Batatins I and II, Ester-Type Dimers of Acylated Pentasaccharides from the Resin Glycosides of Sweet Potato†

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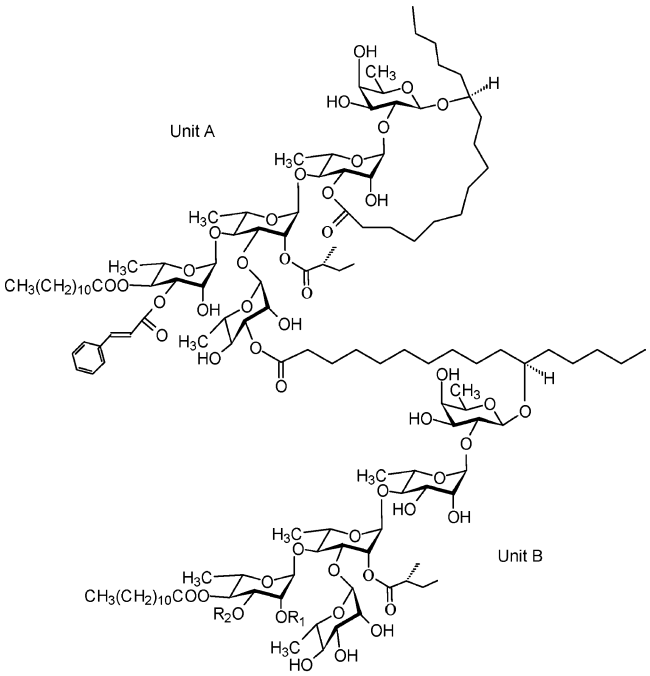
Batatins I (**1**) and II (**2**), two ester-type dimers of acylated pentasaccharides, have been isolated by recycling HPLC from the hexane-soluble extract of sweet potato (*Ipomoea batatas* var. *batatas*). The structures were elucidated by a combination of high-resolution NMR spectroscopy and mass spectrometry. Complete analysis and unambiguous assignments of the ^{1}H and ^{13}C NMR spectra of **1** and **2** were achieved by 2D shift correlation measurements. The glycosidic acid forming each branched pentasaccharide monomeric unit was confirmed as simonic acid B. Three different fatty acids esterify this core at the same positions in both batatins: C-2 on the second rhamnose unit and C-4 and C-2 (or C-3) on the third rhamnose moiety. The acylating residues were identified as (+)-(2*S*)-methylbutanoic, dodecanoic (lauric), and cinnamic acids. The site of lactonization by the aglycon in unit A was placed at C-3 of the second saccharide. The position for the ester linkage for monomeric unit B on the macrocylic unit A was identified as C-3 of the terminal rhamnose‴. Through spectroscopic simulation of these complex oligosaccharides, the chemical shifts and coupling constants were deduced for the overlapped proton resonances with a non-first-order resolution. The experimental NMR spectroscopic values registered for batatinoside I (**3**), a new polyacylated macroyclic pentasaccharide also isolated from this plant, were used as the starting point for spectral simulation of **1** and **2**. Both polymers **1** and **2** represent dimers of compound **3**.

Native to tropical America, *Ipomoea batatas* (L.) Lam. var. *batatas* (sweet potato, colloquially called "camote"[1]) is a perennial morning-glory vine that has been cultivated for over 2000 years for its edible tubers in Mexico, Central and lowland South America, and the West Indies. It is thought that the cultivated varieties originated in Mexico[2] from a naturally occurring hybridization event involving *I. trifida* (H.B.K.) G. Don and *I. tiliacea* (Willd.) Choisy, which have similar lobed leaves and growth habits but are not tuberous. Columbus introduced sweet potatoes to Europe, and immediately after the 16th Century Spanish Conquest, they were not just imported but cultivated in Spain and Portugal, where they became staples long before the 18th century popularization of the common potato (*Solanum tuberosum* L.) in the rest of Europe.[3] Outside of the Spanish Empire the sweet potato was known only as a herbal remedy or as an exotic garden curiosity during the 16th and 17th centuries, whereas today it is a popular root vegetable that is grown in gardens and as a commercial food crop throughout the world.[4] The People's Republic of China, Indonesia, and Japan lead in the production of sweet potatoes, and this tuber is now a common vegetable throughout the Orient.[5] In addition, some cultivars are propagated as ornamental plants and some are used as ground cover in traditional agriculture in Mexico.[6]

A decoction made from the leaves of this herbal drug is used in folk remedies as gargles to treat mouth and throat tumors. Poultices are prepared for inflammatory tumors.[7] In Mexico, traditional healers have long used leaf decoctions, considered to be of "cold-nature",[8] to reduce excessive body heat,[1] contemporarily defined as such illnesses as fever, heart disease, dysentery, diarrhea, stomach distress, gastrointestinal infection, and parasites. Previous to this investigation, the only known study of the resin glycosides of sweet potato was conducted in Japan using the roots of a locally grown Brazilian cultivar (cv. Simon), from which the structures of one tetrasaccharide and four pentasaccharides (the simonin series)[9] were elucidated.



	R_1	R_2
1	*trans*-cinnamoyl	H
2	H	*trans*-cinnamoyl

Results and Discussion

The present investigation describes the isolation of two ester-type dimers, to which the names batatins I (**1**) and II (**2**) have been given, and they were obtained from the hexane-soluble resin glycosides of the high-starch content, white-fleshed, and white-skinned staple-type cultivar "Picadito". The new polyacylated pentasaccharide batatinoside I (**3**), which was also isolated from this mixture, forms the monomeric units described for both batatins. A combination of high-resolution ESIMS, FABMS, and NMR methods[10] was used to elucidate the complex chemical nature of these polymers. The registered values for compound **3** were used

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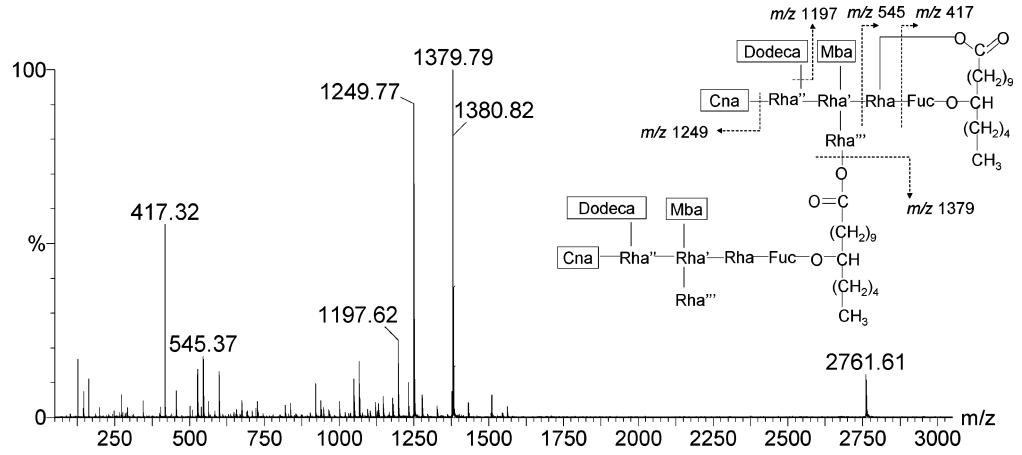
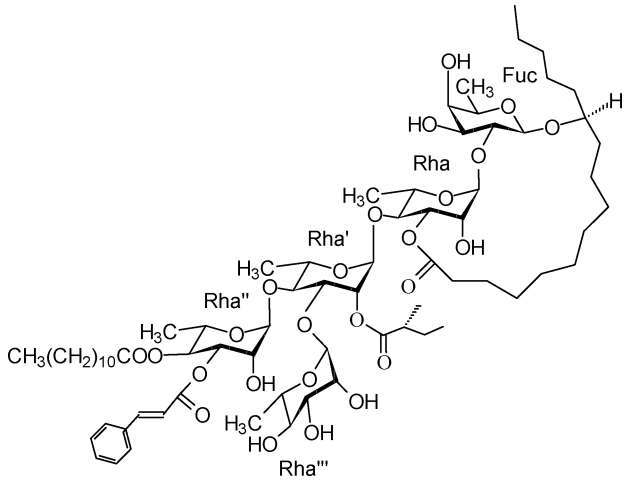


Figure 1. Negative-ion MS/MS ESI-mass spectrum of batatin I (**1**). This technique provides an easily detectable $[M - H]^-$ peak (*m*/*z* 2761.61). All the characteristic ions resulting from glycosidic cleavage are clearly observed. The high-mass ion $[M/2 - H]^-$ (*m*/*z* 1379.79) corresponds to the ester cleavage of the dimeric structure.

as the starting point to compare this macrocyclic pentasaccharide with the two dimeric units of batatins I (**1**) and II (**2**), as well as providing the chemical shifts and coupling constant values for the ^{1}H NMR simulation work[11] presented here.



3

Pure compounds **1**−**3** were submitted to saponification and yielded a water-soluble glycosidic acid and an organic solvent-soluble acidic fraction. The glycosidic acid was characterized as a branched pentasaccharide and identified as simonic acid B: (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnpyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside, which has been previously identified in the resin glycosides of *I. batatas*,[9] *I. stolonifera*,[12] *I. murucoides*,[13] and *I. pes-caprae*.[14] The liberated fatty acids were identified as 2*S*-methylbutanoic, *n*-dodecanoic, and cinnamic acids.

In the negative ESIMS, batatins I (**1**) and II (**2**) showed the same iodine adduct at *m*/*z* 2889.47 $[M + I]^-$, which permitted the calculation of the molecular formula $C_{144}H_{232}O_{50}$, indicating that these natural products represent a pair of diastereoisomers. A quasimolecular ion was detected for both batatins at *m*/*z* 2761.61 $[M - H]^-$ and revealed that the molecular formula for these compounds corresponds to two monomeric units of batatinoside I (**3**, $C_{72}H_{117}O_{25}$ $[M + H]^+$, *m*/*z* 1381.78). To facilitate discussion, each monomer was arbitrarily named unit A (macrocyclic moiety) and unit B. Both compounds **1** and **2** shared the same peak at *m*/*z* 1379.79 $[M/2 - H]^-$ in their ESIMS (Figure 1), representing a fragmentation pathway resulting from the cleavage of an ester-type dimer linkage[15] to form the high-mass fragment ion for the two monomeric units.[16] Other shared fragments were produced by the elimination of the lipophilic esters[14] and observed at *m*/*z* 1249 $[M/2 - H - C_9H_6O]^-$, *m*/*z* 1197 $[M/2 - H - C_{12}H_{22}O]^-$, and *m*/*z* 1067 $[1249 - C_{12}H_{22}O]^-$. Each fragment represents the loss of cinnamoyl (130 amu) and dodecanoyl (182 amu) residues, respectively. A second major fragmentation for compounds **1** and **2** was produced by glycosidic cleavage[14,17] of the sugar moieties, yielding the shared peaks at *m*/*z* 921 $[1067 - C_6H_{10}O_4$ (methylpentose)$]^-$, *m*/*z* 837 $[921 - C_5H_8O$ (Mba) $- C_6H_{10}O$ (methylpentose)$]^-$, *m*/*z* 691 [837 − 146 (methylpentose)]$^-$, *m*/*z* 545 [691 − 146 (methylpentose)]$^-$, and *m*/*z* 417 $[545 - 128 (C_6H_8O_3)]^-$.

A combination of ^{1}H NMR spectra (Table 1) and 2D homonuclear techniques (DQF-COSY and TOCSY)[10] allowed all C-bonded protons to be sequentially assigned within each ring system (Table 1). The use of the ^{1}H-detected {^{1}H, ^{13}C} one-bond correlation experiment (HMQC)[18] assigned all of the resonances in the ^{13}C NMR spectra (Table 2), where nine diagnostic signals in the anomeric region (ca. 98 to 105 ppm) were registered. All the signals assignable to the fatty acid groups, as well as those for the fucose anomeric carbons of units A and B, plus those for the 2-methylbutanoate carbonyl carbons, were overlapped in both **1** and **2**, giving, respectively, only one signal each in the ^{13}C NMR spectra. The diagnostic resonances observed in the downfield region δ 4.80−6.40 (Table 1) were assigned to the anomeric protons because their multiplicity for each signal is a doublet. A group of paramagnetically shifted nonanomeric protons reflected the presence of eight sites of esterification. The remaining region, where the majority of the methine resonances for the oligosaccharide core were found, was difficult to assign due to partial spectroscopic overlap. However, expansion of the TOCSY spectrum for the region δ 3.4−5.9 showed that at least one proton signal for each monosaccharide unit was completely resolved. Therefore, edited ^{1}H NMR subspectra[10,15] for each individual monosaccharide moiety were obtained and permitted the assignment of all resonances in both monomeric units A and B. All NMR spectral data for compound **3** were entirely superimposed with those registered for compounds **1** and **2** except for those downfield shifted resonances where there was a difference in the pattern of esterification in the oligosaccharide cores.

The HMBC technique[18] was used to locate the ester residues on each monomeric unit, the position of lactonization on unit A, and the position for the ester linkage forming the dimer-type structure. The shielded carbonyl resonance at δ 174.7 was clearly assignable to the lactone functionality because of its observed ^2J-coupling with the C-2 diastereotopic methylene protons of the aglycon unit A at δ_H 2.25 and 2.84. The site of lactonization was corroborated at C-3 of the second monosaccharide unit (Rha) by the observed ^3J-coupling between this lactone carbon and its geminal proton (δ_H

Table 1. ^{1}H NMR Data of Compounds **1**−**3** (500 MHz)[a]

	1		**2**		**3**
proton[b]	unit A	unit B	unit A	unit B	
Fuc-1	4.82 d (7.8)	4.81 d (7.9)	4.83 d (7.9)	4.83 d (7.9)	4.82 d (7.8)
2	4.52 dd (9.5, 7.8)	4.53 dd (9.6, 7.9)	4.54 dd (9.5, 7.9)	4.54 dd (9.5, 7.9)	4.52 dd (9.5, 7.8)
3	4.20*	4.20*	4.20*	4.20*	4.19 dd (9.5, 3.4)
4	3.92 d (2.5)	3.92 d (2.0)	3.92 d (2.5)	3.92 d (2.5)	3.92 d (3.4)
5	3.82 q (6.4)	3.82 q (6.4)	3.82 q (6.4)	3.82 q (6.5)	3.82 q (6.3)
6	1.52 d (6.4)	1.52 d (6.4)	1.52 d (6.4)	1.52 d (6.4)	1.52 d (6.3)
Rha-1	6.34 bs	6.34 bs	6.35 d (1.5)	6.35 d (1.5)	6.33 d (1.3)
2	5.31 bs	5.31 bs	5.32 bs	5.32 bs	5.31 bs
3	5.63 dd (9.2, 1.6)	4.42 dd (9.5, 3.1)	5.64 dd (9.7, 2.4)	4.43 dd (9.5, 3.1)	5.63 dd (9.9, 2.7)
4	4.66 dd (9.2, 9.2)	4.67 dd (9.5, 9.5)	4.67 dd (9.7, 9.5)	4.67 dd (9.5, 9.5)	4.66 dd (9.9, 9.9)
5	5.02 dq (9.2, 6.3)	5.02 dq (9.5, 6.3)	5.04 dq (9.5, 6.3)	5.04 dq (9.5, 6.2)	5.03 dq (9.9, 6.1)
6	1.59 d (6.3)	1.60 d (6.3)	1.60 d (6.2)	1.60 d (6.2)	1.59 d (6.1)
Rha′-1	5.62 d (1.5)	5.64 d (1.8)	5.63 bs	5.65 d (1.8)	5.62 d (1.5)
2	5.81 dd (3.2, 1.5)	5.82 dd (3.0, 1.8)	5.82 dd (3.0, 1.8)	5.82 dd (3.0, 1.8)	5.81 dd (3.0, 1.5)
3	4.62 dd (9.5, 3.2)	4.59 dd (9.2, 3.0)	4.63 dd (9.4, 3.0)	4.63 dd (9.4, 3.0)	4.62 dd (9.3, 3.0)
4	4.27 dd (9.5, 9.5)	4.29 dd (9.2, 9.2)	4.27 dd (9.6, 9.4)	4.27 dd (9.6, 9.4)	4.27 dd (9.3, 9.3)
5	4.40 dq (9.5, 6.0)	4.36 dq (9.2, 6.0)	4.40 dq (9.6, 5.9)	4.40 dq (9.6, 6.0)	4.39 dq (9.3, 6.1)
6	1.61 d (6.0)	1.63 d (6.0)	1.62 d (5.9)	1.62 d (6.0)	1.61 d (6.1)
Rha″-1	5.93 d (1.2)	5.80*	5.94 d (1.5)	5.94 d (1.5)	5.93 d (1.3)
2	4.91*	5.99 dd (3.2, 2.2)	4.91*	4.91*	4.91*
3	5.85 dd (9.9, 3.0)	4.70 dd (9.3, 3.2)	5.85 dd (9.9, 3.0)	5.84 dd (9.6, 3.2)	5.83 dd (9.9, 3.1)
4	6.08 dd (9.9, 9.9)	5.77 dd (9.3, 9.3)	6.08 dd (9.9, 9.9)	5.78 dd (9.6, 9.6)	6.07 dd (9.9, 9.9)
5	4.48 dq (9.9, 6.3)	4.41*	4.48 dq (9.9, 6.3)	4.44*	4.48 dq (9.9, 6.3)
6	1.46 d (6.3)	1.53 d (6.9)	1.46 d (6.3)	1.46 d (6.3)	1.46 d (6.3)
Rha‴-1	5.66 d (1.0)	5.62 d (0.9)	5.66 d (1.1)	5.63 bs	5.65 d (1.2)
2	4.92*	4.77 bs	4.75 bs	4.75 bs	4.76 dd (3.3, 1.2)
3	5.62 dd (7.6, 2.7)	4.42 dd (9.2, 3.1)	5.62 dd (9.8, 2.4)	4.43 dd (9.3, 3.1)	4.42 dd (9.5, 3.3)
4	4.23*	4.20*	4.23*	4.20*	4.20 dd (9.5, 9.5)
5	4.27 dq (9.5, 6.0)	4.25 dq (9.2, 6.2)	4.27*	4.25*	4.25 dq (9.5, 5.9)
6	1.72 d (6.0)	1.72 d (6.2)	1.72 d (6.0)	1.72 d (6.0)	1.71 d (5.9)
Jal-2	2.25 ddd (14.7, 7.1, 2.7)	2.25 ddd (14.9, 6.6, 2.8)	2.33−2.43*	2.33−2.48*	2.26 ddd (15.1, 7.0, 3.7)
	2.84 ddd (14.7, 11.2, 2.4)	2.86 ddd (14.9, 10.4, 1.7)	2.83 ddd (14.5, 11.0, 2.4)	2.33−2.48*	2.83 ddd (15.1, 10.9, 2.5)
11	3.89 m	3.89 m	3.89 m	3.89 m	3.89 m
16	1.00 t (6.9)	1.00 t (7.0)	1.00 t (6.9)	1.00 t (6.9)	1.00 t (6.9)
Mba-2	2.40 tq (7.0, 6.9)	2.42 tq (7.0, 6.8)	2.40 tq (7.1, 6.9)	2.40 tq (7.1, 6.9)	2.40 tq (7.0, 6.7)
2 Me	1.15 d (7.0)	1.18 d (7.0)	1.15 d (7.1)	1.15 d (7.1)	1.15 d (7.0)
3 Me	0.90 t (7.4)	0.92 t (7.4)	0.90 t (7.4)	0.90 t (7.4)	0.90 t (7.4)
Cna-2	6.55 d (16.0)	6.52 d (16.2)	6.56 d (16.0)	6.56 d (16.0)	6.55 d (16.0)
3	7.84 d (16.0)	7.75 d (16.2)	7.85 d (16.0)	7.85 d (16.0)	7.84 d (16.0)
Dodeca-2	2.46 t (7.3)	2.46 t (7.3)	2.45 t (7.2)	2.45 t (7.2)	2.46 t (7.2)
12	0.88 t (7.2)	0.87 t (7.1)	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.3)

[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 = singulet, 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; Jal = 11-hydroxyhexadecanoyl; Mba = 2-methylbutanoyl; Dodeca = dodecanoyl; Cna = *trans*-cinnamoyl.

5.63) in the pyranose ring.[10] Esterification of macrocyclic unit A by the acyclic unit B was identified by the observable ^3J connectivity between the carbonyl group for the ester at δ 174.8 and the proton at C-3 of the terminal rhamnose (Rha‴, δ_H 5.62).

In batatin I (**1**), a nonanomeric proton signal (doublet of doublets, Rha″ H-2) paramagnetically shifted to δ_H 5.99 indicated the presence of esterification at a different site than that observed in compounds **2** and **3**, as well as the ^{13}C−^{1}H long-range connectivity ($^3J_{CH}$) between the carbonyl carbon (δ_C 166.8) of the *trans*-cinnamoyl residue with this proton resonance in unit B. In batatin II (**2**), ^{1}H NMR signals of units A and B were almost superimposable, indicating that the esterification sites in monomers A and B are the same, so the conclusion was made that a *trans*-cinnamoyl residue is located in the same Rha″ position of both monomeric units. Batatin II (**2**) represents a homodimer of the new batatinoside I (**3**).

Spectroscopic simulation was used to duplicate definitively the registered ^{1}H NMR (500 MHz) data and thus permit the correct assignment for the chemical shifts and coupling constants of all superimposed protons in **1** and **2**. Chemical shifts were estimated from TOCSY, COSY, and HMQC experiments. ^{1}H NMR simulation for each monosaccharide unit provided the correct chemical shift and coupling constant values for the tightly coupled protons.[10] In the case of the nonsuperimposed protons, as many of the coupling constants as possible were estimated from the ^{1}H NMR spectrum. In some cases, such as the triplet at ca. 6.08 ppm, the multiplicity pattern was evident by visual inspection. In other cases, such as the four-line pattern centered at ca. 5.81 ppm, an anomeric rhamnose and two overlapping H-2 doublets of doublets for rhamnose signals were uncovered by careful analysis. Where coupling constants could not be estimated directly from the spectrum, rhamnose chemical shifts and coupling constants from compound **3** were used as initial estimates for the second-order analysis. The parameters were varied until an optimal agreement between measured and calculated spectra was achieved, as shown in Figure 2 for compound **1**. This methodology employed the MestRe-C program. A system of 24 nuclei was simulated for the proton signals located in the downfield region between δ 4.75 to 6.40 for compound **1**. NMR simulation was applied where a visual NMR analysis was impossible, as in the example of the multiple signal centered at δ 5.58−5.65. Here the anomeric protons in both units A and B of Rha′ (blue) and Rha‴ (red) as well as the doublet of doublets for unit A proton H-3 in Rha (purple) and Rha‴ (red) moieties overlapped and produced a non-first-order resolution. The superposition of the simulated patterns for each monosaccharide unit duplicated this complex signal in the measured ^{1}H NMR spectrum (Figure 2). The chemical shifts and coupling constants used to calculate the final dimeric oligosaccharide spectra are summarized in Table 1.

Table 2. ^{13}C NMR Data of Compounds **1**−**3** (125.7 MHz)[a]

	1		2		3
carbon[b]	unit A	unit B	unit A	unit B	
Fuc-1	101.6	101.6	101.6	101.6	101.6
2	73.4	73.4	73.4	73.4	73.5
3	76.6	76.6	76.6	76.6	76.6
4	73.6	73.6	73.6	73.6	73.6
5	71.3	71.3	71.2	71.2	71.3
6	17.2	17.2	17.2	17.2	17.2
Rha-1	100.2	100.3	100.3	100.3	100.2
2	69.8	69.8	69.8	69.8	69.8
3	78.0	72.5	78.0	72.5	78.0
4	77.2	77.2	77.2	77.2	77.2
5	67.9	67.9	67.9	67.9	67.9
6	19.2	19.3	19.3	19.3	19.2
Rha′-1	99.0	98.8	99.0	98.8	99.0
2	72.8	72.8	72.8	72.8	72.8
3	79.8	79.1	79.8	79.8	79.8
4	80.0	80.0	80.0	80.0	80.0
5	68.4	68.5	68.4	68.5	68.4
6	18.8	18.8	18.8	18.8	18.8
Rha″-1	103.7	100.5	103.7	103.8	103.7
2	70.0	74.0	70.0	70.0	70.0
3	73.3	68.1	73.3	73.3	73.3
4	71.7	75.1	71.7	72.7	71.7
5	68.2	68.4	68.2	68.4	68.2
6	17.8	18.0	17.8	17.8	17.8
Rha‴-1	104.6	104.5	104.6	104.5	104.5
2	72.2	72.7	72.5	72.7	72.7
3	78.0	72.4	78.0	72.4	72.4
4	73.6	73.6	73.6	73.6	73.6
5	70.6	70.8	70.6	70.8	70.8
6	18.9	18.8	18.8	18.8	18.8
Jal-1	174.7	174.8	174.7	174.8	174.7
2	33.9	33.8	33.9	33.9	33.9
11	79.5	79.4	79.5	79.5	79.5
16	14.5	14.5	14.5	14.5	14.5
Mba-1	175.4	175.4	175.4	175.4	175.4
2	41.4	41.4	41.4	41.4	41.4
2-Me	16.9	16.9	16.9	16.9	16.9
3-Me	11.8	11.8	11.8	11.8	11.8
Cna-7	118.4	118.4	118.4	118.4	118.4
8	128.6	128.6	128.6	128.7	128.6
	129.2 (× 2)	129.0 (× 2)	129.2 (× 2)	129.2 (× 2)	129.2 (× 2)
	130.7 (× 2)	130.5 (× 2)	130.7 (× 2)	130.5 (× 2)	130.7 (× 2)
	134.7	134.7	134.7	134.7	134.7
	145.4	145.6	145.4	145.6	145.4
9	166.2	166.8	166.2	166.8	166.2
Dodeca-1	173.2	173.5	173.2	173.5	173.2
2	34.6	34.6	34.6	34.6	34.6
12	14.3	14.3	14.3	14.3	14.3

[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; Jal = 11-hydroxyhexadecanoyl; Mba = 2-methylbutanoyl; Dodeca = dodecanoyl; Cna = *trans*-cinnamoyl.

The glycolipid ester-type dimeric structure assigned for batatin I (**1**) was (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-[3-*O*-(11*S*-hydroxyhexadecanoyl-11-*O*-α-L-rhamnopyranosyl-{2-*O*-(*trans*-cinnamoyl)-4-*O*-(*n*-dodecanoyl)}-(1→4)-*O*-α-L-{2-*O*-(2-*S*-methylbutanoyl) (1→3)-*O*-α-L-rhamnopyranosyl}rhamnopyranosyl-(1→4)-*O*-α-L-r-hamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside]-rhamnopyrano-syl-{3-*O*-(*trans*-cinnamoyl)-4-*O*-(*n*-dodecanoyl)}-(1→4)-*O*-α-L-{2-*O*-(2*S*-methylbutanoyl)-(1→3)-*O*-α-L-rhamnopyranosyl}rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone). The structure established for its isomer batatin II (**2**) was (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-[3-*O*-(11*S*-hydroxyhexadecanoyl-11-*O*-α-L-rhamnopyranosyl-{3-*O*-(*trans*-cinnamoyl)-4-*O*-(*n*-dodecanoyl)}-(1→4)-*O*-α-L-{2-*O*-(2-*S*-methylbutanoyl)(1→3)-*O*-α-L-rhamnopyranosyl}-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside]rhamnopyranosyl-{3-*O*-(*trans*-cinnamoyl)-4-*O*-(*n*-dodecanoyl)}-(1→4)-*O*-α-L-{2-*O*-(2*S*-methylbutanoyl)-(1→3)-*O*-α-L-rhamnopyranosyl}rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone). This type of dimeric resin glycosides has been identified in only two other morning-glory species. The first of these yielded merremin, isolated from the roots of *Merremia hungaiensis*,[16] a Chinese medicinal plant. The second yielded tricolorins H−J from the aerial parts of *I. tricolor*, a species used as a cover crop in traditional agriculture in the tropical zones of Mexico.[15]

It has been hypothesized that, in actuality, the resin glycosides of *Ipomoea* species could form large, high molecular weight polar polymers.[16,19] Difficulties in their investigation have arisen from their poor solubility in less polar organic solvents, which makes isolation and purification problematic, except for more highly acylated lipophilic resin glycosides such as the batatins. It is also possible that these polymers aggregate in aqueous solutions and form micelles, which could perturb cell membranes through an ion-driven efflux provoking surface interactions with target cells. A clue to this possibility is found in the example of the crystalline state of tricolorin A.[20]

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. ^1H (500 MHz) and ^{13}C (125.7 MHz) NMR experiments were conducted on a Bruker DMX-500 instrument. The NMR techniques were performed according to previously described methodology.[15] The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600E multisolvent delivery system equipped with a photodiode UV detector (Waters 996) at 254 nm. Positive-ion LRFABMS were recorded using a matrix of triethanolamine on a JEOL SX102A spectrometer. Negative- and positive-ion ESIMS experiments were performed on a VG Quattro triple quadruple instrument (Micromass, Beverly, MA). Nitrogen was used as both nebulizing and drying gas. Mass spectra were acquired over a range of 100−3000 Da in 5 s/scan using electrospray ionization with cone voltage set to 30 V. The sample was dissolved in 1:1 MeOH−H_2O with 0.1% formic acid and infused via syringe. High-resolution FTMS data were acquired using a Bruker-Daltonics APEX II Fourier transform mass spectrometer, equipped with a 9.4 T passively shielded superconducting magnet and an external ESI ion source. 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 gradient to 300 °C 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. NMR spectroscopic simulation was carried out with the MestRe-C 4.0 program (Mestrelab Research, Santiago de Compostela, Spain).

Plant Material. The roots of *I. batatas* var. *batatas* (cv. Picadito) were collected in plantations in Salvatierra, Guanajuato, Mexico, in 1999. The plant material was identified by Dr. Robert Bye. A voucher specimen (R. Bye FB 1314) was deposited in the Ethnobotanical Collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation. The powdered dry roots (2.6 kg) of *I. batatas* var. *batatas* were extracted by maceration at room temperature with hexane to give, after removal of the solvent, a dark orange syrup (13.1 g). The crude extract prepared from the herbal drug was subjected to column chromatography over silica gel (150 g) using gradients of CH_2Cl_2 in hexane and Me_2CO in CH_2Cl_2. A total of 185 fractions (200 mL each) were collected and combined to give several pools containing mixtures of resin glycosides. The most lipophilic fractions (59−64), eluted with CH_2Cl_2−Me_2CO (7:3) and rich in resin glycosides, were pooled and subjected to column chromatography over silica gel (30 g) using a gradient of MeOH in $CHCl_3$ to eliminate the pigmented residues. The process was monitored by TLC, and a total of 20 fractions (20 mL each) were collected and combined, yielding from the fraction eluted with $CHCl_3$−MeOH (9:1) a mixture of lipophilic oligosaccharides (945 mg).

Recycling HPLC Separation. The analytical HPLC separations were done on a Symmetry C_{18} column (Waters; 5 mm, 4.6 × 250 mm)

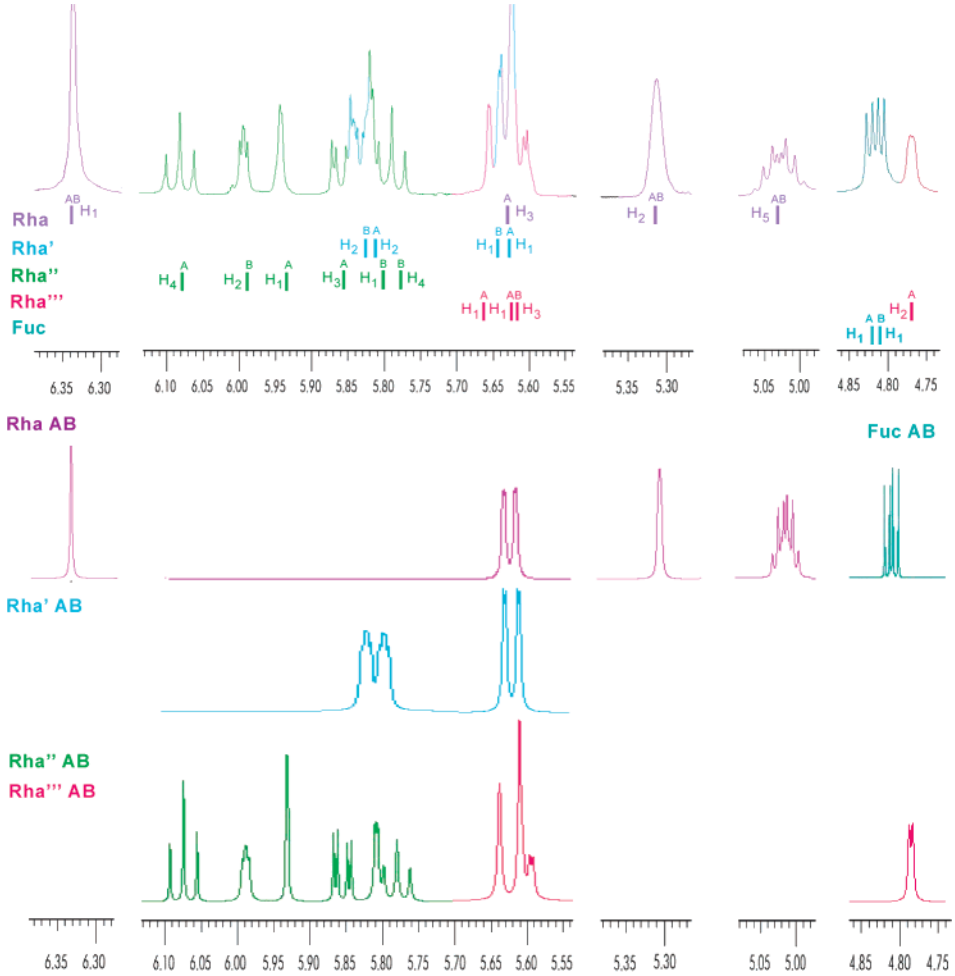


Figure 2. Simulation of the anomeric region of the ^{1}H NMR spectrum of batatin I (**1**). The top trace is the measured 500 MHz spectrum, resolution enhanced. Peaks are color coded for each rhamnose and fucose monomers of units A and B. Actual chemical shifts are depicted along the bottom of the top plots. Below are the simulated spectra for each of the units. The H_n descriptors denote a specific position for each sugar unit.

with an isocratic elution of $CH_3CN-MeOH$ (9:1), a flow rate of 0.7 mL/min, and a sample injection of 20 mL (3 mg/mL). The crude fraction was subjected to preparative HPLC on a reversed-phase C_{18} column (7 mm, 19 × 300 mm). The elution was isocratic with $CH_3CN-MeOH$ (9:1) using a flow rate of 9 mL/min. Eluates across the peaks with t_R of 20.2 min (peak I), 21.6 min (peak II), and 22.9 min (peak III) were collected by the technique of heart cutting and independently reinjected in the apparatus operated in the recycle mode[21] to achieve total homogeneity after 10−20 consecutive cycles employing the same isocratic elution. These techniques afforded pure compounds **1** (17.3 mg) from peak II; **2** (14.6 mg) from peak I, and **3** (6.7 mg) from peak III.

Batatin I (1): white powder; mp 117−120 °C; $[\alpha]_D$ −38 (*c* 1.0, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative HRESIMS *m/z* 2760.5654 $[M - H]^-$ (calcd for $C_{144}H_{231}O_{50}$ +4.40 ppm).

Batatin II (2): white powder; mp 124−130 °C; $[\alpha]_D$ −33 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative HRESIMS *m/z* 2760.5602 $[M - H]^-$ (calcd for $C_{144}H_{231}O_{50}$ +2.50 ppm).

Batatinoside I (3): white powder; mp 126−130 °C; $[\alpha]_D$ −24 (*c* 1.0, MeOH); ^{1}H and ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 1379 $[M - H]^-$, 1249 $[M - H - C_9H_6O]^-$, 921, 837, 545, 417; positive HRESIMS *m/z* 1381.7878 $[M + H]^+$ (calcd for $C_{72}H_{117}O_{25}$ +1.72 ppm).

Alkaline Hydrolysis of Compounds 1−3. Individual solutions of compounds **1**−**3** (10 mg) in 5% $KOH-H_2O$ (3 mL) were refluxed at 95 °C for 2 h. The reaction mixtures were acidified to pH 4.0 and extracted with $CHCl_3$ (10 mL). The organic layers were washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. Each residue was directly analyzed by GC-MS, and three peaks were detected: 2-methylbutanoic acid (t_R 7.2 min), *m/z* $[M]^+$ 102 (3), 87 (33), 74 (100), 57 (50), 41 (28), 39 (8); cinnamic acid (t_R 16.5 min), *m/z* $[M]^+$ 148 (100), 147 (96), 131 (25), 103 (40), 102 (20), 77 (25), 74 (8), 51 (20), 50 (8), 39 (5), 38 (4); 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 (30). The preparation and identification of 4-bromophenyacyl (2*S*)-2-methylbutyrate[21] from compounds **1**−**3** were performed according to previously reported[22] procedures: mp 40−42 °C; $[\alpha]_D$ +18 (*c* 1.0, MeOH); GC-MS (t_R 4.75 min) *m/z* $[M + 2]^+$ 272 (6.8), $[M]^+$ 270 (7.3), 254 (3.8), 252 (3.8), 186 (2.1), 172 (8.6), 171 (100), 70 (9.7), 169 (88.7), 90 (13.9), 89 (23.4), 85 (11.5), 63 (5.3) 57 (19), 51 (2.3), 50 (2.9), 41 (8.5), 39 (9.4). The aqueous phase from each reaction was extracted with *n*-BuOH (5 mL) and concentrated to give a colorless solid. The residue was methylated with CH_2N_2 to yield simonic acid B methyl ester. The physical and spectroscopic constants (^{1}H and ^{13}C NMR) registered for this derivative[9] were identical in all aspects to those previously reported: 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).

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References and Notes

(1) The sweet potato is called "camote" in Mexico, the word deriving from the Nahuatl "camohtli" and refering to an edible root or potato of the family Convolvulaceae. In the Aztec treasury of herbal remedies "De la Cruz-Badianus Manuscript" (1552) or "Little Book of Indian Medicinal Herbs" (*Libellus de Medicinalibus Indorum Herbis*) seven species of morning-glories were included with their therapeutic descriptions in Latin. One of these illustrates (Folio 28 verso) a bindweed called "*Tlacacamohtli*" (Nahuatl, noble edible root, "*tlaca*", "*tlacatl*" = noble) prescribed to cure heat in the heart (*contra cordis calorem*) and also to reduce excessive heat of the body. The illustration represents a tuberous rooted red-flowered *Ipomoea* species, the sweet potato, as its indigenous name indicates. See: Emmart, E. W. *The Badianus Manuscript (Codex Barberini, Latin 241). An Aztec Herbal of 1552*; The Johns Hopkins Press: Baltimore, 1940; pp 252−253.

(2) Dr. Francisco Hernández (1515−1587), King Phillip II's chief medical officer in the Spanish colonies in the New World (1570−1577), was the first trained scientist to gather ethnobotanical information directly from Aztec healers and to assess the medicinal usefulness of the natural resources found in the central area of Mexico. He described the nutritious properties and the manner of cultivation of "*cacamotli*" and mentioned four varieties named long ago on the basis of their skin and flesh colors: "The root is sometimes red on the outside and white inside, and is called "*acamotli*". If the outer skin is purple and the inner part white, it is called "*ihaicamotli*". If the outside is white and the inside yellow with a reddish tinge, it is called "*xochicamotli*". There are times when both the inside and the outside are red or completely white, and then it is called "*camocpalcamotli*" or "*poxcauhcamotli*": names bestowed many centuries ago according to the variety of the colors." In contemporary Mexico and Brazil, there are four cultivated varieties recognized by their different skin colors: white, yellow, red, and purple. See: Hernández, F. *The Mexican Treasury: The Writings of Dr. Francisco Hernández*; Varey, S., Ed.; Stanford University Press: Stanford, 2000; p 184.

(3) The awareness of the role played by the sweet potato in Europe was not properly appreciated until recently due partially to a linguistic confusion perpetuated by herbals. Whereas the native word "batata" and "patata" were used in Spanish to indicate the sweet potato and the common one, respectively, in English they were joined together under the word "potato", which was used to indicate both species. Perhaps, the best known reference reinforcing this confusion comes from John Gerard's *General History of Plants* (1597), where he asserted an Andean origin to the sweet potato (*Sisarum peruvianum*) while claiming that the ordinary potato originated in the English colony of Virginia (*Battata virginiana*). The potato that William Shakespeare mentions in the *Merry Wives of Windsor* is the sweet potato. See: Gerard, J. *The Herbal or General History of Plants*; Dover: New York, 1975; pp 925−928.

(4) In the United States, two varieties are common: the dry, mealy, "yellow sweet potato", and the watery, orange "yam", which in reality is not a true yam (*Dioscorea* spp.). This root crop is generally eaten boiled, baked, fried, or dried, and ground into syrup or flour to make biscuits, bread, pastries, and candy. Tubers are also dehydrated into "chips", cooked, frozen, creamed, and used as pie fillings in the same manner as pumpkins. The foliage and the tuber are also valued as feed for farm animals. The yellow and orange pigments in the pulp of the tuber are carotenoids and therefore a rich source of vitamin A. Over one-fourth is carbohydrates, mostly starch, but the sweet taste indicates that sucrose is also present, especially in the watery yams (3−6% w/w).

(5) Sweet potatoes are currently believed to have been brought to Polynesia by sea from South America around 1000 B.C. Historical records indicate that sweet potatoes were introduced into India, Malaysia, Indonesia, and the Philippines in the 16th century, reaching Fujian, mainland China in 1584. They first reached Japan at Miyakojima Island, in 1597. Asia accounts for 90% of the world production of 130 million tons, with China alone producing 86%.

(6) Mata, R.; Pereda-Miranda, R.; Lotina-Hennsen, B. In *Secondary Metabolites from Mexican Plants: Chemistry and Biological Properties*; Rodríguez-Hann, L., Pandalai, S. G., Eds.; Research Signpost: Trivandrum, India, 1996; pp 59−68.

(7) Hartwell, J. L. *Lloydia* **1968**, *31*, 71−170.

(8) In Mexico, traditional healers classify illnesses and herbal remedies 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, 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.

(9) Noda, N.; Yoda, S.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1992**, *40*, 3163−3168.

(10) Pereda-Miranda, R.; Bah, M. *Curr. Top. Med. Chem.* **2003**, *3*, 111−131.

(11) (a) Podányi, B.; Reid, R. S.; Kocsis, A.; Szabó, L. *J. Nat. Prod.* **1989**, *52*, 135−142. (b) Allerding, E.; Ralph, J.; Schatz, P. F.; Gniechwitz, D.; Steinhart, H.; Bunzel, M. *Phytochemistry* **2005**, *66*, 113−124.

(12) (a) Noda, N.; Takahashi, N.; Kawasaki, T.; Miyahara, K.; Yang, C.-R. *Phytochemistry* **1994**, *36*, 365−371. (b) Noda, N.; Takahashi, N.; Miyahara, K.; Yang, C.-R. *Phytochemistry* **1998**, *48*, 837−841.

(13) Chérigo, L.; Pereda-Miranda, R. *J. Nat. Prod.* **2006**, *69*, 595−599.

(14) Pereda-Miranda, R.; Escalante-Sánchez, E.; Escobedo-Martínez, C. *J. Nat. Prod.* **2005**, *68*, 226−230.

(15) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007−9022.

(16) Noda, N.; Tsuji, K.; Kawasaki, T.; Miyahara, K.; Hanazono, H.; Yang, C.-R. *Chem. Pharm. Bull.* **1995**, *43*, 1061−1063.

(17) Lee, J.-B.; Hayashi, T.; Hayashi, K.; Sankawa, U. *J. Nat. Prod.* **2000**, *63*, 136−138.

(18) Duus, J. Ø.; Gotfredsen, H.; Block, K. *Chem. Rev.* **2000**, *100*, 4589−4614.

(19) MacLeod, J. K.; Ward, A. *J. Nat. Prod.* **1997**, *60*, 467−471.

(20) Rencurosi, A.; Mitchell, E. P.; Cioci, G.; Pérez, S.; Pereda-Miranda, R.; Imberty, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5918−5922.

(21) Pereda-Miranda, R.; Hernández-Carlos, B. *Tetrahedron* **2002**, *58*, 3145−3154.

(22) Miyahara, K.; Du, X.-M.; Watanabe, M.; Sugimura, C.; Yahara, S.; Nohara, T. *Chem. Pharm. Bull.* **1996**, *44*, 481−485.

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