Hepta-, Hexa-, Penta-, Tetra-, and Trisaccharide Resin Glycosides from Three Species of *Ipomoea* and their Antiproliferative Activity on Two Glioma Cell Lines

Ismael León-Rivera,[a*] Federico del Río-Portilla,[b] Raúl G. Enríquez,[b] Edgar Rangel-López,[c] Juana Villeda,[d] María Yolanda Rios,[a] Gabriel Navarrete-Vázquez,[e] Israel Hurtado-Díaz,[a] Ulisés Guzmán-Valdivieso,[a] Verónica Núñez-Urquiza,[a] Carolina Martínez-Escobedo[f]

*Correspondence to: Ismael León-Rivera, Centro de Investigaciones Químicas, IICBA, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Estados Unidos Mexicanos.

Email: ismaelr@uaem.mx

[a] Centro de Investigaciones Químicas, IICBA, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Estados Unidos Mexicanos.

[b] Instituto de Química, Universidad Nacional Autónoma de México, Ciudad de México, Estados Unidos Mexicanos.

[c] Laboratorio de Amino Ácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía, Ciudad de México, Estados Unidos Mexicanos.

[d] Laboratorio de Patología, Instituto Nacional de Neurología y Neurocirugía, Ciudad de México, Estados Unidos Mexicanos.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mrc.4476

[e] Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Estados Unidos Mexicanos.

[f] Departamento de Farmacia, Universidad de Guanajuato, Guanajuato, Guanajuato, Estados Unidos Mexicanos.

ABSTRACT

Six new partially acylated resin glycosides were isolated from convolvulin of *Ipomoea purga, Ipomoea stans,* and *Ipomoea murucoides* (Convolvulaceae). The structures of compounds **1** - **6** were elucidated by a combination of NMR spectroscopy and mass spectrometry. The structure of jalapinoside B (**1**) consists of a hexasaccharide core bonded to an 11-hydroxytetradecanoic (convolvulinic) acid forming a macrolactone acylated by a 2-methylbutanoyl, a 3-hydroxy-2-methylbutanoyl, and a quamoclinic acid B units. Purgic acid D (**2**) contains a hexasaccharide core bonded to a convolvulinic acid acylated by a 3-hydroxy-2-methylbutanoyl unit. Stansin A (**4**) is an ester-type heterodimer, and consists of two stansoic acid A (**3**) units, acylated by 2-methylbutanoic and 3-hydroxy-2-methylbutanoic acids. The site of lactonization was located at C-3 of Rhamnose, and the position for the ester linkage of the monomeric unit B on the macrolactone unit A was established as C-4 of the terminal rhamnose. Compounds **5** and **6** are glycosidic acids. Murucinic acid II (**5**) is composed of a pentasaccharide core bonded to an 11-hydroxyhexadecanoic (jalapinolic) acid, acylated by an acetyl unit. Stansinic acid I (**6**) is a tetrasaccharide core bonded to a jalapinolic acid, acylated by 2-methylbutanoyl and 3-hydroxy-2-methylbutanoyl units. Preliminary testing showed the cytotoxicity of compounds **1** – **6** toward OVCAR and UISO-SQC-1 cancer cell lines. In addition, compound **1** showed an antiproliferative activity on glioma C6 and RG2 tumor cell lines.

Keywords: Resin glycoside, *Ipomoea stans*, *Ipomoea purga*, *Ipomoea murucoides*, cytotoxicity, glioma.

Introduction

The so-called resin glycosides have been isolated principally from the family *Convolvulaceae*. Resin glycosides were classified into an ether-soluble fraction (jalapin) and an ether-insoluble one (convolvulin). These resin glycosides consist of a mixture of glycolipids. The chemical structure of glycolipids contains a hydroxy-fatty acid bonded to an oligosaccharide core, partially acylated by short chain acids. In Mexico, the genus *Ipomoea* of the family *Convolvulaceae* includes a great number of ornamental, medicinal, and nutritional species.[1]

Ipomoea purga is a vine native to Mexico. In Mexico it is called "raíz de jalapa", and has been used in Mexican traditional medicine: as a mild cathartic since pre-Hispanic times, a decoction of the aerial parts is recommended as a galactogogue, and the aqueous extract of the root is used as a diuretic.[2] The phytochemical analysis of the aereal part has reported the isolation and characterization of pentasaccharides (purginosides I – IV) and ester-type dimer of operculinic acids A and B (purgins I – III).[3,4] A new macrocyclic bisdesmoside from the root of *I. purga* has shown an activity as modulator of multidrug resistance in human cancer cells.[5]

The root of *Ipomoea stans* Cav. (Convolvulaceae) is popularly known in Mexico as "raíz de tumbavaqueros" (cowboy stunner), probably due to the action on central nervous system (CNS). It has been used in Mexican traditional medicine: for treating epileptic seizures, as anticonvulsant, sedative, purgative, abortive, hypotensor, and for the treatment of renal disorders.[6] Organic-soluble extracts and pure compounds from the root of this plant have

showed effects on central nervous system.[7,8,9,10] From the root of this plant has been isolated glycolipids which contain an 11-hydroxy-hexadecanoic acid bonded to a tetrasaccharide core (partially acylated by short chain acids) forming a macrolactone ring.[11,12,13]

Ipomoea murucoides is one of the thirteen tree-like species in the genus *Ipomoea* series *Arborescentes*, native to Mexico, and is commonly called "cazahuate". Decoctions of flowers, leaves, and bark of *I. murucoides* are applied on skin to treat itching, rashes, inflammation, rheumatism, and other infections. In case of scorpion bite the decoction of this medicinal plant complex is drunk, and for alleviating tootache gargles are recommended.[14] Besides the cytotoxic activity of murucins,[15] some murucoidins from flowers of *I. murucoides* showed antimicrobial activity against SA-1199B a norfloxacin-resistant strain that over-expresses the NorA MDR efflux pump.[16] Murucoidins have also showed effects on the growth of *Spodoptera frugiperda*.[17] The glycolipids isolated from the root and flowers of *I. murucoides* consist of a pentasaccharide or tetrasaccharide unit bonded to an 11-hydroxyhexadecanoic acid forming a macrolactone.[15,16,17,18,19]

Resin glycosides have shown cytotoxic activities in several types of cancer cell lines.[5,16,20,21] The C6 and RG-2 cells are *in vitro* models for studying a form of malignant tumor called gliomas; whereas HCT-15, UISO-SQCX-1, and OVCAR-5 here studied are representative cell lines of several types of carcinomas. The present study details the isolation and structure elucidation of six new partially acylated glycolipids from *Ipomoea stans, Ipomoea purga*, and *Ipomoea murucoides.* The study also determines the cytotoxicity showed by compounds **1** – **6** towards some tumor and cancer cell lines.

Results and discussion

The dried roots of *Ipomoea purga* were extracted with methanol. The methanol-soluble extract was separated in fractions by column chromatography, leading to the isolation of

convolvulin. Convolvulin was chromatographed on normal and inverse phase silica gel column, yielding jalapinoside B (**1**).

Alkaline hydrolysis of compound **1** with NaOH produced a nonpolar fraction, which after being analyzed by GC-MS, showed the presence of 2-methylbutanoic and 3-hydroxy-2-methylbutanoic acids as the acylating residues. The polar fraction was fractionated by inverse and normal phase column chromatography, leading to the separation of two glycosidic acids **A** and **B**. Both acids were hydrolyzed in acid solution by separated, and their nonpolar fractions were analyzed by GC-MS, allowing the determination of ethyl (7S)-hydroxydecanoate (for **A**) and ethyl (11S)-hydroxytetradecanoate (for **B**), which were identified by comparison of optical rotation and spectroscopic (NMR and MS) data with published values.[22,23] The monosaccharide mixtures were analyzed by HPLC, allowing the identification of quinovose in **A**, and rhamnose, fucose, quinovose, and glucose for **B**, and. The chemical structure of **A** (quamoclinic acid B) and **B** (purgic acid A) was assigned with the above results, and 1D and 2D NMR spectra.

The MS analysis of compound **1** was conducted by HRFABMS and ESIMS in the positive ion detection mode. HRFABMS identified the adduct ion at *m/z* 1656.8342 $[M + Na]^+$ ($C_{76}H_{128}NaO_{37}$). LC-ESIMS/MS provided ions resulting from glycosidic cleavage and eliminations of the esterifying groups with observed ions at *m/z* = 1633 $[M + H]^+$, 1533 $[(M + H)^+ -$ hydroxymethylbutanoyl (100)$]^+$, 1387 [1633 − 146 (methylpentose) − 100]$^+$, 1316 [1633 − 146 − 171 (hydroxydecanoyl)]$^+$, 1225 [1387 − 162 (hexose unit)]$^+$.

Structure elucidation of jalapinoside B (**1**) was accomplished by 1D- and 2D-NMR experiments. In the low-field region of ^1H, ^{13}C, and HSQC NMR spectra, seven anomeric signals were confirmed at: δ_H 4.71, δ_C 102.7 (Quinovose-1); δ_H 5.79, δ_C 101.5 (Glucose-1); δ_H 5.60, δ_C 97.2 (Rhamnose-1); δ_H 6.29, δ_C 101.7 (Glucose′-1); δ_H 5.12, δ_C 107.1 (Quinovose′-1); δ_H 5.93, δ_C 102.7 (Fucose-1); and δ_H 4.79, δ_C 103.6 (Quinovose″- 1). Therefore, seven

separate spin systems for sugar skeletons were readily distinguished in the $^{1}H–^{1}H$ COSY and TOCSY spectra. 2D HSQC NMR experiments were used to assign the ^{13}C NMR signals (Table 1). With these experiments it was possible to identify: three quinovopyranosyl, one rhamnopyranosyl, one fucopyranosyl, and two glucopyranosyl units in compound **1.** The anomeric configuration in each sugar unit was deduced from a 2D $^{1}J_{CH}$ NMR experiment. For D-sugars in the $^{4}C_{1}$ conformation, the$^{1}J_{CH}$ values for quinovose (157 Hz), glucose (156 Hz), glucose (159 Hz), and fucose (159 Hz) supported their β-anomeric configurations. The α-anomeric configuration was deduced for the L-rhamnopyranosyl unit ($^{1}J_{CH}$ 171 Hz). The inter-glycosidic connectivities were established on the basis of long-range heteronuclear coupling correlations $^{3}J_{CH}$ by HMBC experiments, the following connectivities were observed between: H-1 (δ_H 4.71, Qui) and C-11 (δ_C 80.6, convolvulinic acid); H-1 (δ_H 4.79, Qui″) and C-7 (δ_C 78.7, 7-OH-decanoyl); H-1 (δ_H 5.79, Glu) and C-2 (δ_C 80.1, Qui); H-2 (δ_H 4.20, Glu) and C-1 (δ_C 97.2, Rha); H-1 (δ_H 6.29, Glu′) and C-3 (δ_C 74.3, Rha); H-1 (δ_H 5.12, Qui′) and C-2 (δ_C 86.6, Glu′); and H-4 (δ_H 4.57, Rha) and C-1 (δ_C 102.7, Fuc). The locations of the acyl residues were established by the following HMBC correlations: H-3 (δ_H 5.75, Glu) and the carbonyl of convolvulinic acid (δ_C 173.8); H-2 (δ_H 5.64, Rha) and carbonyl of a quamoclinic acid B residue (δ_C 174.1); H-4 (δ_H 5.35, Qui′) and C-1 of a 3-hydroxy-2-methylbutyryl moiety (δ_C 175.3); and H-4 (δ_H 5.65, Fuc) with the C-1 of a 2-methylbutyryl group (δ_C 176.9). Therefore, the structure of jalapinoside B was elucidated as (11S)-hydroxytetradecanoic acid 11-O-β-D-quinovopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→3)-O-[2-methylbutanoyl-(1→5)]β-D-fucopyranosyl-(1→4)],[(7S)-hydroxydecanoic acid 7-O-β-D-quinovopyranosyl -(1→2)]-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-O-[3-hydroxy-2-methylbutanoyl-(1→4)]β-D-quinovopyranosyl- 1,3″-lactone (**1**). A similar compound (jalapinoside) a bidesmoside having an oligosaccharide core linked at C-11 of the aglycone (ipurolic acid) was reported recently.[5] Consequently, jalapinoside B (**1**)

represents a new macrocyclic resin glycoside isolated from convolvulin of *I. purga*.

Saponification of compound **1** with $NaHCO_3$ afforded purgic acid D (**2**). The molecular formula of compound **2** ($C_{55}H_{96}O_{31}$) was determined by positive-ion HRFABMS of the pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1276.3218. In the negative-ion FABMS the following peaks were detected: the quasimolecular ion $[M - H]^-$ at *m*/*z* 1251; at *m*/*z* 1151 $[(M - H)^+ - 100]^-$; at *m*/*z* 1004 [1151 − deoxyhexose]$^-$; and the characteristics peaks of glycolipids at *m*/*z* = 842, 575, 413, and 243 (11−hydroxytetradecanoic acid). These data corroborated the presence of an 11-hydroxytetradecanoic acid moiety glycosidically linked to a hexasaccharide core consisting of four deoxyhexose, two hexoses, and one niloyl units.

The ^1H, ^{13}C, and HSQC NMR data of compound **2** showed in the low-field region of the spectrum, six anomeric signals at: δ_H 4.30, δ_C 105.6 (Quinovose-1); δ_H 4.93, δ_C 100.9 (Glucose-1); δ_H 5.33, δ_C 99.0 (Rhamnose-1); δ_H 5.21, δ_C 100.1 (Glucose′-1); δ_H 4.68, δ_C 103.6 (Quinovose′-1); and δ_H 4.94, δ_C 101.9 (Fucose-1). The ^1H and ^{13}C chemical shifts for the sugar units were assigned by the COSY, TOCSY, and HSQC NMR spectra. With these experiments it was possible to identify: two quinovopyranosyl, one rhamnopyranosyl, one fucopyranosyl, and two glucopyranosyl units. The β-anomeric configuration for quinovose, glucose, and fucose, and α-anomeric configuration for rhamnose was deduced from a 2D $^1J_{CH}$ NMR experiment. The inter-glycosidic connectivities were established on the basis of long-range heteronuclear coupling correlations $^3J_{CH}$ by HMBC experiment: H-1 Qui and C-11 convolvulinoyl; H-1 Glu and C-2 Qui; H-2 Glu and C-1 Rham; H-1 Glu′ and C-3 Rham; H-4 Rham and C-1 Fuc; and H-1 Qui′ and C-2 Glu′. The location of the acyl residue was established by the following HMBC correlation: H-4 (δ_H 4.39, Qui′-4) and C-1 (δ_C 175.3) of a 3-hydroxy-2-methylbutanoyl moiety. Compound **2** was identified as (11S)-hydroxytetradecanoic acid 11-O-β-D-quinovopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→3)-O-[β-D-fucopyranosyl-(1→4)]-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-

glucopyranosyl-(1→2)-O-[3-hydroxy-2-methylbutanoyl-(1→4)]β-D-quinovopyranoside´. Compound **2** represents a new hexasaccharide glycosidic acid derivative of purgic acid A.

The dried roots of *Ipomoea stans* were extracted with methanol. The methanol-soluble extract was separated by column chromatography leading to the isolation of convolvulin. Convolvulin was chromatographed on normal and inverse phase silica gel column, yielding compounds **4** and **6**. Compound **4** was hydrolyzed with KOH, producing a water-soluble glycosidic acid derivative and an organic solvent-soluble acidic fraction. GC-MS analysis of the organic solvent-soluble acidic fraction permitted the identification of 2-methylbutanoic and 3-hydroxy-2-methylbutanoic as the liberated short chain carboxylic acids by co-elution with authentic samples. A fraction of the glycosidic acid (**3**) was subjected to acid hydrolysis and the polar fraction was analyzed by HPLC for its sugar content, determinig the presence of D-glucose, D-quinovose, and L-rhamnose, and their naturally occurring form was confirmed by their optical rotation measurements. The nonpolar fraction was analyzed by GC-MS, allowing the determination of ethyl (11S)-hydroxyhexadecanoate, which was identified by comparison of optical rotation and MS data with published values.[24] The structure of this glycosidic acid was assigned as a trisasaccharide bonded to 11-hydroxyhexadecanoic acid, and was named stansoic acid A (**3**). Compound **3** is similar to tricoloric acid C.[25]

The molecular formula of stansin A (**4)** $C_{78}H_{136}O_{33}$ was determined by HRFABMS from the adduct ion $[M + Na]^+$ at m/z 1624.8831. For compound **4**, the negative-ion FABMS produced: the pseudomolecular ion $[M - H]^-$ at *m/z* 1599, the high-mass fragment ions at *m/z* 707 (unit B) and 827 (unit A) resulting from the ester–type heterodimer cleavage, and the cleavage of the glycosidic linkages of the sugar moieties at *m/z* 561 $[707 - C_6H_{10}O_4]^-$, 417 $[561 - C_6H_8O_4]^-$, and 271 $[417 - C_6H_{10}O_4]^-$.

The anomeric region of the ^1H, ^{13}C, and HSQC NMR spectra of compound **4** showed the presence of six sugar units. The HSQC experiment for compound **4** allowed the identification of

chemical shifts for the anomeric positions in unit A: at δ_H 4.58, δ_C 104.1 (Quinovose-1); δ_H 4.27, δ_C 101.4 (Glucose-1); δ_H 5.10, δ_C 97.2 (Rhamnose-1); and in unit B: at δ_H 4.46, δ_C 102.1 (Quinovose-1); δ_H 4.25, δ_C 101.4 (Glucose-1); δ_H 4.26, δ_C 101.5 (Rhamnose-1). Doublet signals observed in the region 1.1 - 1.4 ppm, corroborated the presence of methylpentoses in **4**. The nonanomeric signals of each monosaccharide unit were sequentially assigned (Table 1) by a combination of COSY and TOCSY experiments. The HSQC spectrum allowed the assignment of all carbon signals by the one bond ^{1}H-^{13}C correlations. These procedures allowed the identification of two glucopyranosyl, two rhamnopyranosyl, and two quinovoyranosyl units in **4**. The anomeric configurations for the sugar units were assigned as β for glucopyranosyl, β for quinovopyranosyl, and α for rhamnopyranosyl, from their $^{3}J_{H1\text{-}H2}$ and $^{1}J_{C1\text{-}H1}$ values. The interglycosidic connectivities in compound **4** were confirmed by the long-range heteronuclear coupling correlations ($^{3}J_{CH}$) observed in the HMBC NMR spectrum, for unit A: C-1 (δ_C 104.1) of Qui with H-11 (δ_H 3.62) of aglycon, C-1 (δ_C 101.4) of Glu with H-2 (δ_H 3.54) of Qui, C-1 (δ_C 97.2) of Rha with H-2 (δ_H 3.45) of Glu; and for unit B, C-1 (δ_C 102.1) of Qui with H-11 (δ_H 3.62) of aglycon, C-1 (δ_C 101.4) of Glu with H-2 (δ_H 3.52) of Qui, and C-1 (δ_C 101.5) of Rha with H-2 (δ_H 3.45) of Glu. The HMBC spectrum also permitted to locate the esterifying positions on each monomeric unit using the long-range heteronuclear correlations between the carbonyl carbon of an ester group with a nonanomeric proton: (a) the unit A macrolactonization site was identified at C-3 of Rha, by the correlation between the carbonyl carbon (δ_C 175.5, unit A) of jalapinolic acid with H-3 (δ_H 5.11) of Rha and the C-2 methylene protons (δ_H 2.26 and 2.41) of the aglycon in unit A, (b) the ester-type linkage of the trisaccharide core in macrocyclic unit A with the aglycone of acyclic unit B was identified by the correlation between the carbonyl carbon (δ_C 176.0) of acyclic aglycon (unit B) with H-4 (δ_H 4.23) of Rha unit A, (c) C-6 of Glu in unit B (δ_H 4.20, 4.40) was esterified by a 3-hydroxy-2-methylbutanoic acid (δ_C 177.5) group, (d) a 2-

methylbutanoic acid was esterified in unit B (δ_C 175.5) at C-2 (δ_H 4.64) of Rha. Stansin A (**4**) is the first glycolipid ester-type heterodimer isolated from the convolvulin of *Ipomoea stans*. The chemical structure of compound **4** is similar to those reported for glycolipid ester-type dimers from *Ipomoea tricolor*.[25]

The exudates from the bark of *Ipomoea murucoides* were extracted with MeOH. The methanol-soluble extract was fractionated by column chromatography. Purification of the less polar chromatographic fraction by HPLC yielded murucin 1. Basic hydrolysis of murucin 1 with $NaHCO_3$ yielded murucinic acid II (**5**).

The molecular formula of compound **5** ($C_{48}H_{84}O_{25}$) was determined by HRFABMS. The negative-ion FABMS of the new glycosidic acid showed the following peaks at: *m*/*z* 1059 the pseudomolecular ion $[M - H]^-$, *m*/*z* 913 [1059 - 146 (methylpentose unit)]$^-$, *m*/*z* 579 [871 - methylpentose - hexose]$^-$, *m*/*z* 417 [579 - hexose]$^-$, the further loss of a methylpentose unit gave a peak at *m*/*z* 271, corresponding to 11-hydroxyhexadecanoic acid (jalapinolic acid). These data corroborated that compound **5** was a jalapinolic acid moiety bonded through a glycosidic bond to a linear pentasaccharide core consisting of four deoxyhexose units and one hexose unit.

The ^1H and ^{13}C NMR spectra of compound **5** allowed the identification of five anomeric positions at: δ_H 4.32, δ_C 101.0 (Quinovose-1); δ_H 5.25, δ_C 103.0 (Rhamnose-1); δ_H 4.85, δ_C 102.1 (Rhamnose'-1); δ_H 5.15, δ_C 103.0 (Rhamnose''-1); δ_H 4.42, δ_C 105.5 (Glucose-1). COSY and TOCSY spectra permitted to assign the signals of the nonanomeric protons in each monosaccharide unit. Carbon signals of each saccharide unit were assigned by the ^1H - ^{13}C HSQC NMR experiment. With these experiments it was possible to identify: one quinovopyranosyl, one glucopyranosyl, and three rhamnopyranosyl units in compound **5**. The anomeric configurations for the sugar units were assigned as β for Glucose and Quinovose, and α for Rhamnose from their $^3J_{H1\text{-}H2}$ and $^1J_{C1\text{-}H1}$ values. The inter-glycosidic connectivities

were established on the basis of long-range heteronuclear coupling correlations ($^3J_{CH}$) in the HMBC NMR spectrum between: C-1 (δ_C 104.0) of Qui with H-11 (δ_H 3.56) of jalapinolic acid, C-1 (δ_C 103.0) of Rha with H-2 (δ_H 3.60) of Qui, C-1 (δ_C 102.1) of Rha' with H-4 (δ_H 3.58) of Rha, C-1 (δ_C 103.0) of Rha'' with H-4 (δ_H 3.95) of Rha', C-1 (δ_C 105.5) of Glu with H-2 (δ_H 3.17) of Rha''. The position of the esterification was established by the correlation between C=O (δ_C 173.8) of the acetyl group and H-4 (δ_H 4.56) of Rha'' in the HMBC NMR spectrum. Compound **5** represents a new glycosidic acid derivative.

Compound **6** was hydrolyzed with KOH, producing a water-soluble glycosidic acid derivative and an organic solvent-soluble acidic fraction. GC-MS analysis of the organic solvent-soluble acidic fraction permitted the identification of 2-methylbutanoic and 3-hydroxy-2-methylbutanoic as the liberated short chain carboxylic acids by co-elution with authentic samples. A fraction of the glycosidic acid was subjected to acid hydrolysis and the polar fraction was analyzed by HPLC for its sugar content, determinig the presence of D-glucose, D-quinovose, and L-rhamnose, and their naturally occurring form was confirmed by their optical rotation measurements. The nonpolar fraction was analyzed by GC-MS, allowing the determination of ethyl (11S)-hydroxyhexadecanoate, which was identified by comparison of optical rotation and MS data with published values.[24] The structure of this glycosidic acid was assigned as scammonic acid A (a tetrasaccharide moiety bonded to 11-hydroxyhexadecanoic acid), by comparisson of their physical and spectroscopic data.[26]

The molecular formula of stansinic acid I (**6**) ($C_{60}H_{78}O_{23}$) was determined by the HRFABMS ion at m/z 1190.2395 $[M + Na]^+$. The negative-ion FABMS of **6** showed the following peaks: the quasimolecular ion peak at *m*/*z* 1165 $[M - H]^-$, at *m*/*z* 1065 $[(M - H)^- - 100]^-$, at *m*/*z* 979 $[1067 - 84]^-$, and the characteristics peaks of glycolipids at *m*/*z* = 842, 579, 417, and 271 (11-hydroxytetradecanoic acid).

The ^{1}H, ^{13}C, and HSQC NMR spectra of compound **6**, confirmed the presence of four anomeric signals at δ_H 4.32, δ_C 101.0 (Quinovose-1); δ_H 4.42, δ_C 105.5 (Glucose-1), δ_H 5.25, δ_C 103.0 (Rhamnose-1); and δ_H 4.85, δ_C 102.1 (Quinovose'-1). The same spectra allowed the identification of one glucopyranosyl, one rhamnopyranosyl, and two quinovopyranosyl units in compound **6.** The anomeric configurations for the sugar moieties were assigned as β for glucose and quinovose, and α for rhamnose from their $^{3}J_{H1\text{-}H2}$ and $^{1}J_{C1\text{-}H1}$ values. The interglycosidic connectivities were determined by the long range HMBC correlations between: H-11 (δ_H 3.56) of 11-hydroxyhexadecanoyl moiety with C-1 (δ_C 103.2) of Qui, H-2 (δ_H 3.56) of Qui with C-1 (δ_C 101.9) of Glu, H-2 (δ_H 3.40) of Glu with C-1 (δ_C 101.2) of Rha, and H-4 (δ_H 3.59) of Rha with C-1 (δ_H 105.2) of Qui´. The HMBC NMR spectrum also permitted the determination of the position of esterification by the correlation between C=O (δ_C 178.0) of the 2-methylbutanoyl group and H-2 (δ_H 4.33) of Rhamnose, C-4 of Qui´ (δ_H 4.40) and C=O of a 3-hydroxy-2-methylbutanoic acid (δ_C 1767.5) group. The triplet signal at 2.27 ppm for the methylene protons at C-2 of the aglycon unit and the absence of correlations between ^{13}C=O of aglycon and protons for the sugar units in the HMBC spectrum, confirmed the acyclic molecular structure of stansinic acid I (**6**). Compound **6** has a chemical structure similar to those reported for tyrianthinic acids I, II, and VI.[24,26]

Compounds **1** - **6** were subjected to a cytotoxic assay using cultured cells as models representatives of colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1), and ovarian carcinoma (OVCAR-5). Compounds **1**, **2**, **3**, **5**, and **6** showed a discrete effect against the proliferative rate on HCT-15 (ED50 > 24.0 µg/mL) and OVCAR (ED50 > 25.0 µg/mL). Compound **4** exhibited a little more activity against the proliferation of OVCAR (ED_{50} 5.5 µg/mL) but it showed less cytotoxicity toward HCT-15 and UISO-SQC-1.

When compound **1** was assessed on two glioma cell lines, it was observed a significant reduction of viability in both cell lines. At 24 hours of interaction, compound **1** diminished

the cell viablitiy approximately in a 40% and 54% at 1 μg/mL in C6 and RG2 cell lines respectively. However, at 48 hours of interaction, even at low concentrations (0.1 μg/mL), we observed a deleterious effect on cell viability eliciting a 73% of death cell at 1 μg/ml in C6 cell line. Compound **1** also inhibited the cell proliferation in RG2 cell line approximately in a 78% but just when 1 μg/mL was assessed by 48 hours in RG2 cell line (Figure 1). These data strongly support the hypothesis that jalapinoside B (**1**) exerts notorious effects on cell viability in glioma cell lines, as it has been previously reported on several mammalian cancer cells, where authors suggest that these extracts could act as inhibitors of multidrug efflux pumps, particularly on glycoprotein-P, which is responsible for the MDR phenotype.[5]

Experimental

General

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 Vectra22. The 1D and 2D NMR spectra were recorded on a Varian Mercury 400 and Varian 700 spectrometer. Positive- and negative-ion FABMS were recorded using *m*-nitrobenzyl alcohol as matrix on a JEOL MStation JMS700 mass spectrometer. Mass spectra were acquired over the range 400 - 2300 Da. Agilent 1290 Infinity LC system coupled with an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system equipped with an Agilent Jet Stream dual electrospray ionization (ESI) source. HPLC purification was achieved on a system comprised of an Agilent 1100 chromatograph using a semipreparative C18 reverse phase column (Ultrasil-ODS column; 10 mm i.d. x 300 mm, 10 μm, Altex), eluting with a mixture of CH_3CN-H_2O (7:3), at a flow rate 1 mL/min at 25 ºC, and detection with UV at 215 nm. The GC-MS system consisted of an Agilent 6890 gas chromatograph and an Agilent 5970 mass selective detector in the electron-ionization

mode. GC conditions: 25 m x 0.2 mm HP-5 column (Hewlett Packard): He, 1 mL/min; 40 ºC isothermal for 2 min, linear gradient to 250 ºC at 15 ºC/min, final temperature hold for 10 min; split 1:20; MS conditions: ionization energy 70 eV; ion source temperature 280 ºC; interface temperature 300 ºC; mass range 30 - 600 amu. For column chromatography, Merck silica gel (100 - 200 mesh) was used. TLC was performed on precoated silica gel aluminum sheets (silica gel 60 F254, 0.20 mm, Merck). Fractions and pure compounds were monitored by UV (254 nm) and by a sulfuric acid solution followed by heating.

Nuclear magnetic resonance spectra

The 1D (^{1}H, ^{13}C) and 2D (COSY, TOCSY, HSQC, HSQC-TOCSY, and HMBC) NMR spectra were recorded on a Varian Mercury 400 and Varian 700 spectrometer equipped with a 5-mm inverse detection pulse field gradient probe at 25ºC. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS), with 20 mg of each glycolipid being dissolved in ca. 0.60 mL of deuterated solvents. TOCSY spectra were obtained with different mixing times to facilitate the detection of all the signals for each individual monosacharide unit. 2D NMR data were processed with forward linear prediction and zero filling.[25]

Plant Material

Roots of *Ipomoea stans* were collected in the state of Puebla (December 2004), Mexico. Botanical classification was carried out by M. Sc. Abigail Aguilar, Head of the Instituto Mexicano del Seguro Social Herbarium in Mexico City (IMSSM), and a voucher specimen (number 15077) is deposited at IMSSM.

Exudates from the bark of *Ipomoea murucoides* were collected manually in the campus of the Universidad Autónoma del Estado de Morelos (UAEM), in Cuernavaca, Morelos, México in December 2008. The plant material was identified by Biol. Alejandro Flores, and a voucher

specimen (No. 22444) was deposited at the Herbarium of the Centro de Investigación en Biodiversidad y Conservación, UAEM.

Roots of *Ipomoea purga* were authenticated and donated by M. Sc. Abigail Aguilar in 2012, Head of the Instituto Mexicano del Seguro Social Herbarium in Mexico City (IMSSM). A voucher specimen (number 16180) is deposited at IMSSM.

Extraction and isolation of jalapinoside B (1), murucoidin XIX, stansin A (4), and stansinic acid I (6)

The dried, powdered roots of *Ipomoea purga* and *Ipomoea stans* (250.0 g each one) were extracted by maceration with CH_3OH (500 mL x 3), to obtain a dark syrup (25.0 g *I. purge*, and 20.4 g *I. stans*). The dark syrups were extracted with distilled water (3 × 50 mL) and CH_2Cl_2 (3 × 50 mL), to afford a dark solid (9.3 g *I. purga*, and 7.6 g *I. stans*). The dark solids (1.0 g *I. purga*, 1.1 g *I. stans*) were submitted to a C18 column (Supelco, 10 × 15 mm) with a gradient of CH_3OH − H_2O (0:100 to 100:0, at increments of 10%/mL), fractions were collected and pooled. After elimination of the solvent, a brown resinous material (0.7 g *I. purga*, 0.6 g *I. stans*) was obtained. The resinous solids were percolated on an activated charcoal column, eluting with CH_3OH. Fractions of 5 mL were collected and reunited giving the convolvulin (0.42 g *I. purga*, and 0.32 g *I. stans*). Convolvulin of *I. purga* was chromatographed on normal and inverse phase silica gel column, using as mobile phase $CH_2Cl_2/CH_3OH/H_2O$ (84:14:2) respectively with CH_3OH gradient, yielding 80 mg jalapinoside B (**1**). And, from convolvulin of *I. stans* in the same conditions 30 mg of stansin A (**4**) and 27 mg of stansinic acid I (**6**) were obtained.

Exudates from the bark of *Ipomoea murucoides* (15 g) were dried, ground, and disolved in MeOH to give, after filtration and removal of the solvent, a brown solid material (10 g). The brown solid was dissolved in a mixture of $CHCl_3$-CH_3OH (9:1). This solution was

subsequently subjected to passage over a silica gel column eluted with a gradient system of $CHCl_3$ - CH_3OH (from 9:1 to 7:3), leading to the separations of two chromatographic fractions. Purification of the less polar chromatographic fraction was carried out by preparative HPLC. Eluates with t_R value of 23.5 min were collected and reinjected into the HPLC system to achieve pure murucoidin XIX.

Preparation of compounds 2 and 5

Individual solutions of resin glycosides (jalapinoside B (**1**), murucoidin XIX, 100 mg each one) in 1% $NaHCO_3$ (2.5 mL H_2O + 2.5 mL MeOH) were heated until reflux for 30 min. Then the solution was allowed to reach room temperature, the reaction mixture was acidified to pH 4 and then extracted with CH_2Cl_2 (5 mL). The aqueous phase from each reaction was lyophilized, and the residue was extracted with n-butanol (5 mL), and was concentrated giving a resinous solid (40 mg). Each residue was further analyzed by HPLC and this procedure showed that compounds **2** and **5** were pure.

Acid Hydrolysis of Resin Glycosides

Individual solutions (50 mg each one) of compounds **1** - **6** in 2.0 N HCl (5 mL of water - ethanol) were heated until reflux for 2.0 h. The reaction mixtures were taken to pH 4 with NaOH solution, and the solutions were extracted with CH_2Cl_2 (2 x 5 mL). The organic solutions were washed with water, dried over Na_2SO_4, and analyzed by GC-MS allowing the identification of the following acid derivatives: ethyl acetate (t_R = 4.2 min), *m*/*z* $[M]^+$ 88 (20.5), 73 (34), 43 (80), 29 (100); ethyl 2-methylbutanoate (t_R = 6.6 min), *m*/*z* $[M]^+$ 130 (0.5), 102 (34), 85 (26), 57 (80), 29 (100); ethyl 3-hydroxy-2-methylbutanoate (t_R = 8.65 min), *m*/*z* $[M]^+$ 146 (5.0), 87 (20), 73 (100), 45 (40); ethyl 7-hydroxydecanoate (t_R = 15.57 min), *m*/*z* $[M]^+$ 218 (4), 189 (5.0), 145 (42), 73 (47), 83 (45), 57 (100), 73 (20), 45 (40); ethyl 11-

hydroxytetradecanoate (t_R = 18.21 min), *m*/*z* $[M]^+$ 272 (3), 227 (3.0), 199 (50), 73 (50), 83 (45), 57 (100), 73 (20), 45 (40); and ethyl 11-hydroxyhexadecanoate (t_R = 19.57 min), *m*/*z* $[M]^+$ 300 (1), 255 (3.0), 199 (50), 101 (60), 83 (45), 57 (100), 73 (20), 45 (40).

Another aliquot of each dichloromethane solution for compounds **1 - 6** were submitted to normal phase column chromatography, using gradients of CH_3OH in CH_2Cl_2 (0:100 to 95:5) to give 4 mg of ethyl 11-hydroxyhexadecanoate. The observed optical rotation $[\alpha]^{25}_D$ +0.42°) was similar to that reported for the *S* enantiomer of the ethyl ester of jalapinolic acid $[\alpha]^{25}_D$ +0.45°).[24] A solution of compounds **1** and **2** following the procedure described above afforded 3 mg of ethyl 11-hydroxytetradecanoate, which showed a optical rotation $[\alpha]^{25}_D$ +6.9°) value similar to that reported for the *S* enantiomer of the ethyl ester of convolvulinic acid $[\alpha]^{25}_D$ +7.3°).[23]

The aqueous phase from compounds **1 - 6** were neutralized with Na_2CO_3 solution and lyophilized to give a colorless powder. The residue was dissolved in CH_3OH and analyzed by HPLC: Alltech Nucleosil 100 NH_2 column (5 μm, 250 x 4.6 mm), using an isochratic elution of CH_3CN-H_2O (8:2), at a flow rate of 1 mL/min, and a sample injection of 200 μL (sample concentration 10 mg/mL), affording four compounds which were identified by coelution with standard L-rhamnose (t_R = 7.9 min), D-fucose (t_R = 8.2 min), D-quinovose (t_R = 8.9 min), and D-glucose (t_R = 15.2 min).

Cytotoxicity Assays

Glioma C6 cells (ATTC® CCL-107™) and glioblastoma RG2 cells (ATTC® CRL-2433™) derived from rats, were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and used in this study. These tumor cells were seeded in DMEM media supplemented with 10% of fetal bovine serum (FBS) and 1% of an antibiotic solution. Cells were maintained at 37 °C in an atmosphere enriched with 5% of CO_2. Cells from

confluent bottles were seeded at a density of 1 X 10^4 cells/cm^2 in 96-well plates. After 24 hours, the culture media was substituted by DMEM + SFB medium and cells were exposed to several concentrations (from 0.001 to 1 μg of intact glycosidic acid extract to determine their concentration-effect curves on cell viability. Each experiment was carried out in triplicate, considering at least three repeats per experiment.

Cell viability assay

Cell viability was estimated in tumor cell lines at 24 and 48 hours of exposure to the glycosidic acid extract, using the exclusion of crystal violet (CV) as the marker to detect viable cells since dead cells are detached and washed out. After exposure to compound **1**, cells were washed and fixed with 100 μl per well of 1% paraformaldehyde (Sigma) for 15 min. Then, cells were whased with PBS and stained with 0.1% CV (Sigma) for 30 min, washed twice with PBS and finally, CV was extracted from cells with 150 μl of 10% acetic acid. The amount of eluted CV was determined using an imaging reader at 570/595 nm (Cytation 3, Biotek, United States). Wells without cells were considered as blanks.

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 were 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 (from 0.5 to 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 (ED50). The values were estimated from a semilog plot of the drug concentration (μg/ mL) against the percent of viable cells.

Compounds

Jalapinoside B (**1**)

Amorphous powder; mp 156 - 158 ºC; $[\alpha]^{25}_{D}$ -22.0 (*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 Table 1; positive-ion FABMS *m/z* 1655 $[M + Na]^+$; positive LC-ESIMS *m/z* 1633 $[M + H]^+$, 1533, 1387, 1315, and 1225; HRFABMS *m/z* 1656.8342 $[M + Na]^+$ (calcd for $C_{76}H_{128}NaO_{37}$, requires 1656.8351).

Purgic acid D (**2**)

Amorphous powder; mp 156 - 158 ºC; $[\alpha]^{25}_{D}$ -18.0 (*c* 1.9 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 Table 2; positive-ion FABMS *m/z* 1275 $[M + Na]^+$; negative-ion FABMS *m/z* 1251 $[M - H]^-$, 1151, 1004, 842, 533, 389, and 243; HRFABMS *m/z* 1276.3209 $[M + Na]^+$ (calcd for $C_{55}H_{96}NaO_{31}$, requires 1260.3218).

Stansoic acid I (**3**)

Amorphous powder; mp 162 - 165 ºC; $[\alpha]^{25}_{D}$ -15.8 (*c* 1.4 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 Table 4; positive-ion FABMS *m/z* 749 $[M + Na]^+$; negative-ion FABMS *m/z* 725 $[M - H]^-$, 579, 417, and 271; HRFABMS *m/z* 749.8373 $[M + Na]^+$ (calcd for $C_{34}H_{62}NaO_{16}$, requires 749.8361).

Stansin A (4)

Amorphous powder; mp 144 - 146 ºC; $[\alpha]^{25}_{D}$ –13.9 (*c* 1.4 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 Table 3; positive-ion FABMS *m/z* 1623 $[M + Na]^+$; negative-ion FABMS *m/z* 1599 $[M – H]^-$, 827,

707, 561, 417, and 271; HRFABMS *m/z* 1624.8831 $[M + H]^+$ (calcd for $C_{95}H_{161}O_{40}$, requires 1624.8840).

Murucinic acid II (**5**)

Amorphous powder; mp 146 - 148 ºC; $[\alpha]^{25}_D$ -15.1 (*c* 1.1 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C-H), 1740 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Table 4 and 5; positive-ion FABMS *m/z* 1083 $[M + Na]^+$; negative-ion FABMS *m/z* 1059 $[M - H]^-$, HRFABMS *m/z* 1084.1670 $[M + Na]^+$ (calcd for $C_{48}H_{84}NaO_{25}$ requires 1084.1655).

Stansinic acid I (**6**)

Amorphous powder; mp 150 - 152 ºC; $[\alpha]^{25}_D$ -15.8 (*c* 1.4 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 Table 4 and 5; positive-ion FABMS *m/z* 1189 $[M + Na]^+$; negative-ion FABMS *m/z* 1165 $[M - H]^-$, 871, 579, 417, and 271; HRFABMS *m/z* 1190.2384 $[M + Na]^+$ (calcd for $C_{60}H_{78}NaO_{23}$, requires 1190.2395).

Acknowledgments

This research was supported by CONACyT (CB2011-168569, and LANEM 232693). I. Hurtado-Díaz and V. Núñez-Urquiza are grateful to CONACyT for graduate student scholarships (18304 and 19849). We thank V. Labastida (CIQ, UAEM) for recording the FABMS, and M. Medina (CIQ, UAEM) for recording the GC-MS and IR spectra. We also thank V. M. Mondragón (Agilent Technologies) for recording HPLC-ESIMS spectra.

References

[1] C. Mannich, P. Schumann. *Arch. Pharm. Ber. Dtsch. Pharm*. **1938,** *276*, 211 - 213.

[2] R. Pereda-Miranda, M. Bah. *Current Topics in Med. Chemistry*. **2003**, *3*, 111 - 131.

[3] J. Castañeda-Gómez, R. Pereda-Miranda. *J. Nat. Prod*. **2011**, *74*, 1148 - 1153.

[4] J. Castañeda-Gómez, G. Figueroa-González, N. Jacobo, R. Pereda-Miranda. *J. Nat. Prod.* **2013**, *76*, 64 - 71.

[5] E. Bautista, M. Fragoso-Serrano, R. Pereda-Miranda. *J. Nat. Prod*. **2015**, *78*, 168 - 172.

[6] J. L. Díaz. (1976) Usos de las Plantas Medicinales de México. Monografías Científicas II. IMEPLAM, México, pp 329.

[7] C. Contreras, L. Chacón, R. G. Enríquez. *Phytomedicine*. **1996**, *3*, 41 – 44.

[8] A. Navarro-Ruiz, G. P. de la Mora, M. T. Villanueva, J. R. Domínguez-Rodríguez. *Phytotherapy Res.* **1996**, *10*, 242 - 44.

[9] M. Herrera-Ruiz, C. Gutiérrez, J. E. Jiménez-Ferrer, J. Tortoriello, G. Mirón, I. León. *J. Ethnopharmacol*. **2007**, 112, 243 - 47.

[10] R. G. Enríquez, I. León, F. Pérez, F. Walls, K. A. Carpenter, F. V. Puzzuolli, W. F. Reynolds. *Can. J. Chem*. **1992**, *70*, 1000 - 1008.

[11] W. F. Reynolds, M. Yu, R. G. Enriquez, H. Gonzalez, I. Leon, G. Magos, M. L. Villarreal. *J. Nat. Prod*. **1995**, *58*, 1730 - 34.

[12] I. León, R. G. Enríquez, D. Gnneco D, M. L. Villarreal, D. Alonso, W. F. Reynolds, M. Yu. *J. Nat. Prod*. **2004**, *65*, 1552 - 56.

[13] I. León-Rivera, J. Villeda-Hernández, V. Campos-Peña, A. Aguirre-Moreno, S. Estrada-Soto, G. Navarrete-Vázquez, M. Y. Rios, B. Aguilar-Guadarrama, P. Castillo-España, J. C. Rivera-Leyva. *Bioorg. Med. Chem. Letters*. **2014**, *24*, 3541 - 45.

[14] A. Argueta, L. Cano, M. Rodarte. (1994) Atlas de las Plantas de la Medicina Tradicional Mexicana. Tomo II. Instituto Nacional Indigenista, México, pp 233.

[15] I. León, R. Enríquez, D. Nieto, W. F. Reynolds, E. Aranda, D. A. Cortés, J. Villa. *J. Nat. Prod*. **2005**, *68*, 1141 - 1146.

[16] L. Chérigo, R. Pereda-Miranda, S. Gibbons. *Phytochemistry*. **2009**, *70*, 222 -227.

[17] I. León Rivera, L. G. Vera Curzio, F. del Río Portilla, E. Aranda, V. M. Hernández Velázquez, P. Guevara Fefer, E. Montiel, P. Castillo, D. O. Salinas Sánchez. *J. Entomol*. **2013**, *10*, 24 - 34.

[18] L. Chérigo, R. Pereda-Miranda. *J. Nat Prod*. **2006**, *69*, 595 - 599.

[19] L. Chérigo, R. Pereda-Miranda, M. Fragoso-Serrano, N. Jacobo-Herrera, G. W. Kaatz, S. Gibbons. *J. Nat. Prod*. **2008**, *71*, 1037 - 1045.

[20] G. Mirón-López, M. Herrera-Ruiz, S. Estrada-Soto, F. Aguirre-Crespo, G. Vázquez-Navarrete, I. León-Rivera. *J. Nat. Prod*. **2007**, *70*, 557 - 562.

[21] I. León-Rivera, M. Herrera-Ruiz, S. Estrada-Soto, M. C. Gutiérrez, I. Martínez-Duncker, G. Navarrete-Vázquez, M. Y. Rios, B. Aguilar, P. Castillo-España, A. Aguirre-Moreno. *J. Ethnopharm*. **2011**, *135*, 434 - 439.

[22] M. Ono, Y. Takagi-Taki, F. Honda-Yamada, N. Noda, K. Miyahara. *Chem. Pharm. Bull*. **2010**, *58*, 666 - 672.

[23] R. Pereda-Miranda, M. Fragoso-Serrano, E. Escalante-Sánchez, B. Hernández-Carlos, E. Linares, R. Bye. *J. Nat. Prod*. **2006**, *69*, 1460 - 1466.

[24] I. León-Rivera, G. Mirón-López, G. M. Molina-Salas, M. Herrera-Ruiz, S. Estrada-Soto, M. C. Gutiérrez, D. Alonso-Cortes, G. Navarrete-Vázquez, M. Y. Rios, S. Said-Fernández. *J. Nat. Prod*. **2008**, *71*, 1686 - 1691.

[25] M. Bah, R. Pereda-Miranda. *Tetrahedron*. **1997**, *53*, 9007 - 9022.

[26] I. León-Rivera, J. M. Castro, G. Mirón-López, F. del Río-Portilla, R. Enríquez, W. F. Reynolds, S. Estrada-Soto, P. Rendón-Vallejo, M. C. Gutiérrez, M. Herrera-Ruíz, A. Mendoza, G. Vargas. *J. Nat. Med*. **2014**, *68*, 655 - 6567.

Table 1. NMR data of compound **1** in pyridine-d_5,^{1}H (400 MHz), ^{13}C (100 MHz), δ in ppm, *J* in Hertz.

position[a]	δ_H	δ_C	position	δ_H	δ_C
Qui-1	4.71 d (7.5)	102.7	Qui´-1	5.12 d (7.6)	107.1
2	4.19*	80.1	2	4.07 dd (9.0, 7.5)	77.5
3	4.58*	78.9	3	5.35 dd (9.5, 9.5)	77.2
4	3.49 dd (8.5, 8.5)	77.3	4	4.22*	75.4
5	3.77 dd (9.0, 6.0)	72.9	5	4.07 dd (9.0, 7.5)	77.5
6	1.54 d (5.5)	18.7	6	1.73 d (6.0)	18.8
Glu-1	5.79 d (7.5)	101.5	Fuc-1	5.93 d (7.5)	102.7
2	4.20*	75.8	2	4.26*	73.9
3	5.75 dd (9.5, 9.0)	79.8	3	4.36*	73.0
4	4.15 dd (9.0, 8.5)	70.5	4	5.65*	74.5
5	3.72 dd (9.0, 9.0)	77.7	5	4.40*	73.0
6a	4.40*	62.6	6	1.44 d (6.0)	17.1
6b	4.26*		Quamoclinic acid B		
Rha-1	5.60 d (1.6)	97.2	Qui´´-1	4.79 d (7.5)	103.6
2	5.64*	74.5	2	3.97 dd (9.0, 8.0)	75.9
3	5.30 dd (9.0, 3.0)	74.3	3	4.15 dd (9.0, 8.5)	78.6
4	4.57 dd (9.0, 9.0)	79.9	4	3.72 dd (9.0, 9.0)	77.3
5	5.07*	68.0	5	3.75*	73.1
6	1.93 d (6.0)	19.5	6	1.63 d (6.0)	19.4
Glu´-1	6.29 d (8.4)	101.7	7-OH Dec-1		174.1
2	3.99 dd (8.4, 8.4)	86.6	2	2.37*, 2.27*	44.2
3	4.49*	77.3	7	3.90*	78.7
4	3.92*	72.7	10	1.10 t (6.2)	14.1
5	4.18*	78.3			
6a	4.47 dd (11.5, 9.0)	63.1			
6b	4.07 dd (9.0, 7.5)				
Nil-1		175.3	Conv-1		173.8
2	2.90 q (7.0)	49.4	2a	2.60*	35.6
2-Me	1.36 d (7.0)	13.7	2b	2.69*	
3	4.36*	69.9	11	3.70*	80.6
4	1.43	21.5	14	1.06 t (6.0)	14.6
Mba-1		176.9			
2	2.74-2.61*	41.8			
2-Me	1.33 d (7.0)	17.3			
3	1.9,* 1.66*	27.3			
4	1.06 t (7.5)	12.2			

[a]*Qui*-quinovopranosyl, *Glu*-glucopyranosyl, *Rha*-rhamnopyranosyl, *Fuc*-fucopyranosyl, Niloyl-3-hydroxy-2-methylbutanoyl, *Conv*-11-hydroxytetradecanoyl, *7-OHDec*-7-hydroxydecanoyl, *mba*-2-methylbutanoyl. Chemical shifts marked with asterisk (*) indicate overlapped signals.

Table 2. NMR data of compound **2** in CD_3OD,[1]H (400 MHz), [13]C (100 MHz), δ in ppm, *J* in Hertz.

position[a]	δ_H	δ_C	position	δ_H	δ_C
Qui-1	4.30 d (7.6)	105.6	Qui´-1	4.68 d (7.6)	103.6
2	3.50 dd (9.0, 7.6)	78.8	2	3.32 *	74.7
3	3.79 dd (9.0, 9.0)	77.3	3	3.33 dd (9.0, 9.0)	76.1
4	3.28 dd (9.0, 7.0)	75.6	4	3.00 dd (9.1, 9.0)	75.7
5	3.77 dd (9.0, 6.0)	71.6	5	4.39 dd (9.1, 6.4)	71.7
6	1.28 d (7.0)	16.8	6	1.32 d (6.4)	17.3
Glu-1	4.93 d (7.5)	100.9	Fuc-1	4.94 d (7.5)	101.9
2	3.41 dd (9.1, 7.7)	75.9	2	4.26*	76.1
3	3.50 dd (9.0, 9.1)	78.6	3	4.36*	73.5
4	3.17 dd (9.0, 9.0)	71.1	4	5.65*	76.1
5	3.23 dd (9.0, 6.5)	76.6	5	4.40*	71.9
6a	3.51 dd (9.0, 6.5)	62.3	6	1.44 d (6.0)	15.9
6b	3.85 dd (9.0, 3.3)		Conv-1		175.3
Rha-1	5.33 d (1.4)	99.0	2	2.23 t (7.0)	35.3
2	4.10 dd (9.0, 1.4)	70.5	11	3.58*	81.8
3	4.26 dd (9.3, 9.0)	77.7	14	0.93 t (7.8)	13.1
4	3.89 dd (9.0, 9.3)	78.0	Nil-1		180.7
5	4.39 dd (9.0, 6.5)	66.8	2	2.40 m	47.1
6	1.27 d (6.5)	16.9	3	3.82*	69.4
Glu´-1	5.21 d (7.8)	100.1	4	0.98 t (6.3)	174.1
2	3.45 dd (9.0, 7.8)	83.2			
3	3.70 dd (9.1, 9.0)	70.0			
4	3.40 dd (9.0, 9.1)	70.3			
5	4.40 dd (9.0, 6.7)	66.8			
6a	3.66 dd (9.0, 3.4)	61.2			
6b	3.89 dd (9.0, 6.7)				

[a]*Qui*-quinovopranosyl, *Glu*-glucopyranosyl, *Rha*-rhamnopyranosyl, *Fuc*-fucopyranosyl, Niloyl-3-hydroxy-2-methylbutanoyl, *Conv*-11-hydroxytetradecanoyl. Chemical shifts marked with asterisk (*) indicate overlapped signals.

Table 3.- NMR data of compound **4** in CD_3OD, ^1H (400 MHz), ^{13}C (100 MHz), δ in ppm, *J* in Hertz.

.

	4			
	Unit A		**Unit B**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
Qui				
1	4.58 d (7.6)	104.1	4.46 d (7.6)	102.1
2	3.54 dd (9.0, 7.6)	79.4	3.52 dd (9.0, 7.6)	79.4
3	3.42 dd (9.0, 9.0)	78.3	3.40 dd (9.0, 9.0)	78.3
4	3.05 *	76.9	3.06 *	76.9
5	3.24 dd (9.0, 7.0)	72.1	3.24 dd (9.0, 7.0)	72.1
6	1.16 d (7.0)	18.1	1.16 d (7.0)	18.1
Glu				
1	4.27 d (7.7)	101.4	4.25 d (7.7)	101.4
2	3.45 dd (9.1, 7.7)	78.8	3.45 dd (9.1, 7.7)	78.8
3	3.60 *	76.5	3.60 *	76.5
4	3.35 dd (9.0, 9.0)	72.2	3.35 dd (9.0, 9.0)	72.2
5	3.50 dd (9.0, 6.5)	70.6	3.50 dd (9.0, 6.5)	70.6
6	3.75 dd (9.3, 9.0)	63.1	4.20 dd (9.3, 9.0)	63.1
6b	3.59 *		4.40 *	
Rha				
1	5.10 d (1.4)	97.2	4.26 d (1.4)	101.5
2	4.84 b	71.4	4.64 *	71.4
3	5.11 dd (9.3, 3.2)	72.1	4.89 dd (9.3, 3.2)	72.1
4	4.23 dd (9.0, 9.3)	78.4	3.75 dd (9.0, 9.3)	78.4
5	3.75 dd (6.5, 9.0)	68.6	4.23 dd (6.5, 9.0)	68.6
6	1.21 d (6.5)	18.3	1.21 d (6.5)	18.3
Jal				
1		175.8		176.0
2	2.26 ddd (14, 7, 2)	34.6	2.35 t (7.2)	34.6
2a	2.41 ddd (14, 8, 2)			
11	3.62 *	79.6	3.62 *	79.5
16	0.88 t (7.0)	14.4	0.88 t (6.9)	14.4
Mba				
1				175.5
2			2.46 q (7.0)	41.6
2-Me			1.17 d (7.2)	16.9
3			1.70 m	19.4
3a			1.55 m	
3-Me			0.99 dd (7.2, 8.3)	11.8
Nil				
1				177.5
2			2.41 d (10.0)	14.3
3			3.85 m	77.9
3-Me			1.02 d (7.2)	12.2

Qui = quinovopyranosyl, *Glu* = glucopyranosyl, *Rha* = rhamnopyranosyl, *Jal* = 11-hydroxyhexadecanoyl, N*il* = 3-hydroxy-2-methylbutanoyl, *Mba* = 2-methylbutanoyl. Chemical shifts marked with asterisk (*) indicate overlapped signals.

Table 4.- NMR spectral data of oligosaccharide core in compounds **3**, **5,** and **6** in CD_3OD, ^1H (400 MHz), ^{13}C (100 MHz), δ in ppm, *J* in Hertz.

	3		**5**		**6**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
Qui						
1	4.32 d (7.6)	101.0	4.32 d (7.6)	101.0	4.30 d (7.6)	103.2
2	3.60 *	79.4	3.60 dd (9.0, 7.6)	79.4	3.56 dd (9.0, 7.6)	79.0
3	3.30 *	78.3	3.30 dd (9.0, 9.0)	78.3	3.27 dd (9.0, 9.0)	78.5
4	3.24 *	77.6	3.24 dd (9.0, 9.0)	77.7	2.99 dd (9.0, 9.0)	77.4
5	3.33 *	72.3	3.33 dd (9.0, 7.7)	72.3	3.99 dd (9.0, 7.7)	72.6
6	1.27 d (7.0)	18.4	1.27 d (7.0)	18.4	1.17 d (7.0)	16.8
Glu						
1	4.95 db (7.7)	101.9	4.42 d (7.7)	105.5	4.42 d (7.7)	105.5
2	3.40 dd (9.1, 7.7)	77.7	3.45 dd (9.1, 7.7)	78.0	3.45 dd (9.1, 7.7)	78.0
3	3.48 dd (9.0, 9.1)	79.1	3.65 *	79.4	3.65 *	79.4
4	3.10 dd (9.0, 9.0)	72.4	3.10 dd (9.0, 9.0)	72.7	3.10 dd (9.0, 9.0)	72.7
5	3.27 dd (9.0, 6.5)	77.9	3.25 dd (9.0, 6.5)	77.3	3.25 dd (9.0, 6.5)	77.3
6	3.51 dd (9.0, 6.5)	62.6	3.63 *	63.7	3.60 *	63.7
6a	3.85 dd (9.0, 3.3)		3.90 dd (10.0, 3.5)		3.90 dd (10.0, 3.5)	
Rha						
1	4.28 d (1.4)	101.2	5.25 d (1.4)	97.0	4.25 d (1.4)	103.0
2	3.83 d	72.1	3.95 dd (9.3, 1.3)	72.0	3.95 dd (9.3, 1.3)	72.0
3	3.93 dd (9.3, 3.2)	71.9	4.06 dd (9.1, 9.3)	72.2	4.06 dd (9.1, 9.3)	72.2
4	3.59 dd (9.0, 9.3)	83.1	3.58 *	83.8	3.58 *	83.8
5	4.03 dd (9.0, 6.5)	68.2	3.40 dd (9.0, 6.5)	68.2	3.40 dd (9.0, 6.5)	68.2
6	1.28 d (6.5)	16.7	1.30 d (6.5)	18.3	1.30 d (6.5)	18.3
Qui’						
1					4.54 d (7.6)	102.1
2					3.05 dd (9.0, 7.6)	79.2
3					3.36 dd (9.0, 9.0)	71.7
4					3.99 dd (9.1, 9.0)	74.4
5					3.25 dd (9.0, 6.9)	70.2
6					1.32 d (6.9)	17.3
Rha’						
1			4.85 d (1.2)	102.1		
2			3.19 dd (9.0, 1.2)	79.1		
3			3.34 dd (9.0, 9.0)	73.4		
4			3.95 dd (9.0, 9.0)	78.6		
5			3.20 dd (9.0, 6.9)	70.0		
6			1.32 d (6.9)	17.3		
Rha’’						
1			5.15 d (1.3)	103.0		
2			3.17 dd (9.0, 1.3)	76.7		
3			3.31 dd (9.0, 9.0)	75.2		
4			4.56 dd (9.0, 9.0)	78.6		
5			3.20 dd (9.0, 6.9)	70.0		
6			1.34 d (6.9)	19.0		

Qui-quinovopyranosyl, *Glu*-glucopyranosyl, *Rha*-rhamnopyranosyl. Chemical shifts marked with asterisk (*) indicate overlapped signals.

Table 5.- NMR spectral data of carboxylic acids in compounds **3, 5**, and **6** in CD_3OD, ^1H (400 MHz), ^{13}C (100 MHz), δ in ppm, *J* in Hertz.

	3		**5**		**6**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
Jal						
1		177.0		176.9		177.0
2	2.27 t (7.4)	33.2	2.27 t (7.4)	33.2	2.30 t (7.3)	33.0
11	3.56 *	77.3	3.56 *	77.3	3.58 *	77.2
16	0.92 t (7.1)	14.3	0.92 t (7.0)	14.3	0.91 t (7.1)	14.2
Mba						
1						178.1
2					2.46 m	41.5
2-Me					1.19 d (7.7)	17.7
3					1.72, 1.56 *	27.5
4					1.00 t (7.0)	12.1
Ac						
1				173.8		
2			2.15 s	48.5		
Nil						
1						178.3
2					2.43 m	40.3
3					3.58 *	78.8
4					1.13 d (7.8)	13.1

Jal-11-hydroxyhexadecanoyl, *Nil*-3-hydroxy-2-methylbutanoyl, *Mba*-2-methylbutanoyl, *Ac*-acetyl. Chemical shifts marked with asterisk (*) indicate overlapped signals.

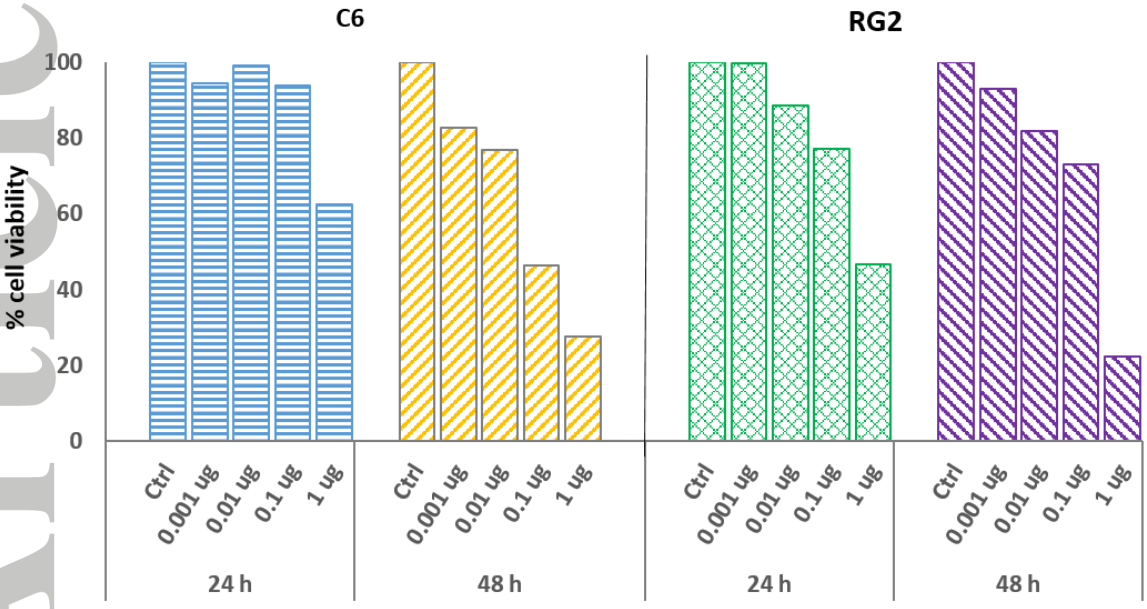


Figure 1. Influence of compound **1** on the proliferation of Glioma C6 and RG2 cell lines. Both cell lines were exposed for 24 and 48 hours to compound **1** (from 0.001 to 1 μg/ml). Then, the cytotoxic effect was determined by elution of crystal violet from surviving cells. At 24 hours, RG2 cell line were more sensible to compound **1** compared to C6, but at 48 hours of interaction, C6 cell line showed an inhibitory effect on viability even at low concentrations compared of RG2.

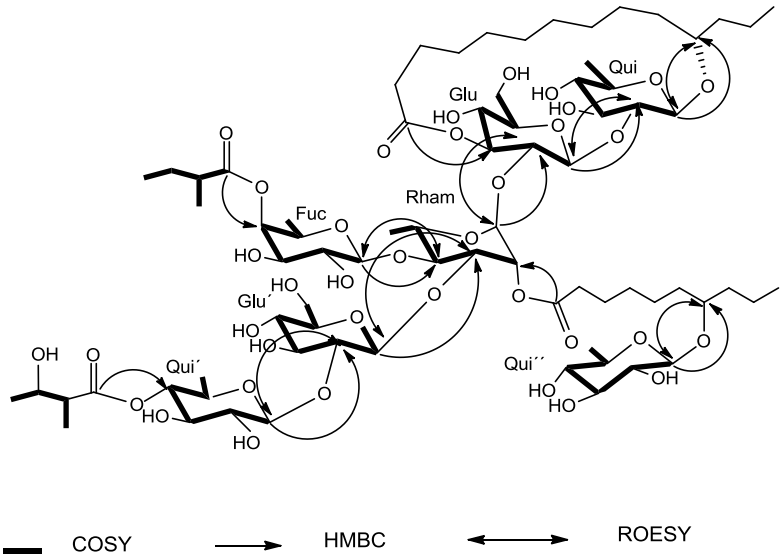
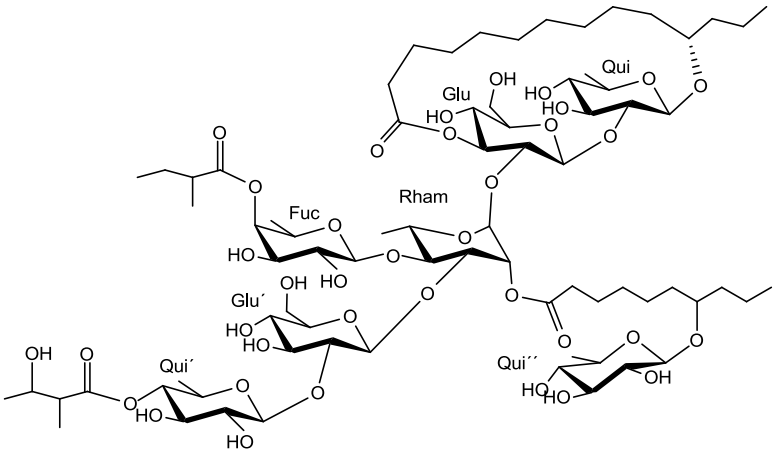
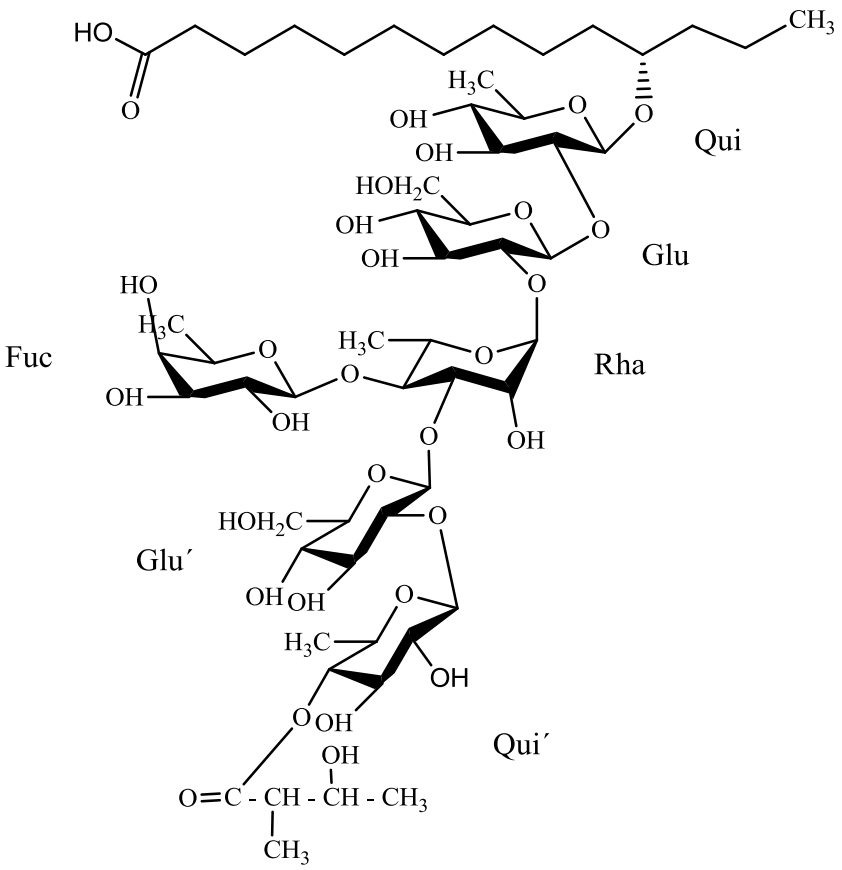


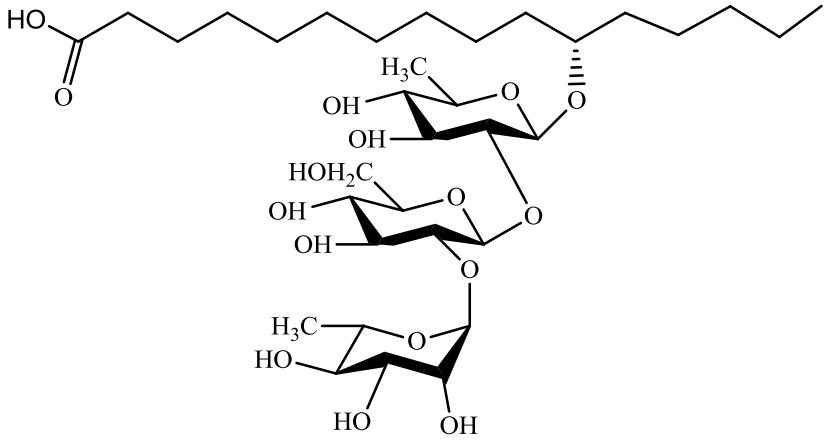
Figure 2.- Key HMBC, COSY, and ROE correlations of **1**.



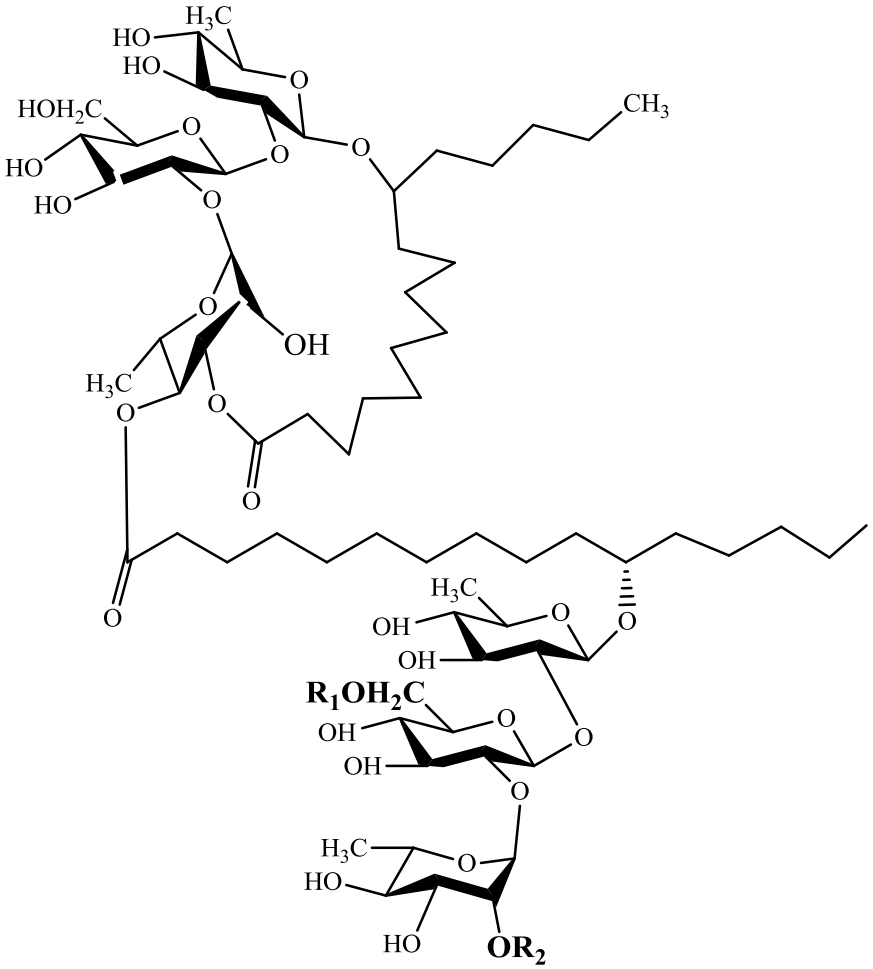
Jalapinoside B (**1**)



Purgic acid D (**2**)

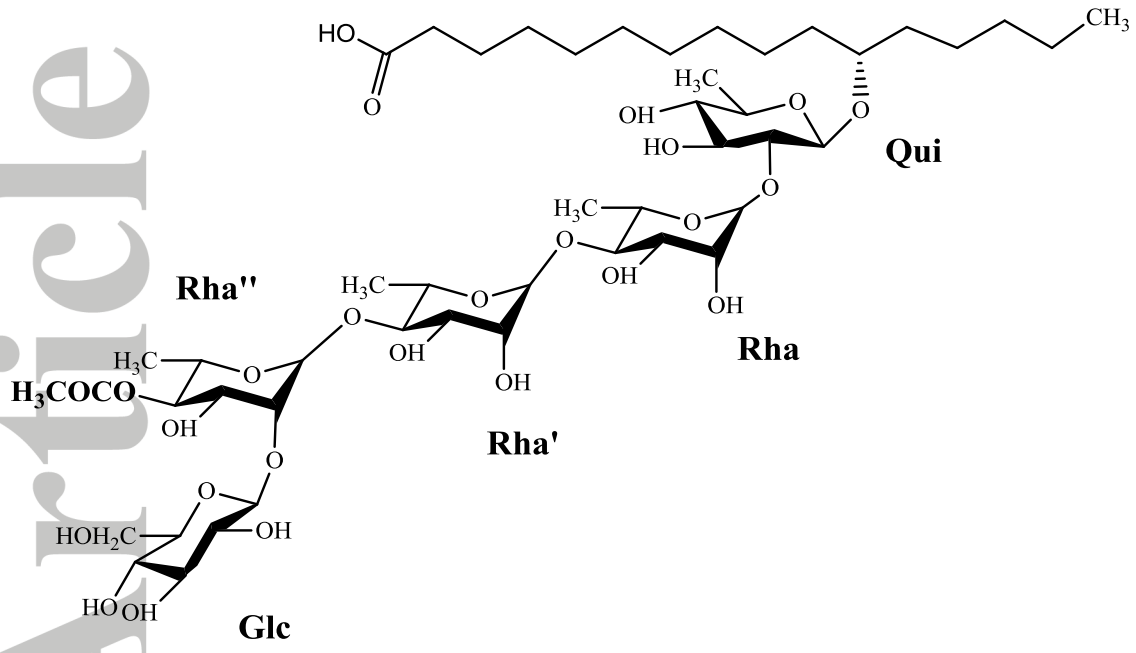


Stansoic acid A (**3**)

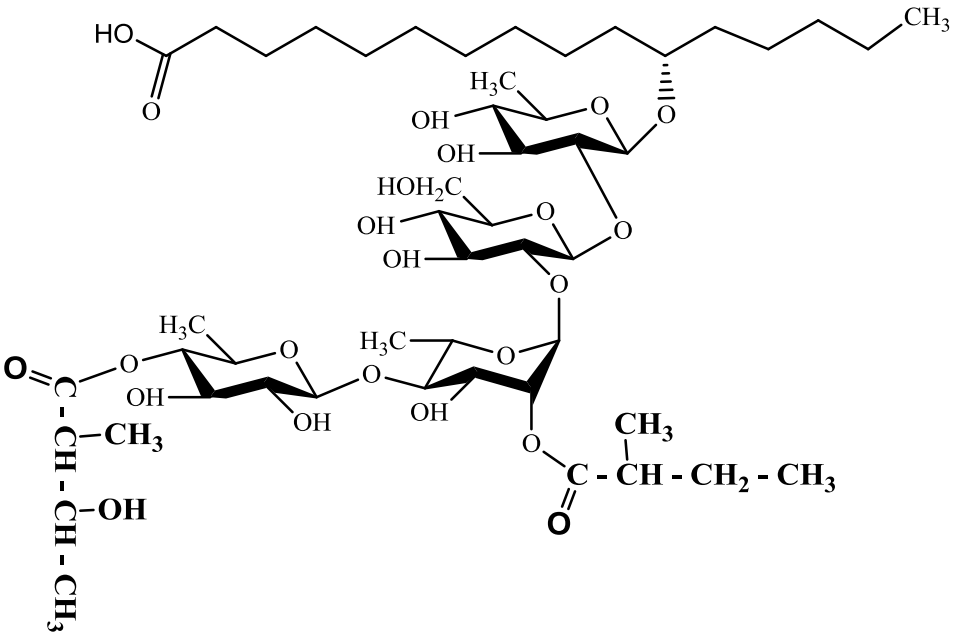


Stansin A (**4**)

R_1 = 3-hydroxy-2-methylbutanoyl
R_2 = 2-methylbutanoyl



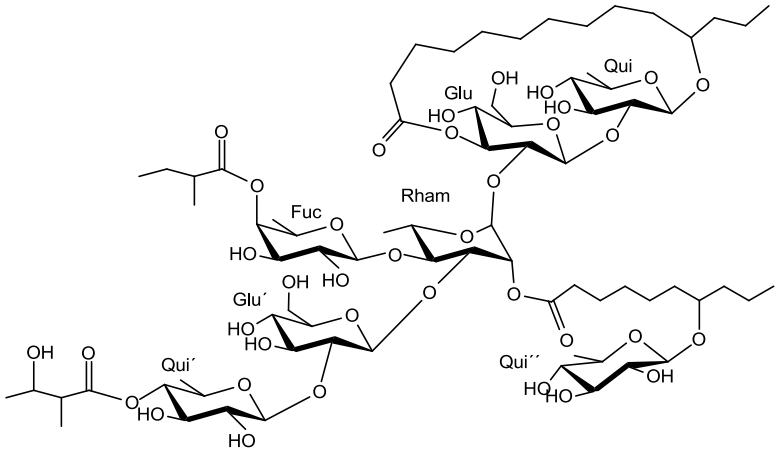
Murucinic acid II (**5**)



Stansinic acid I (**6**)

Hepta-, Hexa-, Penta-, Tetra-, and Trisaccharide Resin Glycosides from Three Species of *Ipomoea* and their Antiproliferative Activity on Two Glioma Cell Lines

Ismael León-Rivera,* Federico del Río-Portilla, Raúl G. Enríquez, Edgar Rangel-López, Juana Villeda, María Yolanda Ríos, Gabriel Navarrete-Vázquez, Israel Hurtado-Díaz, Ulises Guzmán-Valdivieso, Verónica Núñez-Urquiza, Carolina Martínez-Escobedo.



Six new partially acylated resin glycosides were isolated from convolvulin of *Ipomoea purga*, *Ipomoea stans*, and *Ipomoea murucoides* (Convolvulaceae). Preliminary testing showed the cytotoxicity of the six compounds toward OVCAR and UISO-SQC-1 cancer cell lines. In addition, compound **1** showed an antiproliferative activity on glioma C6 and RG2 tumor cell lines.