Isolation and Characterization of Five New Tetrasaccharide Glycosides from the Roots of *Ipomoea stans* and Their Cytotoxic Activity

Ismael León,*,† Raúl G. Enríquez,‡ Dino Gnecco,§ María L. Villarreal,⊥ Daniel Alonso Cortés,⊥ William F. Reynolds,∥ and Margaret Yu∥

Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa 62210 Cuernavaca, Morelos, México, Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México D. F., 04510 México, Centro de Química, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla, Puebla, México, Centro de Investigación Biomédica del Sur, IMSS, Argentina 1, Xochitepec, Morelos, México, and Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 3H6, Canada

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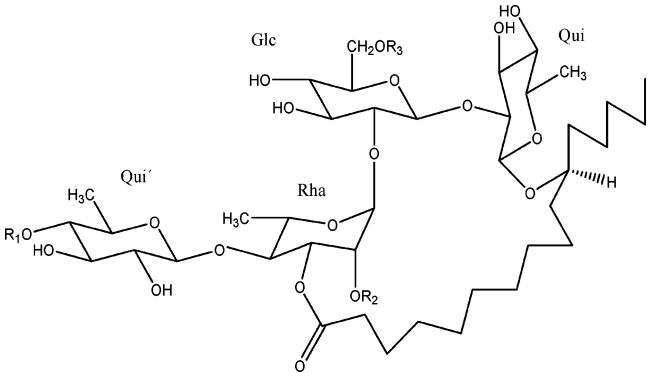
Five new tetrasaccharide glycosides, stansins 1−5 (**1**−**5**), were isolated from the roots of *Ipomoea stans*, and their structures were elucidated using spectroscopic and chemical methods. Preliminary testing showed the cytotoxicity of **5** toward the OVCAR and UISO-SQC-1 cancer cell lines.

Aqueous infusions of *Ipomoea stans* Cav. (Convolvulaceae) roots have been used in Mexican traditional medicine for treating epileptic seizures.[1] In previous investigations,[2,3] we reported the isolation of two chromatographic fractions of different polarity from the roots of *I. stans*. The less polar fraction contains hetero-tetrasaccharides forming a macrocyclic ester with the aglycon 11-hydroxyhexadecanoic acid.[2,3] The more polar fraction showed anticonvulsant properties similar to valproic acid.[4] The present study describes the isolation and characterization of five new tetrasaccharides of jalapinolic acid, stansins 1−5 (**1**−**5**), from the ethyl acetate extract of the roots of *I. stans*.

The roots of *I. stans* were dried, pulverized, and macerated with ethyl acetate, and the extract was fractionated by column chromatography on silica gel. The more polar chromatographic fraction was subjected to preparative HPLC in the reversed-phase mode, with the chromatographic peaks collected and reinjected until compounds **1**−**5** were purified.

Results and Discussion

The more polar chromatographic fraction was hydrolyzed in an aqueous/ethonolic acid medium, producing an organic fraction together with a water-soluble mixture of carbohydrates. The analysis of the organic fraction by GC−MS permitted the identification of 2-methylbutanoic, 3-hydroxy-2-methylbutanoic, methylpropanoic, and 11-hydroxyhexadecanoic ethyl esters by comparison with the mass spectra and retention times of the ethyl esters of authentic samples. The GC−MS analysis of the silylated sugar residues in the aqueous phase allowed the identification of D-quinovopyranose, L-rhamnopyranose, and D-glucopyranose. The basic hydrolysis of the more polar chromatographic fraction produced an organic acid fraction and a water-soluble glycosidic acid derivative. The structure of this glycosidic acid was determined by ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HMQC, HMBC, and ROESY experiments



	R_1	R_2	R_3
1	nla	mpa	nla
2	nla	3-mba	nla
3	nla	mba	nla
4	mpa	mba	H
5	mba	mba	H

3-mba = 3-Methylbutanoyl
nla = 3-Hydroxy-2-methylbutanoyl
mpa = 2-Methylpropanoyl
mba = 2-Methylbutanoyl

as scammonic acid A [(11*S*)-hydroxyhexadecanoic acid 11-*O*-*β*-D-quinovopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-quinovopyranoside], previously obtained from *Convolvulus scammonia*,[5] *I. stans*,[2] and *Ipomoea orizabensis*.[7]

Stansin 1 (**1**), an amorphous powder, gave a quasi-molecular ion at *m*/*z* 1147 $[M + Na]^+$ in the positive FABMS, which suggested the molecular formula $C_{55}H_{94}O_{24}$. The ^{1}H NMR spectrum of **1** showed nine doublet methyl signals and a methyl triplet signal. The basic hydrolysis of **1** provided a glycosidic acid, and comparison of the physical properties and NMR data of the glycosidic acid matched, within experimental error, those reported for scammonic acid A.[2] The HMQC spectrum of **1** indicated four anomeric carbons at 100.6, 100.5, 96.6, and 104.6 ppm, which showed correlations with the anomeric protons at

* To whom correspondence should be addressed. Tel: (777) 329 79 97. Fax: (777) 329 79 98. E-mail: ismaelr@ciq.uaem.mx.
† Centro de Investigaciones Químicas, UAEM.
‡ Instituto de Química, UNAM.
§ Centro de Química, Instituto de Ciencias, BUAP.
⊥ Centro de Investigación Biomédica del Sur, IMSS.
∥ Department of Chemistry, University of Toronto.

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

position	**1**	**2**	**3**	**4**	**5**
Qui[a]					
1	4.53 d (7.4)[b]	4.42 d (7.5)	4.51 d (7.7)	4.43 d (7.8)	4.44 d (7.9)
2	3.56 dd (9.1, 7.4)	3.52 dd (9.0, 7.5)	3.53 dd (8.9, 7.7)	3.53 dd (9.0, 7.8)	3.52 dd (9.1, 7.9)
3	3.57 dd (9.0, 9.1)	3.52 dd (8.9, 9.0)	3.53 dd (9.0, 8.9)	3.42 dd (9.0, 9.0)	3.42 dd (8.9, 9.1)
4	3.02 dd (9.1, 9.0)	2.98 dd (9.0, 8.9)	3.01 dd (9.1, 9.0)	3.02 dd (8.9, 9.0)	3.02 dd (9.1, 8.9)
5	3.25 m	3.20 m	3.23 m	3.20 m	3.21 m
6	1.23 d (7.0)	1.19 d (7.2)	1.22 d (7.0)	1.21 d (7.0)	1.22 d (7.1)
Glc					
1	5.10 d (7.0)	5.07 d (7.0)	5.08 (7.3)	5.02 d (7.0)	5.02 d (7.0)
2	3.47 dd (9.1, 7.0)	3.43 dd (9.0, 7.0)	3.46 dd (9.0, 7.3)	3.46 dd (9.1, 7.0)	3.46 dd (9.0, 7.0)
3	3.62 dd (9.0, 9.1)	3.58 dd (9.0, 9.0)	3.58 dd (9.0, 9.0)	3.58 dd (9.0, 9.1)	3.60 dd (9.0, 9.0)
4	3.39 dd (9.0, 9.0)	3.35 dd (9.1, 9.0)	3.37 dd (9.5, 9.0)	3.35 dd (9.0, 9.0)	3.36 dd (9.5, 9.0)
5	3.51 ddd (9.0, 7.0, 3.3)	3.47 ddd (9.1, 6.5, 3.5)	3.50 ddd (9.5, 7.0, 3.5)	3.31 ddd (9.0, 6.5, 3.0)	3.32 ddd (9.5, 7.0, 3.5)
6	4.19 dd (11.0, 3.3)	4.16 dd (11.5, 3.5)	4.18 dd (12.0, 3.5)	3.62 dd (11.5, 3.0)	3.63 dd (11.0, 3.5)
6'	4.46 dd (11.0, 7.0)	4.41 dd (11.5, 6.5)	4.43 dd (12.0, 7.0)	3.82 dd (11.5, 6.5)	3.84 dd (11.0, 7.0)
Rha					
1	5.40 d (1.5)	5.36 d (1.5)	5.39 d (1.5)	5.49 d (1.5)	5.50 d (1.5)
2	5.55 dd (3.5, 1.5)	5.50 dd (3.0, 1.5)	5.54 dd (3.0, 1.5)	5.64 dd (3.5, 1.5)	5.67 dd (3.5, 1.5)
3	5.48 dd (9.0, 3.5)	5.44 dd (9.5, 3.0)	5.50 dd (9.5, 3.0)	5.50 dd (9.0, 3.5)	5.49 dd (9.0, 3.5)
4	3.77 dd (9.5, 9.0)	3.72 dd (9.0, 9.5)	3.76 dd (9.5, 9.5)	3.77 dd (9.0, 9.0)	3.75 dd (9.0, 9.0)
5	4.27 dd (9.0, 6.5)	4.20 dd (9.0, 6.0)	4.25 dd (9.5, 6.0)	4.21 dd (9.0, 6.5)	4.21 (9.0, 6.5)
6	1.30 d (6.5)	1.28 d (6.0)	1.30 d (6.0)	1.29 d (6.5)	1.29 d (6.5)
Qui′					
1	4.46 d (7.0)	4.43 d (7.5)	4.45 d (7.5)	4.45 d (7.0)	4.46 d (7.0)
2	3.26 dd (8.5, 7.0)	3.22 dd (8.0, 7.5)	3.25 dd (8.5, 7.5)	3.23 dd (9.0, 7.0)	3.25 dd (9.0, 7.0)
3	3.53 dd (9.0, 8.5)	3.50 dd (8.9, 8.0)	3.52 dd (9.0, 8.5)	3.52 dd (9.0, 9.0)	3.52 dd (9.0, 9.0)
4	4.61 dd (9.0, 9.0)	4.57 dd (9.0, 8.9)	4.58 dd (9.0, 8.5)	4.58 dd (9.0, 8.9)	4.60 dd (9.0, 8.9)
5	3.54 m	3.48 m	3.49 m	3.49 m	3.49 m
6	1.18 d (6.0)	1.12 d (6.5)	1.12 d (6.0)	1.12 d (6.0)	1.15 d (6.0)
Jal					
2a	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)	2.25 ddd (14.0, 7.0, 3.5)
2b	2.38*	2.36*	2.40*	2.36*	2.37*
11	3.59*	3.65*	3.60*	3.65*	3.60*
16	0.90 t (7.0)	0.87 t (7.0)	0.89 t (7.0)	0.92 t (7.0)	0.93 t (7.0)
mba					
2			2.46*	2.46*	2.47*
3			1.55,1.77	1.57, 1.71	1.55, 1.71
mba′					
2					2.39*
3					1.45, 1.69
mpa					
2	2.47*			2.56*	
3	1.22 d (7.0)			1.15 d (7.0)	
nla					
2	2.52*	2.52*	2.47*		
3	3.96*	3.96*	3.89*		
nla′	2.48*	2.48*	2.48*		
2					
3	3.89*	3.89*	3.89*		
3-mba					
2a		2.25 dd (7.0, 7.0)			
2b		2.09 dd (7.0, 7.0)			
3		0.99 d (6.5)			
4		0.99 d (6.5)			

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

4.53 (d, J = 7.4 Hz), 5.10 (d, J = 7.0 Hz), 5.40 (d, J = 1.5 Hz), and 4.46 (d, J = 7.0 Hz) ppm, and these results suggested that part of the molecule was a tetrasaccharide. A combination of one- and two-dimensional ^{1}H NMR techniques allowed all protons to be sequentially assigned within each saccharide system, and this NMR analysis led to the identification of one glucopyranosyl, one rhamnopyranosyl, and two quinovopyranosyl units as the monosaccharides present in **1**. The anomeric configuration for the sugar moieties were defined as β for glucopyranosyl, β for quinovopyranosyl, and α for rhamnopyranosyl from their coupling constants of 7.4, 7.0, and 1.5 Hz, respectively. Using the ^{1}H detected heteronuclear one-bond correlation (HMQC) NMR spectrum all the resonances in the ^{13}C NMR spectrum were assigned. HMBC spectra were used to assign unambiguously the linkage sites within the tetrasaccharide core with the following correlations: C-1 of quinovose (δ_C 100.6) and H-11 of the aglycon (δ_H 3.59), C-1 of glucose (δ_C 100.5) and H-2 of quinovose (δ_H 3.56), C-1 of rhamnose (δ_C 96.6) and H-2 of glucose (δ_H 3.47), and C-1 of quinovose′ (δ_C 104.6) and H-4 of rhamnose (δ_H 3.77). The ^{13}C NMR spectrum of **1** showed four carbonyl signals indicating the presence of three short-chain acids. The HMBC spectrum of **1** permitted the unambiguous assignment of the esterified positions of the oligosaccharide core by the correlations between the carbonyl ester group with their corresponding vicinal proton ($^2J_{CH}$) and the pyranose ring proton ($^3J_{CH}$). In **1**, a 2-methylpropanoyl was placed at C-2 of rhamnose, as shown by the correlation between the carbonyl at δ_C 176.2 with H-2 at δ_H 2.47 and the

Table 2. ^{13}C NMR Data for Compounds **1**−**5** (125 MHz, CD_3COCD_3)

carbon	1	2	3	4	5
Qui					
1	100.6	100.7	100.4	100.8	100.9
2	79.5	79.3	79.2	79.6	79.7
3	78.3	78.1	78.2	78.3	78.4
4	77.2	77.0	77.1	77.0	77.2
5	72.2	72.1	72.2	72.1	72.4
6	18.2	18.1	18.2	18.2	18.2
Glc	100.5	100.3	100.9	101.4	101.6
1					
2	79.3	79.2	78.2	78.8	79.0
3	77.4	77.1	77.8	77.0	77.2
4	72.4	72.2	72.3	72.3	72.6
5	74.6	74.5	75.6	76.5	76.7
6	64.6	64.4	64.5	63.2	63.3
Rha					
1	96.6	96.4	96.86	96.40	96.40
2	70.0	69.8	70.24	69.00	69.20
3	71.9	71.4	71.75	71.45	71.61
4	78.7	78.5	78.33	78.38	78.57
5	68.7	68.6	68.59	68.59	68.83
6	18.5	18.4	18.35	18.45	18.51
Qui′					
1	104.6	104.4	104.4	104.4	104.6
2	75.2	75.0	75.0	75.3	75.5
3	75.4	75.2	75.3	75.3	75.3
4	76.8	76.6	76.6	76.1	76.3
5	70.6	70.5	70.4	70.5	70.7
6	17.9	17.9	17.2	17.9	18.1
Jal					
1	173.8	173.7	173.8	173.8	173.8
2	34.4	34.3	34.4	34.4	34.3
11	79.5	79.4	79.5	79.5	79.4
16	14.1	14.1	14.1	14.0	14.0
mba					
1			175.6	175.3	175.8
2			41.6	41.6	41.7
3			27.7	27.7	27.8
4			17.9	17.3	17.3
mba′					
1					176.2
2					42.1
3					27.3
4					17.2
mpa					
1	176.2			176.2	
2	34.6			34.7	
3	19.7			19.3	
nla					
1	175.7	175.7	175.6		
2	48.7	48.7	48.5		
3	69.7	69.7	69.8		
4	20.7	20.7	20.9		
nla′					
1	175.1	175.1	175.1		
2	48.3	48.3	48.7		
3	70.3	70.3	69.8		
4	21.0	21.0	20.9		
3-mba					
1		172.2			
2		43.6			
3		26.4			
4		22.6			

pyranose proton at δ_H 5.55; a niloyl (3-hydroxy-2-methylbutanoyl) residue was located at C-4 of quinovose′, as shown by the correlation between the carbonyl at δ_C 175.7 with H-2 of niloyl at δ_H 2.52 and the proton at δ_H 4.61 of C-4 of quinovose′; a second niloyl residue was placed at C-6 of the glucose as shown by the correlations between the carbonyl at δ_C 175.1 with H-2 of niloyl′ at δ_H 2.48 and the methylene protons at δ_H 4.19, 4.46; the most shielded carbonyl resonance (δ_C 173.8) was assigned to the lactone by the long-range correlations with the methylene protons of H-2 (δ_H 2.24, 2.38) and H-3 of rhamnose (δ_H 5.48).

The FABMS of stansin 2 (**2**) gave a quasi-molecular ion at *m*/*z* 1161 $[M + Na]^+$ in accordance with the molecular formula $C_{55}H_{94}O_{24}$. The ^1H NMR spectrum of **2** showed nine doublet methyl signals and a methyl triplet signal. The ^{13}C NMR spectrum of **2** showed four anomeric signals indicating the compound was a tetrasaccharide and four carbonyl signals suggesting the presence of three short chain acids. The basic hydrolysis of **2** provided scammonic acid A.[2] The HMBC spectrum of **2** permitted the unambiguous assignment of the esterified positions of the oligosaccharide core, by the correlations between the carbonyl ester groups with their corresponding vicinal proton ($^2J_{CH}$) and the pyranose ring proton ($^3J_{CH}$). For **2**, a 3-methylbutanoyl was attached at C-2 of rhamnose, a niloyl residue was located at C-4 of quinovose′, a second niloyl residue was placed at C-6 of the glucose, and the carbonyl of jalapinolic acid was esterified at C-3 of rhamnose.

Stansin 3 (**3**) gave a quasi-molecular ion at *m*/*z* 1161 $[M + Na]^+$, corresponding to the molecular formula $C_{55}H_{94}O_{24}$. The ^1H NMR and ^{13}C NMR spectrum of **3** were almost identical to those of **2**. The basic hydrolysis of **3** provided scammonic acid A.[2] For **3**, a 2-methylbutanoyl was attached at C-2 of rhamnose, a niloyl residue was located at C-4 of quinovose′, a second niloyl residue was placed at C-6 of the glucose, and the jalapinolic acid was esterified at C-3 of rhamnose.

Stansin 4 (**4**) gave a quasi-molecular ion at *m*/*z* 1031 $[M + Na]^+$ in the positive FABMS, corresponding to the molecular formula $C_{49}H_{84}O_{21}$. The ^{13}C NMR spectrum of **4** showed in the carbonyl region three signals, indicating the presence of only two short-chain acids. The basic hydrolysis of **4** provided scammonic acid A.[2] For **4**, a 2-methylbutanoyl was attached at C-2 of rhamnose, a methylpropanoyl residue was located at C-4 of quinovose′, and the jalapinolic acid was esterified at C-3 of rhamnose.

Stansin 5 (**5**) exhibited a quasi-molecular ion at *m*/*z* 1031 $[M + Na]^+$ corresponding to the molecular formula $C_{50}H_{86}O_{21}$. The ^{13}C NMR spectrum of **5** showed in the carbonyl region only three signals, indicating the presence of only two short-chain acids. The basic hydrolysis of **5** provided scammonic acid A.[2] For **5**, 2-methylbutanoyl residues were attached at C-2 of rhamnose and at C-4 of quinovose′, and the jalapinolic acid was esterified at C-3 of rhamnose.

Compounds **1**−**5** from the more polar chromatographic fraction are macrocycles differing from the previously reported glycosides of the less polar chromatographic fraction of *I. stans* in the type of short-chain acids esterified to the sugar moieties, while compounds **4** and **5** do not contain a short-chain acid in the glucose unit. Noda et al.[5] and Hernández-Carlos et al.[6] reported the isolation of tetrasaccharide glycosides with the same macrolactone structure of compounds **1**−**5**, but their structures are different in the short-chain acid esterified at C-4 of the terminal quinovose.

Compounds **1**−**5** were subjected to a cytotoxic assay using cultured cells representative of colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1), and ovarian carcinoma (OVCAR-5). Compounds **1**, **2**, and **3** were inactive[7] against HCT-15 (ED_{50} > 24.0 μg/mL) and OVCAR (ED_{50} > 25.0 μg/mL). Compound **4** exhibited weak activity against OVCAR (ED_{50} 5.5 μg/mL) but was inactive toward HCT-15 and UISO-SQC-1. Compound **5** was active against OVCAR (ED_{50} 1.5 μg/mL) and UISO-SQC-1 (ED_{50} 4.0 μg/mL) cells, but inactive against HCT-15 (ED_{50} 24.0 μg/mL)

cells. The cytotoxicity of orizabins[6] was an order of magnitude lower than most of the compounds isolated from *I. stans*. The different cytotoxicity of tetrasaccharide glycosides from *Ipomoea* species requires a more detailed structure−activity investigation.

Experimental Section

General Experimental Procedures. All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a Bruker spectrophotometer model Vectra 22. The GC−MS system consisted of a gas chromatograph HP 5890 and a mass selective detector HP 5970. NMR spectra for **1**, **2**, and **5** were recorded on a Varian Unity 500 spectrometer equipped with a 5 mm inverse detection probe at 25 °C. COSY, TOCSY, HMQC, HMBC, and ROESY experiments were carried out using standard Varian software. Compounds **3** and **4** were analyzed on a Varian Unity INOVA 500 spectrometer equipped with a 5 mm inverse pulse field gradient probe at 25 °C. The pulse field gradient COSY, HSQC, HMBC, and ROESY experiments were carried out using standard Varian software. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS). Each oligosaccharide (10 mg) was dissolved in ca. 0.75 mL of acetone-d_6. MS were recorded on a JEOL JMS-SX102A spectrometer, using a *m*-nitrobenzyl alcohol matrix. 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 a Varian 9010 ternary pump, a Varian variable-wavelength UV−vis 9050 detector, and a Rheodyne injector.

Plant Material. Samples of *Ipomoea stans* were collected near km 93 on the Puebla-Orizaba highway, in the state of Puebla, Mexico. Botanical classification was carried out by Dr. M. Martínez, Instituto de Biología, UNAM, and a voucher specimen (No. 2691) is deposited at the IMSSM Herbarium in Mexico City.

Extraction and Isolation. Dried and ground roots were defatted with hexane at room temperature. The residual material was extracted exhaustively with ethyl acetate to give, after removal of the solvent, a resinous material. The ethyl acetate extract showed two spots by TLC on silica gel eluted with $CHCl_3$−CH_3OH (9:1) (R_f about 0.34 and 0.45). The resinous extract (50.0 g) was subjected to column chromatography over silica gel (500 g) in a gravity column using a gradient of CH_3OH in $CHCl_3$, separating two chromatographic fractions. Further separation of the more polar oligosaccharide fraction was carried out by HPLC using a MCH-10 column (4 mm i.d. × 250 mm, 5 μm, Varian) for preparative HPLC, with a mixture of CH_3CN−H_2O (6:4), a flow rate of 2 mL/min at 35 °C, and detection at λ 220 nm. Compounds **1** (t_R 12.5 min), **2** (t_R 13.5 min), **3** (t_R 16.1 min), **4** (t_R 18.8 min), and **5** (t_R 25.3 min) were collected by the technique of heart-cutting, and aliquots were reinjected until compounds had a purity better than 95%. The percentages of integrated areas (by normalization) were **1** (10%), **2** (10%), **3** (20%), **4** (40%), and **5** (20%).

Compound 1: amorphous white powder; mp 125−128 °C; $[\alpha]^{25}_D$ −32.0° (*c* 7.7 $CHCl_3$); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C═O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1147.5845 $[M + Na]^+$ (calcd for $C_{54}H_{92}O_{24}Na$, requires 1147.5876).

Compound 2: amorphous white powder; mp 127−130 °C; $[\alpha]^{25}_D$ −30.7° (*c* 1.0 $CHCl_3$); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C═O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1161.6024 $[M + Na]^+$ (calcd for $C_{55}H_{94}O_{24}Na$, requires 1161.6032).

Compound 3: amorphous white powder; mp 126−129 °C; $[\alpha]^{25}_D$ −29.7° (*c* 4.5 $CHCl_3$); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C═O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1161.6024 $[M + Na]^+$ (calcd for $C_{55}H_{94}O_{24}Na$, requires 1161.6032).

Compound 4: amorphous white powder; mp 130−133 °C; $[\alpha]^{25}_D$ −18.1° (*c* 7.5 $CHCl_3$); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C═O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1031.5360 $[M + Na]^+$ (calcd for $C_{49}H_{84}O_{21}Na$, requires 1031.5366).

Compound 5: amorphous white powder; mp 131−134 °C; $[\alpha]^{25}_D$ −16.9° (*c* 1.3 $CHCl_3$); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C═O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1045.5518 $[M + Na]^+$ (calcd for $C_{50}H_{86}O_{21}Na$, requires 1045.5522).

Acid Hydrolysis of the More Polar Chromatographic Fraction. The more polar chromatographic fraction (50 mg) was refluxed in 1.0 N HCl (10 mL of water−ethanol) for 1.5 h. The reaction mixture was taken to pH 5 with NaOH solution, and the solution extracted with $CHCl_3$ and analyzed by GC−MS (25 m × 0.2 mm HP-1 column; He, 1 mL/min; 40−250 °C, Δ 15 °C/min, 250 °C 10 min; split 1:50) giving the following acid derivatives: ethyl 2-methylpropionate t_R 4.9 min; *m/z* $[M]^+$ 116 (7), $[M - OC_2H_5]^+$ 71 (37), 43 (100), 29 (54); ethyl 2-methylbutyrate t_R 6.6 min; *m/z* $[M]^+$ 130 (0.5), $[M - C_2H_4]^+$ 102 (34), $[M - OC_2H_5]^+$ 85 (26), 57 (80), 29 (100); ethyl 3-methylbutyrate t_R 6.9 min; *m/z* $[M]^+$ 130 (0.5), $[M - C_2H_4]^+$ 102 (35), $[M - OC_2H_5]^+$ 85 (26), 57 (80), 29 (100); ethyl 3-hydroxy-2-methylbutyrate t_R 8.65 min; *m/z* $[M]^+$ 132 (5.0), $[M - OC_2H_5]^+$ 87 (20), 73 (100), 45 (40); and ethyl 11-hydroxyhexadecanoate t_R 19.57 min; *m/z* $[CH_3-(CH_2)_4-CH-(OH)-(CH_2)_9CO_2C_2H_5]^+$ 300 (1), $[CH_3-(CH_2)_4-CH-(OH)-(CH_2)_9CO]^+$ 255 (3.0), $[(CH_2)_9CO_2C_2H_5]^+$ 199 (50), $[CH_3-(CH_2)_4-CH-OH]^+$ 101 (60), 83 (45), 57 (100), 73 (20), 45 (40).

The aqueous phase of each reaction mixture 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 (25 m × 0.2 mm HP-1 column; He, 1 mL/min; 40−250 °C, Δ 15 °C/min, 250 °C 10 min; split 1:50) allowed the identification of the following TMS-sugars by coelution with authentic samples: tetra-TMS rhamnose, t_R 10.2 and 12.3 min; tetra-TMS quinovose, t_R 14.3 and 16.3 min; penta-TMS glucose, t_R 17.9 and 20.3 min.

Alkaline Hydrolysis of the More Polar Chromatographic Fraction. The more polar chromatographic fraction (250 mg) was refluxed in 0.1 N NaOH (10 mL) for 30 min. The reaction mixture was acidified to pH 5 and extracted with ether. 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 in methanol, and a white solid (glycosidic acid) was obtained after removal of the solvent. The characterization of the glycosidic acid by NMR allowed the identification as scammonic acid A:[2] mp 150−153 °C; $[\alpha]^{25}_D$ −50.2° (*c* 1.0 MeOH); positive FABMS *m/z* 895 $[M + Na]^+$.

Cytotoxicity Assay. The HCT-15, UISO-SQC-1, and OVCAR-5 cell lines were maintained in RPMI culture medium with 10% fetal bovine serum (FBS), and all cell lines cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at a log phase of their growth were treated in triplicate at various concentrations of the compounds (0.5−100 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentrations were determined by protein analysis. Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). The values were estimated from a semilog plot of the drug concentration (μg/ mL) against the percent of viable cells.

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