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Resin Glycosides from *Ipomoea murucoides* and their Effects on Growth of *Spodoptera frugiperda*

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ABSTRACT

Spodoptera frugiperda is a major polyphagous pest in Mexico: it is the responsible of considerable economic loss to some agricultural crops, including *Zea mays*. The aim of this study was to evaluate the biological activity of two new resin glycosides (murucoidins XIX (1) and XX (2)) isolated from exudates of *I. murucoides* on *S. frugiperda* larvae. The new glycolipids (1 and 2) were incorporated into the diet and fed to first instar larvae of *S. frugiperda*. The survived larvae were weighed at day seven and pupae incubated until moths emerged. The fecundity of females was determined. The chemical structures of the new compounds 1 and 2 consist of a pentasaccharide core bonded to an 11-hydroxyhexadecanoic unit forming a macrolactone-type structure, with the lactonization site of the aglycone placed at C-2 of the second sugar and both compounds contain the same esterifying residues (n-dodecanoic and acetic acids). The results showed that compounds 1 and 2: did not cause significant mortality on *Spodoptera frugiperda* larvae but reduced larval weight, increased the time for pupation and in reaching adult stage and reduced the number of eggs laid and its viability. Compounds 1 and 2 can be considered as a protective potential against *S. frugiperda* larvae.

Key words: *Ipomoea murucoides*, resin glycosides, insect growth, *Spodoptera frugiperda*

INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a pest of corn, *Zea mays* (L.) and other crops in the warmer part of Mexico (Foster, 1989). On corn, it attacks the above ground part of the plant, for that reason, Mexican farmers call this insect "gusano cogollero del maiz".

Plants produce many secondary metabolites which affect feeding behavior and growth of insects. It has been considered that plant protection chemicals from plants are more environmentally benign than synthetic pesticides (Scott *et al.*, 2005).

Morning glory family produce two types of compounds, alkaloid and resin glycoside (mixture of amphipathic glycolipids) which are considered the responsible of the biological activities of these plants (Eich, 2008).

Peterson *et al.* (1998) reported that resin glycosides from *Ipomoea batatas* were toxic to second instar of diamondback moth, *Plutella xylostella*. Subsequently, Jackson and Peterson (2000) probed the same resin in first instar of *P. xylostella,* resulting in significant correlations between resin glycoside level and survival. Weight of larvae and life time fecundity also was affected at sub-lethal doses of resin glycoside.

Ipomoea murucoides Roem et Schult (Convolvulaceae) is a tree-like morning glory which flowers on winter and is popularly called "cazahuate" in Mexico. In some communities of Mexico the smoke from this burned tree is used to repel mosquitoes; the flowers are used on skin infections and as decoctions for rheumatism, inflammation and muscular pain (Monroy and Castillo, 2000).

Resin glycosides have been isolated from flowers and roots of *I. murucoides* and the characterization of the individual components have shown that their chemical structures consist of macrolactones of four known glycosidic acids identified as simonic acids A and B, operculinic acid A and murucinic acid with different fatty acids esterifying the pentasaccharide core (Leon *et al.*, 2005; Cherigo and Pereda-Miranda, 2006). Some of these glycolipids have shown marginal cytotoxicity activity against OVCAR cells and exerted a potentiation effect of norfloxacin against the NorA over-expressing *Staphylococcus aureus* strain SA-1199B (Cherigo *et al.*, 2008).

A study with methanol-soluble extracts from *I. murucoides* showed a 95% of mortality in neonatal larvae of *S. frugiperda* (Vera-Curzio *et al.*, 2009). In another study, Guzman-Pantoja *et al.* (2010) reported sub-lethal effects of organic-soluble extracts of *Ipomoea pauciflora* on larvae of *S. frugiperda*.

The aim of the present study was to determine the biological activity of the two new resin glycosides (murucoidins XIX (1) and XX (2)) isolated from exudates of *I. murucoides* on *S. frugiperda* larvae.

MATERIALS AND METHODS

Plant material: The exudates were manually collected on the bark of *I. murucoides* in the campus of Universidad Autonoma del Estado de Morelos (UAEM), Cuernavaca, in the state of Morelos, Mexico in December 2008. The plant material was identified by Biol. Alejandro Flores and a voucher specimen (No. 22444) has been deposited at the Herbarium of the Research Center of Biodiversity and Preservation, UAEM.

Extraction and isolation of compounds 1 and 2: The exudates (15 g) were dried, powdered and extracted by maceration at room temperature with MeOH to afford after removal of the solvent a brown solid material (10 g). The crude extract (10 g) was fractionated by open column chromatography over silica gel 60 (70-230 mesh Merck, Darmstadt, Germany, 100 g), using a gradient of MeOH in $CHCl_3$ (9:1-7:3). TLC was carried out and spots were visualized by spraying the plates with 10% H_2SO_4 solution followed by heating. A total of 50 fractions (20 mL each) were collected, examined by TLC (on precoated Kieselgel 60 F_{254} 0.25 mm thick, Merck, Darmstadt, Germany plates) and combined as 15 fractions (1-15) containing a mixture of resin glycosides. Purification of murucoidins XIX (1) and XX (2) from fraction 5, was performed on an Agilent HPLC

system, fitted a 1100 series pump, Rheodyne injector, 1100 variable wavelength UV-Vis detector at 210 nm and Ultrasil-ODS column (Altex, 300×10 mm; 10 μm). A 250 μL volume sample (50 mg mL^{-1}) was injected each time for a run time of 40 min. CH_3CN:H_2O (7:3 v/v) at a flow rate of 1 mL min^{-1} was chosen for optimum separation. The eluates across the peaks with t_R values of 23.5 min (peak 1) and 26.4 min (peak 2) were collected by the technique of heart cutting and independently reinjected in the HPLC system to achieve total homogeneity, employing the same conditions. This technique afforded pure compound 1 from peak 1 and compound 2 from peak 2.

Isolation of glycosidic acids from compounds 1 and 2: Both solutions of compounds 1 and 2 (100 mg each) were refluxed with 5% KOH:H_2O (10 mL) for 90 min. The solution was cooled to room temperature. After acidification (pH = 4) the solution with HCl 0.5 N was extracted with CH_2Cl_2 (2×5 mL). The aqueous phases of each basic hydrolysis were concentrated under reduced pressure to give a colorless solid. The residues were further dissolved in MeOH and were subjected to open column chromatography on a reverse-phase C18 column (Altech, 50×10 mm; 100 μm). The elution was isocratic with MeOH, giving after solvent elimination, the glycosidic acids as white solids (50 mg each).

Identification of carboxylic acids: The organic phases from the basic hydrolysis were washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, the residues were directly analyzed by GC-MS. 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: HP-5 column (Hewlett Packard, 25 m×0.2 mm): He, 1 mL min^{-1}; 40°C isothermal for 2 min, linear gradient to 250°C at 15°C min^{-1}, final temperature hold for 10 min; split 1:20. MS conditions: ionization energy 70 eV; ion source temperature 280°C; interface temperature 300°C; mass range 30-600 amu. GC-MS data: ethyl acetate (t_R 3.0 min): m/z $[M]^+$ 88 (7), 61 (14), 43 (100), 29 (24); and ethyl dodecanoate (t_R 15.0 min): m/z $[M]^+$ 228 (10), 183 (10), 101 (45), 88 (100), 73 (15), 43 (18).

Analysis of glycosidic acids: Both solutions of glycosidic acids (40 mg each) in methanol were refluxed with 2 N HCl-MeOH for 2 h. After hydrolysis was complete, the reaction mixtures were diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (2×5 mL). The organic phase residues of glycosidic acids were analyzed by GC-MS. The aqueous phases from the acid hydrolysis reactions were neutralized with Na_2CO_3 solution and lyophilized to give white solids. Furthermore, the residues were dissolved in CH_3CN and analyzed by the HPLC system fitted with NH_2 column (Supelco, 250×10 mm; 10 μm), an isocratic elution of CH_3CN:H_2O (85:15 v/v), at a flow rate of 1 mL min^{-1} and a sample injection of 100 μL (4 mg mL^{-1}). Identification of the sugar components was realized by coelution experiments with standard carbohydrates samples: rhamnose (t_R 8.2 min), fucose (t_R 9.7 min) and glucose (t_R 12.1 min). Each one of these eluates was individually collected, concentrated and dissolved in H_2O. Optical rotations were measured with a Perkin-Elmer model 341 polarimeter, allowing establishing the configurations L for rhamnose and D for fucose and glucose.

Characterization of glycosidic acids and murucoidins XIX (1) and XX (2): ^1H (400 MHz), ^{13}C (100 MHz) and 2D NMR (COSY, TOCSY, HSQC, HSQC-TOCSY and HMBC) experiments were obtained on a Varian Unity 400 spectrometer. Samples were dissolved in methanol-d_4; chemical shifts are reported in parts per million as δ relative to tetramethylsilane (internal standard). Low

and high resolution FAB-MS were recorded using a matrix of m-nitrobenzyl alcohol on a JEOL MStation JMS700 spectrometer, mass spectra were acquired over the range 400-2300 Da. All melting points were determined on a Fisher-Johns apparatus and are uncorrected.

Insect rearing: *Spodoptera frugiperda* larvae were collected in Yautepec, state of Morelos, Mexico, in 2004. This colony has been reared continuously since then on a premixed and modified meridic diet. The *S. frugiperda* colonies were kept in individual petri dishes (60 mm diameter) which were placed in a biological incubator at a photoperiod of 16:8 (L:D) h, 27±1°C) and 50% relative humidity. Adults were fed on a 10% sucrose solution administered through a saturated cotton dental roll (2 cm diameter). Females of *S. frugiperda* oviposited on foil paper arranged around the cage. Eggs were placed on petri dishes (60 mm diameter) with a cotton roll saturated with distilled water for two or three days until occlusion of larvae.

Bioassay of resin glycosides against *Spodoptera frugiperda*: An aliquot of each resin glycoside (exudates, compound 1 or 2) for each treatment concentration was placed on the bottom of polystyrene plates (Cell Wells, Corning) with 12 wells. Then 1 mL of hot artificial diet (meridic) was added to each well. The diet-resin glycoside mixture was stirred and the diet was allowed to solidify at room temperature. Similar procedures were applied to the preparation of methanol-diet mixture used as negative control and Fosdrim-diet mixture used as positive control. Larvae with similar size were chosen. One experimental unit consisted of 24 well plates with 24 neonatal larvae (one larva/well). Each treatment was performed in four replicates. The plates with larvae were held at constant temperature and relative humidity (27±1.5°C and 60%) and a 16:8 (L:D) photoperiod for seven days. Larvae were checked every day and at day seven were counted the number of larvae dead.

At 8th day of incubation, the surviving larvae were fed with normal diet (free of compounds 1 and 2). The weight of larvae was recorded at 9 and 13 days of incubation. The date of pupation and pupal weight were determined for each individual. Emerging males and females were placed in plastic vessels (1 L) covered with foil paper and left until duration of the experiment or male died. The date of emergence and date of death from natural causes was recorded for each adult. The foil papers were collected daily and the number of eggs per female was recorded.

Data analysis: For the comparison of 3rd and 5th instar weights, HOVTEST_LEVENE option SAS to account for homogeneity of variance and normality (SAS, 2008) and means were separated using the Duncan's Multiple range test at 5%, before normalizing the data (angular transformation).

RESULTS

Isolation of murucoidins XIX (1) and XX (2); The exudates from the bark of *I. murucoides* were extracted with methanol and the residue was fractionated by column chromatograpy over silica gel. The fraction 5 was analyzed by preparative reversed-phase HPLC. The chromatogram of this fraction was comprised of two major glycolipids (85%) and the rest were detected as minor constituents. From the peak with t_R value of 23.5 min (peak 1) compound 1 (100.7 mg) was isolated and compound 2 (60.3 mg) was obtained from the peak 2 at 26.4 min.

Murucoidin XIX (1): White powder; MP 141-143°C; $[\alpha]^{25}_{D}$ -19.0° (c 4.0 CH_3OH); IR V_{max} 3376, 2985, 1735, 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H-NMR (CD_3OD 400 MHz) and ^{13}C-NMR (CD_3OD 100 MHz) data, see Table 1; negative-ion FABMS m/z 1223 $[M-H]^-$, 1041

Table 1: NMR data of compounds 1 and 2

	Compound 1 (ppm of TMS)		Compound 2 (ppm of TMS)	
Position	δ_C	δ_H	δ_C	δ_H
Rhamnose-1	99.0	5.04 d (1.7)	99.0	5.04 d (1.7)
Rhamnose-2	74.2	5.12 dd (3.7, 1.7)	74.2	5.12 dd (3.7, 1.7)
Rhamnose-3	70.2	4.23 dd (9.1, 3.7)	70.2	4.23 dd (9.1, 3.7)
Rhamnose-4	81.9	3.45*	82.0	3.45*
Rhamnose-5	69.1	3.89*	69.1	3.89*
Rhamnose-6	18.9	1.26*	18.9	1.26*
Rhamnose'-1	96.2	5.09 d (1.7)	100.4	5.09 d (1.7)
Rhamnose'-2	73.7	5.54 dd (3.6, 1.6)	73.7	5.54 dd (3.6, 1.7)
Rhamnose'-3	72.3	4.06 dd (9.0, 3.6)	80.8	4.06 dd (9.0, 3.6)
Rhamnose'-4	71.2	3.63*	79.2	3.63*
Rhamnose'-5	69.0	3.86*	69.0	3.86*
Rhamnose'-6	19.3	1.25*	19.3	1.25*
Rhamnose"-1	103.3	5.28 d (1.7)	103.3	5.23 d (1.7)
Rhamnose"-2	72.2	4.06 dd (3.6, 1.7)	72.2	4.05 dd (3.6, 1.7)
Rhamnose"-3	78.5	3.76 dd (8.9, 3.6)	70.5	3.78 dd (8.9, 3.6)
Rhamnose"-4	70.7	3.65 dd (9.3, 8.9)	75.7	4.86 dd (9.3, 8.9)
Rhamnose"-5	69.7	3.84*	68.7	3.83*
Rhamnose"-6	16.3	1.16 d (6.0)	16.3	1.15 d (6.0)
Rhamnose"'-1			105.3	4.34 d (1.7)
Rhamnose"'-2			80.2	3.46 dd (3.6, 1.7)
Rhamnose"'-3			74.1	3.66 dd (8.9, 3.6)
Rhamnose"'-4			74.7	3.55 dd (9.3, 8.9)
Rhamnose"'-5			73.7	3.64*
Rhamnose"'-6			17.3	1.26*
Glucose-1	105.6	4.45 d (7.0)	105.5	4.44 d (7.0)
Glucose-2	74.4	3.25 dd (7.0, 9.0)	75.4	3.45 dd (7.0, 9.0)
Glucose-3	78.4	3.42*	78.4	3.31*
Glucose-4	71.4	3.19 dd (9.0, 9.1)	71.4	3.18 dd (9.0, 9.1)
Glucose-5	77.6	3.49*	77.6	3.28*
Glucose-6	63.3	3.60, 3.81	63.3	3.61, 3.80
Fucose-1	105.5	4.31 d (7.1)		
Fucose-2	80.4	3.44 dd (9.0, 1.6)		
Fucose-3	74.0	3.52 dd (8.9, 9.0)		
Fucose-4	71.6	3.59*		
Fucose-5	68.5	3.80*		
Fucose-6	17.1	1.25*		
11-hydroxyhexadecanol-1	175.2		175.2	
11-hydroxyhexadecanol-2a	35.0	2.25 m	35.0	2.25 m
11-hydroxyhexadecanol-2b	35.0	2.58 m	35.0	2.58 m
11-hydroxyhexadecanol-11	84.1	3.60*	84.2	3.60*
11-hydroxyhexadecanol-16	14.6	0.9 t (7.0)	14.6	0.9 t (7.0)
Acetyl-1	172.6		172.6	
Acetyl-2	42.6	2.1 s	42.6	2.1 s
Dodecanoyl-1	175.3		175.3	
Dodecanoyl-2	35.1	2.36 t (6.6)	35.1	2.36 t (6.6)
Dodecanoyl-12	16.3	1.20 t (7.0)	16.3	1.20 t (7.0)

Data recorded in CD_3OD (400 MHz and 100 MHz). The spin coupling (J) is given in parentheses,*Overlapped signals, Spin coupled patterns are; s: Singlet, d: Doublet, t: Triplet, m: Multiplet, All assignments are based on ^1H-^1H COSY and TOCSY experiments, TMS: Trimethylsilane

$[M\text{-}H\text{-}C_{12}H_{22}O]^-$, 999 $[1041\text{-}C_2H_2O]^-$, 837, 691, 545, 417 and 271; positive-ion HRFABMS m/z 1247.6772 $[M+Na]^+$ (CALCD for $C_{60}H_{104}NaO_{25}$, requires 1247.6764).

Murucoidin XX (2): White powder; mp 142-144°C; $[\alpha]^{25}_D$ -21.0° (c 3.4 CH_3OH); IR V_{max} 3376, 2985, 1735, 1450, 1370, 1200, 1090 cm^{-1}; ^1H NMR (CD_3OD 400 MHz) and ^{13}C NMR (CD_3OD 100 MHz) data, see Table 1; negative-ion FABMS m/z 1223 $[M\text{-}H]^-$, 1041 $[M\text{-}H\text{-}C_{12}H_{22}O]^-$, 999 $[1041\text{-}C_2H_2O]^-$, 853, 707, 561, 433 and 271; positive-ion HRFABMS m/z 1247.6770 $[M+Na]^+$ (CALCD for $C_{60}H_{104}NaO_{25}$, requires 1247.6764).

Effect of compounds 1 and 2 on *Spodoptera frugiperda*: Exudates (50 and 200 ppm), compound 1 (50 and 100 ppm) or compound 2 (50 and 100 ppm) did not produce mortality on first instar larvae of *S. frugiperda*. Fosdrim (positive control) caused a mortality of 100% on larvae. There was no significant mortality attributable to compounds 1 and 2 for pupae or adults.

Resin glycosides lowered food consumption and reduced larval growth rate which was measured in larval weight. Compounds 1 and 2 produced statistically the same weight reduction in larvae of *S. frugiperda* at 3rd and 5th instar (Table 2).

In the development of larvae, compounds 1 and 2 (50 and 100 ppm) extended the pupal period in one day compared with control larvae. Murucoidins also affected the days needed to reach the adult stage prolonging it in one day (Table 3). This increment in time affected the development of adults which was manifested as malformations.

Females of *S. frugiperda* fed with compounds 1 and 2 oviposited 50% less than control group and lowered lifetime fecundity. In addition, only 40% of eggs oviposited by females fed with compounds 1 or 2 were viable.

Table 2: Weight and weight reduction of *S. frugiperda* 3rd and 5th instar larvae treated with compounds 1 and 2 from *I. murucoides*

	3rd instar larvae			5th instar larvae		
Treatment	n	Weight (mg)	Reduction (%)	n	Weight (mg)	Reduction (%)
Control	72	23.5±1.5[a]	0.0	72	486.8±9.5[a]	0.0
Com. 1 (50 ppm)	54	15.4±1.9[b]	33.8	54	338.5±20.2[b]	30.6
Com. 1 (100 ppm)	59	16.0±1.9[b]	30.3	59	337.6±22.6[b]	30.7
Com. 2 (50 ppm)	55	17.6±2.1[b]	27.3	55	323.0±24.0[b]	33.6
Com. 2 (100 ppm)	56	15.3±1.3[b]	33.3	56	321.5±19.6[b]	34.0

3rd instar p = 0.005; 5th instar p = 0.005. F-values = 4.84, df = 3, Values (Mean±SE) followed by the same letter are not significantly different using Tukey test at p = 0.05

Table 3: Days required for *S. frugiperda* larvae to reach pupation and adult stage after feeding on compounds 1 and 2 of *I. murucoides*

	Pupation		Adult stage	
Treatment	n	Days	n	Days
Control	65	16.3±0.1[a]	65	10.5±0.1[a]
Com. 1 (50 ppm)	43	17.2±0.2[b]	43	11.7±0.2[b]
Com. 1 (100 ppm)	46	17.9±0.3[b]	46	11.6±0.3[b]
Com. 2 (50 ppm)	45	17.9±0.2[b]	55	11.3±0.2[b]
Com. 2 (100 ppm)	46	17.8±0.3[b]	56	11.5±0.2[b]

p = 0.005, F-values = 46.94, df = 6, Values (Mean±SE) followed by the same letter are not significantly different using Tukey test at p = 0.05

DISCUSSION

Structure elucidation: The exudates from the bark of *I. murucoides* were powdered and extracted with methanol. The extract was fractionated by column chromatograpy over silica. The more abundant fraction (5) was submitted to purification of the individual constituents by preparative reverse-phase HPLC. This procedure allowed the purification of two compounds named murucoidins XIX and XX (1 and 2).

Compound 1 was subjected to alkaline hydrolysis using NaOH yielding a water-soluble glycosidic acid and a mixture of organic solvent-soluble acids. GC-MS analysis of the acid fraction permitted the identification of acetic and dodecanoic acids, as the esterifying moieties in the oligosaccharide core. On hydrolysis with mineral acid, the glycosidic acid was completely hydrolyzed and yielded free monosaccharides identified as D-glucose, L-rhamnose and D-fucose by coelution with authentic samples (HPLC). The analysis of the organic phase from the acid hydrolysis allowed the identification of the aglycon in compound 1 as 11(S)-hydroxyhexadecanoic acid (jalapinolic acid) by comparison of their physical and spectroscopic (GC-MS and NMR) constants with those published (Enriquez *et al.*, 1992). The glycosidic acid from compound 1 was identified by NMR and MS spectroscopic techniques as operculinic acid A, (11S)-hydroxyhexadecanoic acid 11-O-**β**-glucopyranosyl-(1-3)-O-[**α**-rhamnopyranosyl-(1-4)]-O-[**α**-rhamnopyranosyl-(1-4)]-O-[**α**-rhamnopyranosyl-(1-2)]-**β**-fucopyranoside, previously obtained from *Ipomoea operculata* (Ono *et al.*, 1989), *Ipomoea intrapilosa* (Bah *et al.*, 2007) and *Ipomoea murucoides* (Leon *et al.*, 2005; Cherigo and Pereda-Miranda, 2006; Cherigo *et al.*, 2008).

The HRFAB mass spectrum for murucoidin XIX (1) displayed the metal adduct peak $[M+Na]^+$ at m/z 1247.6772, corresponding to the molecular formula $C_{60}H_{104}NaO_{25}$. The negative-ion FABMS of 1 showed the quasi-molecular ion peak $[M-H^+]^-$ at m/z 1223. The peak from the consecutive elimination of one acetyl and one dodecanoyl group $[M-H^+-42\ (C_2H_2O)-182\ (C_{12}H_{22}O)]^-$ was detected at m/z 999, the acylating residues on the oligosaccharide core. It further showed the fragment ion detected at m/z 837 [m/z 999-162 $(C_6H_{10}O_5)]^-$ was attributed to the loss of the glucose moiety. Further, three fragment ions detected at m/z 691 [m/z 837-146 $(C_6H_{10}O_4)]^-$, 545 [m/z 691-146 $(C_6H_{10}O_4)]^-$ and 417 [m/z 545-128 $(C_6H_8O_3)]^-$ as the result of the consecutive loss of three rhamnose moieties. The fragment ion at m/z 271 [m/z 417-146 $(C_6H_{10}O_4)]^-$ is characteristic of the 11-hydroxyhexadecanoic acid (jalapinolic acid).

The ^1H and ^{13}C NMR spectra of compound 1 (Table 1) allowed the identification of five anomeric signals at 5.28, 5.09, 5.04, 4.45 and 4.31 ppm, corresponding to the carbon signals at 99.0, 96.2, 103.3, 105.6 and 105.5 ppm, corroborating the presence of a pentasaccharide core. All protons of each saccharide unit were sequentially assigned by a combination of COSY and TOCSY (edited) NMR techniques and all carbon atoms of the saccharide units were assigned with the HSQC spectrum. These procedures allowed the identification of one glucopyranosyl, one fucopyranosyl and three rhamnopyranosyl units in compound 1. The coupled HSQC NMR spectrum allowed the determination of the $^1J_{H\text{-}1,C\text{-}1}$ values for the saccharide units. The value of 160 Hz indicated a **β**-anomeric configuration for fucose and glucose and a value of $^1J_{H\text{-}1,C\text{-}1}$ = 170 Hz for rhamnose units was indicative of an **α**-anomeric configuration.

The interglycosidic connectivities were determined by the long-range heteronuclear coupling correlations ($^3J_{CH}$) observed in the HMBC NMR spectrum and were the same as those for its glycosidic acid. For compound 1, C-1 (δ_C 105.5) of Fuc with H-11 (δ_H 3.60) of aglycone, C-1 (δ_C 99.0) of Rha with H-2 (δ_H 3.44) of Fuc, C-1 (δ_C 100.4) of Rha' with H-4 (δ_H 3.45) of Rha, C-1 (δ_C 103.3) of Rha" with H-4 (δ_H 3.63) of Rha' and C-1 (δ_C 105.6) of Glc with H-3 (δ_H 4.06) of Rha'.

The HMBC NMR spectrum also permitted to locate the sites for the ester linkages using the long range heteronuclear correlations between carbonyl carbon of an ester group with a non-anomeric proton. For murucoidin XIX (1), C-1 (172.6 ppm) of acetyl group with H-4 (4.95 ppm) of Rha", C-1 (175.2 ppm) of n-dodecanoyl with H-2 (5.54 ppm) of Rha', the carbonyl resonance for the lactone (175.2 ppm) was identified through the long range heteronuclear correlations with the C-2 diastereotopic methylene protons at 2.25 and 2.58 ppm of the 11-hydroxyhexadecanoic group (4) and with H-2 (5.12 ppm) of Rha.

The alkaline hydrolysis of compound 2 using NaOH yielded a water-soluble glycosidic acid and acetic and dodecanoic acids. The glycosidic acid was completely hydrolyzed using HCl yielding free monosaccharides identified as D-glucose and L-rhamnose. The aglycon in compound 2 was identified as 11(S)-hydroxyhexadecanoic acid (jalapinolic acid). The glycosidic acid from the basic hydrolysis of compound 2 was identified by NMR and MS as simonic acid A, (11S)-hydroxyhexadecanoic acid 11-O-**β**-rhamnopyranosyl-(1-3)-O-[**α**-rhamnopyranosyl-(1-4)]-O-[**α**-rhamnopyranosyl-(1-4)]-O-[**α**-rhamnopyranosyl-(1-2)]-**β**-glucopyranoside, previously obtained from *Ipomoea batatas* (Noda *et al.*, 1992).

The HRFAB mass spectrum of murucoidin XX (2) showed the same metal adduct peak $[M+Na]^+$ as that for compound 1 at m/z 1247.6772, corresponding to the molecular formula $C_{60}H_{104}NaO_{25}$ indicating both compounds are isomers. In the negative-ion FABMS of 2 was detected the quasi-molecular $[M-H^+]^-$ ion peak at m/z 1223. The fragment ion detected at m/z 999 was attributed to the consecutive elimination of one acetyl and one dodecanoyl residue $[M-H^+ -42 (C_2H_2O) -182 (C_{12}H_{22}O)]^-$. The fragment ion peaks corresponding to the loss of three rhamnose units were detected at m/z 853 $[m/z\ 999-146]^-$, 561 $[m/z\ 853-154-146]^-$ and 433 $[m/z\ 561-128]^-$. The fragment ion peak attributed to jalapinolic acid was detected by the peak at m/z 271 [m/z 433-162 (glucose)]$^-$.

The ^1H and ^{13}C-NMR spectra of compound 2 (Table 1) allowed the identification of five anomeric signals, corroborating the presence of a pentasaccharide core. The protons and carbon atoms of each saccharide unit were sequentially assigned by a combination of COSY, TOCSY and HSQC NMR techniques. These procedures allowed the identification of one **β**-glucopyranosyl and four **α**-rhamnopyranosyl units in compound 2.

The interglycosidic connectivities in compound 2 were determined by the long-range heteronuclear coupling correlations observed in the HMBC NMR spectrum: C-1 of Glc with H-11 of aglycone, C-1 of Rha with H-2 of Glc, C-1 of Rha' with H-4 of Rha, C-1 of Rha" with H-4 of Rha' and C-1 of Rha"' with H-3 of Rha'. The sites for the ester linkages for murucoidin XX (2) were also determined using the correlations in the HMBC NMR spectrum: C-1 of acetyl group with H-4 of Rha", C-1 of n-dodecanoyl with H-2 of Rha', the site for the aglycon lactonization was placed at C-2 of Rha, by the correlation of C-1 of aglycon with H-2 of Rha.

Murucoidins XIX (1) and XX (2) are new resin glycosides (Fig. 1 and 2) but differing in the sugar unit bonded to the aglycone. These new compounds differ in the type and position of the short chain acids from the before reported murucoidin series (Leon *et al.*, 2005; Cherigo and Pereda-Miranda, 2006).

Previously, we found that methanol-soluble extracts from *I. murucoides* induced a 95% of mortality in neonatal larvae of *S. frugiperda*. The present study demonstrates that individual murucoidins XIX (1) and XX (2) did not induce mortality on *S. frugiperda* larvae (Vera-Curzio *et al.*, 2009).

Compounds 1 and 2 (50 and 100 ppm) lowered food consumption and as a consequence larval growth was reduced by 30% at third and fifth instar. This result is similar to that reported by

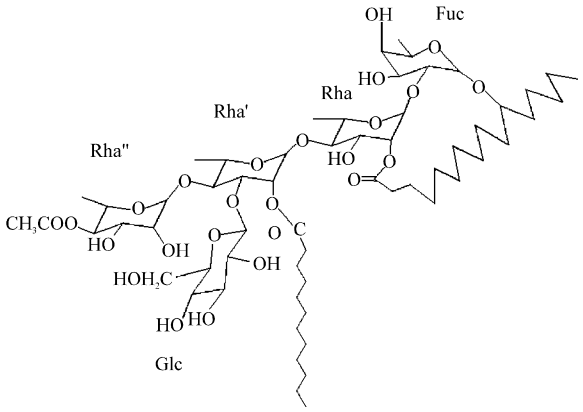

Fig. 1: Structure of murucoidin XIX (1), Rha: Rhamnose, Fuc: Fucase, Glc: Glucose

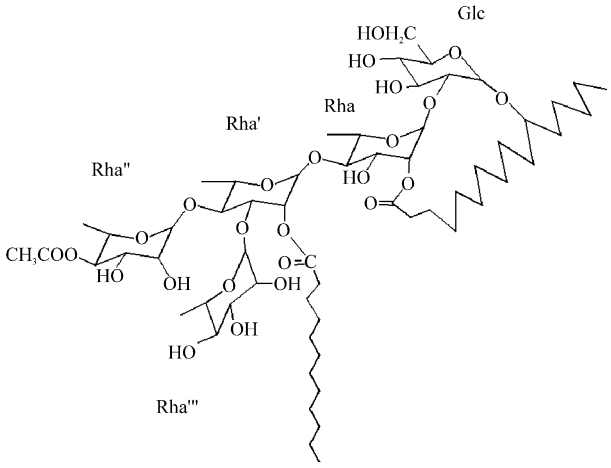

Fig. 2: Structure of murucoidin XX (2), Rha: Rhamnose, Fuc: Fucase, Glc: Glucose

Jackson and Peterson (2000), with a reduction of 50% in larval weight of first instar of *P. xylostella* by resin glycosides of *I. batatas*. *Diploknema butyracea* saponins, another type of glycosides with molecular weights similar to those of murucoidins XIX (1) and XX (2), lowered food consumption and reduced larval growth rate by 30% on larvae of *Spodoptera litura* (Saha *et al.*, 2010). The results suggest that compounds 1 and 2 short or suppress the feeding process.

The effect on female oviposition was not dependent on the concentration of compounds 1 and 2. At both concentrations (50 and 100 ppm) of compounds 1 and 2, females produced 50% less eggs than control group. A similar result was reported by Weathersbee and McKenzie (2005) using a neem biopesticide. Although, there is not any report about the mechanism of reduction on the number of eggs produced by females, it is believed that hormone production is affected by ingested molecules (Mordue (Luntz) and Nisbet, 2000). Vera-Curzio *et al.* (2009) reported that chromatographic fractions containing resin glycosides from organic-soluble extracts of *I. murucoides* affected the number of eggs produced by females. Our results showed that individual glycolipids (1 and 2) are the responsible of the reduction in the number of eggs produced by females of *S. frugiperda*.

The mechanism of insect growth regulatory activity of glycolipids from *Ipomoea species* is not known. On a cellular level, there is some evidence that glycolipids increased the membrane permeability for the ions K^+, Na^+ and Cl^- in a dose-dependent manner, suggesting a membrane disruption induced by the amphiphilic properties of these molecules, from the results reported of glycolipids from *Ipomoea tricolor* with Sf9 cell membranes of *S. frugiperda*. It seems that the activity of glycolipids from *Ipomoea* species on *S. frugiperda* is due to the presence of a macrolactone-type structure as shown by the results obtained with compounds 1, 2 and tricolorins and the fact that glycolipids without the macrolactone-type structure did not show activity on *S. frugiperda* cells (Pereda-Miranda *et al.*, 2009).

The results obtained represent the first report of biological activities of pure resin glycosides (murucoidins XIX (1) and XX (2)) on *S. frugiperda* larvae, since other studies on the insecticidal potential of resin glycosides used the mixture of this type of compounds. The results obtained suggest that pure compounds 1 and 2 offers potential activity against *S. frugiperda* larvae.

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