purified by column chromatography packed with reversed-phase silica gel, leading to the separation of two chromatographic fractions. The more polar chromatographic fraction was subjected to separation over a column packed with Sephadex LH-20 and by preparative TLC, and compounds **1**−**4** were purified by semipreparative HPLC.

The more polar chromatographic fraction was hydrolyzed in an aqueous/methanolic acid medium, producing an organic fraction together with a water-soluble mixture of carbohydrates. Analysis of the organic fractions by GC-MS permitted the identification of propanoic, 2-methylbutanoic, 3-hydroxy-2-methylbutanoic (nilic), 2-methyl-2-butenoic (tiglic), and 11-hydroxyhexadecanoic methyl ester units by comparison with the mass spectra and retention times of these methyl esters with authentic samples. HPLC analysis of the carbohydrates present in the aqueous phase and GC-MS analysis of the trimethysilyl derivatives of these carbohydrates allowed the identification of quinovose, rhamnose, and glucose.

Basic hydrolysis of the more polar chromatographic fraction produced an organic acid fraction and a water-soluble glycosidic acid derivative. The structure of the glycosidic acid was assigned as scammonic acid A, which has been previously identified in resin glycosides of *Convolvulus elongatus*,[14] *I. stans*,[15] *I. orizabensis*,[1,2] and *I. tyrianthina*.[3]

The negative and positive FABMS of tyrianthinic acid I (**1**) showed quasimolecular ion peaks at m/z 1055 [M − H]$^-$ and m/z 1079 [M + Na]$^+$, consistent with the molecular formula, $C_{50}H_{88}O_{23}$. This molecular formula was supported by HRMALDITOFMS (m/z 1058.2310 [M + H]$^+$; 1058.2278 calcd for $C_{50}H_{88}O_{23}H^+$). The negative-ion FABMS showed a quasimolecular ion at m/z 1055 [M − H]$^-$, along with other fragments at m/z 955 [m/z 1055 − 100 ($C_5H_8O_2$)]$^-$ and m/z 871 [m/z 955 − 84 (C_5H_8O)]$^-$, representing the loss of niloyl and 2-methylbutanoyl residues, respectively. The glycosidic cleavage of the sugar moieties yielded peaks at m/z 725 [m/z 871 − 146 ($C_6H_{10}O_4$)]$^-$, 579 [m/z 725 − 146 ($C_6H_{10}O_4$)]$^-$, 417 [m/z 579 − 162 $C_6H_{10}O_5$)]$^-$, and 271 [m/z 417 − 146 ($C_6H_{10}O_4$)]$^-$.

The ^{1}H NMR spectrum of compound **1** (Table 1) was completely assigned by a combination of COSY, TOCSY, and HSQC experiments. COSY and TOCSY experiments revealed the presence of four spin systems, three assigned to 6-deoxyhexoses and one to a hexose, and four anomeric protons were observed at δ_H 4.30 (d, J = 7.6 Hz), 4.95 (d, J = 7.6 Hz), 5.22 (d, J = 1.6 Hz), and 4.55 (d, J = 7.8 Hz) of a tetrasaccharide core (Table 1). The ^{13}C NMR spectrum of **1** (Table 2) showed 50 carbon resonances, of which 24 were assigned to the saccharide units. Analysis of the HSQC spectrum allowed the assignments of each carbon resonance in the four spin systems through the corresponding one-bond ^{13}C−^{1}H correlations. The coupling constants of the protons and the corresponding carbon resonances, together with three doublet methyl

signals at δ_H 1.25, 1.24, and 1.22, indicated that the tetrasaccharide core of **1** was composed of one glucose, two quinovose, and one rhamnose moiety. The anomeric configurations for the sugar moieties were assigned as β for glucopyranosyl, β for quinovopyranosyl, and α for rhamnopyranosyl from the coupling constants of 7.6 and 7.8 Hz for anomeric protons of the quinovose and glucose units and 1.6 Hz for the anomeric proton of rhamnose. Moreover, the coupling constants between H-1 and C-1 determined in the coupled HSQC experiment, of 170 Hz for rhamnose and 160 Hz for glucose and quinovose, supported these assignments. The sugar sequence was determined on the basis of a 2D-NMR HMBC experiment. In the HMBC experiment of compound **1**, ^{1}H−^{13}C long-range correlations were observed between H-2 (3.56 ppm) of quinovose and C-1 (101.9 ppm) of glucose, H-2 (3.36 ppm) of glucose and C-1 (101.2 ppm) of rhamnose, and H-4 (3.56 ppm) of rhamnose and C-1 (105.2 ppm) of quinovose.[1]

The position of the 11-hydroxyhexadecanoic acid (aglycon) moiety in the oligosaccharide was determined by the correlation between aglycon H-11 (3.62 ppm) and quinovose H-1 (4.30 ppm) in a 2D-NMR ROESY spectrum. The triplet-like signal at 2.32 ppm for the methylene protons at C-2 of the aglycon unit and the absence of correlations between ^{13}C=O of the aglycon and protons of sugar units in the HMBC spectrum confirmed the acyclic molecular structure proposed for compound **1**.

On the basis of the GC-MS analysis of the base-hydrolyzed products recovered from the organic fraction of tyrianthinic acid I (**1**), two residues, 2-methylbutanoic acid and nilic acid, were identified. The acyl residues in the oligosaccharide core were determined in the 2D-NMR HMBC spectrum of compound **1**, by the ^{1}H−^{13}C long-range correlation between the carbonyl resonance at δ_C 179.3 ppm of a niloyl group with H-4 (4.32 ppm) of quinovose[1] and from the further long-range correlation ^{13}C=O (178.5 ppm) of a 2-methylbutanoyl group with H-2 (3.88 ppm) of rhamnose.

Compound **2** gave the molecular formula $C_{50}H_{86}O_{22}$, which was determined from the positive-ion HRMALDITOFMS (m/z [M + H]$^+$ 1040.2151; calcd for $C_{50}H_{86}O_{22}H^+$, requires 1040.2125). The negative-ion FABMS showed a quasimolecular ion at m/z 1037 [M − H]$^-$ and the fragments for the loss of tigloyl and 2-methylbutanoyl residues at m/z 955 and 871, respectively, and the glycosidic cleavage of the sugar moieties yielded the peaks at m/z 725, 579, 417, and 271.

The ^{1}H and ^{13}C NMR spectra for compound **2** were very similar to those of **1**, except for the absence of the signals of a niloyl group. Additional signals due to a methine at δ_H 6.90 (q, J = 7.0 Hz, H-3) and two methyl resonances at δ_H 1.84 (s, H-2) and δ_H 1.82 (d, J = 7.0 Hz, H-4) were apparent. The ^{13}C NMR spectrum exhibited the corresponding carbon resonances at δ_C 139.1 (C-3), 129.5 (C-2), 12.4 (C-2), and 14.6 (C-4), plus a carbonyl (C-1) signal at δ_C 168.8. These data were in good accordance with those of a tigloyl unit. The above observations, plus a molecular mass difference of 18 amu between the two compounds, clearly suggested that **2** contains a tigloyl group instead of the niloyl unit in compound **1**. GC-MS analysis of the products recovered from the acid hydrolysis of tyrianthinic acid II (**2**) corroborated the presence of 2-methylbutanoic acid, tiglic acid, and 11-hydroxyhexadecanoic acid.

The positions of the acyl residues in the oligosaccharide core of tyrianthinic acid II (**2**) were determined from the HMBC spectrum, showing a ^{1}H−^{13}C long-range correlation between the carbonyl resonance (168.8 ppm) of a tigloyl group and H-4 (4.32 ppm) of quinovose[1] and between the carbonyl resonance (178.4 ppm) of a 2-methylbutanoyl group and H-2 (3.90 ppm) of rhamnose. The position of the 11-hydroxyhexadecanoic acid moiety in the oligosaccharide core of **2** was determined by the correlation between H-11 (3.60 ppm) of aglycon and the quinovose H-1 (4.32 ppm) in a 2D-NMR ROESY spectrum. The triplet-like signal (2.30 ppm)

Table 1. ^{1}H NMR Data (400 MHz, CD_3OD) for Compounds **1−4**[a]

	1	**2**	**3**	**4**
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
Qui				
1	4.30 d (7.6)	4.32 d (7.6)	4.37 d (7.6)	4.38 d (7.6)
2	3.56*	3.56*	3.47*	3.48*
3	3.63 dd (9.0, 9.0)	3.60 dd (9.0, 9.0)	3.37 dd (9.0, 9.0)	3.38 dd (9.0, 9.0)
4	2.98 dd (8.9, 9.0)	2.95 dd (8.9, 9.0)	2.96 dd (8.9, 9.0)	2.98 dd (8.9, 9.0)
5	3.27 m	3.26 m	3.23 m	3.24 m
6	1.25 d (7.0)	1.26 d (7.0)	1.20 d (7.0)	1.23 d (7.0)
Glc				
1	4.95 d (7.6)	4.97 d (7.6)	5.02 d (7.5)	5.03 d (7.5)
2	3.36 dd (9.1, 7.5)	3.40 dd (9.1, 7.5)	3.46 dd (9.1, 7.5)	3.45 dd (9.1, 7.5)
3	3.43 dd (9.0, 9.1)	3.45 dd (9.0, 9.1)	3.52 dd (9.0, 9.1)	3.53 dd (9.0, 9.1)
4	3.06 dd (9.0, 9.0)	3.11 dd (9.0, 9.0)	3.24 dd (9.0, 9.0)	3.24 dd (9.0, 9.0)
5	3.22*	3.23*	3.35*	3.35*
6	3.55*	3.55*	3.61 m	3.61 m
6′	3.84 dd (11.5, 6.5)	3.83 dd (11.5, 6.5)	3.85 dd (11.5, 6.5)	3.86 dd (11.5, 6.5)
Rha				
1	5.22 d (1.6)	5.23 d (1.6)	5.50 d (1.6)	5.50 d (1.6)
2	3.88 dd (3.2, 1.6)	3.90 dd (3.2, 1.6)	5.55 dd (3.2, 1.6)	5.55 dd (3.2, 1.6)
3	3.99 dd (9.3, 3.2)	4.01 dd (9.3, 3.2)	5.59 dd (9.3, 3.2)	5.58 dd (9.3, 3.2)
4	3.56*	3.59*	3.76 dd (9.3, 9.0)	3.77 dd (9.3, 9.0)
5	4.29 dd (9.0, 6.5)	4.30 dd (9.0, 6.5)	4.23 dd (9.0, 6.5)	4.22 dd (9.0, 6.5)
6	1.24 d (6.5)	1.27 d (6.5)	1.24 d (6.5)	1.22 d (6.5)
Qui[1]				
1	4.55 d (7.8)	4.57 d (7.8)	4.41 d (7.8)	4.42 d (7.8)
2	3.19 dd (9.0, 7.6)	3.18 dd (9.0, 7.6)	3.21 dd (9.0, 7.6)	3.22 dd (9.0, 7.6)
3	3.34 dd (9.0, 9.0)	3.33 dd (9.0, 9.0)	3.51*	3.51*
4	4.32 dd (9.0, 8.9)	4.32 dd (9.0, 8.9)	3.52*	3.53*
5	3.88*	3.87*	3.46*	3.47*
6	1.22 d (6.4)	1.25 d (6.4)	1.13 d (6.4)	1.15 d (6.4)
Jal				
2	2.32 t	2.30 t	2.28 m	2.30 m
2[1]			2.45 m	2.47 m
11	3.62*	3.60*	3.55*	3.54*
16	0.87 t (7.0)	0.88 t (6.9)	0.91 t (7.0)	0.91 t (6.9)
mba				
2	2.38 m	2.38 m		
3	0.85 t (7.5)	0.85 t (7.5)		
nil				
2	2.50 m			
3	3.91*			
prop				
2				2.45*
3				1.21 t (7.2)
tgl				
3		6.90 q (7.0, 1.3)	6.90 q (7.0, 1.3)	
4		1.82 d (1.3)	1.82 d (1.3)	

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, Jal = 11-hydroxyhexadecanoyl, mba = 2-methylbutanoyl, tigl = 2-methyl-2-butenoyl, nil = 3-hydroxy-2-methylbutanoyl, and prop = propanoyl. Chemical shifts marked with an asterisk indicate overlapped signals.

for the methylene protons at C-2 of the aglycon unit and its correlation with the carbonyl resonance (176.9 ppm) of the 11-hydroxyhexadecanoyl moiety confirmed the acyclic molecular structure of compound **2**, as shown.

The negative and postive FABMS of tyrianthin 8 (**3**) showed quasimolecular ion peaks at *m/z* 953 [M − H]⁻ and 977 [M + Na]⁺, respectively, indicating a molecular formula of $C_{45}H_{78}O_{21}$. The negative-ion FABMS showed fragment peaks at *m/z* 953 [M − H⁺]⁻, 871 [(M − H⁺)⁻ − (tigloyl)], besides fragmentation peaks produced by glycosidic cleavage of the sugar moieties at *m/z* 707, 561, 417, and 271.

The ^{13}C NMR spectrum of compound **3** showed 45 carbon resonances, including two carbonyl signals and four anomeric signals, suggesting a partially acylated tetrasaccharide compound. A combination of the 1D- and 2D-homonuclear NMR techniques revealed in compound **3** the presence of four spin systems, assigned to 6-deoxyhexoses and one to a hexose of the tetrasaccharide core. The sugar sequence in compound **3** was determined in the same way as for compounds **1** and **2** on the basis of a 2D-NMR HMBC experiment. In this experiment, [1]H−[13]C long-range correlations

were observed between H-2 (3.47 ppm) of quinovose and C-1 (102.0 ppm) of glucose, H-2 (3.46 ppm) of glucose and C-1 (96.7 ppm) of rhamnose, and H-4 (3.76 ppm) of rhamnose and C-1 (104.2 ppm) of quinovose.[1] GC-MS analysis of the products recovered from acid hydrolysis of compound **3** established the presence of tiglic acid and 11-hydroxyhexadecanoic acid. The position of the aglycon in the oligosaccharide was determined by the correlation between H-11 (3.55 ppm) and quinovose H-1 (4.37 ppm) in a ROESY NMR spectrum. The HMBC NMR spectrum of **3** permitted the esterification sites to be established through the conectivities between carbonyl resonance at δ_H 168.8 of a tigloyl group with H-2 (5.55 ppm) of rhamnose and the carbonyl resonance at δ_C 174.9 of 11-hydroxyhexadecanoyl with H-3 (5.59 ppm) of rhamnose. The correlation between the carbonyl resonance at δ_C 174.9 with signals at δ_H 2.28 and 2.45 of the nonequivalent protons at C-2 of the aglycon indicated that the structure of tyrianthin 8 (**3**) was of the macrocyclic lactone type, and is shown.

Tyrianthin 9 (**4**) gave a quasimolecular ion [M + Na]⁺ at *m/z* 951 in the positive-ion FABMS. The molecular formula of **4** ($C_{43}H_{76}O_{21}$) was determined from the positive-ion HRM-

Table 2. ^{13}C NMR Data for Compounds **1**−**4** (CD$_3$OD, δ ppm)^a

position	δ_C, mult.			
	1	**2**	**3**	**4**
Qui				
1	103.1, CH	103.0, CH	102.1, CH	102.1, CH
2	79.4, CH	79.5, CH	79.2, CH	79.3, CH
3	78.0, CH	78.1, CH	78.7, CH	78.5, CH
4	77.2, CH	77.3, CH	77.3, CH	77.2, CH
5	72.2, CH	72.1, CH	71.9, CH	72.1, CH
6	18.2, CH$_3$	18.2, CH$_3$	18.1, CH$_3$	18.2, CH$_3$
Glc				
1	101.9, CH	102.0, CH	102.0, CH	101.9, CH
2	79.2, CH	79.2, CH	78.9, CH	79.1, CH
3	77.4, CH	77.3, CH	77.9, CH	77.7, CH
4	72.4, CH	72.3, CH	72.2, CH	72.3, CH
5	74.6, CH	74.5, CH	75.4, CH	75.5, CH
6	63.6, CH$_2$	63.3, CH$_2$	63.4, CH$_2$	63.5, CH$_2$
Rha				
1	101.2, CH	101.0, CH	96.7, CH	96.6, CH
2	72.2, CH	70.8, CH	69.6, CH	69.7, CH
3	72.2, CH	72.7, CH	71.2, CH	71.2, CH
4	78.0, CH	78.3, CH	78.6, CH	78.7, CH
5	68.0, CH	68.9, CH	69.6, CH	69.5, CH
6	18.2, CH$_3$	18.4, CH$_3$	18.3, CH$_3$	18.4, CH$_3$
Qui^1				
1	105.2, CH	105.3, CH	104.2, CH	104.2, CH
2	76.2, CH	76.1, CH	73.1, CH	73.1, CH
3	73.1, CH	73.2, CH	73.0, CH	73.3, CH
4	77.0, CH	76.9, CH	72.2, CH	71.9, CH
5	70.2, CH	70.3, CH	75.4, CH	75.5, CH
6	18.2, CH$_3$	18.2, CH$_3$	17.7, CH$_3$	17.8, CH$_3$
Jal				
1	177.9, qC	177.9, qC	174.9, qC	174.8, qC
2	34.4, CH$_2$	34.4, CH$_2$	34.7, CH$_2$	34.7, CH$_2$
11	83.6, CH	83.5, CH	82.3, CH	82.2, CH
16	12.5, CH$_3$	12.4, CH$_3$	14.6, CH$_3$	14.6, CH$_3$
mba				
1	178.5, qC	178.4, qC		
2	41.7, CH	41.7, CH		
3	27.8, CH$_2$	27.7, CH$_2$		
4	13.7, CH$_3$	13.6, CH$_3$		
nil				
1	179.3, qC			
2	48.7, CH			
3	69.7, CH			
4	20.7, CH$_3$			
prop				
1				176.7, qC
2				34.7, CH$_2$
3				18.4, CH$_3$
tig				
1		168.8, qC	168.8, qC	
2		129.5, CH	129.5, CH	
3		139.1, qC	139.0, qC	
4		18.6, CH$_3$	18.5, CH$_3$	

^{a} Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, tig = 2-methyl-2-butenoyl, mba = 2-methylbutanoyl, nil = 3-hydroxy-2-methylbutanoyl, prop = propanoyl, and Jal = 11-hydroxyhexadecanoyl.

ALDITOFMS. The negative-ion FABMS of **4** showed the fragment peaks at *m/z* 927 [M − H$^+$]$^-$, 871, 707, 561, 417, and 271. The ^{1}H and ^{13}C NMR spectra of compound **4** were very similar to those of **3**, except for signals due to a propanoyl group instead of signals for a tigloyl residue. GC-MS analysis of the products recovered from acid hydrolysis of tyrianthin 9 (**4**) established the presence of propanoic acid and 11-hydroxyhexadecanoic acid. The HMBC NMR spectra of **4** permitted the esterification sites to be established through the conectivities between carbonyl and ^{1}H NMR signals of the monosaccharides: a propanoyl group was bonded at H-2 of rhamnose, and a 11-hydroxyhexadecanoyl group was located at C-3 of rhamnose. Also in the HMBC NMR spectrum of **4** was observed a correlation between the carbonyl resonance and the methylene

protons at C-2 of the aglycon unit, which confirmed the macrolactone structure of compound **4**.

Tyrianthinic acids I (**1**) and II (**2**) represent a third type of naturally acylated glycosidic acids related to the macrocyclic ester structures of the Convolvulaceous resin glycosides, in addition to two previously acylated glycosidic acids named pescaprosides[16,17] and cus-1 and -2.[18] Cryptophilic acids A−C are also another group of tetrasaccharide glycosidic acids, but were isolated from *Scrophularia crypthophila*.[19]

The amount of each methanolic extract produced by the roots of *I. tyrianthina* collected in three different growing locations was in general the same and independent of the region and season of collection (Table S1, Supporting Information). However, variations in the concentration levels of **1**−**4** were found to be dependent on the plant location growth. Similar results were obtained for the dicholoromethane extract of *I. tyrianthina*.[3] The dependence of the amount of methanolic extract produced by the root of *Ipomoea* collected in different growing locations was also observed by Pereda-Miranda et al.[20,21]

Thyrianthinic acids I (**1**) and II (**2**) showed the same moderate activity (MIC 25 μg/mL) against *M. tuberculosis* as tyrianthins 8 (**3**) and 9 (**4**). This activity has also been reported for five macrolactones from *I. tricolor* (MICs 16−32 μg/mL).[16]

Compounds **1**−**4** were subjected to cytotoxicity evaluation using nasopharyngeal carcinoma (KB), colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1), and ovarian carcinoma (OVCAR-5) cells. These compounds were active against KB cell line (ED$_{50}$ 2.6, 2.8, 2.2, and 2.5 μg/mL respectively), but inactive against the other cell lines (ED$_{50}$ >5 μg/mL).

The new compounds **1**−**4** administered ip (10 mg/kg) to mice led to an increase in the latency and duration of hypnosis induced by pentobarbital (30 mg/kg). The administration of 40 mg/kg of compounds **1**−**4** induced protection rates in the range 70−100% against seizures induced by pentylenetetrazole, in a similar way to those reported for tetasaccharide macrolactones.[3,13] The administration of compounds **3** and **4** (4 μg/mL) to mouse brain slices induced a maximal increment of GABA at 90 s, with such behavior similar to that observed for the dichloromethane extract of *I. stans*.[13] Compounds **1**−**4** released glutamic acid gradually from 30 to 180 s, at a dose of 4 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 341 digital polarimeter. IR spectra were recorded using a Bruker model v22 spectrometer. NMR spectra were obtained on a Varian Unity 400 NMR spectrometer, equipped with a 5 mm inverse detection pulse field gradient probe at 25 °C, and a Bruker 400 NMR spectrometer. The 2D NMR spectra were processed using forward linear prediction and zero filling for increasing resolution. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS), with 20 mg of each oligosaccharide being dissolved in ca. 0.70 mL of methanol-d_4 or pyridine-d_5. Positive- and negative-ion LRFABMS were recorded using *m*-nitrobenzyl alcohol as matrix on a JEOL MStation JMS700 mass spectrometer. High-resolution MALDITOFMS data were acquired using a Voyager DE-PRO mass spectrometer, equipped with a laser of nitrogen at 337 nm. The analyte was concentrated using Zip Tip C$_{18}$ (Millipore), eluted with a solution (80% v/v CH$_3$CN/0.5% trifluoroacetic acid) saturated with *R*-cyano-4-hydroxycinnamic acid (Sigma), applied on the metallic sample plate, dried, and analyzed in mode reflector. The GC-MS system consisted of a HP 6890 gas chromatograph and a HP 5970 mass selective detector in the electron-ionization mode. Silica gel (70−230 mesh, Merck) was used for column chromatography. TLC was carried out on precoated Kieselgel 60 F$_{254}$ (0.25 mm thick; Merck, Darmstadt, Germany) plates, and spots were visualized by spraying the plates with 10% H$_2$SO$_4$ solution followed by heating. Preparative TLC was carried out on precoated Kieselgel 60 F$_{254}$ (20 × 20 cm; 1.0 mm thick; Merck, Darmstadt, Germany) plates. HPLC was performed using a system

comprised of an Agilent 1100 binary pump, an Agilent variable-wavelength UV−vis 1100 detector, and a Rheodyne injector.

Plant Material. Roots of *I. thyrianthina* were collected in the states of Puebla (August 2000 and December 2002), Morelos (December 2004 and August 2005), and Distrito Federal (May 2003 and July 2004), Mexico. Botanical classification was carried out by Biol. M. Castro, Facultad de Ciencias, UNAM, and voucher specimens (numbers 15073, 15004, 15077, 15076, 15074, and 15075, respectively) are deposited at the Instituto Mexicano del Seguro Social Herbarium in Mexico City.

Extraction and Isolation. Dried and ground roots (100 g) of the three locations of growth were extracted exhaustively with hexane and CH_2Cl_2. The residual vegetal materials were extracted exhaustively by methanol to give, after removal of the solvent, brown solids (ca. 20 g). The methanolic extracts were subjected to gravity column chromatography over reversed-phase (C_{18}) silica gel (50 g), using a gradient of CH_3OH in H_2O for elution, leading to brown resinous solids (10 g each) in the 100% methanol fractions. The resinous materials were subjected to separation by a column packed with Sephadex LH-20 (20 g) and by preparative TLC, obtaining two chromatographic fractions. The components of the more polar chromatographic fraction (2 g) were purified by preparative HPLC using an Ultrasil ODS column (10 mm i.d. × 300 mm, 5 μm, Altex), eluting with a mixture of CH_3CN-H_2O (7:3), at a flow rate of 1 mL/min at 25 °C, and detection with UV at 215 nm. Chromatographic peaks were collected and reinjected until pure. This technique afforded pure compounds from the three samples. Puebla sample: compounds **1** (40 mg, t_R 13.5 min), **2** (700 mg, t_R 15.2 min), **3** (50 mg, t_R 23.4 min), and **4** (40 mg, t_R 25.6 min). Distrito Federal sample: compounds **1** (400 mg, t_R 13.5 min), **2** (100 mg, t_R 15.2 min), **3** (70 mg, t_R 23.4 min), and **4** (80 mg, t_R 25.6 min). Morelos sample: compounds **1** (50 mg, t_R 13.5 min), **2** (60 mg, t_R 15.2 min), **3** (90 mg, t_R 23.4 min), and **4** (76 mg, t_R 25.6 min).

Tyrianthinic acid I (1): amorphous, white powder; mp 136−138 °C; $[\alpha]^{25}_D$ −20.1 (*c* 1.1 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 1079 [M + Na]$^+$; negative-ion FABMS *m/z* 1055 [M − H]$^-$; HRMALDITOFMS *m/z* 1058.2310 [M + H]$^+$ (calcd for $C_{50}H_{88}O_{23}H^+$ 1058.2278).

Tyrianthinic acid II (2): amorphous, white powder; mp 140−142 °C; $[\alpha]^{25}_D$ −18.1 (*c* 1.3 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 1061 [M + Na]$^+$; negative-ion FABMS *m/z* 1037 [M − H]$^-$; HRMALDITOFMS *m/z* 1040.2151 [M + H]$^+$ (calcd for $C_{50}H_{86}O_{22}H^+$, 1040.2125).

Tyrianthin 8 (3): amorphous, white powder; mp 133−135 °C; $[\alpha]^{25}_D$ −16.5 (*c* 1.6 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 977 [M + Na]$^+$; negative-ion FABMS *m/z* 953 [M − H]$^-$; HRMALDITOFMS *m/z* 956.0952 [M + H]$^+$ (calcd for $C_{45}H_{78}O_{21}H^+$, 956.0931).

Tyrianthin 9 (4): amorphous, white powder; mp 143−145 °C; $[\alpha]^{25}_D$ −13.0 (*c* 1.2 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 951 [M + Na]$^+$; negative-ion FABMS *m/z* 927 [M − H]$^-$; HRMALDITOFMS *m/z* 930.0680 [M + H]$^+$ (calcd for $C_{43}H_{76}O_{21}H^+$, 930.0653).

Acid Hydrolysis of the Chromatographic Fraction. The more polar chromatographic fraction (100 mg) was refluxed with 1.0 N HCl (10 mL of water−methanol) for 2.0 h. The reaction mixture was taken to pH 5 with NaOH solution, and the solution was extracted with CH_2Cl_2 and analyzed by GC-MS, allowing the identification of the following acid derivatives: methyl propanoate (t_R 3.0 min) *m/z* [M]$^+$ 88 (15), [M − CH$_3$]$^+$ 73 (27), [M − OCH$_3$]$^+$ 57 (52), 29 (100); methyl 2-methyl-2-butenoate (t_R 3.8 min) *m/z* [M]$^+$ 88 (10), [M − CH$_3$]$^+$ 89 (4), [M − OC$_2$H$_5$]$^+$ 69 (12), 55 (60), 29 (100); methyl 2-methylbutanoate (t_R 6.6 min) *m/z* [M]$^+$ 130 (0.5), [M − C$_2$H$_4$]$^+$ 102 (34), [M − OC$_2$H$_5$]$^+$ 85 (26), 57 (80), 29 (100); methyl 3-hydroxy-2-methylbutanoate (t_R 8.65 min) *m/z* [M]$^+$ 132 (5.0), [M − OC$_2$H$_5$]$^+$ 87 (20), 73 (100), 45 (40); and methyl 11-hydroxyhexadecanoate (t_R 19.57 min) *m/z* [CH$_3$ − (CH$_2$)$_4$ − CH − (OH) − (CH$_2$)$_9$CO$_2$ C$_2$H$_5$]$^+$ 300 (1), [CH$_3$ − (CH$_2$)$_4$ − CH − (OH) − (CH$_2$)$_9$CO]$^+$ 255 (3.0), [(CH$_2$)$_9$CO$_2$ C$_2$H$_5$]$^+$ 199 (50), [CH$_3$ − (CH$_2$)$_4$ − CH − OH]$^+$ 101 (60), 83 (45), 57 (100), 73 (20), 45 (40).

Carbohydrate Analysis. The aqueous phase of the acid hydrolysis reaction was neutralized with Na_2CO_3 solution and lyophilized to give a colorless powder. The residue was dissolved in 1.0 mL of dry pyridine and treated with 1 mL of hexamethyldisilazane and 0.5 mL of

chlorotrimethylsilane at 60 °C for 50 min. GC-MS analysis gave three peaks, t_R 14.1, 18.2, and 22.2 min, which coeluted with TMSi ethers of standard α-L-rhamnose, 6-deoxy-β-D-glucose (quinovose), and β-D-glucose, respectively. An aliquot of the hydrolysis mixture was subjected to preparative HPLC on a Nucleosil 100 NH$_2$ column (Alltech; 5 μm, 250 × 4.6 mm), with an isocratic elution of CH_3CN-H_2O (8:2), at a flow rate of 1 mL/min, and a sample injection of 200 μL, affording three compounds, which were identified by coelution with standard α-L-rhamnose (1 mg, t_R 7.9 min), 6-deoxy-β-D-glucose (quinovose, 2 mg, t_R 8.9 min), and β-D-glucose (1 mg, t_R 15.2 min). The absolute configurations of the collected sugars were determined by their optical rotations: glucose, $[\alpha]^{25}_D$ +105 (*c* 0.9 CH_3OH); rhamnose, $[\alpha]^{25}_D$ −6.0 (*c* 0.9 CH_3OH); and quinovose, $[\alpha]^{25}_D$ +64.9 (*c* 1.8 CH_3OH).

Alkaline Hydrolysis of the Chromatographic Fraction. The more polar chromatographic fraction (100 mg) was refluxed in 0.2 N NaOH (10 mL) for 60 min. The reaction mixture was acidified to pH 5 and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The aqueous layer was lyophilized, the residue was dissolved with methanol, and a white solid (glycosidic acid) was obtained after removal of solvent. The glycosidic acid was characterized by NMR and mass spectrometry as scammonic acid A.^{14} The glycosidic acid was refluxed in 1.0 N HCl (5 mL of water−ethanol) for 1.0 h. The reaction mixture was taken to pH 5 with NaOH solution, and the solution extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The observed optical rotation ($[\alpha]^{25}_D$ +0.42) for the *S* enantiomer of the ethyl ester of jalapinolic acid was closely comparable to that previously reported ($[\alpha]^{25}_D$ +0.45).^{9}

Anti-*Mycobacterium tuberculosis* Assay. The activity of plant extracts and pure glycolipids against *M. tuberculosis* strains was tested using the microplate Alamar Blue assay (MABA) modified by Molina-Salinas and co-workers.^{6} The strain of *M. tuberculosis* used for this assay was H$_{37}$Rv (ATTC 27294), a strain sensitive to five first-line anti-TB drugs (streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide). Organic extracts and pure compounds for the anti-*M. tuberculosis* bioassay were prepared at a concentration of 4 mg/mL in 100% DMSO. The concentrations for plant extract and pure compounds ranged from 100 to 3.13 μg/mL. Rifampin (Sigma-Aldrich Co., St. Louis, MO) was used as a positive control with concentration ranging from 2.0 to 0.062 μg/mL. All assays were performed in duplicate.

Cytotoxicity Assays. The protocol applied was the same as described previously for the dichloromethane extract from the root of *I. arborescens*.^{22} Camptothecin was used as a positive control with concentrations less than 1.0 μg/mL.

CNS-Related Assays. The protocols applied were the same as for the dichloromethane extract from the root of *I. tyrianthina*.^{3}

Statistical Analysis. The statistical analysis of the results for CNS-related assays was performed with the SPSS 11.0 program and based on an analysis of variance (ANOVA) followed by the Dunnet test, in which a significant difference was established among groups when the *p* value was less than 0.05.

Supporting Information Available: Table S1 and Figures S1−S17 are available free of charge via the Internet at http://pubs.acs.org.

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