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Seven New Aminoacyl Sugars in *Ipomoea batatas*

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An analysis of the polar extracts from sweet potato *Ipomoea batatas* (Convolvulaceae) led to the isolation of seven unknown aminoacyl sugars. On the basis of 1D, 2D NMR, and mass spectrometry data, the structures of the compounds were elucidated as: β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-valyl]-glucopyranoside (**1**), β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-tyrosyl]-glucopyranoside (**2**), β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-threonyl]-glucopyranoside (**3**), β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-hystidyl]-glucopyranoside (**4**), 2-β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-alanyl]-glucopyranoside (**5**), β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-tryptophanyl]-glucopyranoside (**6**), and β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-glycyl]-glucopyranoside (**7**).

KEYWORDS: Camote; sweet potato; Convolvulaceae; NMR; MS

INTRODUCTION

Ipomoea batatas Lam (Convolvulaceae), more famous as sweet potato or camote, is among the world's most important, versatile, and underexploited food crops. With more than 133 million tons in annual production, sweet potato currently ranks as the fifth most important food crop on a fresh-weight basis in developing countries after rice, wheat, maize, and cassava. Considered as a "small" farmer's crop, sweet potatoes grow in many farming conditions. The crop has relatively few natural enemies, which means that pesticides are rarely used to produce it, and can be grown in poor soils with little fertilizer. More than 95% of the global sweet potato crop is produced in developing countries, where it is the fifth most important food crop. Camote is not related to the potato because it belongs to the Convolvulaceae family, not to the Solanaceae family. Nearly all the sweet potato produced is used for animal feed, with the remainder primarily used for human consumption, either as fresh or processed products (candies and alcohol) (*1*, *2*). The camote is an important source of vitamins A, B6, and E, and iron, potassium, and fiber. It contains virtually no fat and is low in sodium. It is also a substantial source of dietary fiber, especially when eaten with the skin (*3*). Nowadays sweet potato tubers are used in Africa to counteract a widespread vitamin A deficiency that results in blindness and even death for 250 000−500 000 African children per year. Therefore, it seems of interest to study the phytochemicals, plant secondary bioactive compounds, since they generally play a key role in the survival of the organisms producing them and on which humans have always been dependent for medicines, flavors, and pigments for foods (*4*, *5*). In the present study, the analysis of the MeOH extracts from camote tubers revealed the presence of seven new aminoacyl sugars in significant concentration. Aminoacylated sugars generally have characteristic taste, some of which are sweet and proposed as sweeteners (*6*−*8*). Sweetener use has largely increased in the past 20 years. Nowadays they occupy a large portion of commercial space on supermarket shelves worldwide. These products are available mainly for people who are diabetic or who are looking for low calorie materials. Sweeteners can be natural or artificial (*9*). Some studies relate the use of artificial sweeteners (saccharin and cyclamate) in large doses with bladder cancer occurrence in laboratory animals, while others present negative results. On the other hand, natural sweeteners showed low or no toxic effects (*10*, *11*). The finding of aminoacyl sugars may widen the availability of natural sweeteners.

MATERIALS AND METHODS

General Experimental Procedures. FABMS spectra (recorded in a glycerol matrix) were measured on a Prospec Fisons mass spectrometer (Danvers, NJ). ESIMS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole mass spectrometer (Warrington, Cheshire, U.K.). The spectra were recorded by infusion into the ESI source using MeOH as the solvent. NMR spectra were determined at 25 °C on Varian Unity Inova 500 NMR spectrometers (Palo Alto, CA) and processed using the Varian VNMR software package; chemical shifts were referenced to the residual solvent signal (CH_3OD: δ_H 3.34, δ_C 49.0). Homonuclear ^1H connectivities were determined by COSY experiments. The reverse-detected, gradient-enhanced single-quantum heteronuclear correlation (HSQC) spectra were optimized for an average $^1J_{CH}$ of 140 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiments were optimized for a $^3J_{CH}$ of 8 Hz. HPLC analysis in linear gradient mode was performed on a Hewlett-Packard HP 1100 series apparatus with a UV-photodiode detector (λ 200 nm) (Waldbronn, Germany), equipped with a (300 × 7.8 mm i.d.) μ-Bondapack C_{18} column (Waters, Milford, MA). Optical rotations were determined on a Jasco P-100 polarimeter (Tokio, Japan) equipped with a sodium lamp (589 nm) and a 10-mm microcell. Droplet counter current chromatography (DCCC) separation was performed on a Buchi apparatus (Flawil, Switzerland) equipped with 300 tubes (2.0 mL vol, 2.2 mm i.d.).

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Table 1. ^1H and ^{13}C NMR Data of the Glycosidic Portion of **1**–**7**[a]

	^1H	^{13}C
	Fructose	
1	3.75 (1H, d, 12.2 Hz)	63.4
	3.78 (1H, d, 12.2 Hz)	
2		105.2
3	4.11 (1H, d, 8.5 Hz)	79. 4
4	4.02 (1H, dd, 8.5, 8.2 Hz)	75.8
5	3.64 (1H, m, 4.9, 6.2, 8.2 Hz)	83.8
6	3.65 (1H, dd, 4.9, 11.8 Hz)	64.1
	3.70 (1H, dd, 6.2, 11.8; Hz)	
	Glucose	
1	5.38 (1H, d, 3.6 Hz)	93.6
2	3.40 (1H, dd, 3.6, 10.0 Hz)	73.2
3	3.81 (1H, dd, 10.0, 6.2 Hz)	74.4
4	3.36 (1H, dd, 10.0, 6.2 Hz)	71.4
5	3.70 (1H, ddd, 2.2, 6.2, 10 Hz)	74.7
6	3.80, 3.90 (2H, dd, 11.2, 6.2 Hz)	62.3

[a] Spectra were measured in CD_3OD.

Plant Material. The plant materials were supplied by Dr. Santiago Antúnez de Mayolo, Casella Postale 18-1125, Lima 18, Peru, and reference specimens were deposited at the Dipartimento di Chimica delle Sostanze Naturali, Napoli, Italy.

Extraction and Isolation. The flour from camote tubers (1 kg) was extracted with MeOH (2 L), MeOH/H_2O, 80:20 (2 L), and MeOH/H_2O, 90:10 (2 L) to give 156.4, 105.5, and 28.8 g of extract, respectively.

Compounds 1 and 2. The MeOH extract (156.4 g) was partitioned between *n*-BuOH and H_2O (600 mL). The butanolic extract (6.6 g) was evaporated and further separated by DCCC using *n*-BuOH/Me_2-CO/H_2O (60:12:28) as stationary phase and *n*-BuOH/Me_2CO/H_2O (14:12:74) as descending phase. DCCC fractions (9.0 mL) 52−73 (560.7 mg) containing the crude amino acidic mixture were chromatographed by reversed-phase HPLC with linear gradient elution from H_2O to MeOH in 20 min at a flow rate of 2.0 mL/min to yield two pure compounds: **1** (6.1 mg) and **2** (0.5 mg).

Compounds 3 and 4. The MeOH/H_2O (90:10) extract (28.8 g) was dissolved in a mixture of $CHCl_3$/MeOH/H_2O (2:2:1) and refrigerated to give a residue (2.85 g) that was dissolved in MeOH and further purified by reversed-phase HPLC with linear gradient elution from H_2O to MeOH in 20 min at a flow rate of 2.0 mL/min to yield two pure compounds: **3** (921.7 mg) and **4** (2.2 mg).

Compounds 5, 6, and 7. The MeOH/H_2O (80:20) extract (105.5 g) was partitioned between *n*-BuOH and H_2O. The aqueous extract (88.4 g) was evaporated and chromatographed on a silica gel column (30 × 2.0 cm), with *n*-BuOH/Me_2CO/H_2O (24:6:10) as eluent. Fractions (9 mL) were collected and checked by TLC (Si gel plates). Fractions 47−150 (31.8 g) and 251−300 (4.39 g) containing amino acidic derivatives were evaporated and chromatographed on a strong cationic exchange column (10 g) with H_2O (100 mL) and then with 30% NH_4OH (100 mL) as eluent. The NH_4OH fraction (1.02 g) containing the crude amino acidic mixture was further purified by reversed-phase HPLC with linear gradient elution from H_2O to MeOH in 20 min at a flow rate of 2.0 mL/min to yield three pure compounds: **5** (10.1 mg) and **6** (6.0 mg) from silica gel fraction 47−150 and **7** (7.9 mg) from silica gel fraction 251−300.

Compound 1: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-valyl]-glucopyranoside. $[\alpha]^{25}_D$ +0.04° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 442.19256 $[M + H]^+$; calculated for $C_{17}H_{31}NO_{12}$ *m*/*z* 441.42754. ESI-MS (positive ion): *m*/*z* 442 $[M + H]^+$, *m*/*z* 341, *m*/*z* 280, *m*/*z* 118, *m*/*z* 73, and *m*/*z* 57. NMR data: **Tables 1** and **2**; **Figure 1**.

Compound 2: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-tyrosyl]-glucopyranoside. $[\alpha]^{25}_D$ −0.84° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 506.18722 $[M + H]^+$; calculated for $C_{21}H_{31}NO_{13}$ *m*/*z* 505.46974. ESI-MS (positive ion): *m*/*z* 506 $[M + H]^+$, fragments: *m*/*z* 341, *m*/*z* 369, and *m*/*z* 219. NMR data: **Tables 1** and **2**; **Figure 1**.

Table 2. ^1H and ^{13}C NMR Data of the Aminoacyl Portion of **1**–**7**[a]

	^1H	^{13}C
	Valine (**1**)	
α	3.46 (1H, d, 4.4 Hz)	61.8
β	2.31 (1H, dd, 4.4 Hz)	30.1
COO		173.6
CH_3	1.06 (3H, d, 7.0 Hz)	19.2
CH_3	1.03 (3H, d, 7.0 Hz)	17.8
	Tyrosine (**2**)	
α	4.64 (1H, d, 4.0 Hz)	57.3
β	3.57, 3.47 (2H, d, 4.0 Hz)	37.5
1		138.0
2	7.10 (1H, d, 7.2 Hz)	130.5
3	6.81 (1H, d, 7.2 Hz)	117.5
4		156.3
5	6.81 (1H, d, 7.2 Hz)	117.5
6	7.10 (1H, d, 7.2 Hz)	130.5
COO		175.0
	Threonine (**3**)	
α	4.43 (1H, d, 4.2 Hz)	67.9
β	4.72 (1H, d, 4.2 Hz)	61.1
COO		173.6
CH_3	1.60 (1H, d, 7.1 Hz)	20.0
	Hystidine (**4**)	
α	4.60 (1H, d, 4.0 Hz)	58.4
β	3.87, 3.77 (2H, d, 4.0 Hz)	29.8
1		130.2
2	7.43 s	118.7
4	8.36 s	135.1
COO		180.0
	Alanine (**5**)	
α	3.79 (1H, d, 7.2 Hz)	47.7
β	1.86 (3H, d, 5.4 Hz)	20.1
COO		173.5
	Tryptophan (**6**)	
α	3.79, 3.58 (1H, d, 4.0 Hz)	27.6
β	4.65 (1H, d, 4.0 Hz)	56.4
1		108.8
2	7.27 s	126.1
4	7.40 (1H, d, 7.6 Hz)	123.4
5	7.15 (1H, t, 7.6 Hz)	119.6
6	7.01 (1H, t, 7.6 Hz)	120.8
7	7.61 (1H, d, 7.6 Hz)	113.3
8		137.8
9		127.8
COO		175.5
	Glycine (**7**)	
α	3.57 s	43.6
COO		173.6

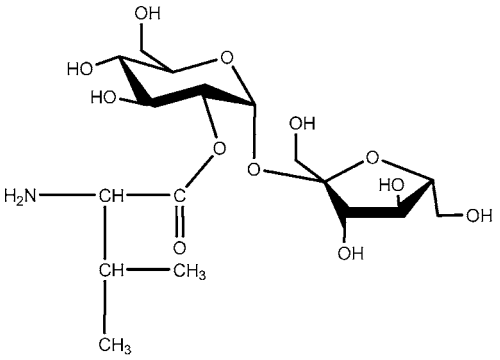
[a] Spectra were measured in CD_3OD.

Compound 3: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-threonyl]-glucopyranoside. $[\alpha]^{25}_D$ −0.50° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 444.17188 $[M + H]^+$; calculated for $C_{16}H_{31}NO_{13}$ *m*/*z* 443.40036. ESI-MS (positive ion): *m*/*z* 444 $[M + H]^+$, fragments: *m*/*z* 341, *m*/*z* 281, *m*/*z* 197, and *m*/*z* 120. NMR data: **Tables 1** and **2**; **Figure 2**.

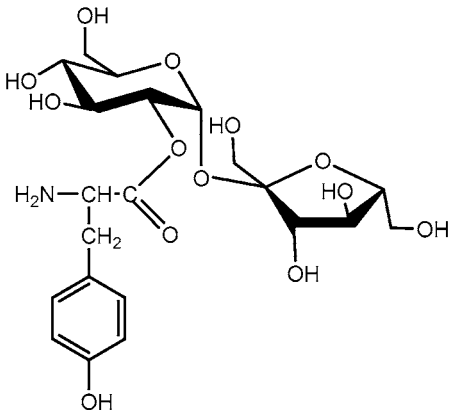
Compound 4: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-hystidyl]-glucopyranoside. $[\alpha]^{25}_D$ +0.09° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 480.18308 $[M + H]^+$; calculated for $C_{18}H_{29}N_3O_{12}$ *m*/*z* 479.43576. ESI-MS (positive ion): *m*/*z* 480 $[M + H]^+$, fragments: *m*/*z* 341, *m*/*z* 323. NMR data: **Tables 1** and **2**; **Figure 2**.

Compound 5: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-alanyl]-glucopyranoside. $[\alpha]^{25}_D$ +0.03° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 414.16130 $[M + H]^+$; calculated for $C_{15}H_{27}NO_{12}$ *m*/*z* 413.37438. ESI-MS (positive ion): *m*/*z* 414 $[M + H]^+$ and fragment: *m*/*z* 341, *m*/*z* 111. NMR data: **Tables 1** and **2**; **Figure 3**.

Compound 6: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-tryptophanyl]-glucopyranoside. $[\alpha]^{25}_D$ −0.02° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 529.20348 $[M + H]^+$; calculated for $C_{23}H_{32}N_2O_{12}$ *m*/*z*

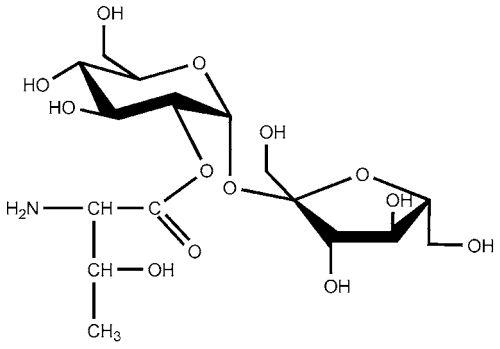


1, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-valyl]-glucopyranoside

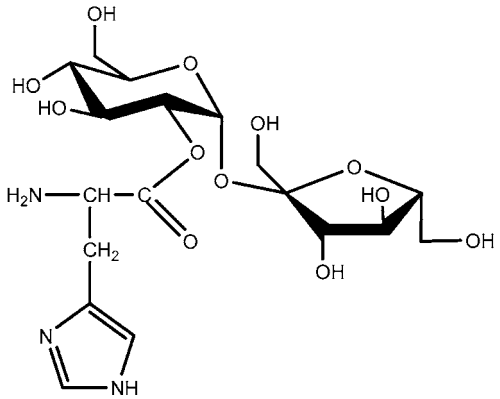


2, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-tyrosyl]-glucopyranoside

Figure 1. Compounds **1** and **2** isolated from *I. batatas* tubers.



3, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-threonyl]-glucopyranoside



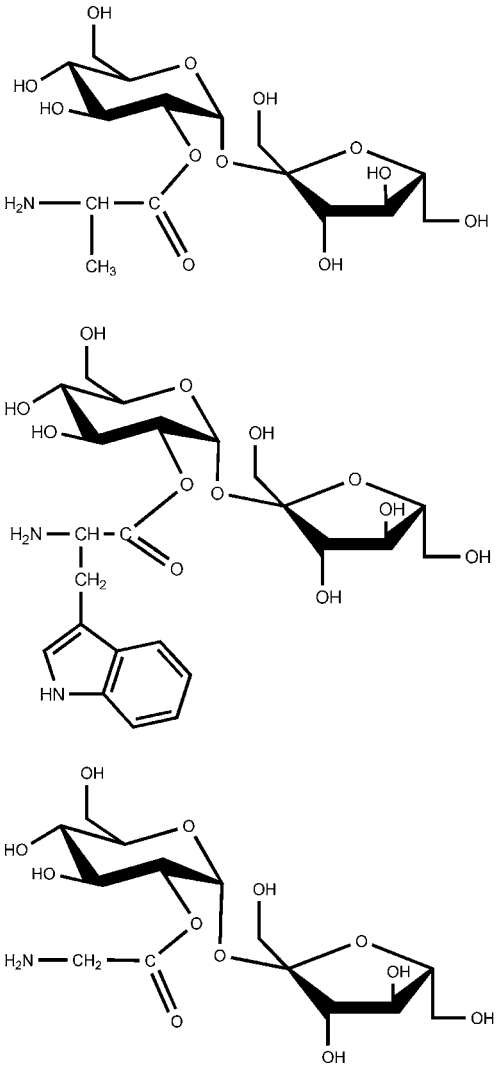
4, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-hystidyl]-glucopyranoside

Figure 2. Compounds **3** and **4** isolated from *I. batatas* tubers.

528.50638. ESI-MS (positive ion): *m*/*z* 529 $[M + H]^+$, fragments: *m*/*z* 341, *m*/*z* 367, and *m*/*z* 134. NMR data: **Tables 1** and **2**; **Figure 3**.

Compound 7: β-D-Fructofuranosyl-(2 → 1)-α-D-[2-*O*-glycyl]-glucopyranoside. $[\alpha]^{25}_D$ + 0.07° (*c* 0.003 in MeOH); HRFABMS positive ion found *m*/*z* 400.14564 $[M + H]^+$; calculated for $C_{14}H_{25}NO_{12}$ *m*/*z* 399.34780. ESI-MS (positive ion): *m*/*z* 400 $[M + H]^+$, fragments: *m*/*z* 341, *m*/*z* 218, and *m*/*z* 202. NMR data: **Tables 1** and **2**; **Figure 3**.

Acid Hydrolysis of Compounds 1−7: Monosaccharide Composition. A solution of each compound (1 mg) in 10% H_2SO_4/EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The resulting monosaccharides were reacted with TRISIL-Z (Pierce) and analyzed by GC−MS on a 25 m × 0.32 mm × 25 m



5, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-alanyl]-glucopyranoside

6, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-tryptophanyl]-glucopyranoside

7, β-D-fructofuranosyl-(2→1)-α-D-[2-*O*-glycyl]-glucopyranoside

Figure 3. Compounds **5**–**7** isolated from *I. batatas* tubers.

l-Chirasil-Val column (Alltech, Deerfield, IL). The temperature for the injector and detector was 200 °C, and the temperature gradient system for the oven was as follows: 100 °C for 1 min, raised to 180 °C at 5 °C/min. Retention times were identical to those of the authentic trimethylsilylated sugars.

RESULTS AND DISCUSSION

The flour from camote tubers was exhaustively extracted with MeOH, MeOH/H_2O (90:10), and MeOH/H_2O (80:20) to give seven new pure compounds. The extracts were partitioned among solvents and then purified by sequential chromatographic techniques, affording the aminoacyl sucrose derivatives.

The positive ESI MS spectrum of compound **1** showed a pseudomolecular ion peak at *m*/*z* 442 $[M + H]^+$. High-resolution measurements on the pseudomolecular ion peak indicated the molecular formula $C_{17}H_{31}NO_{12}$. Diagnostic resonances in the ^{1}H NMR spectrum of **1** (CD_3OD, **Tables 1** and **2**) were those attributed to two methyls (δ 1.06, 1.03), two methynes (δ 3.46, 2.31), and one anomeric proton (δ 5.38). The ^{13}C NMR spectrum (**Tables 1** and **2**) showed 17 resonance lines, supporting the molecular formula deduced from MS, five of them attributed to the aglycon and 12 to two monosaccharides. All the proton resonances of each compound were unambiguously associated with the relevant carbon atoms by using the HSQC spectrum. The proton sequence within each spin system was elucidated by the series of cross-peaks of the COSY spectrum, while data arising from the HMBC experiment were used to interconnect the partial structures. Analysis of 2D COSY, HSQC, and HMBC spectra of **1** allowed assignment of all the signals belonging to the aglycon moiety, which was identified as a valine. Concerning the sugar portions of the molecule, COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides. All proton resonances were correlated with those of their corresponding carbons from the HSQC experiment. The first step of the analysis was the association of the anomeric carbon (δ 93.6) with the relevant anomeric proton signal (δ 5.38), through the HSQC experiment. Starting from the anomeric proton at δ 5.38 (H-1 Glu), we identified the sequence of a hexopyranose unit. The small coupling constants observed for H-1 Glu/H-2 Glu indicated its α-glucopyranose nature. Starting from the H-6 of the second sugar at δ 3.65, with the same type of analysis, it was identified as a *β*-fructofuranose (*12*). The interglycosidic and the sequential arrangements of the disaccharide moiety linked at COOH of the aglycon were deduced from the HMBC spectra. HMBC cross-peaks were detected between the anomeric proton (H-1) of a glucose at δ 5.38 and the fructose signal at δ 105.2 (C-2) as well as between the glucose (H-2) signal at δ 3.40 and the signal at δ 173.6 of aglycon COOH, which indicated the linkage of a glucose to fructose and to the carboxylic group of the aglycon. To confirm the nature of the sugar units and to determine their absolute configuration, **1** was

subjected to acid hydrolysis (1 N HCl), followed by trimethylsilylation and GC analysis in comparison with authentic trimethylsilylated sugars. By this procedure, the sugars were identified as D-glucose and D-fructose. This procedure was applied to all of the new isolated compounds. From these data, the structure of **1** was established as β-D-fructofuranosyl-(2 → 1)-α-D-[2-*O*-valyl]-glucopyranoside (**Figure 1**).

The structure elucidation of compounds **2**−**7** was aided by comparison with the MS and NMR data obtained for **1**. The MS and NMR profiles of each compound (**Tables 1** and **2**) appeared to be superimposable with those of **1** for the signals relative to the sugar portion. The aglycon unit was identified on the basis of MS profile, chemical shifts, multiplicity of the signals, absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data (**Tables 1** and **2**) and by comparison with literature data (*13*). Diagnostic HMBC cross-peaks between the glucose (H-2) signal at approximately δ 3.40 and carboxylic group of aglycons **2**−**7** at δ 175.0, 173.6, 180.0, 173.5, 175.5, 173.6, respectively, indicated the same substitution position on sucrose for each compound.

In conclusion, the present study reports the isolation of seven new aminoacyl sucrose derivatives. These findings are important because very little is known about amino acid sugar esters from natural sources. Moreover, it is possible to suppose their use as sweeteners because all of the seven compounds show the presence of L-aminoacyl groups at C-2 and the α-configuration at C-1 of glucose that seems to be the structural prerequisite for sweetness in this group of compounds (*6*). From a phytochemical point of view, these compounds may be used for a variety of identification because their occurrence has not been reported thus far.

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