Resin Glycosides from the Flowers of *Ipomoea murucoides*†

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The $CHCl_3$-soluble extract from the flowers of the Mexican medicinal plant *Ipomoea murucoides*, through preparative-scale recycling HPLC, yielded murucoidins I−V (**1**−**5**), which are new pentasaccharides of jalapinolic acid, as well as the known stoloniferin I (**6**). Saponification of the crude resin glycoside mixture yielded two glycosidic acids, simonic acid B (**9**) and operculinic acid A (**10**), and their esterifying residues were composed of the two short-chain fatty acids, 2-methylpropanoic and (2*S*)-methylbutyric acids. All the isolated compounds (**1**−**6**) were characterized through high-field NMR spectroscopy. Compound **4** exhibited marginal cytotoxicity against Hep-2 cells (ED_{50} 4 μg/mL).

Ritual and therapeutic uses[1,2] of psychoactive convolvulaceous plants, as confirmed by the stylized depiction of a morning glory in a 500 A.D. Teotihuacan mural, were amply diffused throughout the entire pre-Hispanic world. This painting depicts Xochiquetzal, Mother Goddess of terrestrial waters, backed by a morning glory now interpreted as the sacred "ololiuhqui" of the Aztecs[3] (*Rivea corymbosa* or *Ipomoea tricolor*, hallucinogenic species containing lysergic acid derivatives[4]). In reality, the flowering plant with largely exaggerated branches seems to better resemble one of the arborescent species of *Ipomoea* only found in Mesoamerica and Mexico,[5] where in the State of Morelos they are called by the vernacular name of "cazahuate" (Nahuatl language, "tree for curing mange"), e.g., *Ipomoea murucoides* Roem. et Schult.

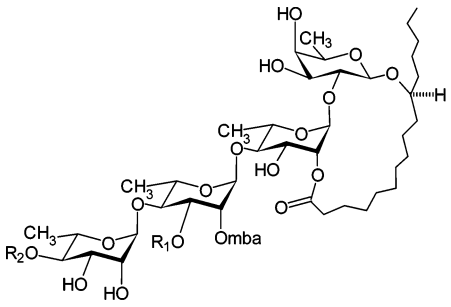
Two characteristics relate these species to the mural: (1) even today these tree-like plants are considered magical, for they flower exuberantly during the dry season, and according to popular legends, announce the onset of the rains in early summer; (2) traditional healers continue to use decoctions of this plant considered to be of "cold-nature"[6] to reduce excessive body heat[7] and relieve uncomfortable "water and cold" symptoms believed to be produced by abrupt climatic changes resulting in diseases considered to be "hot", e.g., pain caused by rheumatic conditions and inflammation.

One of the seven morning glory species illustrated in the Badianus Manuscript of 1552 (*Little Book of Indian Medicinal Herbs*) is a small tree-like plant called "xiuhamolli" (Nahuatl language, "soap plant") which was prescribed by Aztec healers as a decoction made in dog or deer urine to combat hair loss.[8] The miniature appears to represent a white-flowered arborescent *Ipomoea*, and the Latin legend beneath reads "capillorum profluvium" (hair loss). This therapeutic description suggests its botanical identification[8,9] as *I. murucoides* and appears to still be valid today, since the bark of the tree is continued to be boiled in water to prepare a lotion for rubbing onto the head.[10] The given name of "amole" in the manuscript[8] provides additional support for its identification, and in contemporary rural Mexico and Guatemala, "cazahuate" ashes are employed as a substitute for soap in washing clothes.[8,9]

Ipomoea murucoides has long been used to treat skin diseases[6] by washing the affected parts with a decoction of flowers, leaves, stem, and bark, and the same decoction is drunk to treat scorpion stings. The latex is applied directly to snake bites. Rheumatic complaints, bruises, and inflammation are treated with plasters or poultices of only flowers and leaves in a manner similar to that of "toloache" (*Datura stramonium*, jimsonweed). For abdominal swelling and stomachache, "cazahuate" leaves are boiled in water that could also be combined with the wood of "palo mulato" (*Zanthoxylum fagara*) and "cuajiote" (*Pseudosmodingium perniciosum*) and drunk before breakfast for 3 days.[10] To treat dropsy, "cazahuate" bark is boiled in water, whereas for a cough remedy one leaf of eucalyptus (*Eucalyptus camaldulensis* or *E. globulus*), another of "palo prieto" (*Ehretia tinifolia*), and the bark of "cuachalalate" (*Amphipterygium adstringens*) are added to the drink.[10] Hot baths of leaves are used for paralysis.[11] The smoke from burning the tree is used to repel mosquitoes.

Previous to this investigation, the only known study of the resin glycosides of *I. murucoides* was conducted from roots collected in the southern Mexican state of Oaxaca. In that study the structures of five pentasaccharides of jalapinolic acid (the murucin series) were elucidated with their linear oligosaccharide core assigned as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-quinovopyranoside (murucinic acid).[12]

As part of a continuing effort to elucidate the structural diversity of the convolvulaceous resin glycosides,[1] this paper describes the isolation of six lipophilic pentasaccharides of jalapinolic acid from the flowers of *I. murucoides*, the new murucoidins I−V (**1**−**5**), and the known stoloniferin I (**6**).



	R_1	R_2
1	Rhamnopyranosyl	H
2	Rhamnopyranosyl	2-Methylpropanoyl
3	Rhamnopyranosyl	(2*S*)-Methylbutanoyl = mba
4	Glucopyranosyl	(2*S*)-Methylbutanoyl

Results and Discussion

Flowers of *I. murucoides* were macerated with $CHCl_3$, and the extract was fractionated by column chromatography on silica gel. The major fraction rich in resin glycosides was saponified to liberate an organic solvent-soluble acid as well as a water-soluble glycosidic acid. GC-MS analysis of the hydrolyzed products recovered from

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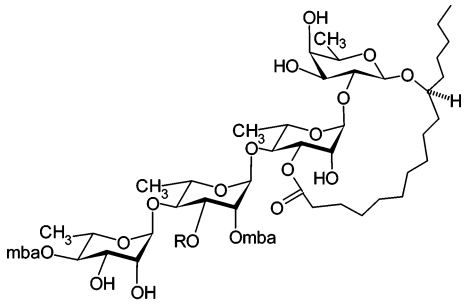
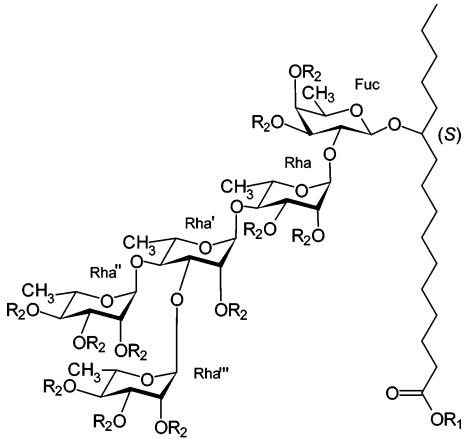
the organic-soluble fraction revealed the presence of 2-methylpropanoic (isobutyric) and (2*S*)-methylbutyric acids, which were identified by comparison of their mass spectra and retention times with those of authentic samples.[13]



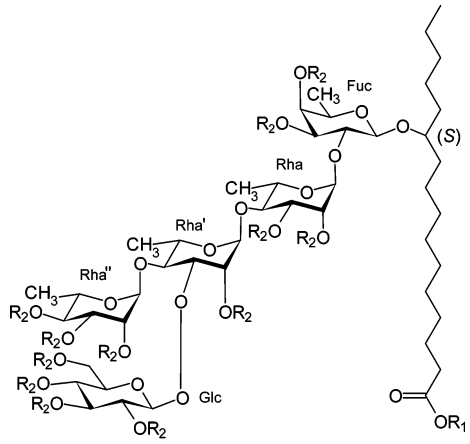
	R
5	Glucopyranosyl
6	Rhamnopyranosyl

The glycosidic acid fraction was methylated and further acetylated to give a residue that was separated on C_{18} reversed-phase HPLC, affording compounds **7** and **8**. The structures of these glycosidic acid derivatives were confirmed by ^{1}H and ^{13}C NMR spectroscopy. Compound **7** was identified as the peracetylated methyl ester derivative of simonic acid B, (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside (**9**), previously isolated from *I. batatas*[14] and *I. stolonifera*,[15] while compound **8** was identified as the corresponding derivative of 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 (**10**), previously obtained from *I. operculata*[16] and *I. leptophylla*.[17]



	R_1	R_2
7	CH_3	Ac
9	H	H

Negative-ion FABMS of compounds **1**−**5** gave pseudomolecular $[M - H]^-$ ions.[13,18] Murucoidin I (**1**) afforded a peak at *m*/*z* 1067, indicating a molecular formula of $C_{51}H_{88}O_{23}$, in contrast to the ion detected at *m*/*z* 1137 ($C_{55}H_{94}O_{24}$) for murucoidin II (**2**), corresponding to a difference of C_4H_6O between these compounds. Murucoidin III (**3**) showed the $[M - H]^-$ ion at *m*/*z* 1151 ($C_{56}H_{96}O_{24}$), a difference of one methylene group from its homologue **2**. Murucoidins IV (**4**) and V (**5**) showed the same $[M - H]^-$ ion at *m*/*z* 1167, and therefore these glycolipids could be assigned as diastereoisomeric compounds of molecular formula $C_{56}H_{96}O_{25}$. Common fragment peaks produced by glycosidic cleavage, similar to those reported for the pescaprein series,[13] were observed in all mass spectra, confirming the branched pentasaccharide core of murucoidins I−III (**1**−**3**) as that previously described[15] for simonic acid B (**9**). For murucoidins IV (**4**) and V (**5**), the observed difference of 16 mass units in the *m*/*z* 1167 $[M - H]^-$ and 1083 $[M - H - C_5H_8O]^-$ ions with corresponding peaks in compound **3** (*m*/*z* 1151 and 1067) corroborated their glycosidic core as operculinic acid A (**10**).[16]



	R_1	R_2
8	CH_3	Ac
10	H	H

Substitution patterns on each individual saccharide unit in murucoidins I−V (**1**−**5**) were studied by ^{1}H NMR spectroscopy. COSY and TOCSY techniques[1] made possible the assignment of chemical shift values for all C-bonded protons in each moiety (Table 1). ^{13}C NMR were assigned by HMQC studies[1] (Table 2). HMBC experiments[1] located the ester substituents through links between a specific carbonyl ester group with their vicinal proton resonance ($^2J_{CH}$) and the pyranose ring proton at the site of esterification ($^3J_{CH}$). The following spectroscopic features were observed: (a) the carbonyl resonance of the lactone functionality (δ 173) was assigned by the ^2J-coupling with each of the methylene protons at δ_H 2.2 (1H, ddd) and 2.38 (1H, ddd) on the adjacent C-2 position of jalapinolic acid in compounds **1**−**4**; the lactonization site at C-2 (δ_C 73.9) of the second saccharide (Rha) was established by the observed ^3J-coupling between this carbonyl carbon and its downfield shifted geminal proton (δ 5.9); (b) for compounds **5** and **6**, the lactonization at C-3 (δ_C 78) was corroborated (^3J-coupling) by the significant downfield shift[13] for this geminal proton (δ 5.6) in contrast to the same resonance in compounds **1**−**4**; (c) signals for a methylbutyroyl group esterifying position C-2 of the third saccharide unit (Rha′: δ_H 5.9−6.3; δ_C 72−73) were present in all murucoidins; (d) a second group of signals for one short-chain fatty acid residue esterifying position C-4 at Rha″ (δ_C 73−75) was found; H-2 of these moieties was used as a diagnostic resonance center at δ 2.6 (1H septet) for the isobutyroyl group in murucoidin II (**2**) and at δ 2.5 (1H, tq) for the methylbutyroyl group in compounds **1** and **3**−**6**.

Compounds **1**−**6** were subjected to a cytotoxicity evaluation using nasopharyngeal (KB) and laryngeal carcinoma (Hep-2) cells. Compound **4** exhibited marginal activity againts Hep-2 cells (ED_{50} 4 μg/mL). Compounds **1**−**3**, **5**, and **6** were inactive (ED_{50} >20 μg/mL). These cytotoxicity values were similar to those previously reported for other lipophilic resin glycosides.[12,13] It has been hypothesized that the cytotoxic properties of this type of compound could reflect their ability to perturb cell membranes and the function of multidrug efflux pumps.[19] Probably, the degree and type of acylation could have an influence on this activity, as observed for the more cytotoxic amphiphilic glycolipids (e.g., the tricolorin series).[1,20]

Table 1. ^{1}H NMR Data of Compounds **1**−**5** (500 MHz)[a]

proton[b]	1	2	3	4	5
fuc-1	4.74 d (7.5)	4.73 d (7.5)	4.70 d (7.4)	4.68 d (7.5)	4.82 d (7.9)
2	4.16 dd (7.5, 9.0)	4.16 dd (7.5, 9.5)	4.14 dd (7.4, 9.5)	4.12 dd (7.5, 9.4)	4.50 dd (7.9, 9.4)
3	4.08 dd (3.5, 9.5)	4.07 dd (3.0, 9.0)	4.04 dd (3.5, 9.5)	4.00 dd (3.0, 9.4)	4.19 dd (2.9, 9.4)
4	4.00 d (3.5)	3.99 d (3.0)	3.96 d (3.5)	3.93 d (3.0)	3.90*
5	3.78 dq (0.5, 6.5)	3.77 dq (1.0, 6.5)	3.74 dq (0.7, 6.2)	3.72 dq (0.5, 6.3)	3.82 dq (0.5, 6.3)
6	1.52 d (6.0)	1.51 d (6.0)	1.48 d (6.2)	1.48 d (6.3)	1.50 d (6.3)
rha-1	5.48 d (1.5)	5.48 d (1.5)	5.45 d (1.6)	5.48 brs	6.34 d (1.3)
2	5.95 dd (1.5, 3.0)	5.95 dd (1.5; 3.5)	5.92 dd (1.6, 3.3)	5.90 dd (1.0, 2.5)	5.22 brs
3	5.01 dd (3.0, 9.5)	5.02 dd (3.5, 9.0)	4.98 dd (3.3, 9.5)	4.97 dd (2.5, 9.5)	5.64 dd (2.7, 9.8)
4	4.22 dd (9.5, 9.5)	4.22 dd (9.0, 9.0)	4.18 dd (9.5, 9.5)	4.14 dd (9.4, 9.5)	4.66 dd (9.7, 9.7)
5	4.44 dq (9.5, 6.5)	4.42 dq (6.5, 9.0)	4.40 dq (6.2, 9.5)	4.45 dq (6.0, 9.5)	4.88 brs
6	1.58 d (6.5)	1.62 d (6.0)	1.58 d (6.2)	1.62 d (6.0)	1.60 d (6.1)
rha′-1	6.11 d (1.5)	6.12 d (1.5)	6.08 d (1.7)	5.82 d (1.0)	5.58 d (1.3)
2	5.97 dd (1.5, 3.0)	5.97 dd (1.5, 3.0)	5.94 dd (1.7, 3.0)	6.30 dd (1.0, 3.0)	6.00 dd (1.6, 3.3)
3	4.60 dd (3.0, 9.0)	4.61 dd (3.0, 9.0)	4.58 dd (3.0, 9.0)	4.70 dd (3.0, 9.2)	4.56 dd (3.4, 9.0)
4	4.27 dd (9.0, 9.0)	4.25 dd (9.0, 9.0)	4.24 dd (9.0, 9.0)	4.27 dd (9.2, 9.4)	4.23 dd (9.2, 9.2)
5	4.31 dq (6.0, 9.0)	4.38 dq (6.0, 9.5)	4.32 dq (6.0, 9.0)	4.36 dq (6.1, 9.4)	4.32 dq (6.2, 9.2)
6	1.63 d (6.0)	1.66 d (6.0)	1.62 d (6.0)	1.64 d (6.1)	1.56 d (6.2)
rha″-1	5.92 d (1.0)	5.89 brs	5.86 d (1.0)	6.18 d (1.3)	6.16 d (1.7)
2	4.75 dd (1.0, 4.0)	4.72 d (2.5)	4.68 dd (1.0, 3.0)	4.92 brs	4.88 brs
3	4.46 dd (4.0, 9.0)	4.50 dd (2.5, 9.5)	4.45 dd (3.0, 9.6)	4.52 dd (3.3, 9.2)	4.43 dd (3.2, 9.5)
4	4.30 dd (9.0, 9.0)	5.81 dd (9.5, 9.5)	5.78 dd (9.6, 9.6)	5.74 dd (9.3, 9.4)	5.72 dd (9.3, 9.3)
5	4.37 dq (6.0, 9.0)	4.36 dq (6.0, 9.5)	4.35 dq (6.0, 9.6)	4.36 dq (6.5, 9.4)	4.32 dq (6.6, 9.0)
6	1.66 d (6.0)	1.39 d (6.0)	1.37 d (6.0)	1.40 d (6.5)	1.38 d (6.3)
rha‴-1	5.66 d (1.5)	5.64 d (1.5)	5.60 d (1.5)		
2	4.89 dd (1.5, 2.5)	4.84 brs	4.80 dd (1.5, 2.5)		
3	4.42 dd (2.5, 9.0)	4.41 dd (2.5, 8.5)	4.38 dd (3.0, 8.9)		
4	4.26 dd (9.0, 9.0)	4.27 dd (8.5, 9.0)	4.21 dd (8.9, 9.4)		
5	4.31 dq (6.0, 9.0)	4.24 dq (6.0, 9.0)	4.19 dq (6.0, 9.4)		
6	1.56 d (6.0)	1.56 d (6.0)	1.53 d (6.0)		
glc-1				5.02 d (7.7)	5.05 d (7.6)
2				3.90 dd (8.0, 8.8)	3.90*
3				4.00*	4.12 dd (8.8, 8.9)
4				4.00*	4.14 dd (8.8, 9.1)
5				3.73 ddd (2.4, 5.8, 8.4)	3.84 ddd (3.4, 6.0, 9.0)
6				4.02 dd (5.9, 12.0)	4.31dd (6.1, 12.3)
				4.35 dd (2.4, 12.0)	4.44 dd (3.0, 12.0)
jla-2	2.22 ddd (4.0, 8.0, 13.5)	2.23 ddd (4.0, 8.5, 12.5)	2.21 ddd (3.9, 8.2, 14.2)	2.25 ddd (3.8, 7.9, 14.6)	2.23 ddd (2.8, 7.2, 14.6)
	2.38 ddd (4.0, 8.5, 13.5)	2.37 ddd (4.0, 8.5, 12.5)	2.37 ddd (3.9, 8.7, 14.2)	2.42 dd (3.9, 14.6)	2.55 dd (3.2, 14.6)
11	3.86 m	3.85 m	3.83 m	3.81 m	3.88 m
16	0.88 t (7.0)	0.88 t (7.0)	0.85 t (7.0)	0.85 t (7.0)	0.90 t (7.4)
Mba-2	2.35 tq (7.0, 7.0)	2.35 tq (7.0, 7.0)	2.32 tq (7.1, 7.0)	2.39 tq (6.9, 7.0)	2.44 tq (6.9, 7.0)
2-Me	1.06 d (7.0)	1.07 d (7.0)	1.04 d (7.0)	1.01 d (7.0)	1.10 d (6.9)
3-Me	0.84 t (7.5)	0.85 t (7.0)	0.82 t (7.4)	0.77 t (7.4)	0.83 t (7.4)
Mba′-2			2.47 tq (7.0, 7.0)	2.50 tq (6.9, 7.0)	2.48 tq (6.9, 7.0)
2-Me			1.18 d (7.0)	1.19 d (7.0)	1.18 d (7.0)
3-Me			0.91 t (7.4)	0.92 t (7.4)	0.92 t (7.4)
iba-2		2.63 sept (7.0)			
3		1.19 d (7.0)			
3′		1.17 d (7.0)			

[a] Data recorded in C_5D_5N. 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, brs = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet, sept = septet. All assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jla = 11-hydroxyhexadecanoyl; mba = 2-methylbutanoyl; iba = 2-methylpropanoyl.

Experimental Section

General Experimental Procedures. All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. ^{1}H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted on a Bruker AMX-500 instrument. The NMR techniques were performed according to previously described methodology.[18] Negative-ion LRFABMS and HRFABMS were recorded using a matrix of triethanolamine on a JEOL SX102A spectrometer.

Plant Material. Flowers of *Ipomoea murucoides* were collected at the campus of the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico, in November 1996. The voucher specimens were identified by the botanist Gustavo Soria Rocha and 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 (59 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 several pools containing mixtures of resin glycosides. Fractions 141−220 (21 g), eluted with $CHCl_3$−MeOH (9:1), were subjected to reversed-phase C_{18} column chromatography (330 g), using MeOH (900 mL) as solvent, to eliminate pigmented oily residues. The process was monitored by TLC, and a total of 30 fractions (30 mL each) were collected; these were combined (subfractions 19−25), yielding a mixture of lipophilic pentasaccharides (20 g).

Recycling HPLC Separation. The instrumentation used for preparative HPLC analysis was previously reported.[13] A preliminary separation of the crude fraction was performed on a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm). The elution was isocratic with CH_3CN−MeOH (9:1) using a flow rate of 9 mL/min. Subfractions I−VII across the peaks with t_R values of 7.2 min (peak I), 12.9 min

Table 2. ^{13}C NMR Data of Compounds **1**−**5** (125 MHz)[a]

carbon[b]	1	2	3	4	5
fuc-1	104.3	104.3	104.2	104.3	101.5
2	80.2	80.3	80.2	80.0	73.6
3	73.3	73.3	73.4	73.4	76.6
4	72.9	73.1	73.3	72.9	73.6
5	70.8	70.8	70.8	70.8	71.3
6	17.4	17.4	17.3	17.3	17.2
rha-1	98.8	98.8	98.7	98.5	100.1
2	73.9	73.9	73.9	73.6	70.0
3	69.9	69.9	69.9	69.4	78.3
4	79.9	79.9	79.9	81.4	75.7
5	68.6	68.6	68.6	68.9	68.0
6	19.4	19.5	19.4	19.1	19.2
rha′-1	99.2	99.2	99.2	100.0	99.1
2	73.1	73.1	72.9	73.0	72.2
3	79.6	79.6	79.5	80.2	80.2
4	79.9	80.1	80.1	78.9	78.8
5	68.5	68.2	68.2	68.2	68.1
6	18.7	18.8	18.8	18.9	18.7
rha″-1	103.9	103.8	103.8	103.3	103.4
2	72.8	72.7	72.7	72.5	72.4
3	72.7	70.2	70.2	70.3	70.2
4	73.9	74.9	74.7	75.2	75.2
5	70.8	68.5	68.5	68.4	68.1
6	18.3	17.8	17.8	18.0	18.0
rha‴-1	104.9	104.9	104.8		
2	72.5	72.5	72.5		
3	72.6	72.6	72.6		
4	73.5	73.5	73.4		
5	68.7	70.5	70.5		
6	18.5	18.5	18.5		
glc-1				105.5	105.1
2				75.2	75.2
3				78.5	78.1
4				71.5	70.8
5				78.0	77.8
6				62.9	62.6
jla-1	173.1	173.7	173.1	173.1	174.4
2	34.2	34.3	34.3	34.2	34.3
11	82.3	82.3	82.3	82.3	79.5
16	14.3	14.3	14.3	14.3	14.5
mba-1	175.5	175.5	175.5	176.3	176.1
2	41.5	41.5	41.4	41.2	41.3
2-Me	16.8	16.8	16.8	16.6	16.8
3-Me	11.8	11.8	11.7	11.4	11.6
mba′-1			176.3	176.3	176.3
2			41.5	41.5	41.5
2-Me			16.9	17.0	17.0
3-Me			11.7	11.8	11.7
iba-1		176.7			
2		34.5			
3		19.3			
3′		19.1			

[a] Data recorded in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HMQC and HMBC experiments. [b] Abbreviations: fuc = fucose; rha = rhamnose; glc = glucose; jla = 11-hydroxyhexadecanoyl; mba = 2-methylbutanoyl; iba = 2-methylpropanoyl.

(peak II), 21.7 min (peak III), 24.4 min (peak IV), and 30.1 min (peak V) were collected by the technique of heart cutting and independently reinjected in the apparatus operating in the recycle mode[21] to achieve total homogeneity after 20 consecutive cycles employing the same isocratic elution. These techniques afforded pure compound **1** (2 mg) from peak II; **2** (4 mg) from peak III; **3** (13 mg) from peak IV; and **4** (40 mg) from peak V. An isocratic elution with CH_3CN-H_2O (7:3) was used for the resolution of peak I to afford pure compounds **5** (t_R 26.9 min; 10 mg) and **6** (t_R 17.5 min; 6 mg).

Murucoidin I (1): amorphous white powder; mp 154−156 °C; $[\alpha]_D$ −46 (*c* 0.12, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m*/*z* 1067 $[M - H]^-$, 983 $[M - H - C_5H_8O]^-$, 921 $[M - H - C_6H_{10}O_4]^-$, 837 $[921 - C_5H_8O]^-$, 545, 417, 271; HRFABMS *m*/*z* 1067.5645 $[M - H]^-$ (calcd for $C_{51}H_{87}O_{23}$ requires 1067.5638).

Murucoidin II (2): amorphous white powder; mp 155−157 °C; $[\alpha]_D$ −55 (*c* 0.1, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m*/*z* 1137 $[M - H]^-$, 1065 $[M - H - C_4H_6O]^-$, 1053 $[M - H - C_5H_8O]^-$, 837, 545, 417, 271; HRFABMS *m*/*z* 1137.6058 $[M - H]^-$ (calcd for $C_{55}H_{93}O_{24}$ requires 1137.6056).

Murucoidin III (3): amorphous white powder; mp 150−153 °C; $[\alpha]_D$ −42 (*c* 0.2, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m*/*z* 1151 $[M - H]^-$, 1067 $[M - H - C_5H_8O]^-$, 837, 545, 417, 271; HRFABMS *m*/*z* 1151.6214 $[M - H]^-$ (calcd for $C_{56}H_{95}O_{24}$ requires 1151.6213).

Murucoidin IV (4): amorphous white powder; mp 157−159 °C; $[\alpha]_D$ −29 (*c* 0.15, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m*/*z* 1167 $[M - H]^-$, 1083 $[M - H - C_5H_8O]^-$, 1065 $[M - H - C_5H_8O - H_2O]^-$, 937 $[1083 - C_6H_{10}O_4]^-$, 545, 417, 271; HRFABMS *m*/*z* 1167.6167 $[M - H]^-$ (calcd for $C_{56}H_{95}O_{25}$ requires 1167.6162).

Murucoidin V (5): amorphous white powder; mp 148−150 °C; $[\alpha]_D$ −29 (*c* 0.14, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m*/*z* 1167 $[M - H]^-$, 1083 $[M - H - C_5H_8O]^-$, 1065 $[M - H - C_5H_8O - H_2O]^-$, 937 $[1083 - C_6H_{10}O_4]^-$, 545, 417, 271; HRFABMS *m*/*z* 1167.6162 $[M - H]^-$ (calcd for $C_{56}H_{95}O_{25}$ requires 1167.6162).

Stoloniferin I (6): amorphous white powder; mp 158−160 °C; $[\alpha]_D$ −78 (*c* 0.13, MeOH); HRFABMS *m*/*z* 1151.6215 $[M - H]^-$ (calcd for $C_{56}H_{95}O_{24}$ requires 1151.6213); identified by comparison of NMR data with published values.[15]

Alkaline Hydrolysis of the Resin Glycoside Fraction. A solution of the resin glycoside fraction (400 mg) in 5% KOH−H_2O (8 mL) was refluxed at 95 °C for 2 h. The reaction mixture was acidified to pH 4.0 and extracted with Et_2O (30 mL). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analyzed by GC-MS:[13,20] HP-5MS (5%-phenyl)-methylpolysiloxane column (30 m × 0.31 mm, film thickness 2 μm); He, 2 mL/min; 50 °C isothermal for 3 min, linear gradient to 300 °C at 20 °C/min; with two peaks detected. These were 2-methylpropanoic acid (t_R 4.0 min): *m*/*z* $[M]^+$ 88 (10), 73 (27), 60 (3), 55 (5), 45 (7), 43 (100), 41 (40), 39 (10), 29 (6), 27 (24); and 2-methylbutyric acid (t_R 8.0 min): *m*/*z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8). Previously reported procedures[21] 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 aqueous phase was extracted with *n*-BuOH (30 mL) and concentrated to give a colorless solid (320 mg). The residue (100 mg) was methylated with CH_2N_2 and further acetylated ($Ac_2O-C_5H_5N$, 2:1) to give a residue (120 mg) that was subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 mm). The elution was isocratic with CH_3CN−MeOH (95:5) using a flow rate of 9 mL/min. Eluates across the peaks with t_R values of 11.6 min (peak I) and 12.1 min (peak II) were again collected by heart cutting. Peaks I (15 mg) and II (10 mg) were independently reinjected in the apparatus operated in the recycle mode to achieve total homogeneity after 10 to 20 consecutive cycles employing the same isocratic elution. ^1H and ^{13}C NMR data for the isolated products allowed their identification as peracetylated derivatives of simonic acid B methyl ester (**7**) and operculinic acid A methyl ester (**8**).

Compound 7: mp 81−85 °C; $[\alpha]_D$ −37 (*c* 0.15, MeOH); ^1H NMR (C_5D_5N, 500 MHz) δ 1.21 (3H, d, J = 6.3 Hz, Fuc-6), 4.00 (2H, m, Fuc-5), 4.33 (1H, dd, J = 7.8, 10.0 Hz, Fuc-2), 4.96 (1H, d, J = 7.7 Hz, Fuc-1), 5.47−5.51 (4H, m, Fuc-3), 5.53−5.57 (3H, m, Fuc-4), 1.32 (3H, d, J = 6.2 Hz, Rha-6), 4.24−4.29 (4H, m, Rha-4), 5.47−5.51 (4H, m, Rha-5), 5.47−5.51 (4H, m, Rha-3), 5.53−5.57 (3H, m, Rha-2), 5.65 (1H, brs, Rha-1), 1.42 (3H, d, J = 6.2 Hz, Rha′-6), 4.24−4.29 (4H, m, Rha′-4), 5.53−5.57 (3H, m, Rha′-5), 5.55 (1H, brs, Rha′-1), 5.67 (1H, dd, J = 3.2, 10.2 Hz, Rha′-2), 5.71−5.73 (2H, dd, J = 3.0, 9.5 Hz, Rha′-3), 1.66 (3H, d, J = 6.2 Hz, Rha″-6), 4.20 (1H, dd, J = 9.1, 9.7 Hz, Rha″-4), 4.90 (1H, brs, Rha″-5), 5.41 (1H, brs, Rha″-1), 5.60 (1H, dd, J = 1.7, 3.0 Hz, Rha″-2), 5.71−5.73 (2H, dd, J = 3.0, 9.5 Hz, Rha″-3), 1.58 (3H, d, J = 5.8 Hz, Rha‴-6), 4.24−4.29 (4H, m, Rha‴-4), 4.24−4.29 (4H, m, Rha‴-5), 4.46 (1H, dd, J = 8.6, 3.0 Hz, Rha‴-3), 5.35 (1H, brs, Rha‴-1), 5.47−5.51(4H, m, Rha‴-2), 0.89 (3H, t, J = 6.7 Hz, Jla-16), 2.35 (2H, t, J = 7.5 Hz, CH_2CO_2), 3.60 (3H, s, OCH_3), 4.00 (2H, m, Jla-11); ^{13}C NMR (C_5D_5N, 125 MHz) δ 15.9 (CH_3, Fuc-6), 67.8 (CH, Fuc-5), 71.4 (CH, Fuc-4), 73.0 (CH, Fuc-2), 74.5 (CH, Fuc-3), 99.7 (CH, Fuc-1), 17.1 (CH_3, Rha-6), 67.6 (CH, Rha-5), 71.4 (CH, Rha-3), 72.3 (CH, Rha-2), 78.6 (CH, Rha-4), 99.1 (CH, Rha-1), 17.2 (CH_3, Rha′-6), 67.6 (CH, Rha′-5), 70.5 (CH, Rha′-2), 71.0 (CH, Rha′-3), 80.1 (CH, Rha′-4), 97.4

(CH, Rha′-1), 18.1 (CH_3, Rha″-6), 67.1 (CH, Rha″-5), 71.0 (CH, Rha″-2), 78.6(CH, Rha″-4), 71.0 (CH, Rha″-3), 99.1 (CH, Rha″-1), 18.3 (CH_3, Rha‴-6), 67.6 (CH, Rha‴-5), 72.3 (CH, Rha‴-2), 78.6 (CH, Rha‴-3), 78.6 (CH, Rha‴-4), 99.7 (CH, Rha‴-1), 13.9 (CH_3, Jla-16), 33.9 (CH_2CO_2), 50.8 (OCH_3), 78.0 (CH, Jla-11), 173.6 (C, Jla-1).

Compound 8: mp 79−83 °C; $[\alpha]_D$ −31 (*c* 0.14, MeOH); ^{1}H NMR (C_5D_5N, 500 MHz) δ 1.22 (3H, d, J = 6.4 Hz, Fuc-6), 4.01−4.06 (3H, m, Fuc-5), 4.20−4.26 (3H, m, Fuc-2), 4.97 (1H, d, J = 7.7 Hz, Fuc-1), 5.49−5.52 (4H, m, Fuc-3), 5.55−5.58 (3H, m, Fuc-4), 1.67 (3H, d, J = 6.1 Hz, Rha-6), 4.17 (1H, dd, J = 9.7, 9.7 Hz, Rha-4), 4.85 (1H, brs, Rha-5), 5.48 (1H, brs, Rha-1), 5.55−5.58 (3H, m, Rha-2), 5.71−5.75 (3H, m, Rha-3), 1.56 (3H, d, J = 5.5 Hz, Rha′-6), 4.20−4.26 (3H, m, Rha′-5), 4.55 (1H, dd, J = 3.5, 7.9 Hz, Rha′-3), 5.33 (1H, brs, Rha′-1), 5.49−5.52 (4H, m, Rha′-4), 5.49−5.52 (4H, m, Rha′-2), 1.30 (3H, d, J = 6.2 Hz, Rha″-6), 4.20−4.26 (3H, m, Rha″-5), 5.55−5.58 (3H, m, Rha″-4), 5.65 (1H, dd, J = 3.4, 10.3 Hz, Rha″-3), 5.68 (1H, brs, Rha″-1), 5.71−5.75 (3H, m, Rha″-2), 4.01−4.06 (3H, m, Glc-5), 4.50 (1H, d, J = 12.2 Hz, Glc-6a), 4.78 (1H, dd, J = 3.1, 12.3 Hz, Glc-6b), 5.20 (1H, d, J = 7.8 Hz, Glc-1), 5.35 (1H, d, J = 7.9 Hz, Glc-2), 5.49−5.52 (4H, m, Glc-4), 5.71−5.75 (3H, m, Glc-3), 0.88 (3H, t, J = 6.4 Hz, Jla-16), 2.36 (2H, t, J = 7.5 Hz, CH_2CO_2), 3.60 (3H, s, OCH_3), 4.01−4.06 (3H, m, Jla-11); ^{13}C NMR (C_5D_5N, 125 MHz) δ 16.8 (CH_3, Fuc-6), 68.2 (CH, Fuc-3), 67.9 (CH, Fuc-5), 74.3 (CH, Fuc-4), 77.1 (CH, Fuc-2), 99.8 (CH, Fuc-1), 18.2 (CH_3, Rha-6), 67.3 (CH, Rha-5), 69.9 (CH, Rha-2), 72.4 (CH, Rha-3), 81.2 (CH, Rha-4), 97.8 (CH, Rha-1), 18.5 (CH_3, Rha′-6), 72.0 (CH, Rha′-2), 72.0 (CH, Rha′-4), 77.0 (CH, Rha′-3), 81.2 (CH, Rha′-5), 100.0 (CH, Rha′-1), 17.5 (CH_3, Rha″-6), 67.3 (CH, Rha″-5), 69.0 (CH, Rha″-3), 70.9 (CH, Rha″-4), 72.6 (CH, Rha″-2), 97.7 (CH, Rha″-1), 61.3 (CH_2, Glc-6), 67.9 (CH, Glc-5), 68.3 (CH, Glc-4), 72.0 (CH, Glc-2), 74.3 (CH, Glc-3), 99.9 (CH, Glc-1), 14.0 (CH_3, Jla-16), 34.0 (CH_2CO_2), 50.8 (OCH_3), 77.9 (CH, Jla-11), 173.5 (C, Jla-1).

Cytotoxicity Assay. Nasopharyngeal (KB) 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.[22] 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: ED_{50} (μg/mL) 0.05 (KB); 0.001 (Hep-2).

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