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Mammalian Multidrug Resistance Lipopentasaccharide Inhibitors from *Ipomoea alba* Seeds

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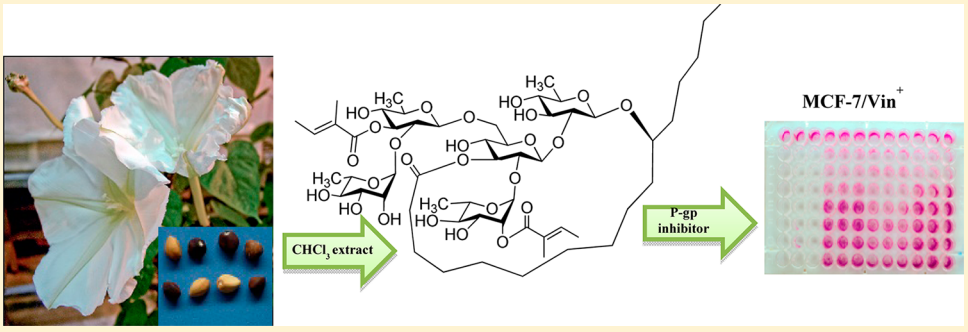
Supporting Information



ABSTRACT: As part of an ongoing project to identify inhibitors of multidrug efflux pumps, three new resin glycosides, albinosides I–III (**1**–**3**), were isolated from a $CHCl_3$-soluble extract from the seeds of moon vine (*Ipomoea alba*). Their structures were established through NMR spectroscopy and mass spectrometry as partially acylated branched pentasaccharides derived from three new glycosidic acids, named albinosinic acids A–C (**4**–**6**). The same oligosaccharide core formed by two D-quinovose, one D-glucose, and two L-rhamnose units was linked to either convolvulinolic or jalapinolic acid for **1** and **3**, respectively. They were partially esterified with (2*R*,3*R*)-3-hydroxy-2-methylbutanoic, acetic, or 2-methyl-2-butenoic acid. Compound **2** has two D-quinovose and three L-rhamnose units, linked to convolvulinolic acid, and its esterifying residues were characterized as two units of 2-methyl-2-butenoic acid. The aglycone lactonization site was located at C-2 of the terminal rhamnose unit (Rha) for **1**, at C-3 of the terminal rhamnose unit (Rha′) for **2**, and at C-3 of the second saccharide unit (Glc) for **3**. Reversal of multidrug resistance by this class of plant metabolites was also evaluated in vinblastine-resistant human breast carcinoma cells (MCF-7/Vin). The noncytotoxic compound **3** exerted the strongest potentiation effect of vinblastine susceptibility to over 2140-fold, while a moderate activity was observed for **1** (3.1-fold) and **2** (2.6-fold) at a concentration of 25 μg/mL.

Morning glory resin glycosides are amphipathic non-cytotoxic glycolipids[1] that have been characterized as substrates for efflux pumps that produce the multidrug-resistant (MDR) phenotype in both Gram-positive[2] and -negative bacteria[3] as well as in mammalian cancer cell lines.[4] In consequence, this class of active glycolipids represents efflux inhibitors that could be used to lower current effective therapeutic doses of drugs, thereby decreasing toxic side effects in refractory malignancies. In this context, the present investigation was undertaken to gain a deeper understanding of the chemical diversity of these MDR reversal agents essentially by isolating major glycolipids from commercial moon vine seeds.

Moon vine or moonflower, *Ipomoea alba* L. (Convolvulaceae), is a species of night-blooming morning glories native to tropical and subtropical regions of America, from Northern Argentina to Mexico and Florida. Formerly known as *Calonyction aculeatum*, it is now properly assigned to the genus *Ipomoea*, subgenus *Quamoclit*, section *Calonyction*.[5] It is a herbaceous climbing plant growing to a height of 5–30 m tall with twining stems, an annual in the North and a perennial in milder regions. The leaves are entire or three-lobed, 5–15 cm long, with a 5–20 cm long stem. The flowers are fragrant, white, and large, with a 8–14 cm diameter. Seeds are ovoid with a smooth hard cover, resembling a brownish nut[6] (Figure S1, Supporting Information). In Mexico, the decoction of the leaves, bark, and flowers of this plant has been used since colonial time to treat paralysis and the swelling of soft tissues due to the accumulation of water.[7] In India, a decoction of the whole

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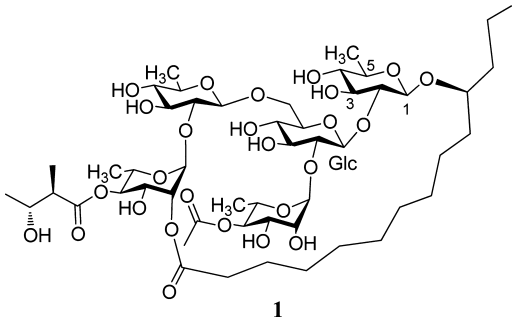
plant is used to treat snake bites.[8] In Africa, the leaves are used as food and as a soap substitute for bathing.[8b] In North America, this species is widely used as an ornamental plant due to its beautiful round shaped white flowers. There are reports of the invasiveness potential of moon vine that can cause problems in agricultural settings.[9,10]

Calonyctin, the natural mixture of resin glycosides extracted from the leaves of moon vine, promotes sweet potato tuber production and increased crop yields of potatoes, yams, peanuts, beans, and wheat.[11] Two glycosides with a tetrasaccharide core, known as calonyctins A_1 and A_2 (Figure S2, Supporting Information), were previously isolated as the active compounds.[12,13] Their structural difference was the length of the fatty acid chain that forms their aglycone moieties, 11*S*-tetradecanoic acid (convolvulinolic acid) for calonyctin A_1[12] and 11*S*-hexadecanoic acid (jalapinolic acid) for calonyctin A_2.[13]

This work describes the isolation, purification, and structure elucidation of three intact resin glycosides, albinosides I–III (**1**–**3**), with a new pentasaccharide core from the $CHCl_3$-soluble extract of moon vine seeds. In addition, saponification of the isolated natural compounds **1**–**3** yielded their constitutive glycosidic acids (**4**–**6**), named albinosinic acids A–C, which displayed new glycosidation sequences. Finally, reversal of multidrug resistance by compounds **1**–**3** was evaluated in vinblastine-resistant human breast carcinoma cells.

RESULTS AND DISCUSSION

$CHCl_3$-soluble extracts of moon vine seeds were subjected to precipitation with MeOH. The resin glycoside fraction was then submitted to preparative recycling reversed-phase HPLC using the techniques of peak shaving and heart cutting.[14] To achieve homogeneity, each peak collected was recycled until overlapped components were separated. This approach allowed the purification of three major compounds, named albinosides I–III (**1**–**3**), which represented resin glycosides of novel structure.



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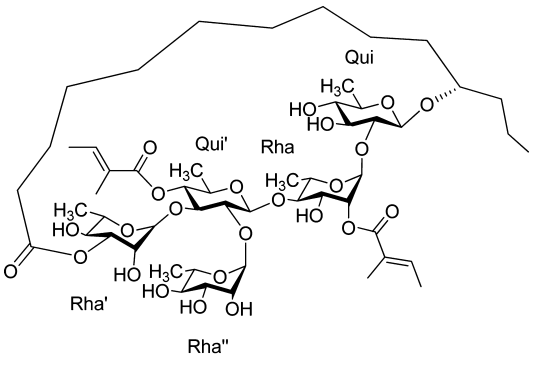
Pure compounds **1**–**3** were each saponified to yield their corresponding water-soluble glycosidic acids (**4**–**6**) and an organic solvent-soluble fraction. The released acids were identified by GC-MS as acetic and (2*R*,3*R*)-3-hydroxy-2-methylbutanoic or nilic (nla) acids from **1**, and 2-methyl-2-butenoic (tga) acid from **2** and **3**. Fractions I–III corresponding to compounds **1**–**3** were hydrolyzed in acid, and their Et_2O-soluble extract was methylated and then silylated. GC-MS analysis of the aglycone for **1** and **2** showed that the mass spectrum corresponded to that of the trimethylsilyl derivative of methyl 11-hydroxytetradecanoate because of the diagnostic R-cleavage ions at *m*/*z* 287 and 145. Compound **3** yielded methyl 11-hydrohexadecanoate (*m*/*z* 287 and 173) as the aglycone.[15]



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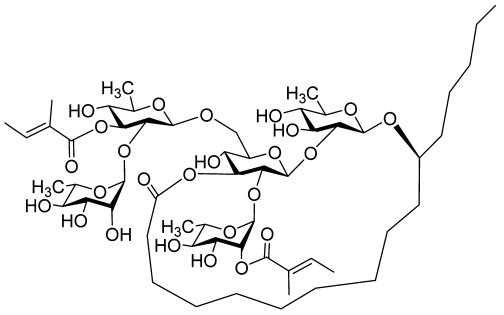

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GC-MS analysis of the water-soluble acid hydrolysates as TMS derivatives led to the identification of rhamnose (Rha), quinovose (Qui), and glucose (Glc) in the approximate ratio 2:2:1 from **1** and **3** by co-elution experiments with retention time identification using standard samples.[15] A ratio of 3:2 for Rha/Qui was found for compound **2** (Figure S36, Supporting Information). The monosaccharide reaction mixtures underwent derivatization with L-cysteine to form thiazolidines, which were identified by GC-MS as their TMS ethers (Figure S37, Supporting Information). This sugar analysis also confirmed the absolute configuration for all monosaccharides as the L-series for rhamnose and the D-series for quinovose and glucose.[16]

Each glycosidic acid (**4**–**6**) was acetylated and methylated to give a residue that was purified by C_{18} reversed-phase HPLC, affording compounds **4a**–**6a**. The main approaches used for the elucidation of these novel oligosaccharide cores involved the use of a combination of high-resolution FABMS and high-field NMR spectroscopy.[1] Negative-ion FABMS for **4** showed a pseudomolecular $[M - H]^-$ at *m*/*z* 989.4799 (Figure S24, Supporting Information), indicating a molecular formula of $C_{44}H_{77}O_{24}$ (calcd error: δ = −0.50), a peak was detected at *m*/*z* 973.4850 (Figure S28, Supporting Information) for **5**, which allowed the calculation of its molecular formula as $C_{44}H_{77}O_{23}$ (calcd error: δ = −0.51), and a peak at *m*/*z* 1017.5112 (Figure S32, Supporting Information) was found for **6** ($C_{46}H_{81}O_{24}$, calcd error: δ = −0.49). Common fragment peaks generated by glycosidic cleavage were observed in all three spectra, confirming the branched pentasaccharide core.[17] For example, the consecutive elimination of sugar units for compound **4** produced the peaks at *m*/*z* 843 $[989 - C_6H_{10}O_4$ (methylpentose unit)$]^-$,

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Table 1. ^{1}H NMR Spectroscopic Data of Albinosides I–III (1–3)[a]

position[b]	1	2	3	position[b]	1	2	3
Qui-1	4.73 d (7.7)	4.75d (4.8)	4.77 d (7.6)	4	3.68 dd (9.0, 8.9)	5.55 brs	4.13 dd (9.2, 9.2)
2	4.48 dd (9.2, 7.8)	4.39 dd (9.2, 4.8)	4.44 dd (10.0,8.0)	5	3.88 dq (9.2, 6.0)	3.66 dq (9.2,6.0)	4.28 dq (9.2, 5.2)
3	4.26–4.30 m*	3.66 dd (9.2,9.2)	4.44 dd (10.0,9.0)	6	1.64 d (6.1)	1.32 d (6.5)	1.68 d (5.6)
4	3.63 m*	4.39 dd (9.2,9.2)	3.55 dd (9.0,8.4)	Rha″-1		5.78 s	
5	3.63 m*	3.66 dq (9.2,5.6)	3.64 dq (8.0,6.0)	2		5.07 brs	
6	1.50 d (5.7)	1.59 d (6.0)	1.59 d (6.0)	3		4.54 dd (9.0, 3.0)	
Glc-1	5.68 d (7.7)		5.94 d (7.2)	4		4.31 dd (9.2,9.6)	
2	4.04 dd (9.0, 7.8)		4.22 t (7.6)	5		4.25 dq (9.6,6.0)	
3	3.95 dd (9.1, 8.9)		5.78 dd (9.6, 8.0)	6		1.67 d (6.5)	
4	3.68 dd (8.9, 8.9)		3.83 m*	Conv 2a	2.64 m*	3.02 dd (10.0,7.2)	
5	3.88 ddd (9.0, 6.0, 3.0)		4.28 ddd (9.6,5.2, 3.0)	2b		2.44 dd (10.0,7.2)	
6a	4.15–4.20 m*		4.30 dd (9.6, 5.2)	11	3.72–3.80 m*	3.82 brs	
6b	4.63 dd (11.7, 2.0)		4.27 dd (9.6,2.0)	14	0.91 t (7.4)	0.89 t (7.2)	
Rha-1	4.94 brs	5.83 s	5.78 s	Jal 2a			3.0 dd (8.8, 7.6)
2	5.60 dd (3.2, 2.1)	5.80 brs	5.09 brs	2b			2.43 dd (8.8, 8.0)
3	3.58 dd (8.4, 2.3)	4.92 dd (9.0, 2.4)	4.38 dd (8.8,2.8)	11			3.83 m*
4	5.58 dd (8.8, 10.0)	4.45 dd (9.0, 9.0)	4.36 dd (8.8, 8.8)	16			0.86 t (6.8)
5	3.63 m*	4.86 dq (9.0,6.8)	3.69 dq (9.2,6.0)	nla-2	2.76 dq (7.2, 7.1)		
6	1.40 d (5.9)	1.91 d (6.5)	1.32 d (6.0)	3	4.28 dq (7.0, 6.5)		
Rha′-1	5.77 d (1.5)	5.69 s	5.74 s	4	1.32 d (7.0)		
2	4.57 brs	4.15 dd (3.0, 1.6)	5.85 brs	5	1.20 d (7.0)		
3	4.69 dd (9.8, 2.8)	5.66 dd (9.0, 2.0)	4.88 dd (9.2,3.2)	Ace-2	1.90 s		
4	5.81 dd (9.8, 9.8)	3.81 dd (9.0, 9.2)	4.46 dd (9.2,9.0)	tga-3		7.09 dq (6.0, 1.2)	7.04 dq (6.8, 0.8)
5	5.18 dq (9.9,6.4)	3.66 dq (9.2, 6.0)	4.85 dq (9.0,6.4)	4		1.51 t (7.2, 6.4)	1.49 d (7.2)
6	1.58 d (6.5)	1.50 d (6.5)	1.92 d (6.0)	5		1.85 s	1.84 s
Qui′-1	5.13 d (7.8)	5.24 d (6.0)	5.24 d (7.6)	tga′-3		7.10 dq (6.0, 0.8)	7.08 dq (7.2, 0.9)
2	3.96 dd (9.0,8.0)	4.24 dd (9.2,7.0)	4.12 dd (9.2,7.0)	4		1.44 d (6.4)	1.41d (7.2)
3	4.19 dd (9.0, 8.9)	4.14 dd (9.2, 9.6)	5.55 dd (9.2,9.2)	5		1.87 s	1.86 s

[a]500 MHz for **1** and 400 MHz for **2** and **3** 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. [b]Abbreviations: qui = quinovose; rha = rhamnose; glc = glucose; conv = 11-hydroxytetradecanoyl; Jal = 11-hydroxyhexadecanoyl; nla = niloyl; ace = acetyl; tga = tigloyl.

697 [843 − $C_6H_{10}O_4$ (methylpentose unit)]$^-$, 551 [697 − $C_6H_{10}O_4$ (methylpentose unit)]$^-$, 389 [551 − $C_6H_{10}O_5$ (hexose unit)]$^-$, and 243 [389 − $C_6H_{10}O_4$ (methylpentose unit)]$^-$, which indicated 11-hydroxytetradecanoic acid, as the aglycone.[15] The difference of 28 mass units (two methylene groups) between compounds **4** and **6** as well as the formation of the same general fragmentation pattern by glycosidic cleavage of each sugar moiety at m/z 1017, 871, 725, 579, 417, and 271 confirmed the same branched pentasaccharide core in both glycosidic acids and 11-hydroxyhexadecanoic acid as the aglycone for **6**. For compound **5**, the peaks at m/z 827, 681, 535, 389, and 243 represented the loss of five methylpentose units, and the peak at m/z 243 indicated 11-hydroxytetradecanoic acid as the aglycone.[15,17]

Common features in both ^{1}H (Table 3; Figures S26, S30, and S34, Supporting Information) and ^{13}C (Table 4; Figures S27, S31, and S35, Supporting Information) NMR spectra of derivatives **4a**–**6a** were noted. In the low-field region of the HSQC spectrum, five anomeric signals were confirmed: for compound **4a**: Qui-1 (δ_H 4.67, δ_C 102.8), Glc-1 (δ_H 5.59, δ_C 100.9), Rha-1 (δ_H 4.89, δ_C 99.8), Rha′-1 (δ_H 5.71, δ_C 98.6), and Qui′-1 (δ_H 5.07, δ_C 100.8); for **5a**: Qui-1 (δ_H 4.68, δ_C 101.0), Rha-1 (δ_H 5.82, δ_C 98.0), Rha′-1 (δ_H 5.48, δ_C 98.5), Rha″-1 (δ_H 5.64, δ_C 99.7), and Qui′-1 (δ_H 5.02, δ_C 99.9); and for **6a**: Qui-1 (δ_H 4.68, δ_C 101.0), Glc-1 (δ_H 5.61, δ_C 101.3), Rha-1 (δ_H 5.82, δ_C 99.7), Rha′-1 (δ_H 5.50, δ_C 97.2), and Qui′-1 (δ_H 5.09, δ_C 101.0). Therefore, five spin systems for each individual monosaccharide moiety were readily distinguished in the ^{1}H–^{1}H COSY and TOCSY spectra. This allowed for the assignment of chemical shift values for all C-bonded protons in the five monosaccharides. The interglycosidic connectivities were established by HMBC studies.[1] For example, the following key correlations were observed for compound **4a**: H-2 (δ_H 4.45) of Qui with C-1 (δ_C 100.9) of Glc, H-2 (δ_H 5.16) of Glc with C-1 (δ_C 98.6) of Rha′, H-6 (δ_H 3.99) of Glc with C-1 (δ_C 100.8) of Qui′, H-1 (δ_H 4.89) of Rha with C-2 (δ_C 78.1) of Qui′, and H-1 (δ_H 4.67) of Qui with C-11 (δ_C 83.1) of convolvulinolic acid. The same glycosidation sequence was found in derivative **6a** but having jalapinolic acid as the aglycone. For compound **5a**, the observed correlations were as follows: H-2 (δ_H 4.14) of Qui with C-1 (δ_C 98.0) of Rha, H-4 (δ_H 5.64) of Rha with C-1 (δ_C 99.9) of Qui′, H-1 (δ_H 5.48) of Rha′ with C-3 (δ_C 78.7) of Qui′, H-2 (δ_H 4.18) of Qui′ with C-1 (δ_C 99.7) of Ram″, and H-1 (δ_H 4.68) of Qui with C-11 (δ_C 83.9) of convolvulinolic acid. Therefore, the structure of albinosinic acid A (**4**) was characterized as (11*S*)-convolvulinolic acid 11-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[-*α*-L-rhamnopyranosyl-(1→2)-6-deoxy-*β*-D-glucopyranosyl-(1→6)]-*O*-*β*-D-glucopyranosyl-(1→2)]-6-deoxy-*β*-D-glucopyranoside; the structure of albinosinic acid B (**5**) was characterized as (11*S*)-convolvulinolic acid 11-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→3)]-*O*-6-deoxy-*β*-D-glucopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-6-deoxy-*β*-D-glucopyranoside; and the structure of albinosinic acid C (**6**) was characterized as (11*S*)-jalapinolic acid 11-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→2)-6-deoxy-*β*-D-glucopyranosyl-(1→6)]-*O*-*β*-D-glucopyranosyl-(1→2)]-6-deoxy-*β*-D-glucopyranoside.

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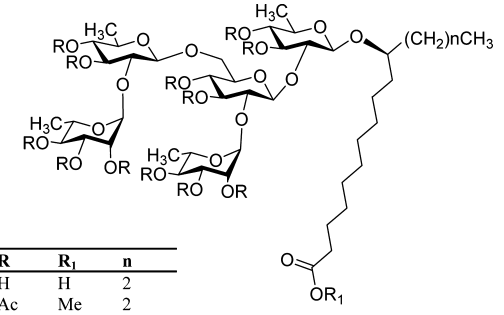
Table 2. ^{13}C NMR Spectroscopic Data of Albinosides I–III (1–3)[a]

position[b]	1	2	3	position[b]	1	2	3
Qui-1	103.5	101.1	103.2	Rha″-1		103.3	
2	78.8	77.4	79.6	2		70.7	
3	78.9	75.7	77.6	3		71.5	
4	76.6	75.5	77.8	4		72.4	
5	72.9	71.2	72.8	5		69.2	
6	18.2	17.1	18.9	6		17.1	
Glc-1	101.2		100.5	Conv-1	173.5	171.1	
2	77.9		81.2	2	33.2	33.8	
3	75.4		79.6	11	81.5	78.8	
4	70.0		77.4	14	14.3	14.3	
5	76.5		74.2	Jal-1			173.0
6	69.6		62.5	2			35.0
Rha-1	100.2	98.4	105.2	11			81.5
2	71.7	72.4	72.6	16			14.7
3	74.2	77.6	71.3	nla-1	174.6		
4	75.6	77.5	79.3	2	48.7		
5	72.2	66.7	70.3	3	68.9		
6	17.4	17.8	17.5	4	20.4		
Rha′-1	101.1	97.0	98.9	5	13.0		
2	71.9	71.7	74.3	Ace-1	170.7		
3	70.1	77.8	79.4	2	25.2		
4	76.1	73.8	79.5	tga-1		166.2	168.1
5	66.5	70.3	68.6	3		136.0	137.9
6	18.1	16.7	19.7	4		12.2	14.5
Qui′-1	104.4	103.1	105.1	5		10.9	12.7
2	81.3	71.4	77.5	tga′-1		166.7	168.6
3	77.7	79.7	74.8	3		136.7	138.7
4	76.7	72.9	73.2	4		12.6	14.5
5	72.5	68.3	71.2	5		10.8	12.7
6	18.4	15.6	18.9				

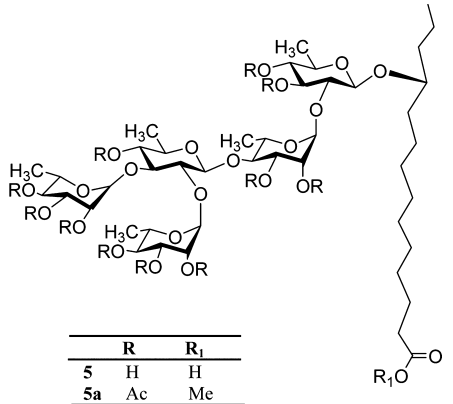
[a]125.7 MHz for **1** and 100 MHz for **2** and **3** in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. Assignments are based on ^{1}H–^{1}H COSY and TOCSY experiments. [b]Abbreviations: qui = quinovose; rha = rhamnose; glc = glucose; conv = 11-hydroxytetradecanoyl; Jal = 11-hydroxyhexadecanoyl; nla = niloyl; ace = acetyl; tga = tigloyl.

Albinoside I (**1**) gave a pseudomolecular $[M - H]^-$ ion at m/z 1113.5320 in the negative-ion FABMS, corresponding to the molecular formula $C_{51}H_{85}O_{26}$ (calcd error: $\delta = -0.80$). Peaks from the consecutive elimination of one niloyl residue $[M - H - 100\ (C_5H_8O_2)]^-$ at m/z 1013 and one acetyl $[1013 - 42\ (C_2H_2O)]^-$ at m/z 971 were also observed for **1** (Figure S3, Supporting Information). For albinoside II (**2**), the mass spectra revealed a $[M - H]^-$ peak at m/z 1119.5582 (Figure S10, Supporting Information) consistent with molecular formula $C_{54}H_{87}O_{24}$ (calcd error: $\delta = -0.44$), in contrast to the $[M - H]^-$ peak detected at m/z 1163.6208 (Figure S17, Supporting Information) for **3** ($C_{57}H_{95}O_{24}$, calcd error: $\delta = -0.43$). The initial loss of a tigloyl residue $[M - H - 82\ (C_5H_6O)]^-$ afforded a peak at m/z 1037 for **2** and at m/z 1081 for **3**, in addition to the peaks at m/z 663 $[1037 - C_6H_{10}O_4 - C_6H_{10}O_4 - 82\ (C_5H_6O)]^-$ and m/z 853 $[1081 - C_6H_{10}O_4 - 82\ (C_5H_6O)]^-$, which indicated the loss of two methylpentose units and one tigloyl residue for compound **2** ad the loss of a methylpentose unit and a tigloyl residue for **3**. Other observed peaks were produced by glycosidic cleavage of the sugar moieties, which are common to all resin glycosides,[17] as the tetrasaccharide members of the calonyctin series.[12,13]

Substitution patterns on each individual saccharide unit in albinosides I–III (**1**–**3**) were studied by ^{1}H NMR spectroscopy (Figures S4, S11, and S18, Supporting Information). COSY and TOCSY techniques[1] made possible the assignment of chemical shift values for all C-bonded protons in each moiety (Table 1,



	R	R_1	n
4	H	H	2
4a	Ac	Me	2
6	H	H	4
6a	Ac	Me	4



	R	R_1
5	H	H
5a	Ac	Me

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Table 3. ^{1}H NMR Spectroscopic Data for Derivatives 4a−6a[a]

position[b]	4a	5a	6a
Qui-1	4.67 d (8.0)	4.68 d (7.5)	4.68 d (7.5)
2	4.45 dd (8.0, 8.0)	4.14 dd (9.5, 8.0)	4.19 dd (10.0, 7.5)
3	5.61 dd (9.0, 8.5)	5.54 t (9.5)	5.54 t (9.5)
4	5.16 dd (9.5, 9.5)	5.08 t (9.5)	5.06 t (9.5)
5	3.68 dq (9.5, 6.0)	4.12 dq (9.5,6.5)	4.08 dq (9.5, 6.5)
6	1.42 d (6.0)	1.35 d (6.0)	1.35 d (6.5)
Glc-1	5.59 d (8.5)		5.61 d (7.5)
2	5.16 dd (9.5, 9.5)		5.82 t (10.5)
3	5.67 t (10.0)		5.64 t (10.5)
4	5.50 dd (9.0, 9.0)		5.83 t (10.5)
5	4.01 ddd (9.0,5.0,3.0)		4.13 m*
6a	3.99 dd (10.0, 5.0)		4.33 dd (12.5, 5.0)
6b	3.98 dd (10.0, 3.0)		4.62 dd (12.0, 4.5)
Rha-1	4.89 brs	5.82 s	5.82 brs
2	5.16 brs	6.12 brs	6.12 dd (3.0, 1.5)
3	5.5 dd (8.5, 2.0)	5.99 dd (10.0, 3.0)	5.99 dd (10.0, 3.0)
4	4.97 dd (9.5, 9.5)	5.64 dd (10.0, 9.0)	5.63 t (9.5)
5	3.48 dq (10.0, 6.0)	4.36 dq (9.5, 6.5)	4.33 m*
6	1.06 d (6.0)	1.35 d (6.0)	1.36 d (6.5)
Rha′-1	5.71 brs	5.48 brs	5.50 brs
2	6.08 dd (4.0, 1.6)	4.81 brs	5.47 dd (3.5, 1.5)
3	5.78 dd (9.0, 4.0)	4.35 dd (9.5, 3.0)	4.77 dd (9.5, 4.0)
4	5.66 dd (10.0, 10.0)	4.55 dd (10.0, 9.5)	4.41 t (9.0)
5	4.76 dq (10.0, 6.5)	4.54 dq (9.5, 6.5)	4.53 dq (9.5, 6.0)
6	1.61 d (6.0)	1.80 d (6.0)	1.80 d (6.0)
Qui′-1	5.07 d (7.5)	5.02 d (7.5)	5.09 d (8.0)
2	4.05 t (8.5)	4.18 dd (8.0, 8.0)	4.18 dd (10.0, 8.0)
3	4.52 t (9.0)	5.65 dd (10.0, 9.0)	5.75 t (9.5)
4	4.93 dd (9.0, 8.0)	5.04 dd (9.5, 7.5)	5.36 t (10.0)
5	3.98 dq (8.0, 6.0)	4.12 dq (8.0, 6.5)	4.1 dq (10.5, 6.5)
6	1.33 d (6.0)	1.12 d (6.5)	1.12 d (6.5)
Rha″-1		5.64 s	
2		5.85 dd (2.8, 2.4)	
3		5.65 dd (9.0, 3.0)	
4		5.03 dd (9.5, 9.0)	
5		3.77 dq (10.0, 6.0)	
6		1.29 d (6.0)	
Conv 2	2.34 t (7.5)	2.35 t (7.5)	
11	3.75 m*	3.65 m*	
14	0.96 t (7.5)	0.93 t (7.5)	
Jal 2a			2.36 t (7.5)
11			3.65 m*
16			0.90 t (7.0)
OCH_3	3.58 m*	3.62 s	3.62 s

[a]500 MHz in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b]Abbreviations: qui = quinovose; rha = rhamnose; glc = glucose; conv = 11-hydroxytetradecanoyl; Jal = 11-hydroxyhexadecanoyl.

Table 4. ^{13}C NMR Spectroscopic Data of Derivatives 4a−6a[a]

position[b]	4a	5a	6a
Qui-1	102.8	101.0	101.0
2	75.6	74.5	73.5
3	76.2	76.1	75.9
4	74.5	74.2	73.9
5	70.4	69.8	69.5
6	17.3	17.7	17.6
Glc-1	100.9		101.3
2	74.6		69.8
3	74.4		71.2
4	73.5		71.8
5	76.1		69.6
6	67.9		63.0
Rha-1	99.8	98.0	99.7
2	73.4	74.2	70.5
3	73.3	76.6	70.4
4	74.1	71.1	71.2
5	70.6	68.8	68.5
6	17.9	17.7	17.6
Rha′-1	98.6	98.5	97.2
2	70.1	71.1	72.8
3	70.4	74.8	79.0
4	71.1	76.7	76.2
5	67.9	67.7	68.0
6	18.0	18.8	18.7
Qui′-1	100.8	99.9	101.0
2	78.1	77.5	77.0
3	81.6	78.7	75.9
4	74.3	71.7	69.8
5	69.9	69.8	72.6
6	17.6	16.8	16.0
Rha″-1		99.7	
2		71.0	
3		73.0	
4		78.8	
5		70.7	
6		17.6	
Conv-1	171.4	171.2	
2	34.4	35.0	
11	83.1	83.9	
14	14.9	14.9	
Jal-1			174.2
2			34.3
11			81.0
16			14.3
OCH_3	51.5	51.5	51.5

[a]125.7 MHz in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. Assignments are based on ^{1}H−^{1}H COSY and TOCSY experiments. [b]Abbreviations: qui = quinovose; rha = rhamnose; glc = glucose; conv = 11-hydroxytetradecanoyl; Jal = 11-hydroxyhexadecanoyl.

Figures S6/S7, S13/S14, and S20/S21, Supporting Information). ^{13}C NMR signals were assigned by HMQC studies[1] (Table 2, Figures S5, S8, S12, S15, S19, and S22, Supporting Information). These natural products proved to be individual macrocyclic lactone-type pentasaccharides since the multiplets (splitting as a ddd) centered ca. δ 2.7−2.8 and 3.2−3.4 showed cross-peaks in their COSY and TOCSY spectra, because these signals correspond to the nonequivalent diastereotopic protons for the methylene C-2 of the aglycone when forming a ring.[1,14,17] The carbonyl carbon corresponding to the aglycone was identified through the $^{2}J_{CH}$ correlations (HMBC) with these protons. The site for the macrolactonization was located by the observed $^{3}J_{CH}$ correlations as follows: for albinoside I (**1**), it was placed at C-2 of the terminal rhamnose unit (Rha) by the observed correlation of H-2 (δ_H 5.60) of Rha with C-1

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Table 5. Modulation of Vinblastine Cytotoxicity in Drug-Sensitive MCF-7 and Multidrug-Resistant MCF-7/Vin by Albinosides I–III (1–3)

	IC_{50} (μg/mL)			reversal fold[c]		
compound[a]	MCF-7/Vin$^-$	MCF-7/Vin$^+$	MCF-7 sens	$RF_{MCF\text{-}7/Vin^-}$	$RF_{MCF\text{-}7/Vin^+}$	$RF_{MCF\text{-}7\ sens}$
vinblastine	1.08 ± 0.06	1.37 ± 0.23	0.047 ± 0.01			
1	0.26 ± 0.002	0.44 ± 0.03	0.022 ± 0.05	4.2	3.1	2.1
2	0.84 ± 0.082	0.54 ± 0.061	0.025 ± 0.002	1.3	2.6	1.9
3	<0.00064	<0.00064	<0.00064	>1687.5	>2140.6	>73.4
reserpine[b]	0.037 ± 0.01	0.31 ± 0.19	0.003 ± 0.001	29.2	4.4	15.7

[a]Serial dilutions from 0.00064 to 10 μg/mL of vinblastine in the presence or absence of glycolipid (25 μg/mL). [b]Reserpine = 5 μg/mL as positive control. [c]RF = IC_{50} vinblastine/IC_{50} vinblastine in the presence of glycolipid. Each value represents the mean ± SD from three independent experiments.

(δ_C 173.5) of convolvulinolic acid; for albinoside II (**2**), the macrolactonization was placed at C-3 of the terminal rhamnose unit (Rha′) by the correlation of H-3 (δ_H 5.66) of Rha′ with C-1 (δ_C 171.1) of convolvulinolic acid; and for albinoside III (**3**), the lactonization was placed at C-3 of the second unit saccharide (Glc) by the correlation between H-3 (δ_H 5.78) of Glc with C-1 (δ_C 173.0) of jalapinolic acid.

HMBC experiments[1] also provided evidence for the location of the ester substituents on the saccharide core 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 ($^3J_{CH}$) (Figures S9, S16, and S23, Supporting Information). The following spectroscopic features were observed: (a) for albinoside I (**1**), H-4 (δ_H 5.58) of Rha correlated with the C-1 (δ_C 174.6) signal of the niloyl group and H-4 (δ_H 5.81) of Rha′ with C-1 (δ_C 170.7) of the acetyl group; (b) for albinoside II (**2**), H-2 (δ_H 5.80) of Rha with C-1 (δ_C 166.2) of the tigloyl group (tga) and H-4 (δ_H 5.55) of Qui′ with C-1 (δ_C 166.7) of the tigloyl group (tga′); (c) for albinoside III (**3**), H-2 (δ_H 5.85) of Rha′ with C-1 (δ_C 168.1) of the tigloyl group (tga) and H-3 (δ_H 5.55) of Qui′ with C-1 (δ_C 168.6) of the tigloyl group (tga′) (Figures S38 and S39, Supporting Information).

Screening for cytotoxicity of compounds **1**–**3** utilized both vinblastine-sensitive and vinblastine-resistant human breast carcinoma cells (MCF-7/Vin).[4] Calculated IC_{50} values are shown in Table S40 (Supporting Information). The lack of cytotoxicity of these compounds (IC_{50} > 20 μg/mL) allowed us to explore their potential as MDR modulators. Results of the modulation assay are shown in Table 5. All tested compounds displayed modulation of vinblastine susceptibility (Figures S41, S42, and S43, Supporting Information) as previously observed for other morning glory resin glycosides.[4] Compound **3** was extremely potent (reversal fold, $RF_{MCF\text{-}7/Vin+}$ > 2140), as it was previously reported for murucoidin V, a proven substrate of glycoprotein P, the chief plasma membrane associated translocase responsible for the MDR phenotype in mammalian cells.[4] Therefore, these results further support the potential of this family of compounds as efflux pump inhibitors for overcoming MDR in cancer therapy, used in combination with already approved antineoplastic drugs.

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 model 341 polarimeter. ^1H (500 and 400 MHz) and ^{13}C (125.7 and 100 MHz) NMR experiments were conducted on a Varian Inova instrument. Negative-ion LRFABMS were recorded using a matrix of triethanolamine on a Thermo DFS spectrometer. Negative-ion HRESIMS experiments were performed on a Bruker MicrOTOF-Q high-resolution quadruple-time-of-flight mass spectrometer. The samples were dissolved in HPLC-grade MeOH (0.4 mg/mL) and infused directly to the ESI source using a syringe pump at a flow rate of 180 μL/h. The nebulizer and drying gas was nitrogen set at 0.4 bar and 4.0 L/min, respectively, with the drying gas temperature being 180 °C. Capillary voltage was 3.2 kV. Mass spectra were acquired over the range 50–3500 Da. The samples were processed using Bruker Data Analysis software. The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA, USA) 600E multisolvent delivery system equipped with a refractive index detector (Waters 410). Control of the equipment, data acquisition, processing, and management of the chromatographic information were performed by Empower 2 software (Waters). GC-MS was performed on a Thermo-Electron instrument coupled to a Thermo-Electron spectrometer. GC conditions: DB-5MS (5% phenyl)-methylpolysiloxane column (30 m × 0.25 mm, film thickness 0.1 μm); He linear velocity, 30 cm/s; 50 °C isothermal for 4 min, linear gradient to 300 at 40 °C/min; final temperature hold, 20 min. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 270 °C; mass range, 45–600 amu.

Chemicals, Cell Lines, and Cell Cultures. RPMI 1640 medium and fetal bovine serum were obtained from Gibco (Life Technologies, Carlsbad, CA, USA). Sulforhodamine B, reserpine, and vinblastine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Drug-sensitive human breast carcinoma cell line MCF-7 was obtained from the American Type Culture Collection (ATCC HTB-22). The resistant counterpart MCF-7/Vin was developed through continuous exposure to vinblastine during three consecutive years as previously reported.[4] All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). To maintain drug resistance, MCF-7/Vin$^+$ cells were cultured in medium containing 0.192 μg/mL vinblastine. At the same time, a stock of MCF-7/Vin cells was maintained in vinblastine-free medium.

Plant Material. Seeds of moon vine (*Ipomoea alba* var. *calonyction*; item 01052-PK-P1) were acquired from Park Seed (Greenwood, SC, USA) in January 2008. For authentication purposes, 20 seeds were germinated in soil, and four seedlings were grown to maturity under greenhouse conditions (Figure S1, Supporting Information). One specimen was deposited at the Ethnobotanical Collection of the National Herbarium (MEXU 1143969), Instituto de Biología, UNAM.

Extraction and Isolation. Dried and milled seeds (363.83 g) were exhaustively extracted by maceration at room temperature with hexane and then with $CHCl_3$ to give, after removal of the solvents, two extracts: an oily residue (8 g) and a dark syrup (6 g). Both extracts were compared by TLC (silica gel 60 F254 aluminum sheets; $CHCl_3$–MeOH, 4:1) with a reference solution of an authentic *I. orizabensis* collection,[15] which confirmed the similar lipophilic resin glycoside mixtures (R_f 0.45) in the $CHCl_3$-soluble extract. This extract was subjected to a precipitation process with MeOH to obtain a resin glycoside crude mixture, which was filtered under reduced pressure, yielding a white solid (5 g). This mixture was resolved by HPLC on a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm) with isocratic

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elution using MeOH−CH_3CN−H_2O (5:4:1), a flow rate of 4 mL/min, sample injection of 500 μL, and concentration of 0.1 mg/μL. Eluates across the peaks with t_R values 23.1 min (peak I), 99.7 min (peak II), and 128.2 min (peak III) were collected by the technique of heart cutting. Each subfraction was independently injected (sample injection, 500 μL; concentration, 0.1 mg/μL) in the apparatus operating in the recycle mode to achieve total homogeneity between 10 and 20 consecutive cycles and employing a Symmetry C_{18} column (Waters; 7 μm, 19 × 300 mm), isocratic elution with MeOH−CH_3CN−H_2O (10:7:3), and a flow rate of 8 mL/min for the first subfraction. For the remaining fractions, isocratic elution with MeOH−CH_3CN (7:3) with a flow rate of 8.5 mL/min was used. These procedures afforded pure compounds **1** (15 mg; t_R 10.8 min) from peak I, **2** (18.1 mg; t_R 8.84 min) from peak II, and **3** (18.5 mg; t_R 9.88 min) from peak III.

*Albinoside I (**1**):* white powder; mp 160−165 °C; $[\alpha]_{589}$ −38.3, $[\alpha]_{578}$ −40.0, $[\alpha]_{546}$ −44.16, $[\alpha]_{436}$ −75.0, $[\alpha]_{365}$ −115.0 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2 (S4 and S5, Supporting Information); negative FABMS *m*/*z* 1113 $[M - H]^-$, 1013 $[M - H - C_5H_8O_2$ (niloyl)$]^-$, 971 $[1013 - C_2H_2O$ (acetyl)$]^-$, 825 $[971 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 679 $[825 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 533 $[679 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[533 + H_2O - C_6H_{10}O_5$ (hexose unit)$]^-$, 243 $[389 - C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRFABMS *m*/*z* 1113.5320 $[M - H]^-$ (calcd for $C_{51}H_{85}O_{26}$ requires 1113.5329).

*Albinoside II (**2**):* white powder; mp 152−155 °C; $[\alpha]_{589}$ −56.0, $[\alpha]_{578}$ −58.0, $[\alpha]_{546}$ −65.0, $[\alpha]_{436}$ −105.0, $[\alpha]_{365}$ −146.0 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2 (S11 and S12, Supporting Information); negative FABMS *m*/*z* 1119 $[M - H]^-$, 1037 $[M - H - C_5H_6O$ (tigloyl)$]^-$, 891 $[1037 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 745 $[891 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 663 $[745 - C_5H_6O$ (tigloyl)$]^-$, 517 $[663 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[517 + H_2O - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 243 $[389 - C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRFABMS *m*/*z* 1119.5582 $[M - H]^-$ (calcd for $C_{54}H_{87}O_{24}$ requires 1119.5587).

*Albinoside III (**3**):* white powder; mp 146−150 °C; $[\alpha]_{589}$ −21.6, $[\alpha]_{578}$ −22.4, $[\alpha]_{546}$ −25.6, $[\alpha]_{436}$ −40.8, $[\alpha]_{365}$ −57.0 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2 (S18 and S19, Supporting Information); negative FABMS *m*/*z* 1163 $[M - H]^-$, 1081 $[M - H - C_5H_6O$ (tigloyl)$]^-$, 935 $[1081 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 853 $[935 - C_5H_6O$ (tigloyl)$]^-$, 707 $[853 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 561 $[707 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 417 $[561 + H_2O - C_6H_{10}O_5$ (hexose unit)$]^-$, 271 $[417 - C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRFABMS *m*/*z* 1163.6208 $[M - H]^-$ (calcd for $C_{57}H_{95}O_{24}$ requires 1163.6213).

Alkaline Hydrolysis of Compounds 1−3. Individual solutions of compounds **1** (8 mg), **2** (9 mg), and **3** (9.5 mg) in 5% KOH−H_2O (0.75 mL) were refluxed at 95 °C for 3 h. Then, the reaction mixtures were acidified to pH 5.0 and extracted with $CHCl_3$ (2 × 5 mL) and Et_2O (2 × 5 mL). The organic layers were combined and washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residues were directly analyzed by GC-MS. For compound **1**, acetic acid (t_R 2.81 min): *m*/*z* $[M]^+$ 60 (65), 45 (80), 43 (100), 29 (19), 15 (25); 3-hydroxy-2-methylbutyric acid (t_R 7.95 min): *m*/*z* $[M]^+$ 118 (2.0), 115 (10), 101 (20), 84 (12), 73 (70), 60 (100). For compounds **2** and **3**, tiglic acid (t_R 6.95 min): *m*/*z* $[M]^+$ 100 (30), 83 (18), 79 (38), 77 (40), 73 (100), 65 (9), 55 (22).

The aqueous phases were extracted with *n*-BuOH (10 mL) and concentrated to give colorless solids. Saponification of compound **1** yielded **4** (7 mg), compound **2** afforded derivative **5** (8.5 mg), and hydrolysis of compound **3** yielded glycosidic acid **6** (8.5 mg).

*Albinosinic acid A (**4**):* white powder; mp 148−150 °C; $[\alpha]_{589}$ −27.6, $[\alpha]_{578}$ −29.2, $[\alpha]_{546}$ −33.0, $[\alpha]_{436}$ −54.6, $[\alpha]_{365}$ −83.0 (*c* 1.0, MeOH), negative FABMS *m*/*z* 989 $[M - H]^-$, 843 $[989 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 697 $[843 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 551 $[697 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[551 - C_6H_{10}O_5$ (hexose unit)$]^-$, 243 $[389 - C_6H_{10}O_4$ (methylpentose unit)$]$; HRFABMS *m*/*z* 989.4799 $[M - H]^-$ (calcd for $C_{44}H_{77}O_{24}$ requires 989.4804).

*Albinosinic acid B (**5**):* white powder; mp 146−148 °C; $[\alpha]_{589}$ −25.0, $[\alpha]_{578}$ −26.6, $[\alpha]_{546}$ −30.0, $[\alpha]_{436}$ −46.6, $[\alpha]_{365}$ −71.6 (*c* 1.0, MeOH), negative FABMS *m*/*z* 973 $[M - H]^-$, 827 $[973 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 681 $[827 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 535 $[681 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 389 $[535 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 243 $[389 - C_6H_{10}O_4$ (methylpentose unit)$]^-$; HRFABMS *m*/*z* 973.4850 $[M - H]^-$ (calcd for $C_{44}H_{77}O_{23}$ requires 973.4855).

*Albinosinic acid C (**6**):* white powder; mp 142−144 °C; $[\alpha]_{589}$ −36.4, $[\alpha]_{578}$ −37.5, $[\alpha]_{546}$ −42.1, $[\alpha]_{436}$ −69.2, $[\alpha]_{365}$ −106.0 (*c* 1.0, MeOH), negative FABMS *m*/*z* 1017 $[M - H]^-$, 871 $[1017 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 725 $[871 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 579 $[725 - C_6H_{10}O_4$ (methylpentose unit)$]^-$, 417 $[579 - C_6H_{10}O_5$ (hexose unit)$]^-$, 271 $[417 - C_6H_{10}O_4$ (methylpentose unit)$]$; HRFABMS *m*/*z* 1017.5112 $[M - H]^-$ (calcd for $C_{46}H_{81}O_{24}$ requires 1017.5117).

Derivatization of 4−6. Each individual glycosidic acid (5 mg of **4**, 6.5 mg of **5**, and 6.0 mg of **6**) was acetylated (Ac_2O−C_5H_5N, 2:1) and methylated with CH_2N_2 to give a residue (8 mg of **4a**, 9 mg of **5a**, and 8.5 mg of **6a**), which was subjected to preparative HPLC on a reversed-phase C_{18} column (7 μm, 19 × 300 mm). The elution was isocratic with CH_3CN−H_2O (95:5) using a flow rate of 8.0 mL/min. For compound **4a**, the eluate across the peak with a t_R value of 11.89 min was collected by heart cutting and independently reinjected in the apparatus operated in the recycle mode to achieve total homogeneity after 10 consecutive cycles employing the same isocratic elution. The same method was used for compounds **5a** (t_R 18.98 min) and **6a** (t_R 21.14 min).

*Peracetylalbinosinic Acid A Methyl Ester (**4a**):* white powder; mp 99−101 °C; $[\alpha]_{589}$ −5.7, $[\alpha]_{578}$ −5.7, $[\alpha]_{546}$ −7.1, $[\alpha]_{436}$ −10.0, $[\alpha]_{365}$ −15.0 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 3 and 4 (S26 and S27, Supporting Information); MALDIMS *m*/*z* $[M + Na]^+$ 1531.

*Peracetylalbinosinic Acid B Methyl Ester (**5a**):* white powder; mp 89−92 °C ; $[\alpha]_{589}$ −2.5, $[\alpha]_{578}$ −2.5, $[\alpha]_{546}$ −5.0, $[\alpha]_{436}$ −10.0, $[\alpha]_{365}$ −18.7 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 3 and 4 (S30 and S31, Supporting Information); MALDIMS *m*/*z* $[M + Na]^+$ 1473.

*Peracetylalbinosinic Acid C Methyl Ester (**6a**):* white powder; mp 78−80 °C; $[\alpha]_{589}$ −4.0, $[\alpha]_{578}$ −6.0, $[\alpha]_{546}$ −8.0, $[\alpha]_{436}$ −8.0, $[\alpha]_{365}$ −12.0 (*c* 1.0, MeOH); ^1H and ^{13}C NMR, see Tables 3 and 4 (S34 and S35, Supporting Information); MALDIMS *m*/*z* $[M + Na]^+$ 1559.

Determination of Configuration of 3-Hydroxy-2-methylbutyrate. Preparation and identification of 4-bromophenacyl (2*R*,3*R*)-3-hydroxy-2-methylbutyrate were performed according to a previously reported procedure: mp 56−59 °C; $[\alpha]_D$ −6.0 (*c* 1.0 $CHCl_3$); GC-MS *m*/*z* 118 (2.0), 115 (10), 101 (20), 84 (12), 73 (70), 60 (100). This transesterification procedure has been used to confirm the absolute configuration for 3-hydroxy-2-methylbutyrate.[14]

Acid Hydrolysis and Sugar Analysis. Fractions I, II, and III (15 mg of each one) in 10 mL of 4 N HCl were refluxed at 90 °C for 2 h. Then, the reaction mixtures were diluted with 5 mL of H_2O and extracted with ether (3 × 10 mL). The organic layer was evaporated to dryness, dissolved in $CHCl_3$ (3 mL), and treated with CH_2N_2. The aqueous phase was neutralized with 1 N KOH and extracted with *n*-BuOH (10 mL), then washed with H_2O (2 × 5 mL) and concentrated to give a colorless solid. The sugar units were converted into volatile derivatives by treatment with chlorotrimethylsilane (Sigma Sil-A) and then analyzed by GC-MS, as previously described,[18] by applying the following conditions: DB-5MS (10 m × 0.18 mm, film thickeness 0.18 μm); He, 2 mL/min; 100 °C isothermal for 3 min, linear gradient to 300 at 20 °C/min. Retention times for TMS derivatives of common sugars were used as standards for GC identification: L-rhamnose t_R 5.25 min, D-fucose t_R 5.47 min, D-quinovose t_R 6.17 min, and D-glucose t_R 7.15 min. D-Quinovose, D-glucose, and L-rhamnose were detected in fractions I and III, while in fraction II only D-quinovose and L-rhamnose were detected.

Aditionally, thiazolidine derivatives of compounds **4−6** were prepared according to previously described procedures.[16] The TMS thiazolidine derivatives were directly analyzed by GC under the same conditions described above. TMS derivatives of D-quinovose, D-glucose, and L-rhamnose were used as standard authentic samples: L-rhamnose,

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t_R 4.58 min; D-quinovose, t_R 4.64 min; and D-glucose, t_R 4.53 min. D-Quinovose, D-glucose and L-rhamnose were detected in fractions I and III, while only D-quinovose and L-rhamnose were detected in fraction II.

Identification of Aglycones. The derivatized (CH_2N_2) organic layer residues obtained from acid-catalyzed hydrolysis of fractions I and II were individually submitted to normal-phase HPLC (ISCO, 21.2 × 250 mm, 10 μm), using isocratic elution [*n*-hexane−$CHCl_3$−Me_2CO (6:3:1)] and a flow rate of 6 mL/min to give 3.0 mg (fraction I) and 3.5 mg (fraction II) of methyl (11*S*)-hydroxytetradecanoate[15] (convolvulinolic acid methyl ester): t_R 18.6 min; mp 27−29 °C; $[\alpha]_D$ +1.5 (*c* 2, $CHCl_3$); ^{13}C NMR 174.4, 71.7, 51.5, 39.6, 37.5, 34.1, 29.6, 29.5, 29.3, 29.2, 29.1, 25.6, 24.9, 18.8, 14.1. An aliquot of this pure sample (2 mg) was derivatized with Sigma Sil-A for 5 min at 70 °C. GC-MS analysis gave one peak (t_R 7 min): *m*/*z* $[M]^+$ 330 (0.3), 315 (3.5), 287 (66.8), 145 (100), 73 (35.4). Treatment of fraction III as described above yielded 2.0 mg of methyl (11*S*)-hydroxyhexadecanoate[15] (jalapinolic acid methyl ester): t_R 16.4 min; mp 42−44 °C; $[\alpha]_D$ +7.3 (*c* 2, $CHCl_3$); ^{13}C NMR 174.4, 72.0, 51.4, 37.5, 37.4, 34.1, 31.9, 29.6, 29.5, 29.4, 29.2, 29.1, 25.6, 25.3, 24.9, 22.6, 14.1. This aglycone (1 mg) was derivatized by treatment with Sigma Sil-A and subjected to GC-MS analysis (t_R 12.8 min): *m*/*z* $[M]^+$ 358 (0.3), 343 (0.5), 311 (10.5), 287 (59.7), 173 (100), 73 (46.3).

Cytotoxicity and Modulation of Multidrug-Resistance Assays. Cytotoxicity and reversal fold of the resin glycosides **1**−**3** were determined by using the SRB assay.[19,20] The cells were harvested at log phase of their growth cycle, treated in triplicate with various concentrations of the test samples (0.2−25 μg/mL), and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. Results are expressed as the concentration that inhibited 50% control growth after the incubation period (IC_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percentage of growth inhibition.[20] Vinblastine was included as a positive control. The reversal effects as modulators were further investigated with the same method. MCF-7 and MDR MCF-7/Vin cells were seeded into 96-well plates and treated with various concentrations of vinblastine (0.00064−10 μg/mL) in the presence or absence of glycolipids at 25 and 5 μg/mL for 72 h as previously described.[4] The ability of glycolipids to potentiate vinblastine cytotoxicity was measured by calculating the IC_{50} as described above. In these experiments, reserpine (5 μg/mL) was used as a positive control. The reversal fold value, as a parameter of potency, was calculated from dividing IC_{50} of vinblastine alone by IC_{50} of vinblastine in the presence of test compounds.

■ ASSOCIATED CONTENT

Supporting Information

Photographs of moon vine (*Ipomoea alba*) (Figure S1). Structures of calonyctins A_1 and A_2 (Figure S2). FABMS and ^1H, ^{13}C, ^1H−^1H COSY, TOCSY, HSQC, and HMBC NMR spectra of albinosides **1**−**3** (Figures S3−S23). FABMS of glycosidic acids **4**−**6** (Figures S24, S28, S32). MALDI-MS and ^1H and ^{13}C NMR spectra of derivatives **4a**−**6a** (Figures S25−S27, S29−S31, S33−S35). GC chromatograms of TMS ethers and thiazolidine derivatives (Figures S36, S37). HMBC correlations for **1**−**3** (Figures S38, S39). Cytotoxicity and modulation assay of vinblastine with **1**, **2**, and **3** (Table S40 and Figures S41−S43). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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