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Bacterial resistance modifying tetrasaccharide agents from *Ipomoea murucoides*

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ABSTRACT

As part of an ongoing project to identify oligosaccharides which modulate bacterial multidrug resistance, the $CHCl_3$-soluble extract from flowers of a Mexican arborescent morning glory, *Ipomoea murucoides*, through preparative-scale recycling HPLC, yielded five lipophilic tetrasaccharide inhibitors of *Staphylococcus aureus* multidrug efflux pumps, murucoidins XII–XVI (**1**–**5**). The macrocyclic lactone-type structures for these linear hetero-tetraglycoside derivatives of jalapinolic acid were established by spectroscopic methods. These compounds were tested for in vitro antibacterial and resistance modifying activity against strains of *Staphylococcus aureus* possessing multidrug resistance efflux mechanisms. Only murucoidin XIV (**3**) displayed antimicrobial activity against SA-1199B (MIC 32 μg/ml), a norfloxacin-resistant strain that over-expresses the NorA MDR efflux pump. The four microbiologically inactive (MIC > 512 μg/ml) tetrasaccharides increased norfloxacin susceptibility of this strain by 4-fold (8 μg/ml from 32 μg/ml) at concentrations of 25 μg/ml, while murucoidin XIV (**3**) exerted the same potentiation effect at a concentration of 5 μg/ml.

1. Introduction

All Mexican medicinal arborescent members of the genus *Ipomoea* (Convolvulaceae) share two therapeutic properties: the raw flowers, used antiseptically, are rubbed directly on skin infections, itching and rashes, and as decoctions, plasters and poultices, and in some instances the leaves, stem and bark are used for rheumatism, inflammation, and muscular pain (Chérigo and Pereda-Miranda, 2006). "Cazahuate", Nahuatl (Aztec language) for "tree to cure mange", is the vernacular name in contemporary Mexican Spanish for this group of arborescent species belonging to the genus *Ipomoea* series Arborescentes, e.g., *Ipomoea murucoides* Roem. et Schult. In the 16th century account of Mexican pre-Hispanic herbolaria "De la Cruz-Badiano Codex" (Emmart, 1940), the antiseptic properties for this medicinal plant complex are confirmed in a description of how Aztec healers used this herbal drug to prevent hair loss.

Murucoidins, a series of related lipophilic pentasaccharides of jalapinolic acid, were first reported in the chemical analysis of resin glycosides from this crude drug (Chérigo and Pereda-Miranda, 2006). A second investigation followed for the identification of new resin glycoside inhibitors of bacterial growth and in some instances inhibitors of multidrug efflux to treat infections resulting from multidrug-resistant *S. aureus* strains. All tested murucoidins exerted a potentiation effect of norfloxacin against the NorA over-expressing strain SA-1199B by increasing the activity 4-fold (8 μg/ml from 32 μg/ml) at concentrations of 5–25 μg/ml (Chérigo et al., 2008). This work was undertaken to increase the recognition of chemical diversity of the target oligosaccharides which modulate bacterial multidrug resistance, basically by isolating compounds from a new plant collection of *I. murucoides* that displayed variations in its resin glycoside composition.

2. Results and discussions

$CHCl_3$-soluble extracts of a new collection of the crude drug "Cazahuate" were compared by C_{18} reversed-phase HPLC with reference solutions of the previously reported resin glycosides from this species (Chérigo et al., 2008). This analysis confirmed a higher complexity in their composition and allowed the detection of the known pentasaccharides stoloniferin I, pescaprein III, intrapilosin I, and murucoidins I–V, as well as five new constituents, murucoidins XII–XVI (**1**–**5**) which were separated and purified by using a recycling HPLC technique (Pereda-Miranda and Hernández-Carlos, 2002). Several NMR techniques and FABMS were used to characterize their structures which were found to be macrolactones of the known operculinic acids C and E, linear hetero-tetraglycosides of jalapinolic acid, with *n*-dodecanoic or (2*S*)-methylbutyric acids esterifying the C-2 or C-3 positions on the second rhamnose unit of the oligosaccharide core and (2*S*)-methylbutyric acid at C-4 on the third rhamnose moiety.

A small portion of the glycosidic mixture was saponified to liberate an H_2O-soluble mixture of five oligosaccharides of jalapinolic acid: the major products were identified as operculinic acid A and

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simonic acids A and B by HPLC retention time comparison with those of authentic samples (Chérigo et al., 2008). Two additional glycosidic acids represented minor constituents and were characterized as operculinic acid C: (11*S*)-hydroxyhexadecanoate 11-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside, and operculinic acid E: (11*S*)-hydroxyhexadecanoate 11-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside, both previously isolated from *I. operculata* (Ono et al., 1989). Evidence for the absolute stereochemistry of the sugars, the configuration of the anomeric linkages as well as the sequence of glycosidation was published when their oligosaccharide cores were first elucidated (Ono et al., 1989, 1990). Sugar analysis confirmed that all monosaccharides were in ther naturally occurring form as previously described (Chérigo et al., 2008).

Negative-ion FAB mass spectra generated by murucoidins XII and XIII (**1–2**) were found to be very similar, with a pseudomolecular $[M–H]^-$ ion at *m/z* 1119, and therefore these constituents represented diastereoisomeric tetrasaccharides of molecular formula $C_{57}H_{100}O_{21}$. Compounds **3** afforded a FAB mass spectrum with the $[M–H]^-$ ion at *m/z* 1103 ($C_{57}H_{99}O_{20}$). Careful analysis of the negative FABMS generated by these tetrasaccharides **1–3** confirmed the nature of the oligosaccharide core for each glycolipid. The observed difference of 16 mass units in all diagnostic fragments for **3** in relation with **1** and **2** indicated the presence of a 6-deoxy-hexose (fucose) instead of the hexose unit (glucose). The initial loss of the dodecanoyl group afforded a peak at *m/z* 937 in **1** and **2** representing $[M–H–C_{12}H_{22}O]^-$, while the same elimination in **3** afforded the peak at *m/z* 921. The subsequent elimination of the methylbutyroyl unit (84 mass units) in **1** and **2** afforded a peak at *m/z* 853 $[937–C_5H_8O]^-$, and the peak *m/z* 837 in **3**. The ions produced by the rupture of each of the glycosidic linkages afforded the series of peaks at *m/z* 707, 561, 433 and 271 in **1** and **2** and at *m/z* 691 $[1067–2 \times 146\ (C_6H_{10}O_4)–C_5H_8O]^-$, 545 $[691–146\ (C_6H_{10}O_4)]^-$, which indicated that the lactonization was located at the first rhamnose unit (Rha), 417 $[545 + H_2O – 146\ (C_6H_{10}O_4)]^-$, and 271

Table 1
^{1}H (500 MHz) NMR spectroscopic data of compounds **1–5** (pyridine-d_5).[a]

Proton[b]	1	2	3	4	5
glc-1	4.99 *d* (8.0)	5.00 *d* (8.0)			
2	4.30* *dd* (8.0, 8.0)	4.30* *dd* (8.0, 8.0)			
3	4.30* *dd* (8.0, 8.0)	4.30* *dd* (8.0, 8.0)			
4	4.17 *dd* (9.0, 9.0)	4.16 *dd* (9.0, 9.0)			
5	3.90*	3.90 *ddd* (2.0, 5.5, 9.0)			
6a	4.38 *dd* (5.6, 12.0)	4.40* *m*			
6b	4.52 *dd* (2.8, 12,0)	4.52 *dd* (3.0, 11.7)			
fuc-1			4.79 *d* (7.5)	4.69 *d* (7.5)	4.73 *d* (7.5)
2			4.54*dd* (7.5, 9.5)	4.14 *dd* (7.5, 9.5)	4.14 *dd* (7.5, 9.5)
3			4.19 *dd* (3.0, 9.5)	4.00 *dd* (3.0, 9.5)	4.07 *dd* (3.0, 9.5)
4			3.92*	3.97 *m*	3.97 *brs*
5			3.82 *dq* (1.0, 6.5)	3.72 *dq* (1.0, 6.5)	3.77 *dq* (1.0, 6.5)
6			1.52 *d* (6.5)	1.50 *d* (6.5)	1.50 *d* (6.5)
rha-1	6.49 *d* (1.0)	6.51 *d* (1.5)	6.39 *d* (2.0)	5.49 *d* (2.0)	5.47 *d* (2.0)
2	5.28 *dd* (1.0, 3.0)	5.25 *dd* (1.5, 3.0)	5.25 *dd* (2.0, 3.0)	5.92 *dd* (2.0, 3.5)	5.90 *dd* (2.0, 3.5)
3	5.59 *dd* (3.0, 9.5)	5.69 *dd* (3.0, 9.5)	5.66 *dd* (2.5, 9.5)	4.99 *dd* (3.5, 9.5)	5.00 *dd* (3.5, 9.5)
4	4.63 *t* (9.5)	4.76 *t* (9.5, 9.5)	4.71 *t* (9.5)	4.20 *t* (9.5)	4.22 *t* (9.5)
5	5.08 *dq* (6.5, 9.5)	5.16 *dq* (6.5, 9.5)	5.08 *dq* (6.0,9.5)	4.45 *dq* (6.5, 9.5)	4.45 *dq* (9.5, 6.5)
6	1.75 *d* (6.5)	1.75 *d* (6.5)	1.59 *d* (6.0)	1.64 *d* (6.5)	1.63 *d* (6.5)
rha′-1	5.57 *d* (1.5)	5.91 *d* (1.5)	5.89 *d* (1.5)	6.01 *d* (2.0)	6.16 *d* (2.0)
2	5.78 *dd* (1.5, 3.5)	4.71 *dd* (1.5, 3.0)	4.70 *dd* (1.5, 3.0)	5.95 *dd* (2.0, 3.5)	4.87 *dd* (2.0, 3.0)
3	4.59 *dd* (3.5, 9.5)	5.72 *dd* (3.0, 9.5)	5.71 *dd* (3.0, 9.5)	4.66 *dd* (3.5, 9.5)	5.74 *dd* (3.0, 9.5)
4	4.26 *t* (9.5, 9.5)	4.57 *t* (9.5, 9.5)	4.56 *t* (9.5, 9.5)	4.24 *t* (9.5, 9.5)	4.58 *t* (9.5, 9.5)
5	4.37 *dq* (6.0, 9.5)	4.43 *dq* (6.5, 9.5)	4.40 *dq* (6.0, 9.5)	4.38 *dq* (6.5, 9.5)	4.42 *dq* (6.5, 9.5)
6	1.69 *d* (6.0)	1.61 *d* (6.5)	1.58 *d* (6.0)	1.71 *d* (6.5)	1.65 *d* (6.5)
rha″-1	6.16 *d* (1.5)	5.68 *d* (1.5)	5.68 *d* (1.5)	6.13 *d* (1.5)	5.75 *d* (1.5)
2	4.77 *dd* (1.5, 3.0)	4.47 *dd* (1.5, 3.0)	4.46 *dd* (1.5, 3.5)	4.81 *brs*	4.53 *brs*
3	4.48 *dd* (3.0, 9.5)	4.40* *m*	4.38 *dd* (3.5, 9.5)	4.55 *dd* (3.0, 9.5)	4.47 *dd* (3.0, 9.5)
4	5.80 *t* (9.5)	5.76 *t* (9.5)	5.75 *t* (9.5, 9.5)	5.83 *t* (9.5, 9.5)	5.79 *t* (9.5, 9.5)
5	4.41 *dq* (6.0, 9.5)	4.32 *dq* (6.5, 9.5)	4.30 *dq* (6.0, 9.5)	4.43 dq (6.5, 9.5)	4.34 *dq* (6.5, 9.5)
6	1.44 *d* (6.0)	1.36 *d* (6.5)	1.35 *d* (6.0)	1.44 *d* (6.5)	1.35 *d* (6.5)
jla-2a	2.24 *ddd* (3.0, 8.0, 11.7)	2.13 *ddd* (3.7, 7.8, 11.5)	2.14 *ddd* (3.5, 7.0, 12.0)	2.22 *ddd* (3.5, 7.0, 12.0)	2.21 *ddd* (3.7, 8.0, 11.5)
2b	2.72 *ddd* (3.0, 8.0, 11.7)	2.25 *ddd* (3.7, 7.8, 11.5)	2.23 *ddd* (3.5, 7.0, 12.0)	2.44 *ddd* (3.5, 7.0, 12.0)	2.35 *ddd* (3.7, 8.0, 11.5)
11	3.90*	3.96 *m*	3.92*	3.84 *m*	3.85 *m*
16	1.00 *t* (7.0)	0.99 *t* (7.0)	0.99 *t* (7.0)	0.88 *t* (7.0)	0.88 *t* (7.5)
mba-2	2.52 *tq* (7.0, 7.0)	2.48 *tq* (7.0, 7.0)	2.47 *tq* (7.0,7.0)	2.40 *tq* (7.0, 7.0)	2.45* tq (7.0, 7.0)
2-Me	1.22 *d* (7.0)	1.20 *d* (7.0)	1.20 *d* (7.0)	1.21 *d* (7.0)	1.19 *d* (7.0)
3-Me	0.95 *t* (7.5)	0.92 *t* (7.5)	0.92 *t* (7.5)	0.94 *t* (7.5)	0.91 *t* (7.5)
mba′-2				2.51 *tq* (7.0, 7.0)	2.45* *tq* (7.0,7.0)
2-Me				1.08 *d* (7.0)	1.13 *d* (7.0)
3-Me				0.85 *t* (7.5)	0.86 *t* (7.5)
dodeca-2a	2.27 *ddd* (7.5, 7.5, 15.0)	2.30 *ddd* (7.5, 7.5, 15.0)	2.27 *ddd* (7.0, 7.0, 15.0)		
2b	2.35 *ddd* (7.5, 7.5, 15.0)	2.39 *ddd* (7.5, 7.5, 15.0)	2.38 *ddd* (7.0, 7.0, 15.0)		
12	0.87 *t* (7.5)	0.87 *t* (7.0)	0.88 *t* (7.5)		

[a] Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (*J*) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, *brs* = broad singlet.
[b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose, jal = 11-hydroxyhexadecanoyl; mba = methylbutanoyl, dodeca = dodecanoyl.