Resin Glycosides from the Roots of *Ipomoea tyrianthina* and Their Biological Activity[#]

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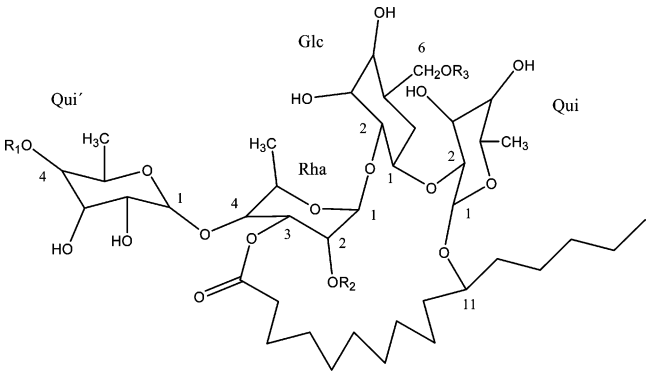
Seven new tetrasaccharide glycosides, tyrianthins 1−7 (**1**−**7**), along with six known glycolipids were isolated from the roots of *Ipomoea tyrianthina*, and their structures were elucidated by spectroscopic and chemical methods. The content of resin glycosides in samples collected in three different regions was analyzed and compared, and the results showed that the flowering or dry season did not have any effect on the chemical composition for the same locality, but the growing location itself did affect the chemical composition of resin glycosides. Intraperitoneal administration to mice of tyrianthin 6 (**6**) resulted in antidepressant activity. Tyrianthin 6 (**6**), scammonin 1 (**8**), and scammonin 2 (**9**) exhibited dose-dependent protective effects against pentylenetetrazole-induced seizures. Also, tyrianthin 6 (**6**) and scammonin 2 (**9**) produced relaxant effects on spontaneous contractions in the isolated rat ileum.

Ipomoea tyrianthina Lindley (Convolvulaceae) is a twining herb that grows in east and central Mexico. This species is known in some states of Mexico as "quiebraplatos", "manto de la virgen", and "cuamoclit". In several communities this plant is used as a purgative.[1] In a continuing investigation on secondary metabolites with biological activity from *Ipomoea* species, we have studied the resin glycosidic content of the roots of *I. tyrianthina* collected in three different regions. We report herein on the isolation and characterization of seven new tetrasaccharides of jalapinolic acid, tyrianthins 1−7 (**1**−**7**), as well as the effects of the location and month of collection on the glycolipid content, and the biological activities of tyrianthin 3 (**3**), tyrianthin 6 (**6**), scammonin 1 (**8**), and scammonin 2 (**9**).

Results and Discussion

The roots of *I. tyrianthina* were dried, pulverized, and macerated in dichloromethane. The CH_2Cl_2 extract was purified by column chromatography on silica gel, leading to the separation of two major chromatographic fractions. Both fractions were subjected to preparative HPLC in the reversed-phase mode, with compounds **1**−**7** being isolated by repetitive chromatography.

These two chromatographic fractions were hydrolyzed independently in an aqueous/ethanolic acid medium, producing an organic fraction together with a water-soluble mixture of carbohydrates. Analysis of both organic fractions by GC-MS permitted the identification of butanoic, 2-methyl-2-butenoic (tyglic), 2-methylbutanoic, 3-hydroxy-2-methylbutanoic (nilic), and 11-hydroxyhexadecanoic ethyl ester units by comparison with the mass spectra and retention times of the ethyl esters of authentic samples. HPLC and GC-MS analysis of the carbohydrates present in the aqueous phase allowed the identification of quinovose, rhamnose, and glucose.



	R_1	R_2	R_3
1	2-methylbutanoyl	niloyl	niloyl
2	tigloyl	niloyl	niloyl
3	tigloyl	niloyl	niloyl
4	tigloyl	butanoyl	niloyl
5	niloyl	niloyl	H
6	2-methylbutanoyl	butanoyl	H
7	H	niloyl	H
8	tigloyl	2-methylbutanoyl	H
9	H	2-methylbutanoyl	H

Basic hydrolysis of both chromatographic fractions produced an organic acid fraction and a water-soluble glycosidic acid derivative. The structure of the glycosidic acid was assigned as scammonic acid A (**10**) by comparison of physical and spectroscopic data.[2]

An accurate mass measurement of the quasi-molecular ion of tyrianthin 1 (**1**) $[M + Na]^+$ in the positive-ion HRFABMS gave *m/z* 1161.8365, consistent with the molecular formula $C_{55}H_{94}O_{24}$. The negative-ion FABMS showed fragment peaks at *m/z* 1137 $[M - H]^-$, 1037 [*m/z* 1137 − 100 $(C_5H_8O_2)]^-$, 937 [*m/z* 1037 − 100 $(C_5H_8O_2)]^-$, 853 [*m/z* 937 − 84 $(C_5H_8O)]^-$, 707 [*m/z* 853 − 146 $(C_6H_{10}O_4)]^-$, 561 [*m/z* 707 − 146 $(C_6H_{10}O_4)]^-$, 417 [*m/z* 561 − 144 $(C_6H_8O_3)]^-$, and 271 [*m/z* 417 − 146 $(C_6H_{10}O_5)]^-$. Basic

[#] This work was taken in part from the Ph.D. thesis of G. Mirón-López.

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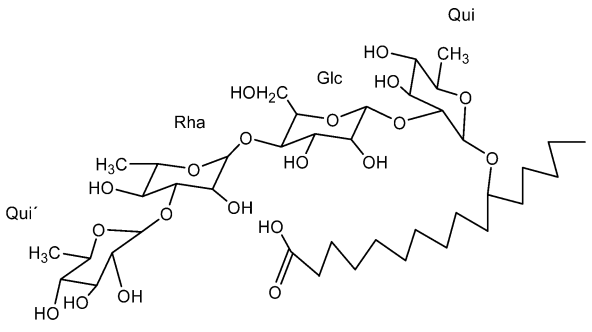

10
Scammonic acid A

hydrolysis of **1** afforded scammonic acid A and 2-methylbutanoic and nilic acids. The ^{13}C NMR spectrum of **1** (Table 1) included four carbonyl signals and four anomeric signals. The ^{1}H NMR spectrum of **1** (Table 2) exhibited three doublet methyl signals of the three 6-deoxyhexose units, overlapped signals at 3.90 (H-3) and 2.50 ppm (H-2) of niloyl groups, and two signals at 2.31 (1H, ddd) and 2.55 (1H, ddd) ppm of the nonequivalent protons of a methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. The position of the jalapinolic acid moiety in the oligosaccharide was determined by the correlation between jalapinolic acid H-11 (3.62 ppm) and quinovose H-1 (4.32 ppm) in a ROESY NMR spectrum. The HMBC NMR spectrum of **1** permitted the esterification sites to be established through the conectivities between carbonyl and ^{1}H NMR signals of the monosaccharides: the ^{13}C═O (175.7 ppm) resonance of a niloyl group correlated with H-6 (4.19 ppm) of glucose; the ^{13}C═O (175.1 ppm) resonance of a second niloyl unit correlated with H-2 (5.65 ppm) of rhamnose; the ^{13}C═O (175.5 ppm) resonance of a 2-methylbutanoyl substituent correlated with H-4 (4.67 ppm) of quinovose′; and the ^{13}C═O (173.8 ppm) resonance of the 11-hydroxyhexadecanoyl group correlated with H-3 (5.48 ppm) of rhamnose.

Compounds **2**−**7** gave quasi-molecular ions $[M + Na]^+$ at *m*/*z* 1177, 1159, 1129, 1077, 1031, and 977, respectively, in their positive-ion FABMS. The molecular formulas of **2** ($C_{55}H_{94}O_{25}$), **3** ($C_{55}H_{92}O_{24}$), **4** ($C_{54}H_{90}O_{23}$), **5** ($C_{50}H_{86}O_{23}$), **6** ($C_{49}H_{84}O_{21}$), and **7** ($C_{44}H_{76}O_{21}$) were determined by their positive-ion HRFABMS. The negative-ion FABMS of **2**−**6** showed the fragment peaks $[M - H^+]^-$ and $[(M - H^+)^- - (\text{acyloyl groups})]^-$, besides the common fragmentation peaks[3] produced by glycosidic cleavage of the sugar moieties. Basic hydrolysis of **2**−**7** afforded scammonic acid A (**10**) as the glycosidic acid present in these compounds. The ^{13}C NMR spectrum of **2**−**7** (Table 1) included four anomeric signals and carbonyl signals. The ^{1}H NMR spectrum of **2**−**7** (Tables 2 and 3) exhibited three doublet methyl signals of the three 6-deoxyhexose units and two signals of the nonequivalent protons of a methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. The position of the jalapinolic acid moiety in the oligosaccharide core of compounds **2**−**7** was determined by the correlation between jalapinolic acid H-11 and quinovose H-1 in their ROESY NMR spectra. The HMBC NMR spectrum permitted the esterification sites to be established through the conectivities between carbonyl and ^{1}H NMR signals of the monosaccharides: thus a tigloyl group was located at C-4 of quinovose′ for **3** and **4**, a 2-methylbutanoyl unit for **6**, and a niloyl substituent for **2** and **5**; a niloyl group was located at C-2 of rhamnose for **2**, **3**, **5**, and **7**, with a butanoyl substituent for **4** and **6**; and a niloyl group was located at C-6 of glucose for **2**−**4**. According to long-range correlations, the jalapinolic acid unit was esterified at C-3 of rhamnose for each of these compounds.

Orizabins I and XVIII, stansins 3 and 5, and scammonins 1 (**8**) and 2 (**9**) were also isolated and characterized by comparison of their physical and spectroscopic data with published data.[4−6]

Table 1. ^{13}C NMR Data for Compounds **1**−**7** (CD_3COCD_3, δ ppm)

carbon	1	2	3	4	5	6	7
Qui							
1	100.9	100.9	100.9	101.0	100.9	100.9	100.9
2	79.6	79.5	79.5	79.6	79.6	79.6	79.5
3	78.3	78.3	78.4	78.3	78.3	78.4	78.3
4	77.2	77.1	77.1	77.2	77.2	77.2	77.2
5	72.2	72.1	72.2	72.1	72.2	72.1	72.2
6	18.1	18.1	18.2	18.2	18.2	18.1	18.2
Glc							
1	101.4	101.3	101.3	101.4	101.4	101.3	101.4
2	79.3	79.2	79.2	79.2	79.3	79.2	79.3
3	77.4	77.3	77.4	77.4	77.3	77.4	77.4
4	72.4	72.3	72.3	72.3	72.4	72.4	72.4
5	74.6	74.5	74.6	74.5	74.5	74.5	74.6
6	63.1	63.2	63.2	63.2	63.2	63.1	63.2
Rha							
1	96.4	96.4	96.5	96.4	96.4	96.4	96.5
2	69.0	69.0	69.1	69.0	69.0	69.1	69.0
3	72.1	72.1	72.1	72.1	72.1	72.2	72.2
4	78.3	78.3	78.3	78.4	78.4	78.3	78.3
5	68.9	68.9	68.8	68.9	68.8	68.9	68.9
6	18.5	18.4	18.4	18.5	18.5	18.4	18.5
Qui′							
1	104.4	104.4	104.4	104.4	104.4	104.4	104.5
2	75.1	75.1	75.1	75.1	75.0	75.0	75.1
3	75.2	75.2	75.3	75.3	75.3	75.2	75.2
4	76.9	76.9	77.0	76.9	76.9	76.9	76.9
5	71.4	71.4	71.4	71.5	71.5	71.5	71.5
6	18.0	17.9	18.0	18.0	18.0	18.0	17.9
Jal							
1	173.8	173.7	173.8	173.8	173.8	173.7	173.8
2	35.4	35.4	35.4	35.4	35.4	35.3	35.4
11	79.3	79.4	79.3	79.3	79.3	79.3	79.3
16	14.4	14.4	14.4	14.4	14.4	14.4	14.4
mba							
1	175.5					175.4	
2	41.6					41.6	
3	27.7					27.7	
4	17.3					17.2	
tgl							
1			167.5	167.6			
2			129.2	129.2			
3			138.0	138.0			
4			14.4	14.3			
nil							
1	175.7	175.7	175.6	175.7	175.6		175.7
2	48.7	48.7	48.5	48.6	48.6		48.7
3	69.7	69.7	69.8	69.7	69.7		69.7
4	20.7	20.7	20.9	20.8	20.7		20.7
nil′							
1	175.1	175.1	175.1		175.1		
2	48.3	48.3	48.7		48.3		
3	70.3	70.3	70.2		70.3		
4	21.0	21.0	20.9		21.0		
nil″							
1		175.3					
2		48.6					
3		70.0					
4		20.8					
ba							
1				175.9		176.0	
2				42.6		42.6	
3				28.7		28.7	
4				17.0		17.0	

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, ba = butanoyl, mba = 2-methylbutanoyl, tgl = 2-methyl-2-butenoyl, nil = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl.

The roots of *I. tyrianthina* were collected in three different growing locations in order to determine the possible variations in the amount of resin glycosides produced by the plant and in the chemical composition of the dichloromethane extract (jalapin). The amount of jalapin produced was in general the same and independent of the region of collection, and the season of collection did

Table 2. ^{1}H NMR Data for Compounds **1**−**4** (CD_3COCD_3, δ ppm, J in Hz)

position	**1**	**2**	**3**	**4**
Qui[a]				
1	4.42 d (7.6)[b]	4.44 d (7.5)	4.41 d (7.7)	4.43 d (7.8)
2	3.56 dd (9.1, 7.6)	3.55 dd (9.1, 7.5)	3.53 dd (8.9, 7.7)	3.53 dd (9.0, 7.8)
3	3.57 dd (9.0, 9.1)	3.57 dd (9.0, 9.1)	3.58 dd (9.0, 8.9)	3.58 dd (9.0, 9.0)
4	3.02 dd (9.1, 9.0)	3.00 dd (9.0, 9.0)	3.01 dd (9.1, 9.0)	3.02 dd (8.9, 9.0)
5	3.25 m	3.25 m	3.23 m	3.20 m
6	1.21 d (6.1)	1.20 d (6.2)	1.22 d (7.0)	1.21 d (7.0)
Glc				
1	5.02 d (7.5)	5.01 d (7.4)	5.02 (7.3)	5.02 d (7.4)
2	3.46 dd (9.1, 7.5)	3.45 dd (9.0, 7.4)	3.46 dd (9.0, 7.3)	3.46 dd (9.1, 7.0)
3	3.62 dd (9.0, 9.1)	3.60 dd (9.0, 9.0)	3.61 dd (9.0, 9.0)	3.61 dd (9.0, 9.1)
4	3.34 dd (9.0, 9.0)	3.35 dd (9.1, 9.0)	3.34 dd (9.5, 9.0)	3.34 dd (9.0, 9.0)
5	3.35 ddd (9.0, 7.0, 3.3)	3.37 ddd (9.1, 6.5, 3.5)	3.35 ddd (9.5, 7.0, 3.5)	3.34 ddd (9.0, 6.5, 3.0)
6	4.19 dd (11.0, 3.3)	4.16 dd (11.5, 3.5)	4.18 dd (12.0, 3.5)	4.18 dd (11.5, 3.0)
6′	4.44 dd (11.0, 7.0)	4.42 dd (11.5, 6.5)	4.43 dd (12.0, 7.0)	4.44 dd (11.5, 6.5)
Rha				
1	5.48 d (1.5)	5.48 d (1.5)	5.49 d (1.5)	5.49 d (1.5)
2	5.65 dd (3.5, 1.5)	5.66 dd (3.0, 1.5)	5.64 dd (3.0, 1.5)	5.64 dd (3.5, 1.5)
3	5.48 dd (9.0, 3.5)	5.49 dd (9.5, 3.0)	5.50 dd (9.5, 3.0)	5.50 dd (9.0, 3.5)
4	3.76 dd (9.5, 9.0)	3.77 dd (9.0, 9.5)	3.76 dd (9.5, 9.5)	3.77 dd (9.0, 9.0)
5	4.23 dd (9.0, 6.5)	4.24 dd (9.0, 6.0)	4.23 dd (9.5, 6.0)	4.23 dd (9.0, 6.5)
6	1.30 d (6.2)	1.31 d (6.2)	1.30 d (6.3)	1.30 d (6.2)
Qui′				
1	4.47 d (7.6)	4.48 d (7.5)	4.47 d (7.5)	4.47 d (7.5)
2	3.27 dd (8.5, 7.6)	3.26 dd (8.0, 7.5)	3.27 dd (8.5, 7.5)	3.27 dd (9.0, 7.5)
3	3.57 dd (9.0, 8.5)	3.58 dd (8.9, 8.0)	3.57 dd (9.0, 8.5)	3.57 dd (9.0, 9.0)
4	4.67 dd (9.0, 9.0)	4.67 dd (9.0, 8.9)	4.67 dd (9.0, 8.5)	4.66 dd (9.0, 8.9)
5	3.54 m	3.53 m	3.54 m	3.54 m
6	1.13 d (6.1)	1.12 d (6.1)	1.12 d (6.0)	1.12 d (6.0)
Jal				
2*a*	2.24 ddd (14.0, 7.0, 3.5)	2.27 ddd (15.0, 7.0, 3.0)	2.29 ddd (15.0, 7.5, 3.0)	2.28 ddd (14.0, 7.0, 3.5)
2*b*	2.38*	2.36*	2.40*	2.36*
11	3.54*	3.55*	3.54*	3.55*
16	0.90 t (7.0)	0.87 t (7.0)	0.89 t (7.0)	0.90 t (7.0)
mba				
2	2.46*			
3	1.59, 1.70*			
tgl				
3			6.85 dq (1.2, 6.9)	6.84 dq (1.2, 6.9)
4			1.78 d (6.9)	1.79 d (6.9)
nil				
2	2.52*	2.52*	2.51*	2.52*
3	3.90*	3.90*	3.89*	3.89*
nil′				
2	2.50*	2.48*	2.50*	
3	3.89*	3.91*	3.91*	
nil″				
2		2.50*		
3		3.89*		
ba				
2				2.25 t (7.0)
3				1.22 m
4				0.99 t (6.5)

[a] Abbreviations: Qui = quinovopyranosyl, Glu = glucopyranosyl, Rha = rhamnopyranosyl, Jal = 11-hydroxyhexadecanoyl, ba = butanoyl, mba = 2-methylbutanoyl, tgl = 2-methyl-2-butenoyl, nil = 3-hydroxy-2-methylbutanoyl. [b]Signal multiplicity and J_{H-H} values (Hz) (shown in parentheses). Chemical shifts marked with asterisk (*) indicate overlapped signals.

not affect the yield of jalapin (Table S1, Supporting Information). The chemical composition of the dichloromethane extract was the same for both the dry and flowering seasons. However, the chemical composition of the glycolipids appeared to be dependent on the growing location, as shown in Table S2, Supporting Information.

In order to establish whether the CH_2Cl_2 extract of *I. tyrianthina* possesses antispasmodic properties, the effect of the extract on spontaneous contractions of rat ileum was evaluated. The extract exhibited inhibition of contractions on the tissue. To determine the effect of some individual components of the extract on rat ileum, compounds **3**, **6**, **8**, and **9** were used in the test. Compounds **6** and **8** induced a significant inhibition of the tone and amplitude of contractions on the ileum. The effect was concentration dependent, and the E_{max} values were 59.1% and 55.1% of relaxation at 250 μg/mL, respectively (Figure S2, Supporting Information). Compounds **3**, **6**, **8**, and **9** were less potent than papaverine, used as the positive control.

The molecular structures of tyrianthins are very similar to those of the glycolipids isolated from *I. stans*.[6] It has been reported that chromatographic fractions of the jalapin from the root of *I. stans* exhibited activity on the central nervous system (CNS) of mice.[7] The above-mentioned data suggest the possibility that compounds isolated from the CH_2Cl_2 extract of *I. tyrianthina* are able to induce effects on the CNS, so we decided to examine the activity of compounds **6**, **8**, and **9** on the CNS of mice, using different standard models such as the forced swimming test (FST), the elevated plus-maze (EPM) test, and pentylenetetrazole-induced seizures.

Tyrianthin 6 (**6**), administered ip (10 mg/kg) to mice, provoked a significant ($p < 0.001$) diminution in immobility time when the animals were subjected to the FST compared with the control group.

Table 3. ^{1}H NMR Data for Compounds **5**−**7** (CD_3COCD_3, δ ppm, J in Hz)[a]

position	5	6	7
Qui[a]			
1	4.42 d (7.6)	4.42 d (7.5)	4.41 d (7.7)
2	3.53 dd (9.0, 7.6)	3.52 dd (9.0, 7.5)	3.53 dd (8.9, 7.7)
3	3.58 dd (9.0, 9.0)	3.52 dd (8.9, 9.0)	3.53 dd (9.0, 8.9)
4	3.02 dd (8.9, 9.0)	2.98 dd (9.0, 8.9)	3.01 dd (9.1, 9.0)
5	3.20 m	3.20 m	3.23 m
6	1.21 d (7.0)	1.19 d (7.2)	1.22 d (7.0)
Glc			
1	5.02 d (7.5)	5.07 d (7.6)	5.08 (7.5)
2	3.46 dd (9.1, 7.5)	3.43 dd (9.0, 7.6)	3.46 dd (9.0, 7.5)
3	3.61 dd (9.0, 9.1)	3.60 dd (9.0, 9.0)	3.60 dd (9.0, 9.0)
4	3.36 dd (9.0, 9.0)	3.35 dd (9.1, 9.0)	3.37 dd (9.5, 9.0)
5	3.31 ddd (9.0, 6.5, 3.0)	3.30 ddd (9.1, 6.5, 3.5)	3.30 ddd (9.5, 7.0, 3.5)
6	3.62 dd (11.5, 3.0)	3.62 dd (11.5, 3.5)	3.62 dd (12.0, 3.5)
6′	3.84 dd (11.5, 6.5)	3.84 dd (11.5, 6.5)	3.84 dd (12.0, 7.0)
Rha			
1	5.49 d (1.5)	5.46 d (1.5)	5.48 d (1.5)
2	5.64 dd (3.5, 1.5)	5.65 dd (3.0, 1.5)	5.64 dd (3.0, 1.5)
3	5.50 dd (9.0, 3.5)	5.49 dd (9.5, 3.0)	5.50 dd (9.5, 3.0)
4	3.77 dd (9.0, 9.0)	3.72 dd (9.0, 9.5)	3.76 dd (9.5, 9.5)
5	4.23 dd (9.0, 6.5)	4.22 dd (9.0, 6.0)	4.25 dd (9.5, 6.0)
6	1.30 d (6.2)	1.29 d (6.1)	1.30 d (6.1)
Qui′			
1	4.47 d (7.6)	4.48 d (7.5)	4.47 d (7.6)
2	3.27 dd (9.0, 7.6)	3.25 dd (8.0, 7.5)	3.25 dd (8.5, 7.6)
3	3.60 dd (9.0, 9.0)	3.58 dd (8.9, 8.0)	3.60 dd (9.0, 8.5)
4	4.60 dd (9.0, 8.9)	4.60 dd (9.0, 8.9)	3.58 dd (9.0, 8.5)
5	3.55 m	3.58 m	3.59 m
6	1.12 d (6.4)	1.12 d (6.5)	1.12 d (6.4)
Jal			
2*a*	2.28 ddd (14.0, 7.0, 3.5)	2.27 ddd (15.0, 7.0, 3.5)	2.29 ddd (15.0, 7.5, 3.4)
2*b*	2.36*	2.36*	2.37*
11	3.55*	3.55*	3.54*
16	0.90 t (7.0)	0.89 t (7.0)	0.89 t (7.0)
mba			
2		2.46*	
3		1.55, 1.78*	
tgl			
2			
3			
nil			
2	2.52*		2.47*
3	3.89*		3.89*
nil′			
2	2.50*		
3	3.91*		
ba			
2*a*		2.25 t (7.0)	
3		1.22 m	
4		0.99 t (6.4)	

[a] Abbreviations: Qui = quinovopyranosyl, Glu = glucopyranosyl, Rha = rhamnopyranosyl. Jal = 11-hydroxyhexadecanoyl, ba = butanoyl, mba = 2-methylbutanoyl, tgl = 2-methyl-2-butenoyl, nil = 3-hydroxy-2-methylbutanoyl. [b] Signal multiplicity and J_{H-H} values (Hz) (shown in parentheses). Chemical shifts marked with asterisk (*) indicate overlapped signals.

The animals that received compounds **8** and **9** did not show any change with respect to the group with Tween 20 (1.0%). Imipramine (15 mg/kg), the antidepressant used in the positive control group, induced a similar modification to that observed with compound **6** ($p < 0.001$).

The ip administration of compounds **6**, **8**, and **9** at doses of 10 mg/kg in ICR mice subjected to the EPM test did not provoke any change ($p > 0.05$) in the percentage time and percentage of entries that mice spent in open arms compared with the negative control. Diazepam, at a dose of 1.0 mg/kg, provoked an increase in the time mice spent in the open arms compared with the negative control ($p < 0.001$), and this drug also increased the percentage of entries into the open arms in comparison with the control group ($p < 0.001$).

All animals treated with diazepam at a dose of 1.0 mg/kg were protected against seizures induced by pentylenetetrazole, and these mice did not show any convulsions after injection of this substance ($p < 0.05$). The administration of 40 mg/kg of compounds **6**, **8**, and **9** induced protection rates of 88%, 100%, and 66%, respectively, while scammonins 1 (**8**) and 2 (**9**) increased significantly the onset of seizures with respect to the negative control group (Table S3, Supporting Information).

Compound **6** showed an antidepressant effect and compounds **8** and **9** an anticonvulsivant effect, and also compounds **6** and **8** induced relaxation on the isolated rat ileum. Apparently, there is a biological correlation between the effects on the CNS and the spasmolytic actions of the compounds analyzed. In the intestine, innervated by an enteric nervous system that can function independently from CNS control, several chemical messengers of different classes (observed also in the brain) are recognized. It is possible that the isolated rat ileum test (which does not represent a depression or convulsive disorder model) permits the prediction of the interaction of the bioactive molecules with the receptors or neurotransmitters present in both tissues.[8] Further experiments are necessary in order to elucidate the mode of action of these compounds.

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. 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 using standard Varian software. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS), with 20 mg of each oligosaccharide being dissolved in ca. 0.75 mL of acetone-d_6, except for scammonic acid A (**10**), which was dissolved in D_2O. Positive- and negative-ion FABMS were recorded on a JEOL MStation JMS700 mass spectrometer using *m*-nitrobenzyl alcohol as matrix. 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, Darmstadt, Germany) 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_2SO_4 solution followed by heating. HPLC was performed using a system comprised of an Agilent 1100 binary pump, an Agilent variable-wavelength UV−vis 1100 detector, an Agilent refractive index detector 1100, 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 IMSSM Herbarium in Mexico City.

Extraction and Isolation. Dried and ground roots (100 g) were defatted with hexane at room temperature. The residual material was extracted exhaustively in CH_2Cl_2 to give, after removal of the solvent, a light yellow resinous material. The resin showed two spots by TLC on silica gel, eluted with CH_2Cl_2−CH_3OH (9:1) (R_f 0.50 and 0.45), and was subjected to gravity column chromatography over silica gel (500 g) using a gradient of CH_3OH in CH_2Cl_2, leading to two chromatographic fractions. Purification of chromatographic fractions was carried out 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. Compounds **7** (t_R 9.6 min), **5** (t_R 11.7 min), **6** (t_R 14.5 min), **2** (t_R 16.2 min), **3** (t_R 20.2 min), **1** (t_R 22.6 min), and **4** (t_R 24.2 min) were collected and reinjected until pure.

Tyrianthin 1 (1): amorphous, white powder; mp 128−130 °C; $[\alpha]^{25}_D$ −20.0 (*c* 2.1 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* 1161 $[M + Na]^+$; negative-ion FABMS *m/z* 1137 $[M - H]^-$, 1037 $[1137 - C_5H_8O_2]^-$, 937 $[1037 - C_5H_8O_2]^-$, 853 $[937 - C_5H_8O]^-$, 707, 561, 417, and 271; HRFABMS *m/z* 1161.7307 $[M + Na]^+$ (calcd for $C_{55}H_{94}O_{24}$, 1138.7402).

Tyrianthin 2 (2): amorphous, white powder; mp 130−131 °C; $[\alpha]^{25}_D$ −22.1 (*c* 1.8 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 1177 $[M + Na]^+$; negative-ion FABMS *m/z* 1153 $[M - H]^-$, 1053 $[1153 - C_5H_8O_2]^-$, 953 $[1053 - C_5H_8O_2]^-$, 853 $[953 - C_5H_8O_2]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 1177.7408 $[M + Na]^+$ (calcd for $C_{55}H_{94}O_{25}$, 1154.7401).

Tyrianthin 3 (3): amorphous, white powder; mp 127−129 °C; $[\alpha]^{25}_D$ −19.9 (*c* 1.8 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 1159 $[M + Na]^+$; negative-ion FABMS *m/z* 1135 $[M - H]^-$, 1035 $[1135 - C_5H_8O_2]^-$, 935 $[1035 - C_5H_8O_2]^-$, 853 $[935 - C_5H_6O]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 1159.7136 $[M + Na]^+$ (calcd for $C_{55}H_{92}O_{24}$, 1136.7244).

Tyrianthin 4 (4): amorphous, white powder; mp 128−130 °C; $[\alpha]^{25}_D$ −18.1 (*c* 2.4 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; positive-ion FABMS *m/z* 1129 $[M + Na]^+$; negative-ion FABMS *m/z* 1105 $[M - H]^-$, 1005 $[1105 - C_5H_8O_2]^-$, 921 $[1005 - C_5H_8O]^-$, 853 $[921 - C_4H_5O]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 1129.6990 $[M + Na]^+$ (calcd for $C_{54}H_{90}O_{23}$, 1106.7087).

Tyrianthin 5 (5): amorphous, white powder; mp 133−135 °C; $[\alpha]^{25}_D$ −20.9 (*c* 2.5 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive-ion FABMS *m/z* 1077 $[M + Na]^+$; negative-ion FABMS *m/z* 1053 $[M - H]^-$, 953 $[1053 - C_5H_8O_2]^-$, 853 $[953 - C_5H_8O_2]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 1077.6678 $[M + Na]^+$ (calcd for $C_{50}H_{86}O_{23}$, 1054.6771).

Tyrianthin 6 (6): amorphous, white powder; mp 130−132 °C; $[\alpha]^{25}_D$ −22.7 (*c* 3.2 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive-ion FABMS *m/z* 1031 $[M + Na]^+$; negative-ion FABMS *m/z* 1007 $[M - H]^-$, 923 $[1007 - C_5H_8O_2]^-$, 853 $[923 - C_4H_6O_2]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 1031.6520 $[M + Na]^+$ (calcd for $C_{49}H_{84}O_{21}$, 1008.6615).

Tyrianthin 7 (7): amorphous, white powder; mp 133−135 °C; $[\alpha]^{25}_D$ −20.1 (*c* 2.2 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive-ion FABMS *m/z* 977 $[M + Na]^+$; negative-ion FABMS *m/z* 953 $[M - H]^-$, 853 $[953 - C_5H_8O_2]^-$, 707, 561, 417, and 271; HRESIMS *m/z* 977.6020 $[M + Na]^+$ (calcd for $C_{45}H_{78}O_{21}$, 954.6140).

Acid Hydrolysis of the Chromatographic Fraction. The resinous extract (150 mg) was refluxed with 1.0 N HCl (10 mL of water−ethanol) for 2.0 h. The reaction mixture was treated by the previously reported procedure,[4] allowing the identification of the following acid derivatives: ethyl 2-butenoate, ethyl butanoate, methyl-2-butenoate, ethyl 2-methylbutanoate, ethyl 3-hydroxy-2-methylbutanoate, and ethyl 11-hydroxyhexadecanoate.

Carbohydrate Analysis. The aqueous phase of the acid hydrolysis reaction was prepared and analyzed by the previously reported procedure,[4] allowing the identification of α-L-rhamnose, d-quinovose, and D-glucose. The sugars were isolated and their absolute configurations were determined by their optical rotations: glucose, $[\alpha]^{25}_D$ +104 (*c* 0.8 CH_3OH), rhamnose, $[\alpha]^{25}_D$ −5.0 (*c* 1.3 CH_3OH), and quinovose, $[\alpha]^{25}_D$ +66.0 (*c* 0.6 CH_3OH).

Alkaline Hydrolysis of the Chromatographic Fractions. The resinous material (200 mg) was refluxed in 0.2 N NaOH (10 mL) for 2.0 h. The reaction mixture treatment and scammonic acid characterization were done according to the previously reported procedure.[4]

Animals and Drug Administration. Male ICR mice with a weight of 32−38 g were used. All animals were purchased from Harlan, Mexico, Mexico, and maintained for three weeks in an animal house with a cycle of 12 h of light and 12 h of darkness and free access to H_2O and food. Three days before testing began, animals were conditioned to the laboratory environment and to the researcher. All experiments were carried out between 8:00 a.m. and 1:00 p.m. and conducted in accordance with the Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture, NOM-062-ZOO-1999, Mexico). The experimental protocols were approved by the Research Committee of Mexican Institute of Social Security. The minimum number of animals and duration of observation required to obtain consistent data were employed. Groups of eight mice per treatment were used, and each group was administered ip (30 min before the experimental session for the anxiolytic and anticonvulsivant tests, and 18, 24, and 30 min before the forced swimming test in a constant volume of 300 μL), with a dose of 10 mg/kg for compounds **6**, **8**, and **9**. Doses of 10 and 40 mg/kg were used for the pentylenetetrazole-induced seizure test. Compounds administered were dissolved in 1% Tween 20. A control group received only the vehicle in the same volume and via the same route. A positive control group was treated (ip) with 1.0 mg/kg of diazepam (Sigma) as an antianxiety and anticonvulsant drug and 15 mg/kg of imipramine (Sigma) as an antidepressant drug.

Elevated Plus-Maze Test. After the administration of test materials, mice were subjected to the elevated plus-maze test, which is a widely used model in mice to determine the anxiolytic activity of different substances.[9] A device made of Plexiglas and consisting of two open arms (30 × 5 cm) and two closed arms (30 × 5 cm) with clear 25 cm walls was utilized. The arms extended from a central square (5 × 5 cm), and the whole device is 38.5 cm high from the floor. Animals were placed in the center of the plus-maze, and during the registration (5 min) period, the number of entrances and the time the animals spent in the open and closed arms, as well as the total exploring activity (number of entrances), were measured. After each session, the device was thoroughly cleaned with a clean paper towel and a solution of 10% EtOH. Each experimental session was videorecorded.

Forced Swimming Test. The forced swimming test is a widely used pharmacological in vivo model for assessing antidepressant activity.[10] The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior.[12] The apparatus consisted of a clear Plexiglas cylinder (20 cm high × 12 cm diameter) filled to a 15 cm depth with water (24 ± 1 °C). In the pretest session, each animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. During the test session, a trained observer registered the immobility time, considered to be when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above water. Immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experimental situation.[11]

Pentylenetetrazole-Induced Seizures. Pentylenetetrazole (75 mg/kg) was injected ip, 30 min after administration of compounds **6**, **8**, and **9**. Following the injection of pentylenetetrazole, mice were placed separately into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of seizures. The time taken before the onset of clonic convulsions and the percentage of mortality protection were recorded.[13]

Isolated Rat Ileum Test. The animals were sacrificed by exposure to diethyl ether. All animal procedures were conducted in accordance with Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture, NOM-062-ZOO-1999, Mexico) and were approved by the Animal Care and Use Committee of the Autonomous State University of Morelos. The ileum was dissected out and placed in Krebs−Henseleit (KH) solution, with the following composition (in mM): NaCl 119, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.5, $NaHCO_3$ 20, and glucose 11.4. Strips (2 cm long) were dissected and mounted in organ baths containing Krebs solution (pH 7.4), gassed with a mixture of O_2-CO_2 (19:1), and continuously recorded for isometric tension with a Grass FTO3 force−displacement transducer and registered on a Grass 7D transductor as previously described.[14] After a stabilization time of 30 min, a 10 min control period was recorded. Compounds were dissolved in DMSO or water and were added to the bath in a volume of 100 μL at different concentrations (cumulative concentration−response curves were obtained for each ileum). The effect of the compounds and positive controls was determined by comparing the areas under the curve (AUC) inscribed by the frequency and the amplitude of the ileum contractions before and after the application of compounds. Areas were calculated from the polygraph tracing, using Acknowledgment software.

Statistical Analysis. The statistical analysis of the results 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 lower than 0.05. For antispasmodic activity, all results are expressed as the mean of six experiments ± SEM. Concentration−response curves (CRC) for extracts and positive control were plotted from the experimental data and were adjusted by the nonlinear curve-fitting program (ORIGIN 6.0). The statistical significance ($p < 0.05$) of differences between means was assessed by an analysis of variance (ANOVA) followed by the Dunnet test.

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Supporting Information Available: Tables S1−S3 and Figures S1 and S2 are available free of charge via the Internet at http://pubs.acs.org.

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