Inhibitors of Bacterial Multidrug Efflux Pumps from the Resin Glycosides of *Ipomoea murucoides*[⊥]

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A reinvestigation of the $CHCl_3$-soluble extract from flowers of the Mexican medicinal arborescent morning glory, *Ipomoea murucoides*, through preparative-scale recycling HPLC, yielded six new pentasaccharides, murucoidins VI−XI (**1**−**6**), as well as the known pescaprein III (**7**), stoloniferin I (**8**), and murucoidins I−V (**9**−**13**). Their structures were characterized through the interpretation of their NMR spectroscopic and FABMS data. Compounds **1**−**6** were found to be macrolactones of three known glycosidic acids identified as simonic acids A and B, and operculinic acid A, with different fatty acids esterifying the same positions, C-2 on the second rhamnose unit and C-4 on the third rhamnose moiety. The lactonization site of the aglycone was placed at C-2 or C-3 of the second saccharide unit. The esterifying residues were composed of two short-chain fatty acids, 2-methylpropanoic and (2*S*)-methylbutyric acids, and two long-chain fatty acids, *n*-dodecanoic (lauric) acid and the new (8*R*)-(−)-8-hydroxydodecanoic acid. For the latter residue, its absolute configuration was determined by analysis of its Mosher ester derivatives. All members of the murucoidin series exerted a potentiation effect of norfloxacin against the NorA overexpressing *Staphylococcus aureus* strain SA-1199B by increasing the activity 4-fold (8 μg/mL from 32 μg/mL) at concentrations of 5−25 μg/mL. Stoloniferin I (**8**) enhanced norfloxacin activity 8-fold when incorporated at a concentration of 5 μg/mL. Therefore, this type of amphipathic oligosaccharide could be developed further to provide more potent inhibitors of this multidrug efflux pump.

In the New World flora there are 13 tree-like morning glory species in the genus *Ipomoea* series *Arborescentes*, most of them are confined to Mexico and nearby Central America.[1] These species have long been of medicinal and economical interest to the native people, who coexist with them in the same ecosystem. In central Mexico, six species collectively called "cazahuate"[2] are a conspicuous floristic element of the seasonal dry tropical forest. They have been used since Prehispanic times[3] and share several morphological features, e.g., trees with large white flowers and funnel-shaped corollas, and the same therapeutic application to treat itching, rashes, and other infections by rubbing the raw flowers directly on the skin. Traditional healers continue to use decoctions of this medicinal plant complex[4−6] considered to be of "cold-nature"[7] to reduce excessive body heat[8] and relieve uncomfortable "water and cold" symptoms believed to be produced by abrupt climatic changes resulting in diseases considered to be "hot", e.g., rheumatism, inflammation, and ear pain.

This paper presents the results of a second investigation based on the chemical analysis of the resin glycoside mixture obtained from the flowers of *Ipomoea murucoides* Roem. et Schult. Six new acylated pentasaccharides of jalapinolic acid, murucoidins VI−XI (**1**−**6**), as well as the known pescaprein III (**7**), stoloniferin I (**8**), and murucoidins I−V (**9**−**13**) were separated and purified by a recycling HPLC technique, and their structures were characterized through the interpretation of NMR spectroscopic and FABMS data. The antimicrobial potential of these complex macrocyclic lactones was evaluated against a panel of *Staphylococcus aureus* strains overexpressing specific efflux pumps as a starting point for the development of new bacterial inhibitors to treat infections resulting from multidrug-resistant *S. aureus* strains.[9,10]

Results and Discussion

A small portion of the resin glycoside fractions, obtained from the $CHCl_3$-soluble extract, was submitted to saponification and yielded a water-soluble solid product that was resolved by HPLC into three glycosidic derivatives of jalapinolic acid and an organic solvent-soluble oily acidic fraction. The glycosidic acids were identified as simonic acid A, (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside,[11] simonic acid B, (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside,[5,11] and operculinic acid A, (11*S*)-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside,[5,12] previously obtained from *Ipomoea batatas*,[11] *I. stolonifera*,[13] *I. operculata*,[14] *I. leptophylla*,[15] *I. murucoides*,[5] and *I. intrapilosa*.[16]

The major liberated fatty acids were identified as 2-methylpropanoic (iba), 2-methylbutanoic (mba), and *n*-dodecanoic acids by GC-MS comparison of their spectra and retention times with those of authentic samples.[5,17] The new (8*R*)-(−)-8-hydroxydodecanoic acid (**14**) was also liberated as one of the hydrolyzed products recovered from the organic-soluble fraction obtained from the saponification of murucoidin X (**5**).

Individual constituents of the remaining portion of these resin glycoside fractions were separated and purified by a recycling HPLC technique,[18] using a semipreparative reversed-phase column. These procedures led to the isolation and structural characterization of six new compounds, for which the names murucoidins VI−XI (**1**−**6**) have been proposed. All compounds displayed the same fragmentation pattern produced by glycosidic cleavage in their negative-ion FABMS, as previously described for the pescaprein[19] and murucoidin[5] series. Common fragment peaks reported for the

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resin glycosides[20,21] were observed in all mass spectra, confirming the branched pentasaccharide core, and the resulting diagnostic peaks also indicated the nature of the esterifying moieties. For example, murucoidin X (**5**) gave a pseudomolecular $[M - H]^-$ ion at *m/z* 1265, indicating a molecular formula of $C_{63}H_{110}O_{25}$. The ions at *m/z* 1181 $[M - H - C_5H_8O]^-$ and 1067 $[M - H - C_{12}H_{22}O_2]^-$ suggested the presence of a methylbutyric acid and a monohydroxydodecanoic acid as esterifying residues. The subsequent losses produced by glycosidic cleavage of each sugar moiety afforded peaks 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 $- [417 - 146\ (C_6H_{10}O_4)]^-$.

Common features in both ^{1}H and ^{13}C NMR spectra of all six new compounds (**1**−**6**) were noted (Tables 1 and 2). All ^{1}H NMR spectra showed significantly downfield shifted signals for the proton on C-2 or C-3 of the first rhamnose unit (rha) and on H-2 and H-3 of the second and third rhamnose units (rha′ and rha″), suggesting esterification at these positions. The multiplets with splitting patterns as ddd centered at δ 2.80 and 2.25 showed cross-peaks in their COSY and TOCSY spectra, revealing the macrocyclic lactone-type structure for all the murucoidins because these signals correspond to the nonequivalent diastereotopic protons of the CH_2-2 of the aglycone (11*S*-hydroxyhexadecanoic acid, jal) when forming a ring.[20,21] The following spectroscopic features were observed: (a) the carbonyl resonance of the lactone functionality (δ 173−174) was assigned by the ^2J coupling with each of the methylene protons on the adjacent C-2 position of jalapinolic acid in all compounds **1**−**6**; (b) the lactonization site at C-3 (δ_C 77) of the second saccharide (rha) was established by the observed ^3J coupling between this carbonyl carbon and its downfield shifted geminal proton (δ 5.6) in murucoidins VI (**1**) and IX−XI (**4**−**6**); for compounds **2** and **3**, the lactonization at C-2 (δ_C 70) was identified by the $^3J_{C-H}$ coupling and corroborated by the significant downfield shift for its geminal proton (δ 5.0) in comparison with the same resonance in the rest of the compounds with the Rha C-3 macrocyclic ring closure; (c) all murucoidins showed signals for one short-chain fatty acid residue esterifying position C-4 at Rha″ (δ_C 73); H-2 of these moieties was used as a diagnostic resonance centered at δ 2.4−2.5 (1H, tq) for the methylbutanoyl group (mba) in murucoidins VI (**1**), VIII (**3**), X (**5**), and XI (**6**) and at δ 2.5−2.6 (1H, septet) for the methylpropanoyl group (iba) in murucoidins VII (**2**) and IX (**4**); (d) the C-2 methylene equivalent protons were observed as a triplet signal at δ 2.4 for the long-chain fatty acid esterifying residues in **1**, **5**, and **6**. In all cases it was possible by HMBC analysis[21,22] to establish the links between a specific carbonyl ester group with their corresponding vicinal proton resonance ($^2J_{CH}$) and the pyranose ring proton at the site of esterification ($^3J_{CH}$). For example, the 8-hydroxydodecanoyl residue in murucoidin X (**5**) was identified through the observed $^2J_{CH}$ coupling between the carbonyl resonance at δ 172.9 with the triplet-like signal for the vicinal methylene protons (δ 2.3, 2H) and its location at C-2 of Rha′ by the $^3J_{CH}$ coupling with the signal at δ 5.8. Therefore, the remaining esterified position (C-4 Rha″ δ_H 4.2; δ_C 73) represented the location of the additional ester linkage for the methylbutanoyl group.

From the TOCSY experiment,[22] edited ^{1}H NMR subspectra for each individual monosaccharide moiety were obtained for all oligosaccharides and permitted the assignment of their resonances (Table 1). Homonuclear spin decoupling experiments were carried out to verify coupling constants. The anomeric configuration in each sugar unit was deduced from 2D $^1J_{CH}$ NMR experiments. For D-sugars in the $^4C_1$ conformation, the α-anomeric configuration (β-equatorial C−H bond) has a $^1J_{CH}$ value of 170 Hz, which is 10 Hz higher than that for the β-anomer (α-axial C−H bond; ca. 160 Hz).[22] From the anomeric signals in the ^{13}C NMR spectra of the glycosidic acids (i.e., simonic acids A and B and operculinic acid C), $^1J_{CH}$ values for fucose (159 Hz) and glucose (164 Hz) supported the β-anomeric configuration. The α-configuration was deduced for the rhamnose units ($^1J_{CH}$ = 170−172 Hz).[20] All monosaccharides were in their naturally occurring form (D-glucose, D-fucose, and L-rhamnose), as confirmed by optical rotation measurements of the acid-liberated individual monosaccharides.

The structure of the new acylating acid residue obtained from the saponification of murucoidin X (**5**) was further supported by its chemical transformations to its silylated methyl ester derivative (**16**).[17] Its mass spectrum indicated that the hydroxyl group was attached at the C-8 position in a fatty acid chain of 12 carbons since the α-cleavage[23] on either side of the trimethylsiloxyl group gave the diagnostic ions at *m/z* 245 $[C_9H_{16}O_3TMS]^+$ and 159 $[C_5H_{10}OTMS]^+$. In compound **5**, the presence of the C-8 hydroxyl group in the aglycone was also recognized by the downfield shift (α-effect ca. +41 ppm)[24] on C-8 (δ_C 75) and the β-effects on C-7 and C-9 (δ_C 38), which resulted in shifts to higher frequency ($\Delta\delta$ = +8 ppm)[24] in comparison with the same resonances in the *n*-dodecanoic acid (δ_C 29−30).[25] To determine the stereochemistry at the C-8 position, compound **15** was converted to the Mosher esters **17** and **18** with (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride, and their ^{1}H NMR spectra were recorded.[26] The chemical shift difference ($\Delta\delta = \delta_S - \delta_R$) between corresponding Me-12 protons was positive ($\Delta\delta$ = +0.059 ppm), allowing the confirmation of a C-8 *R* absolute configuration through the application of the configurational model proposed by Kakisawa and associates (Scheme 1).[27] This result is consistent with the previously reported chemical shift differences ($\Delta\delta$ = +0.057 ppm) at the terminal methyl signals that are separated by as much as four methylene groups from the asymmetric carbon.[28] It is also in accordance with the observation that synthetic monohydroxylated fatty acids with a dextrorotatory optical activity have an *S* absolute configuration and their levoisomers have the opposite, *R* configuration.[29] The acylating 8-(*R*)-hydroxydodecanoic acid of murucoidin X (**5**) represents the first levorotatory monohydroxylated aglycone in the Convolvulaceae family.

Only murucoidins I (**9**), VI (**1**), and VII (**2**) displayed antimicrobial activity at the concentration tested against SA-1199B (MIC 32 μg/mL), a *Staphylococcus aureus* strain that overexpresses the NorA MDR efflux pump. This may be explained by a loss in "fitness" of this strain due to overexpression of this MDR pump when compared to the other strains. All of the murucoidins strongly potentiated the action of norfloxacin against this NorA overexpressing strain[30] in experiments using a subinhibitory concentration of these oligosaccharides (Table 3). They exerted a potentiation effect, which increased the activity of norfloxacin by 4-fold (8 μg/mL from 32 μg/mL) at concentrations of 5−25 μg/mL; stoloniferin I (**8**) enhanced norfloxacin activity 8-fold when incorporated at a concentration of 5 μg/mL. These strong potentiation effects were similar to those previously observed for the amphipathic orizabins,[9] which are tetrasaccharides from the Mexican scammony (*Ipomoea orizabensis*). An ethidium efflux inhibition assay utilizing SA-1199B[31] demonstrated that compound **8** had modest inhibitory activity (Figure 1) in comparison with the strong activity previously reported for orizabin IX, which was more efficacious than reserpine.[9] This decrease in activity could be due to solubility issues, since the murucoidins are extremely nonpolar and at higher concentrations the broth solution became cloudy as a result of test compound precipitation. Compound **11** had no efflux inhibitory activity; the basis for its profound effect on the norfloxacin MIC in the modulation assay currently is unknown and deserves further attention.

The amphipathic properties of these compounds resulting from the acylation of some of the free hydroxyl groups of the oligosaccharide core and the lipophilic alkyl chains of their aglycones would seem to be important in facilitating cellular uptake to its MDR pump target. To verify this hypothesis, the modulatory activity of two

Table 1. ^{1}H NMR Data of Compounds **1**−**6** (500 MHz)[a]

proton[b]	1	2	3	4	5	6
glc-1	5.03 d (7.5)	4.90 d (7.5)	4.91 d (7.5)			
2	4.28 dd (7.5, 8.0)	3.90 dd (7.5, 8.5)	3.90 dd (7.5, 8.5)			
3	4.30 dd (8.0, 9.0)	4.12* dd (8.5, 9.0)	4.16* dd (8.5, 9.0)			
4	4.15 t (9.0, 9.0)	4.12* dd (9.0, 9.5)	4.16* dd (9.0, 9.0)			
5	3.90 ddd (2.4, 5.0, 9.0)	3.88 m	3.88 ddd (2.5, 6.0, 9.0)			
6a	4.33–4.35	4.33 dd (6.0, 12.5)	4.32 dd (6.0, 12.0)			
6b	4.54* dd (3.0, 10.0)	4.47 dd (3.0, 12.5)	4.48 dd (2.5, 12.0)			
fuc-1				4.82 d (8.0)	4.82 d (7.5)	4.82 d (8.0)
2				4.53 dd (8.0, 9.5)	4.55 dd (7.5, 9.5)	4.52 dd (8.0, 9.5)
3				4.20 dd (4.0, 9.5)	4.20 dd (3.5, 9.5)	4.19* dd (3.0, 9.5)
4				3.92 m	3.92 d (3.5)	3.90 m
5				3.82 dq (0.5, 6.5)	3.82 dq (0.7, 6.5)	3.83 q (6.5)
6				1.52 d (6.5)	1.53 d (6.5)	1.52 d (6.5)
rha-1	6.46 d (1.6)	5.57 d (2.0)	5.59 d (1.5)	6.35 d (1.5)	6.35 d (1.5)	6.35 d (1.5)
2	5.32 brs	6.06 dd (2.0, 3.5)	6.07 dd (1.5, 3.5)	5.32 dd (1.5, 2.5)	5.32 dd (1.5, 3.0)	5.25 brs
3	5.62 dd (3.0, 9.8)	5.04 dd (3.5, 9.0)	5.09 dd (3.5, 9.0)	5.60 dd (2.5, 9.8)	5.61 dd (3.0, 9.5)	5.66 dd (3.0, 10.0)
4	4.69 t (9.8)	4.21 dd (9.0, 9.0)	4.24* dd (9.0, 9.5)	4.65 dd (9.8, 9.8)	4.64 dd (9.5, 9.5)	4.68 t (10.0, 10.0)
5	5.10 dq (6.4, 9.8)	4.38* dq (6.0, 9.0)	4.38* dq (6.0, 9.5)	5.00 dq (6.0, 9.8)	5.03 dq (6.0, 9.5)	4.95 brs
6	1.72 d (6.4)	1.58 d (6.0)	1.58 d (6.0)	1.57 d (6.0)	1.58 d (6.0)	1.59 d (6.0)
rha′-1	5.68 d (1.6)	6.13 d (2.0)	6.16 d (1.5)	5.60 d (2.0)	5.66 d (1.5)	5.63 d (1.5)
2	5.84 dd (1.6, 3.0)	5.96 dd (2.0, 3.0)	5.97 dd (1.5, 3.5)	5.78 dd (2.0, 3.0)	5.82 dd (1.5, 3.5)	6.00 dd (1.5, 3.5)
3	4.54* dd (3.0, 3.0)	4.59 dd (3.0, 9.0)	4.61 dd (3.5, 9.0)	4.50 (3.0, 9.0)	4.52* dd (3.5, 9.5)	4.63 dd (3.5, 9.0)
4	4.33 dd (9.0, 9.0)	4.25 dd (9.0, 9.5)	4.27 dd (9.0, 9.5)	4.23 dd (9.0, 9.5)	4.25* dd (9.5, 9.5)	4.35 dd (9.5, 9.5)
5	4.37 dq (6.0, 9.0)	4.32 dq (6.0, 9.5)	4.38* dq (6.0, 9.5)	4.33–4.35* dq (6.0, 9.5)	4.34 dq (6.0, 9.5)	4.31 dq (6.0, 9.5)
6	1.62 d (6.0)	1.64 d (6.0)	1.65 d (6.0)	1.59 d (6.0)	1.60 d (6.0)	1.62 d (6.0)
rha″-1	5.93 brs	5.88 brs	5.91 d (1.0)	5.88 d (1.5)	5.92 brs	6.21 d (2.0)
2	4.63 dd (1.3, 3.3)	4.70 brs	4.71 dd (1.0, 3.0)	4.63 brs	4.63 dd (1.5, 3.5)	4.89 dd (2.0, 3.0)
3	4.42 dd (3.3, 9.7)	4.47 dd (3.0, 9.0)	4.47 dd (3.0, 9.5)	4.42 dd (3.0, 9.5)	4.41 dd (3.5, 9.5)	4.45 dd (3.0, 9.0)
4	5.78 t (9.7)	5.79 t (9.0)	5.82 t (9.5)	5.80 t (9.5)	5.78 t (9.5)	5.73 t (9.0, 9.0)
5	4.33–4.35*	4.38* dq (6.0, 9.0)	4.36 dq (6.5, 9.5)	4.33–4.35* dq (6.0, 9.5)	4.36 dq (6.0, 9.5)	4.37 dq (6.0, 9.0)
6	1.40 d (6.3)	1.38 d (6.0)	1.40 d (6.5)	1.39 d (6.0)	1.39 d (6.0)	1.40 d (6.0)
rha‴-1	5.57 d (1.3)	5.62 d (1.5)	5.65 d (1.0)	5.57 d (1.5)	5.57 d (1.0)	
2	4.79 dd (1.3, 3.1)	4.83 brs	4.84 dd (1.0, 3.5)	4.80 brs	4.79 dd (1.0, 3.5)	
3	4.50 dd (3.1, 9.4)	4.40 dd (3.0, 9.0)	4.41 dd (3.5, 9.0)	4.46 dd (3.0, 9.5)	4.52 dd (3.5, 9.5)	
4	4.23 t (9.4)	4.23 dd (9.0, 9.0)	4.25* dd (9.5, 9.0)	4.26* m	4.25* dd (9.5, 9.5)	
5	4.27 dq (6.0, 9.4)	4.32* dq (6.0, 9.0)	4.38* dq (6.0, 9.5)	4.26* m	4.30 dq (6.0, 9.5)	
6	1.71 d (6.0)	1.55 d (6.0)	1.57 d (6.0)	1.70 d (6.0)	1.71 d (6.0)	
glc-1						5.09 d (7.5)
2						3.96 t (7.5, 9.0)
3						4.14 *dd (9.0,9.0)
4						4.14 * dd (9.0, 9.0)
5						3.88 ddd (3.0, 6.0, 9.0)
6a						4.39 dd (6.0, 12.0)
6b						4.48 dd (3.0, 12.0)
jal-2a	2.25 ddd (3.8, 7.2, 13.6)	2.25 ddd (3.5, 8.0, 12.0)	2.25 ddd (3.5, 8.0, 12.0)	2.24 ddd (3.5, 7.9, 12.5)	2.27 ddd (2.8, 8.0, 11.5)	2.28 ddd (3.0, 7.8, 13.0)
2b	2.91 ddd (3.8, 7.2, 13.6)	2.40 ddd (3.5, 7.0, 12.0)	2.40 ddd (3.5, 8.0, 12.0)	2.83 ddd (3.5, 7.9, 12.5)	2.92 ddd (2.8, 8.0, 11.5)	2.70 dd (2.5, 13.0)
11	3.90 m	3.87 m	3.87 m	3.88 m	3.87 m	3.87 m
16	0.93 t (7.0)	0.83 t (7.0)	0.83 t (7.0)	0.93 t (7.0)	0.95 t (7.0)	0.94 t (7.0)
mba-2	2.50 tq (7.0, 7.0)	2.36 tq (7.0, 7.0)	2.37 tq (7.0, 7.0)	2.48 tq (7.0, 7.0)	2.50 tq (7.0, 7.0)	2.50 tq (7.0, 7.0)
2-Me	1.20 d (7.0)	1.07 d (7.0)	1.08 d (7.0)	1.21 d (7.0)	1.21 d (7.0)	1.20 d (7.0)
3-Me	0.94 t (7.5)	0.85 t (7.5)	0.85 t (7.0)	0.94 t (7.5)	0.94 t (7.5)	0.93 t (7.0)
mba′−2			2.50 tq (7.5, 7.5)			

Table 1. Continued

proton[b]	1	2	3	4	5	6
2-Me			1.20 d (7.0)			
3-Me			0.93 t (7.5)			
iba-2		2.63 sept (7.0, 7.0)		2.54 sept (7.0, 7.0)		
3		1.19 d (7.0)		1.12 d (7.0)		
3′		1.16 d (7.0)		1.17 d (7.0)		
dodeca-2	2.38 t (7.4)					2.42 t (7.5)
12	0.87 t (7.0)					0.87 t (7.5)
8-hydroxydodeca-2					2.42 t (7.5)	
12					0.87 t (7.0)	

[a] Data in C_5D_5. 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 signal, sept = septet. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jal = 11-hydroxyhexadecanoyl; mba = 2-methylbutanoyl; iba = 2-methylpropanoyl; dodeca = dodecanoyl; 8-hydroxydodeca = 8-hydroxydodecanoyl.

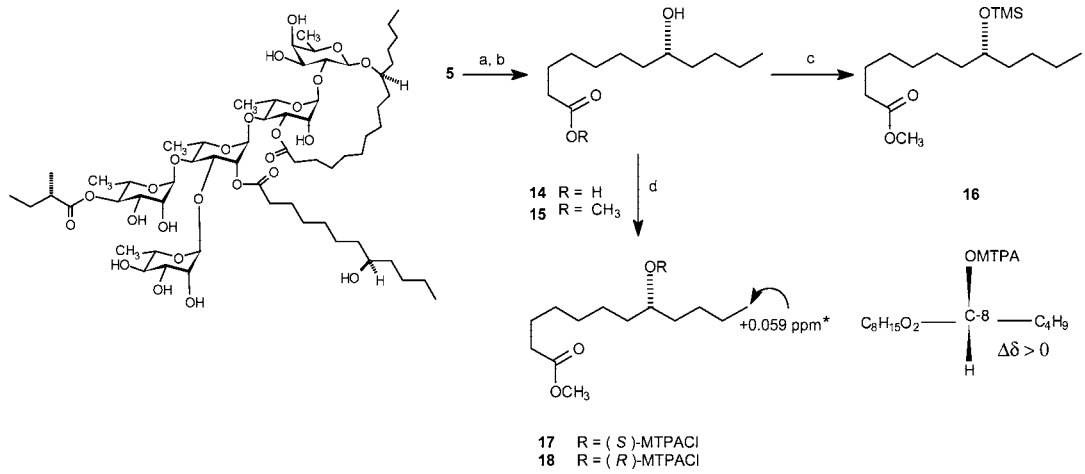
Table 2. ^{13}C NMR Data of Compounds **1**−**6** (125 MHz)[a]

carbon[b]	1	2	3	4	5	6
glc-1	101.4	104.5	104.6			
2	75.3	82.0	82.0			
3	79.8	76.5	76.5			
4	72.1	71.9	71.9			
5	79.8	77.9	78.0			
6	62.8	62.8	62.8			
fuc-1				101.6	101.6	101.5
2				73.4	73.4	73.5
3				76.7	76.7	76.5
4				73.6	73.6	73.5
5				71.3	71.3	71.2
6				17.2	17.2	17.2
rha-1	100.3	98.8	98.8	100.3	100.3	100.1
2	69.7	73.7	73.7	69.8	69.8	69.9
3	77.8	69.9	70.0	77.9	77.8	77.9
4	78.1	79.8	79.8	77.4	78.0	76.5
5	68.0	68.7	68.5	67.9	67.9	68.0
6	19.4	19.3	19.4	19.2	19.2	19.2
rha′-1	99.2	99.3	99.1	99.1	99.2	99.3
2	72.9	73.0	73.1	72.8	73.0	72.3
3	80.0	79.8	79.8	79.8	80.2	80.2
4	79.8	80.1	80.1	79.4	79.2	78.6
5	68.3	68.2	68.2	68.4	68.3	67.9
6	18.7	18.8	18.8	18.8	18.8	18.7
rha″-1	103.7	103.8	103.8	103.9	103.7	103.4
2	72.6	72.7	72.8	72.6	72.6	72.3
3	70.2	70.2	70.2	70.2	70.2	70.2
4	74.8	74.9	74.8	74.8	74.8	75.2
5	68.1	68.5	68.7	68.2	68.2	68.1
6	17.9	17.8	17.9	17.9	17.9	18.0
rha‴-1	104.3	104.9	104.9	104.4	104.3	
2	72.6	72.5	72.5	72.6	72.7	
3	72.5	72.5	72.5	72.6	72.5	
4	73.7	73.5	73.5	73.6	73.7	
5	70.8	68.7	68.7	70.7	70.8	
6	18.7	18.6	18.6	18.8	18.8	
glc-1						104.8
2						75.2
3						78.5
4						70.7
5						79.5
6						62.5
jal-1	174.9	173.4	173.3	174.8	174.9	174.6
2	33.7	34.3	34.3	33.8	33.7	34.1
11	79.4	82.8	82.8	79.4	79.3	79.5
16	14.4	14.5	14.3	14.4	14.4	14.4
mba-1	176.3	175.5	175.5	176.3	176.3	176.3
2	41.6	41.5	41.5	41.6	41.6	41.5
2-Me	17.0	16.1	16.8	17.0	17.0	16.9
3-Me	11.8	11.8	11.7	11.8	11.8	11.7
mba′-1			176.3			
2			41.5			
2-Me			17.0			
3-Me			11.7			
iba-1		176.9		176.0		
2		34.5		34.2		
3		19.3		19.1		
3′		19.1		19.1		
dodeca-1	172.9					173.5
2	34.4					34.5
12	14.3					14.3
8-hydroxydodeca-1					172.9	
2					34.4	
7					38.4	
8					75.2	
9					38.3	
12					14.3	

[a] Data in C_5D_5. Chemical shifts (δ) are in ppm relative to TMS. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jal = 11-hydroxyhexadecanoyl; mba = methylbutanoyl; iba = methylpropanoyl; dodeca = dodecanoyl; 8-hydroxydodeca = 8-hydroxydodecanoyl.

tetrasaccharides, tricolorins A and E (SA-1199B, MIC 8 μg/mL), and their peracetylated derivatives (SA-1199B, MIC >256 μg/mL)

Scheme 1[a]



[a] Key: (a) KOH, (b) CH_2N_2, (c) TMSiCl, pyridine, 70 °C, 15 min, (d) (*R*)-MTPACl or (*S*)-MTPACl, DMAP, pyridine, 70 °C, 5 h. *$\Delta\delta = \delta$(**17**) − δ(**18**).

Table 3. Susceptibility of *Staphylococcus aureus* to Selected Convolvulaceous Oligosaccharides and their Cytotoxicity[a]

	ED_{50} (μg/mL)			MIC (μg/mL)				
							SA-1199B[b]	
compound	KB	Hep-2	HeLa	ATCC 25923	XU-212	EMRSA-15	Nor (−)	Nor (+)
1	>20	>20	>20	>512	512	256	32	8[c]
2	>20	>20	>20	>512	512	256	32	8[c]
3	17.9	14.2	16.4	>512	>512	>512	>512	8
4	>20	10.2	12.4	>512	>512	>512	>512	8
5	15.8	>20	>20	>512	>512	>512	>512	8
6	12.1	>20	>20	>512	>512	>512	>512	32
7	15.7	4.7	>20	>512	>512	>512	>512	32
8	>20	>20	>20	>512	256	512	64	8[c]
9	>20	>20	>20	>512	512	256	32	8[c]
10	>20	>20	16.0	>512	>512	>512	>512	8
11	15.4	10.7	>20	>512	>512	>512	>512	8
12	15.0	4.0	>20	>512	>512	>512	>512	8
13	>20	>20	13.2	>512	256	128	64	8[c]
orizabin IX	>20	>20	5.2	>512	>512	256	512	<2[d]
tricolorin A	1.0	1.6	4.7	16	8	4	8	32[e]
tricolorin E	10.6	>20	1.0	16	8	8	8	32[e]
tetracycline				0.125	64	0.125	0.25	
norfloxacin				0.5	8	0.25		32
reserpine								8[f]
vinblastine	0.003	0.007	0.008					

[a] Abbreviations: KB = nasopharyngeal carcinoma; Hep-2 = laryngeal carcinoma; HeLa = cervix carcinoma; ATCC 25923 = standard *S. aureus* strain; XU-212 = a methicillin-resistant *S. aureus* strain possessing the TetK tetracycline efflux protein; EMRSA-15 = epidemic methicillin-resistant *S. aureus* strain containing the *mecA* gene; SA-1199B = multidrug-resistant *S. aureus* strain overexpressing the NorA efflux pump. [b] Nor (−) = minimum inhibitory concentration (MIC) value determined in the susceptibility testing; Nor (+) = MIC value determined for norfloxacin in the modulation assay at the concentration of 25 μg/mL of the tested oligosaccharide. [c] MIC value for norfloxacin in the modulation assay at the concentration of 5 μg/mL of the tested oligosaccharide. [d] MIC value for norfloxacin in the modulation assay at the concentration of 1 μg/mL of the tested oligosaccharide. [e] MIC value for norfloxacin in the modulation assay at the subinhibitory concentration of 2 μg/mL of the tested oligosaccharide. [f] MIC value for norfloxacin in the modulation assay at the concentration of 20 μg/mL of reserpine, which was used as positive control for an efflux pump inhibitor.

was tested in order to compare their effects with those obtained for the orizabin[9] and murucoidin series. The antimicrobial tricolorins and their peracetylated derivatives exhibited no modulatory activity at the subinhibitory concentration of 2 μg/mL for the natural products and 25 μg/mL for the derivatives. The loss of activity for the highly acylated compounds is a comparable situation to the inactivity recorded for the polar analogue[9] since the cellular uptake is not facilitated, probably in this case, by the formation of complex aggregates or micelles that could induced membrane perturbation, provoking an imbalance in the maintenance of cellular homeostasis.[32] Surprisingly, the antimicrobial tricolorins A and B did not display a potentiation effect in combination with norfloxacin. It seems possible that compounds that are nonpolar will not interact with the membrane efflux pumps and those that are too polar are poorly membrane soluble. Therefore, convolvulaceous plants may elaborate an array of amphipathic mixtures of glycolipids to confer selective advantages against microbial infections through a combination of several mechanisms of action; for example, the tricolorin series are cytotoxic, while the murucoidin series exert their action through inhibition of multidrug resistance pumps (Table 3), as previously reported for the structurally related acylated disaccharides found in the leaf exudates of *Geranium* species (Geraniaceae).[33] The most important result from the standpoint of the murucoidins' potential use as therapeutic agents is that by combining these plant noncytotoxic products with commercial antibiotics, which are substrates for these MDR pumps, the treatment of refractive infections caused by effluxing staphylococci could be alleviated. The use of bacterial resistance modifiers such as this type of complex oligosaccharides as prototypes for new efflux pump inhibitors could facilitate the reintroduction of therapeutically ineffective antibiotics into clinical use (e.g., ciprofloxacin) and might even contribute to the suppression of the emergence of new multidrug-resistant bacterial strains, which are a major cause of clinical infections.[10,34] Extracts that have been standardized on these mixtures of glycosides (e.g., orizabins) would also find utility in the development of antiseptic creams to replace or be used in

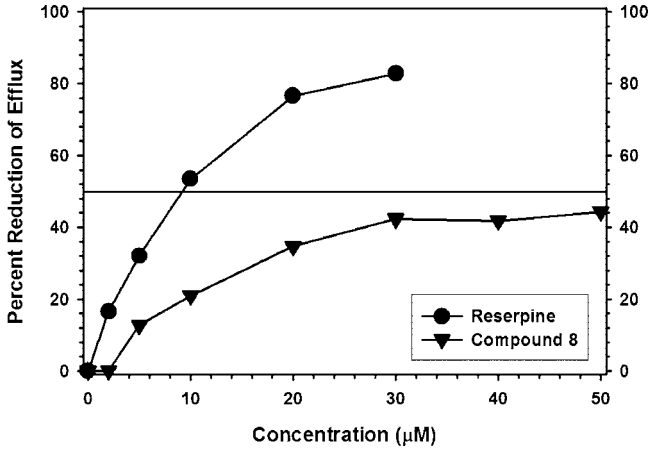


Figure 1. Ethidium efflux inhibition assay against SA-1199B strain cells. The horizontal line indicates 50% efflux inhibition.

combination with mupirocin and fusidic acid, which have wide usage in containment of *S. aureus* and its methicillin-resistant variants in the clinical setting.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR experiments were conducted on a Bruker DMX-500 instrument. The NMR techniques were performed according to a previously described methodology.[18,21] Negative-ion LRFABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX-102A spectrometer. The instrumentation used for HPLC analysis consisted of a Waters 600 E multisolvent delivery system equipped with a Waters 410 differential refractometer detector (Waters Corporation, Milford, MA). Control of the equipment, data acquisition, processing, and management of the chromatographic information were performed by the Empower 2 software program (Waters). GC-MS was performed on a Hewlett-Packard 5890-II instrument coupled to a JEOL SX-102A spectrometer. GC conditions: HP-5MS (5%-phenyl)-methylpolysiloxane column (30 m × 0.25 mm, film thickness 0.25 μm); He, linear velocity 30 cm/s; 50 °C isothermal for 3 min, linear temperature gradient to 300 at 20 °C/min; final temperature hold, 10 min. MS conditions: ionization energy, 70 eV; ion source temperature, 280 °C; interface temperature, 300 °C; scan speed, 2 scans s^{-1}; mass range, 33−880 amu.

Plant Material. Flowers of *Ipomoea murucoides* were collected at the campus of the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico, on December 12, 2004. The voucher specimens were archived at the Departamento de Farmacia, Facultad de Química, UNAM. Macroscopic anatomical features enabled the collected material to be identified by one of the authors (R.P.-M.) through comparison with a voucher specimen deposited at the HUMO herbarium collection (voucher no. 1520).

Extraction and Isolation. The whole plant material (425 g) was powdered and extracted exhaustively by maceration at room temperature with $CHCl_3$ to afford, after removal of the solvent, a dark brown syrup (39 g). The crude mixture of resin glycosides was obtained after fractionation of this extract by open column chromatography over silica gel eluted with a gradient of MeOH in $CHCl_3$. A total of 220 fractions (250 mL each) were collected and combined to give a pool containing a mixture of resin glycosides, which was subjected to fractionation by open column chromatography over reversed-phase C_{18} (330 g) eluted with MeOH to eliminate waxes and pigmented residues. This process provided 30 secondary fractions (30 mL each). Subfractions 19−25 were combined to yield a mixture of lipophilic pentasaccharides (20 g), which were analyzed by reversed-phase C_{18} HPLC using an isocratic elution with CH_3CN−MeOH (9:1). For their resolution, a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm), a flow rate of 9 mL/min, and a differential refractometer detector were used. This analysis allowed the comparison with reference solutions of the previously reported resin glycosides,[5,19] confirming the detection of the following compounds: murucoidin V (**13**, t_R 6.9 min), stoloniferin I (**8**, t_R 7.3 min), murucoidin I (**9**, t_R 13.0 min), pescaprein III (**7**, t_R 16.3 min), murucoidin II (**10**, t_R 21.9 min), murucoidin III (**11**, t_R 24.6 min), and murucoidin IV (**12**, t_R 30.3 min). The eluates across the peaks with t_R values of 7.9 min (peak I), 9.6 min (peak II), 14.3 min (peak III), 17.9 min (peak IV), 18.4 min (peak V), and 22.4 min (peak VI) were collected by the technique of heart cutting and independently reinjected in the apparatus operating in the recycle mode[18,35] to achieve total homogeneity after 15 consecutive cycles. These techniques afforded pure compounds **5** (29 mg) from peak II, **1** (3 mg) from peak III, **2** (3.1 mg) from peak IV, **6** (4.2 mg) from peak V, and **3** (3.0 mg) from peak VI. An isocratic elution with CH_3CN−H_2O (7:3) was used for the resolution of peak I from a complex mixture of related minor oligosaccharide to afford pure major compound **4** (t_R = 24.4 min, 3.2 mg).

Murucoidin VI (1): white powder; mp 143−145 °C; $[\alpha]_D$ −38 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1265 $[M - H]^-$, 1181 $[M - H - C_5H_8O]^-$, 1083 $[M - H - C_{12}H_{22}O]^-$, 853 $[1083 - C_6H_{10}O_4$ (methylpentose) $- C_5H_8O]^-$, 561 $[853 - 2 \times 146 (C_6H_{10}O_4)]^-$, 433 $[561 - 128]^-$, 271 $[Jal - H]^-$; HRFABMS *m/z* 1265.7256 $[M - H]^-$ (calcd for $C_{63}H_{109}O_{25}$ requires 1265.7258).

Murucoidin VII (2): white powder; mp 141−143 °C; $[\alpha]_D$ −39 (*c* 0.11, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1153 $[M - H]^-$, 1083 $[M - H - C_4H_6O]^-$, 937 $[1083 - C_6H_{10}O_4]^-$, 791 $[937 - C_6H_{10}O_4]^-$, 561, 433, 271; HRFABMS *m/z* 1153.6000 $[M - H]^-$ (calcd for $C_{55}H_{93}O_{25}$ requires 1153.6006).

Murucoidin VIII (3): white powder; mp 150−152 °C; $[\alpha]_D$ −20 (*c* 0.09, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1167 $[M - H]^-$, 1083 $[M - H - C_5H_8O]^-$, 937 $[1083 - C_6H_{10}O_4]^-$, 853 $[937 - C_5H_8O]^-$, 561, 433, 271; HRFABMS *m/z* 1167.6159 $[M - H]^-$ (calcd for $C_{56}H_{95}O_{25}$ requires 1167.6162).

Murucoidin IX (4): white powder; mp 156−158 °C; $[\alpha]_D$ −74 (*c* 0.07, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1137 $[M - H]^-$, 1053 $[M - H - C_5H_8O]^-$, 991 $[M - H - C_6H_{10}O_4]^-$, 921 $[991 - C_4H_6O]^-$, 837 $[921 - C_5H_8O]^-$, 545 $[837 - 2 \times 146 (C_6H_{10}O_4)]^-$, 417 $[545 - 128]^-$, 271 $[Jal - H]^-$; HRFABMS *m/z* 1137.6051 $[M - H]^-$ (calcd for $C_{55}H_{93}O_{24}$ requires 1137.6057).

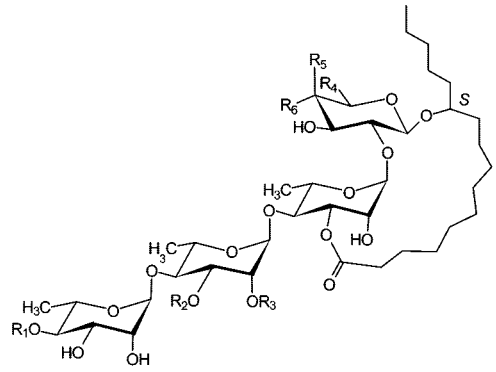
Murucoidin X (5): white powder; mp 128−130 °C; $[\alpha]_D$ −53 (*c* 0.15, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1265 $[M - H]^-$, 1181 $[M - H - C_5H_8O]^-$, 1067 $[M - H - C_{12}H_{22}O_2]^-$, 691 $[1067 - 2 \times 146 (C_6H_{10}O_4) - C_5H_8O]^-$, 545 $[691 - 146 (C_6H_{10}O_4)]^-$, 417, 271; HRFABMS *m/z* 1265.7254 $[M - H]^-$ (calcd for $C_{63}H_{109}O_{25}$ requires 1265.7258).

Murucoidin XI (6): white powder; mp 156−158 °C; $[\alpha]_D$ −50 (*c* 0.32, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1265 $[M - H]^-$, 1181 $[M - H - C_5H_8O]^-$, 1083 $[M - H - C_{12}H_{22}O]^-$, 921 $[1083 - 162 (C_6H_{10}O_5) - C_5H_8O]^-$, 545 $[921 - 2 \times 146 (C_6H_{10}O_4) - C_5H_8O]^-$, 417, 271; HRFABMS *m/z* 1265.7253 $[M - H]^-$ (calcd for $C_{63}H_{109}O_{25}$ requires 1265.7258).

Alkaline Hydrolysis of Resin Glycoside Mixture. A solution of the crude resin glycoside mixture (100 mg) in 5% KOH−H_2O (5 mL) was refluxed at 95 °C for 3 h. The reaction mixture was acidified to pH 4.0 and extracted with $CHCl_3$ (30 mL). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The aqueous phase was extracted with *n*-BuOH (20 mL) and concentrated to dryness. The residue from the organic phase was directly analyzed by GC-MS to allow the detection of three peaks. MS for the minor constituents with <5% of total chromatogram integration were not recorded. The major constituents were identified as 2-methylpropanoic acid (t_R 4.12 min), *m/z* $[M]^+$ 88 (10), 73 (27), 60 (3), 55 (5), 45 (7), 43 (100), 41 (40), 39 (10), 29 (6), 27 (24); 2-methylbutanoic acid (t_R 7.2 min), *m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8); and *n*-dodecanoic acid (t_R 17.8 min), *m/z* $[M]^+$ 200 (15), 183 (2), 171 (18), 157 (40), 143 (10), 129 (48), 115 (20), 101 (15), 85 (33), 73 (100), 60 (80), 57 (30), 55 (47), 43 (44), 41 (30). Previously reported procedures[18] were used for the preparation and identification of 4-bromophenacyl (2*S*)-2-methylbutyrate from the resin glycoside fraction: mp 41−42 °C; $[\alpha]_D$ +18.2 (*c* 1.0, MeOH).

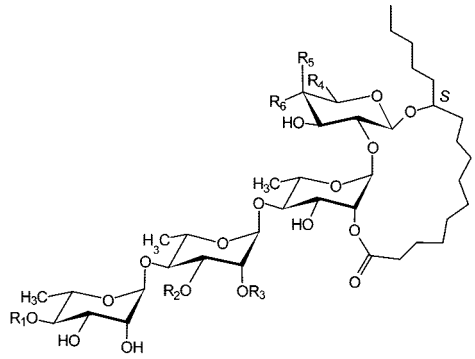
The residue extracted (35 mg) from the aqueous phase was subjected to preparative HPLC on a Waters μBondapak NH_2 column (7.8 × 300 mm; 10 μm). The elution was isocratic with CH_3CN−H_2O (4:1), using a flow rate of 4 mL/min and a sample injection of 500 μL (35 mg/mL). This procedure yielded simonic acid A (6.3 mg, t_R = 4.8 min), operculinic acid C (8.2 mg, t_R = 10.9 min), and simonic acid B (17.0

Chart 1



	R_1	R_2	R_3	R_4	R_5	R_6
1	(2*S*)-methylbutanoyl = mba	α-L-rhamnopyranosyl = rha	*n*-dodecanoyl = dodeca	CH_2OH	H	OH
4	methylpropanoyl = iba	rha	mba	CH_3	OH	H
5	mba	rha	(8*R*)-hydroxydodecanoyl	CH_3	OH	H
6	mba	β-D-glucopyranosyl = glc	dodeca	CH_3	OH	H
7	mba	rha	dodeca	CH_3	OH	H
8	mba	rha	mba	CH_3	OH	H
13	mba	glc	mba	CH_3	OH	H

Chart 2



	R_1	R_2	R_3	R_4	R_5	R_6
2	iba	rha	mba	CH_2OH	H	OH
3	mba	rha	mba	CH_2OH	H	OH
9	H	rha	mba	CH_3	OH	H
10	iba	rha	mba	CH_3	OH	H
11	mba	rha	mba	CH_3	OH	H
12	mba	glc	mba	CH_3	OH	H

mg, t_R = 17.0 min), which were identified by comparison of their physical constants and NMR data with published values.

Sugar Analysis. A solution of fractions IV and V (15 mg) in 4 N HCl (10 mL) was heated at 90 °C for 2 h. The reaction mixture was diluted with H_2O (5 mL) and extracted with Et_2O (30 mL). The aqueous phase was neutralized with 1 N KOH, extracted with *n*-BuOH (30 mL), and concentrated to give a colorless solid (5.7 mg). The residue was dissolved in CH_3CN-H_2O and directly analyzed by HPLC: Waters standard column for carbohydrate analysis (μBondapak NH_2; 3.9 × 300 mm, 10 μm), using an isocratic elution of CH_3CN-H_2O (17:3), a flow rate of 1 mL/min, and a sample injection of 20 μL (sample concentration: 5 mg/mL). Co-elution experiments with standard carbohydrate samples allowed the identification of rhamnose (t_R = 5.9 min), fucose (t_R = 7.7 min), and glucose (t_R = 10.1 min). Each of these eluates was individually collected, concentrated, and dissolved in H_2O. Optical activity was recorded after stirring the solutions for 2 h at room temperature: L-rhamnose $[\alpha]_{598}$ +8, $[\alpha]_{578}$ +8, $[\alpha]_{546}$ +9, $[\alpha]_{436}$ +15, $[\alpha]_{365}$ +21 (*c* 0.1, H_2O); D-fucose $[\alpha]_{598}$ +81, $[\alpha]_{578}$ +83, $[\alpha]_{546}$ +94, $[\alpha]_{436}$ +155, $[\alpha]_{365}$ +236 (*c* 0.1, H_2O); D-glucose $[\alpha]_{598}$ +50, $[\alpha]_{578}$ +51, $[\alpha]_{546}$ +57, $[\alpha]_{436}$ +97, $[\alpha]_{365}$ +150 (*c* 0.1, H_2O).

Alkaline Hydrolisis of Murucoidin X (5). Approximately 22 mg of **5** was dissolved in 1 mL of MeOH and 5% $KOH-H_2O$ (4 mL). The solution was refluxed at 95 °C for 2 h. The reaction mixture was acidified to pH 4.0 and extracted three times with 5 mL of Et_2O. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. A small aliquot was directly analyzed by GC-MS with two peaks detected. These were 2-methylbutyric acid (t_R 8.0 min) and 8-hydroxydodecanoic acid (**14**, t_R 25.0 min): *m/z* $[M - OH - H_2O]^+$ 181 (8), 115 (13), 97 (17), 87 (19), 83 (8), 73 (84), 69 (15), 60 (100), 57 (40), 55 (35), 45 (15), 43 (27), 41 (30). The remaining ether extract was reacted with excess diazomethane, concentrated, and analyzed by normal-phase HPLC; for the resolution of this reaction mixture, a normal-phase column (19 × 150 mm, 10 μm), an isocratic elution with hexane−EtOAc (4:1), a flow rate of 2 mL/min, and a differential refractometer were used. The eluates across the peaks with t_R value of 6 min (2-methylbutyric acid methyl ester, $[\alpha]_D$ +10 (*c* 0.1, $CHCl_3$)) and 12.5 min (8-hydroxydodecanoic acid methyl ester (**15**)) were collected and concentrated to dryness. Compound **15** was dissolved in 0.25 mL of pyridine-d_5 and divided into two portions. An aliquot was derivatized with Sigma Sil-A for 15 min at 70 °C. GC-MS analysis gave one peak (**16**, t_R 5.1 min): 274 $[M - 28]^+$ (1.2), 271 $[M - 31]^+$ (2.3), 255 (7), 245 (29), 216 (10), 159 (65), 141 (26), 103 (18), 95 (37), 75 (40), 73 (100). Half of the second aliquot was treated with 4-(dimethylamino)pyridine (3 mg, previously heated at 70 °C for 3 h) and dry pyridine-d_5 (0.75 mL) in NMR tubes.[25] (*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride was added (20 μL). The reaction was allowed to stand at 70−75 °C for 5 h under an atmosphere of N_2. NMR spectra were then recorded at 500 MHz by acquiring the reaction mixture. Further purification was performed as follows. The mixture was transferred from the NMR tubes into a vial. Saturated aqueous $NaHCO_3$ and Et_2O were added to the mixture and stirred vigorously for 5 min. Water (5 mL) was added and the mixture was extracted with $CHCl_3$. The organic phases were washed with 0.5 N HCl, dried with anhydrous Na_2SO_4, and concentrated. The crude residue was purified by normal-phase HPLC using an isocratic elution with hexane−EtOAc (7:3) and a flow rate of 3 mL/min to give the (*S*)-MTPA ester (**17**, t_R 7.5 min). NMR spectra in $CDCl_3$ were recorded after purification. Treatment of the remaining compound **15** with (*S*)-(−)-MTPA chloride as described above yielded the (*R*)-MTPA ester (**18**, t_R 6.9 min).

The aqueous phase from the saponification of compound **5** was extracted with *n*-BuOH (5 mL) and concentrated to give a colorless solid (5 mg). The residue was methylated with CH_2N_2, and the physical and spectroscopic constants (^1H and ^{13}C NMR) registered for the product were identical in all aspects to those previously reported[11] for simonic

acid B methyl ester: white powder; mp 113−115 °C; $[\alpha]_D$ −82.5 (*c* 1.0, MeOH); HRFABMS *m/z* 1015.5322 $[M - H]^-$ (calcd for $C_{47}H_{83}O_{23}$ requires 1015.5325).

(8*R*)-(−)-8-Hydroxydodecanoic Acid Methyl Ester (15): oil; $[\alpha]_{598}$ −15.6, $[\alpha]_{578}$ −16.3, $[\alpha]_{546}$ −16.8, $[\alpha]_{436}$ −18.8, $[\alpha]_{365}$ −20 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 3.67 (3H, s, OMe), 3.59 (1H, m, H-8), 2.34 (2H, t, $J = 7.5$ Hz, H-2), 1.64 (2H, m, H-3), 1.46−1.07 (14H, m, H_2-4-H_2-7, and H_2-9-H_2-11), 0.90 (3H, t, $J = 7.2$, H-12); HRESIMS *m/z* 229.1805 (calcd for $C_{13}H_{25}O_3$, 229.1803).

Bacterial Strains and Media. *Staphylococcus aureus* EMRSA-15 containing the *mecA* gene was provided by Dr. Paul Stapleton, The School of Pharmacy, University of London. Strain XU-212, a methicillin-resistant strain possessing the TetK tetracycline efflux protein, was provided by Dr. E. Udo.[36] SA-1199B, which overexpresses the NorA MDR efflux protein,[37] and *S. aureus* ATCC 25923 were also used. All strains were cultured on nutrient agar (Oxoid, Basingstoke, UK) before determination of MIC values. Cation-adjusted Mueller-Hinton broth (MHB; Oxoid) containing 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively, was used for susceptibility tests.

Susceptibility Testing. Minimum inhibitory concentration values (MIC) were determined at least in duplicate by standard microdilution procedures, as recommended by the National Committee for Clinical Laboratory Standards guidelines.[38] An inoculum density of 5×10^5 cfu of each of the test strains was prepared in 0.9% saline by comparison with a McFarland standard. MHB (125 μL) was dispensed into 10 wells of a 96-well microtiter plate (Nunc, 0.3 mL volume per well). Glycolipids **1**−**13** were tested at final concentrations ranging from 1 to 512 μg/mL prepared by serial 2-fold dilutions. All test compounds were dissolved in DMSO before dilution into MHB for use in MIC determinations. The highest concentration of DMSO remaining after dilution (3.125% v/v) caused no inhibition of bacterial growth. The MIC was defined as the lowest concentration that yielded no visible growth. Tetracycline and norfloxacin from Sigma (Poole, UK) were also tested as positive drug controls. For the modulation assay, the murucoidins were tested at final concentrations of 25 or 5 μg/mL, and the glycolipids orizabin IX and tricolorins A and E at 2 μg/mL. Serial doubling dilutions of norfloxacin in the range 1−512 μg/mL were added, and the microtiter plates were then interpreted in the same manner as MIC determinations. The activity of reserpine at a concentration of 20 μg/mL was also tested as an efflux pump inhibitor for comparison purposes. All samples were tested in duplicate.

Ethidium Efflux Assay. SA-1199B, which overexpresses NorA, was loaded with EtBr as previously described, and the effect of varying concentrations of compounds **8** and **11** on EtBr efflux efficiency was determined to generate a dose−response profile for each oligosaccharide.[37,39] The effect of reserpine was also determined as a positive control. Assays were performed in duplicate, and mean results were expressed as the percentage reduction of total efflux observed for SA-1199B in the absence of inhibitors. This was calculated as follows: [(efflux in the absence of inhibitor − efflux in the presence of inhibitor)/efflux in the absence of inhibitor] × 100.

Cytotoxicity Assay. Nasopharyngeal (KB), cervix (HeLa), and laryngeal carcinoma (Hep-2) cell lines were maintained in RMPI 1640 (10×) medium supplemented with 10% fetal bovine serum. Cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at log phase of their growth cycle were treated in triplicate with various concentrations of the test samples (0.16−20 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by the NCI sulforhodamine method.[40] Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percentage of viable cells. Vinblastine was included as a positive drug control.

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References and Notes

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(2) The Mexican term "cazahuate" is derived from the Nahuatl ("cuauhzahuatl") words for tree ("quauitl") and mange ("zahuatl"). This medicinal plant complex of arborescent morning glories is composed of six related tree-like *Ipomoea* species: *I. arborescens* (Kunth) G. Don, *I. bracteata* Cav., *I. intrapilosa* Rose, *I. murucoides* Roem. & Schult, *I. pauciflora* Martens & Galeotti, and *I. wolcottiana* Rose.
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(4) A medicinal plant complex consists of an assemblage of herbal drugs that are taxonomically different at the specific, generic, and/or familial level but that share a common name, one or more key morphological features, certain organoleptic characteristics, and one therapeutic application. For examples, see: (a) Linares, E.; Bye, R. *J. Ethnopharmacol.* **1987**, *19*, 153–183. (b) Pereda-Miranda, R.; Fragoso-Serrano, M.; Escalante-Sánchez, E.; Hernández-Carlos, B.; Linares, E.; Bye, R. *J. Nat. Prod.* **2006**, *69*, 1460–1466.
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(8) Indian and mestizo populations in Latin America traditionally classified foods, illnesses, medicine, and people as "hot" or "cold". A hot−cold imbalance must be redressed by the ingestion of contrary elements. For the hot−cold dichotomy, see: (a) López Austin, A. *The Human Body and Ideology. Concepts of the Ancient Nahuas*; University of Utah Press: Salt Lake City, UT, 1988; pp 270−282. (b) Ortiz de Montellano, B. R. *Aztec Medicine, Health, and Nutrition*; Rutgers University Press: New Brunswick, NJ, 1990; pp 213−235. (c) Foster, G. M. *Hippocrates' Latin American Legacy. Humoral Medicine in the New World*; Gordon and Breach: Langhorne, 1994; pp 165−195.
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