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Alkaloidal Components in the Poisonous Plant, *Ipomoea carnea* (Convolvulaceae)

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Natural intoxication of livestock by the ingestion of *Ipomoea carnea* (Convolvulaceae) sometimes occurs in tropical regions of the world. Polyhydroxylated alkaloids were isolated from the leaves, flowers, and seeds of the poisonous plant and characterized. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine (**1**), 2-*epi*-lentiginosine (**2**), calystegines B_1 (**3**), B_2 (**4**), B_3 (**5**), and C_1 (**6**), and *N*-methyl-*trans*-4-hydroxy-L-proline (**7**). The contents of **1** in the fresh leaves and flowers were 0.0029 and 0.0028%, respectively, whereas the contents of **1**, **3**, and **4** in the seeds were ∼10 times higher than those in the leaves and flowers. Alkaloids **3**, **4**, and **6** showed a potent inhibitory activity toward rat lysosomal β-glucosidase, with IC_{50} values of 2.1, 0.75, and 0.84 μM, respectively, and alkaloid **5** was a moderate inhibitor of α- and β-mannosidases. Although alkaloid **1** is known as a powerful inhibitor of lysosomal α-mannosidase (IC_{50} = 0.02 μM), alkaloid **2**, which has been thought to be an intermediate in the biosynthesis of **1**, was also a potent inhibitor of α-mannosidase with an IC_{50} value of 4.6 μM.

KEYWORDS: *Ipomoea carnea*; poisonous plant; intoxication in livestock; polyhydroxylated alkaloids; glycosidase inhibition

INTRODUCTION

Certain poisonous plants often cause serious livestock losses. The Australian legume species, *Swainsona*, are known as "poison peas", and sheep eating them develop a syndrome called "pea struck" (*1*, *2*). There is also the livestock poisoning by the closely related *Astragalus* and *Oxytropis* species, which are found throughout most of the world, and intoxication of livestock by certain of those species known as locoweeds in the western United States is called "locoism" (*2*, *3*). The common clinical symptoms in livestock on ingestion of these poisonous legumes are depression, tremors, nervousness, ema-

ciation, gastrointestinal malfunction, and reproductive alterations (*4*, *5*), and the poisoning is characterized by cytoplasmic vacuolation of neuronal cells due to accumulation of mannose-rich oligosaccharides in lysosomes (*6*). The trihydroxyindolizidine alkaloid swainsonine (**1**) (**Figure 1**) occurs in these legumes and has been identified as a causative agent in locoism (*3*, *7*). Swainsonine is a potent inhibitor of lysosomal (*8*) and Golgi α-mannosidase II (*9*). Lysosomal α-mannosidase and Golgi α-mannosidase II belong to class II α-mannosidases and cleave α1,2-, α1,3-, and α1,6-linked mannose residues (*10−12*). Prolonged ingestion of swainsonine by animals leads to a phenocopy of the genetically induced lysosomal storage disease mannosidosis (*6*). The concentration of swainsonine detected in all plants implicated in poisoning is not high. The yield of swainsonine from the vegetative parts of *Astragalus lentiginosus* was found to be 0.007% (dry weight) (*3*). A threshold of toxicity is difficult to establish for swainsonine, but a conservative approach suggests that levels in excess of 0.001% should be of concern (*13*). Swainsonine is a lysosomotropic compound and

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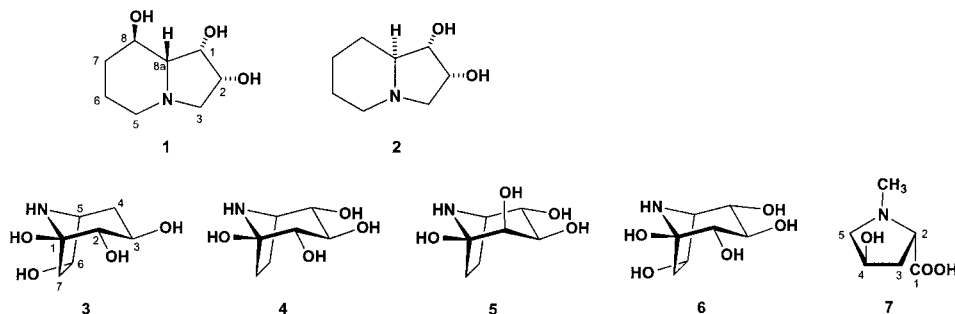⊗ Agricultural Research Service.

Figure 1. Structures of alkaloids isolated from *I. carnea*.

accumulates rapidly in lysosomes of normal human fibroblasts in culture to produce inhibition of intracellular lysosomal α-mannosidase (*14*).

Swainsonine is also found in certain species of the plant family Convolvulaceae. Weir vine [*Ipomoea* sp. Q6 (aff. *calobra*)] grows in a small area of southern Queensland in Australia and is reported to produce neurological disorders in livestock. The clinical symptoms are similar to those caused by swainsonine-containing legumes. Molyneux et al. detected swainsonine in the seeds and estimated the level as 0.058% by gas chromatography−mass spectrometry (GC-MS) (*15*). There are serious livestock poisonings reported for other *Ipomoea* species. *Ipomoea carnea* is a plant of tropical American origin but is now widely distributed in the tropical regions of the world. This plant is erect, densely leafed, and almost unbranched, growing as shrubs to 2−3 m high (*16*). Natural intoxication occurs in livestock that chronically ingest the plant, and the early poisoning reports were from Sudan and India (*17*, *18*). The toxicity has been confirmed in feeding experiments with goats and sheep (*19*, *20*). The nortropane alkaloids calystegines B$_2$ (**4**) and C$_1$ (**6**), together with swainsonine (**1**) (**Figure 1**), have been detected in the leaves collected in Mozambique where goats were intoxicated (*20*). Calystegines B$_2$ and C$_1$ are potent inhibitors of glycosidases (*21*). Hence, whether the poisoning by this plant is due to a single effect of swainsonine or a combination of toxic effects by swainsonine and calystegines is a very important problem (*15*, *20*, *22*).

Poisonous plants are a serious problem for livestock breeding in Brazil, and ∼75 plants have had their toxicity confirmed by experiments with the animal species affected under natural conditions (*23*). *I. carnea* is found in most regions of Brazil and is an evergreen plant blooming throughout the year (**Figure 2**). The animals eat this plant especially in drought periods because it is one of the few plants that stay green. In experimental studies in which the plant was given to adult goats, all animals showed disorders of behaviors and consciousness as well as abnormalities of gait, ability to stand, and posture (**Figure 3**), and one goat died (*23*). The histopathological changes were cytoplasmic vacuolation of the neurons in the central and peripheral nervous system, suggesting a lysosomal storage disorder by lysosomal glycosidase inhibition. In this study we describe the isolation and characterization of alkaloids from the leaves, flowers, and seeds of *I. carnea* in Brazil as well as the alkaloid level in each part, and inhibitory activities of alkaloids toward rat lysosomal glycosidases were investigated.

MATERIALS AND METHODS

General Methods. The purity of samples was checked by HPTLC on silica gel 60F$_{254}$ (E. Merck) using the solvent system PrOH/AcOH/H$_2$O (4:1:1), and a chlorine−*o*-tolidine reagent or iodine vapor was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter (Tokyo, Japan). ^{1}H NMR (500 MHz) and ^{13}C

Figure 2. *I. carnea* growing in a culture at CEPTOX, School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil.

Figure 3. Contracture defects in the front legs of a goat produced by consumption of *I. carnea*.

NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer (Tokyo, Japan). Chemical shifts are expressed in parts per million downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D$_2$O as an internal standard. FABMS were measured using glycerol as a matrix on a JEOL JMS-SX 102A spectrometer.

GC-MS Analysis. Samples were dried and trimethylsilylated by treatment with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide in pyridine

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at 60 °C for 1 h. Analyses were performed on a Hewlett-Packard 5890 series II instrument equipped with a 5971 mass-selective detector operating at 70 eV, an on-column injector, and a 60 m × 0.32 mm i.d. SE-30 fused silica column. The column was temperature programmed from 120 to 300 °C at 10 °C/min.

Plant Materials. Identification of *I. carnea* Jacq. subsp. *fistulosa* (Mart. ex. Choisy) D. Austin was confirmed by taxonomist Rosangela Simão Bianchini of the Instituto de Botanica de São Paulo, Brazil. The leaves and flowers of *I. carnea* were collected in May 2001 and April 2002, respectively, from the culture in the Research Center for Veterinary Toxicology (CEPTOX), School of Veterinary Medicine, University of São Paulo, Brazil. The seeds were collected in July 2002 from the fields of Votuporanga City, São Paulo, Brazil. A voucher specimen was deposited in the Herbarium of the Instituto de Botanica de São Paulo, Brazil (SP-360911).

Extraction and Isolation. The fresh leaves (2 kg) of *I. carnea* were homogenized in 50% aqueous EtOH (6 L) and allowed to stand for a week. The filtrate was evaporated to give a brown syrup (50 g). This syrup was dissolved in water (500 mL) and applied to a column of Amberlite IR-120B (150 mL, H$^+$ form). The 0.5 M NH$_4$OH eluate was concentrated to give a brown syrup (4.5 g), which was further chromatographed over a Dowex 1-X2 (150 mL, OH$^-$ form) column with H$_2$O (1.2 L) as eluant to give a colorless syrup (1.13 g). The syrup was applied to an 87 cm × 1.8 cm Amberlite CG-50 column (NH$_4^+$ form) with H$_2$O as eluant (fraction size = 9.5 mL). The H$_2$O eluate was divided into four pools: I (fractions 12−21); II (fractions 28−38); III (fractions 39−54); and IV (fractions 64−115). The column was eluted with 0.5 M NH$_4$OH, and fractions 4−21 were collected and designated pool V. Total yields of pools I, II, III, IV, and V were 320, 50, 16, 65, and 46 mg, respectively. The successive chromatography of pool I with Dowex 1-X2 (OH$^-$ form) and Amberlite CG-50 (NH$_4^+$ form) gave *N*-methyl-*trans*-4-hydroxy-L-proline (4 mg, **7**) and calystegine C$_1$ (25 mg, **6**). Other pools were similarly chromatographed with Dowex 1-X2 (OH$^-$ form) and Amberlite CG-50 (NH$_4^+$ form) to give calystegine B$_2$ (41 mg, **4**) from pool II, calystegine B$_1$ (13.7 mg, **3**) from pool III, calystegine B$_3$ (7.8 mg, **5**) and swainsonine (55 mg, **1**) from pool IV, and 2-*epi*-lentiginosine (5.7 mg, **2**) from pool V.

In the isolation procedures described above, it was found that most of **7** in the 50% aqueous EtOH extract was adsorbed on the anion-exchange resin Dowex 1-X2 (H$^-$ form). Hence, we improved the isolation method of **7** to determine the content in the fresh leaves. The 50% aqueous EtOH extract (1 g) obtained from 40 g of the fresh leaves was applied to a column of Dowex 50W-X2 (50 mL, H$^+$ form) and eluted with 0.5 M NH$_4$OH. The eluate was concentrated to give a brown syrup (280 mg). This syrup was chromatographed over a Dowex 50W-X8 (50 mL, pyridine form) with 0.1 M pyridinium formate buffer (pH 3.1) as eluant. Fractions 8−15 (fraction size = 5 mL) were concentrated and further chromatographed on an Amberlite CG-50 column (1−65 cm, NH$_4^+$ form) with H$_2$O as eluant to give 24.8 mg (0.062% of fresh weight) of **7**.

The fresh flowers (590 g) and seeds (130 g) were similarly extracted with 50% aqueous EtOH, and the extracts were chromatographed according to the former method to give **1** (16.6 mg), **3** (2.1 mg), and **4** (4.3 mg) from the flowers and **1** (43.1 mg), **3** (6.8 mg), **4** (27.7 mg), **5** (0.8 mg), and **6** (5.2 mg) from the seeds.

Swainsonine (1): ^{13}C NMR (D$_2$O) δ 25.6 (C6), 34.9 (C7), 54.1 (C5), 63.0 (C3), 68.8 (C8), 71.5 (C2), 72.1 (C1), 75.2 (C8a). The trimethylsilyl (TMSi) derivative of **1**, with a GC retention time of 14.21 min, gave the molecular ion at *m/z* 389 and the base peak at *m/z* 185. These data were identical to those of the tri-TMSi derivative of swainsonine.

2-epi-Lentiginosine (2): [α]$_D$ −31.7° (*c* 0.25, H$_2$O); ^{1}H NMR (D$_2$O) δ 1.19 (1H, m, H-6*ax*), 1.31 (1H, m, H-7*ax*), 1.44 (1H, m, H-8*ax*), 1.67 (1H, m, H-8*eq*), 1.83 (1H, m, H-7*eq*), 1.97 (1H, m, H-6*eq*), 2.06 (1H, ddd, *J* = 2.7, 9.1, 11.1 Hz, H-8a), 2.14 (1H, ddd, *J* = 2.7, 11.4, 11.4 Hz, H-5*ax*), 2.19 (1H, dd, *J* = 5.0, 10.5 Hz, H-3), 2.98 (1H, m, H-5*eq*), 3.42 (1H, dd, *J* = 6.9, 10.5 Hz, H-3′), 3.60 (1H, dd, *J* = 6.9, 9.1 Hz, H-1), 4.18 (1H, ddd, *J* = 5.0, 6.9, 6.9 Hz, H-2); ^{13}C NMR (D$_2$O) δ 25.9 (C-7), 27.1 (C-8), 30.6 (C-6), 55.2 (C-5), 63.0 (C-3), 69.1 (C-8a), 69.7 (C-2), 77.4 (C-1). The TMSi derivative of **2**, with a GC retention time of 11.21 min, gave the molecular ion at *m/z* 301

and the base peak at *m/z* 97. These data were identical to those of the di-TMSi derivative of 2-*epi*-lentiginosine.

Calystegine B$_1$ (3): ^{13}C NMR (D$_2$O) δ 38.9 (C-4), 43.6 (C-7), 62.9 (C-5), 72.6 (C-3), 75.9 (C-6), 81.3 (C-2), 93.8 (C-1). The TMSi derivative of **3**, with a GC retention time of 14.93 min, gave [M − CH$_3$]$^+$ at *m/z* 448, [M − HOTMSi] at *m/z* 373, and the base peak at *m/z* 244. These data were identical to those of the tetra-TMSi derivative of calystegine B$_1$.

Calystegine B$_2$ (4): ^{13}C NMR (D$_2$O) δ 24.5 (C-6), 31.5 (C-7), 58.6 (C-5), 77.6 (C-4), 77.7 (C-3), 80.4 (C-2), 93.2 (C-1). The TMSi derivative of **4**, with a GC retention time of 15.97 min, gave the molecular ion at *m/z* 463 and the base peak at *m/z* 217. These data were identical to those of the tetra-TMSi derivative of calystegine B$_2$.

Calystegine B$_3$ (5): ^{13}C NMR (D$_2$O) δ 22.9 (C-6), 34.1 (C-7), 58.2 (C-5), 73.0 (C-3), 75.2 (C-4), 77.2 (C-2), 92.9 (C-1). The TMSi derivative of **5**, with a GC retention time of 14.85 min, gave the molecular ion at *m/z* 463 and the base peak at *m/z* 217. These data were identical to those of the tetra-TMSi derivative of calystegine B$_3$.

Calystegine C$_1$ (6): ^{13}C NMR (D$_2$O) δ 43.6 (C-7), 67.4 (C-5), 71.7 (C-6), 75.5 (C-4), 77.7 (C-3), 79.4 (C-2), 93.6 (C-1). The TMSi derivative of **6**, with a GC retention time of 17.21 min, gave [M − CH$_3$]$^+$ at *m/z* 536 and the base peak at *m/z* 217. These data were identical to those of the penta-TMSi derivative of calystegine C$_1$.

N-Methyl-trans-4-hydroxy-L-proline (7): [α]$_D$ −67.9° (*c* 0.34, H$_2$O); ^{1}H NMR (D$_2$O) δ 2.26 (1H, ddd, *J* = 4.6, 11.0, 14.2 Hz, H-3α), 2.49 (1H, dddd, *J* = 1.8, 2.3, 7.3, 14.2 Hz, H-3β), 3.05 (3H, s, *N*-CH$_3$), 3.22 (1H, ddd, *J* = 1.8, 2.3, 12.8 Hz, H-5β), 3.97 (1H, dd, *J* = 4.6, 12.8 Hz, H-5α), 4.20 (1H, dd, *J* = 7.3, 11.0 Hz, H-2), 4.64 (1H, m, H-4); ^{13}C NMR (D$_2$O) δ 41.1 (C-3), 46.0 (*N*-CH$_3$), 65.5 (C-5), 72.3 (C-4), 73.0 (C-2), 175.8 (C-1); HRFABMS, *m/z* 146.0821 [M + H]$^+$ (C$_6$H$_{12}$NO$_3$ requires 146.0817).

Glycosidase Inhibitory Activities. The rat epididymal fluid was purified from epididymis according to the literature (*11*) and used as the source of lysosomal glycosidases. The reaction mixture consisted of 50 µL of 0.2 M acetate buffer (pH 5.0), 50 µL of 2% Triton X-100 (Sigma Chemical Co.), 30 µL of the enzyme solution, and 20 µL of an inhibitor solution or H$_2$O. The reaction mixture was preincubated at 0 °C for 10 min and started by the addition of 50 µL of 6 mM 4-methylumbelliferyl glycoside (Sigma Chemical Co.) (1 mM in the case of β-galactoside), followed by incubation at 37 °C for 1−2 h. The reaction was stopped by the addition of 2 mL of 0.1 M glycine buffer (pH 10.6). Liberated 4-methylumbelliferone was measured (excitation, 362 nm; emission, 450 nm) with a Hitachi fluorescence spectrophotometer F-4500 (Tokyo, Japan).

RESULTS AND DISCUSSION

GC-MS Analyses of Extracts of *I. carnea*. After 50% aqueous EtOH extracts of the leaves, flowers, and seeds of *I. carnea* were preliminarily purified with Amberlite IR-120B (H$^+$ form) and Dowex 1-X2 (OH$^-$ form), they were trimethylsilylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (*25*). Analysis of the extracts by capillary GC-MS of their trimethyl (TMSi) derivatives demonstrated the presence of swainsonine (**1**) and calystegines B$_1$ (**3**), B$_2$ (**4**), B$_3$ (**5**), and C$_1$ (**6**) from all parts of *I. carnea*. GC analysis of the seed extract is shown in **Figure 4**. The major component in all extracts, with a GC retention time of 14.21 min, gave a molecular ion at *m/z* 389 and a base peak at *m/z* 185. These data were identical to those of the tri-TMSi derivative of swainsonine. The secondary components (15.97 min) in all extracts had a mass spectrum identical in all respects to that of the tetra-TMSi derivative of a standard sample of calystegine B$_2$, with a molecular ion at *m/z* 463 and a base peak at *m/z* 217 characteristic of compounds having three adjacent trimethylsilylated secondary hydroxyl groups. The other two peaks with retention times of 14.93 and 17.21 min had the same fragmentation patterns as those of authentic calystegines B$_1$ and C$_1$, respectively. The tetra-TMSi calystegine B$_1$ and penta-TMSi
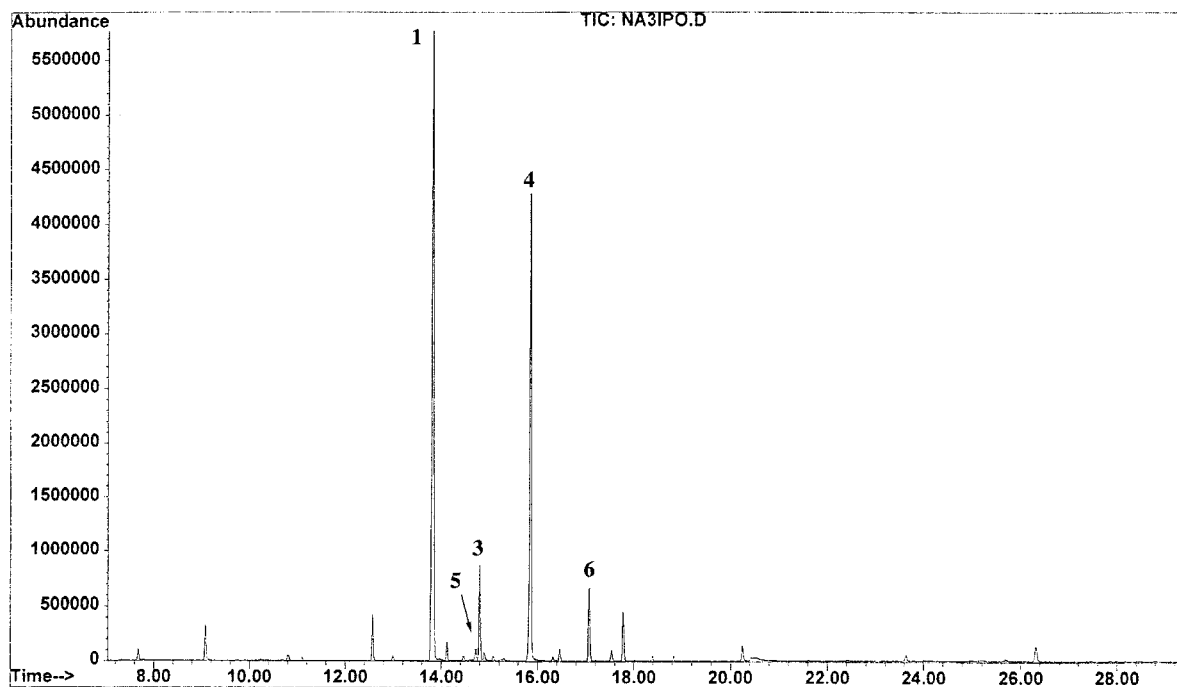
Figure 4. GC-MS analysis of the trimethylsilylated resin-treated extract of the seeds of *I. carnea*. Peaks: 1, swainsonine; 3, calystegine B$_1$; 4, calystegine B$_2$; 5, calystegine B$_3$; 6, calystegine C$_1$.

calystegine C$_1$ showed characteristic fragment ions at *m/z* 448 [M − CH$_3$]$^+$, 373 [M − HOTMSi]$^+$ with a base peak at *m/z* 244, *m/z* 536 [M − CH$_3$]$^+$, and 461 [M − HOTMSi]$^+$ with a base peak at *m/z* 217, respectively. The minor component with a retention time of 14.85 min had a mass spectrum (*m/z* 463 [M]$^+$ and a base peak at *m/z* 217) consistent with those of calystegine B$_3$.

Isolation and Characterization of Alkaloids. The fresh leaves (2 kg) of *I. carnea* were extracted with 50% aqueous EtOH. The chromatographic separation of the extract using various cation and anion ion-exchange resins afforded seven alkaloids: **1** (55 mg), **2** (5.7 mg), **3** (13.7 mg), **4** (41 mg), **5** (7.8 mg), **6** (25 mg), and **7** (4 mg). The GC-MS and ^{13}C NMR spectra of alkaloids **1, 3, 4, 5,** and **6** were in accord with those of swainsonine and calystegines B$_1$, B$_2$, B$_3$, and C$_1$, respectively. The ^{1}H and ^{13}C NMR spectra of **2** were consistent with those of 2-*epi*-lentiginosine isolated from fermentations of the fungus *Rhizoctonia leguminicola* (*26*) and the leaves of the legume *Astragalus lentiginosus* (*27*). GC-MS analysis of **2** gave a retention time (11.21 min) and mass spectrum identical to those of an authentic sample of 2-*epi*-lentiginosine. Comparison of the optical rotations of the natural product [[α]$_D$ −31.7° (*c* 0.25, H$_2$O) from *I. carnea*; [α]$_D$ −32.5° (*c* 0.13, CHCl$_3$) from *A. lentiginosus*] and synthetic sample [[α]$_D$ −39.4° (*c* 0.58, CHCl$_3$)] (*29*) established the structure of **2** as (1*S*,2*R*,8a*S*)-1,2-dihydroxyindolizidine. The ^{1}H and ^{13}C NMR spectra of **7** were superimposable with those of a novel imino acid, *N*-methyl-*trans*-4-hydroxy-L-proline, isolated from the leaves of *Copaifera multijuga* (Caesalpinioideae), and the optical rotation [[α]$_D$ −67.9° (*c* 0.34, H$_2$O)] of **7** was also similar to that [[α]$_D$ −74.0° (*c* 0.01, MeOH)] in the literature (*28*).

The contents of alkaloids in the leaves, flowers, and seeds of *I. carnea* are summarized in **Table 1**. Although a novel imino acid **7** was isolated from the leaves as a minor component, the isolation method using an anion-exchange resin Dowex 1-X2 (OH$^-$ form) was found to cause a significant loss of yield. Hence, we developed a concise isolation method for this imino acid. The 50% EtOH extract was applied to a Dowex 50W-X2

Table 1. Alkaloid Content in Fresh Plant Materials

alkaloid	content (%) in fresh wt^a		
	leaves	flowers	seeds
swainsonine, **1**	0.0029	0.0028	0.0332
2-*epi*-lentiginosine, **2**	0.0003	−^{b}	−
calystegine B$_1$, **3**	0.0007	0.0004	0.0052
calystegine B$_2$, **4**	0.0021	0.0007	0.0213
calystegine B$_3$, **5**	0.0004	−	0.0006
calystegine C$_1$, **6**	0.0013	−	0.0040
N-methyl-*trans*-4-hydroxy-L-proline, **7**	0.0620	nd^c	nd

^{a} [Isolated alkaloid (g)/fresh wt (g) of plant material] × 100. ^{b} −, not detected. ^{c} nd, not determined.

(H$^+$ form) column and eluted with 0.5 M NH$_4$OH to give a total alkaloid fraction. This fraction was applied to a Dowex 50W-X8 (pyridine form) column and eluted with 0.1 M pyridinium formate buffer (pH 3.1) as eluant to give a pure sample of **7**. The content in the fresh leaves was estimated as 0.062%. The contents of swainsonine (**1**) in the fresh leaves and fresh flowers were 0.0029 and 0.0028%, respectively, whereas the contents of **1** and calystegines B$_1$ (**3**) and B$_2$ (**4**) in the seeds were ~10 times higher than those in the leaves and flowers. The contents of **1** in *I. carnea* described above are in fresh weight and exceed the level of 0.001% in dry weight (*13*) estimated to produce neurological damage in livestock, which is derived from lysosomal storage by the chronic inhibition of α-mannosidase.

2-*epi*-Lentiginosine is probably an intermediate in the biosynthesis of swainsonine in *R. leguminicola* because the experimental refeeding of [^{3}H]-2-*epi*-lentiginosine resulted in a very high level (45%) of incorporation of radioactivity into swainsonine (*26*). Very interestingly, Blanco and Sardina have enantiospecifically synthesized 8-*epi*-swainsonine from *trans*-4-hydroxy-L-proline (*30*).

Biological Activities. The 50% EtOH extracts of the leaves, flowers, and seeds were preliminarily treated with Amberlite IR-120B (H$^+$ form) and tested for inhibitory activity of

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Table 2. Concentration of the Resin-Treated Extracts of *I. carnea* Giving 50% Inhibition of Rat Epididymis Glycosidases

enzyme[a]	IC$_{50}$ (μg/mL)		
	leaves	flowers	seeds
α-glucosidase	—[b]	—	—
β-glucosidase	1.7	4.6	0.6
α-galactosidase	290	760	230
β-galactosidase	570	—	—
α-mannosidase	0.51	0.034	0.016
β-mannosidase	730	—	—
α-fucosidase	—	—	—

[a] Activities of rat epididymis glycosidases were determined using 4-methylumbelliferyl glycoside as substrate at pH 5.0. [b] —, no inhibition (<50% inhibition at 1000 μg/mL).

Table 3. Concentration of Alkaloids Isolated from *I. carnea* Giving 50% Inhibition of Rat Epididymis Glycosidases

enzyme[a]	IC$_{50}$ (μM)					
	1	2	3	4	5	6
α-glucosidase	—[b]	—	—	—	—	—
β-glucosidase	—	—	2.1	0.75	20	0.84
α-galactosidase	—	—	—	120	—	—
β-galactosidase	—	—	—	—	—	—
α-mannosidase	0.02	4.6	—	710	150	—
β-mannosidase	—	—	—	—	90	—
α-fucosidase	—	—	—	—	—	—

[a] Activities of rat epididymis glycosidases were determined using 4-methylumbelliferyl glycoside as substrate at pH 5.0. [b] —, no inhibition (<50% inhibition at 1000 μM).

lysosomal glycosidases prepared from rat epididymis. The inhibitory activities were determined fluorometrically by using the appropriate fluorogenic substrates. As shown in **Table 2** all resin-treated extracts showed potent inhibitory activities toward lysosomal β-glucosidase and α-mannosidase and a weak inhibitory activity toward α-galactosidase. In particular, the seed extract powerfully inhibited α-mannosidase and β-glucosidase with IC$_{50}$ values of 0.016 and 0.6 μg/mL, respectively.

The IC$_{50}$ values of alkaloids isolated from *I. carnea* toward rat lysosomal glycosidases are shown in **Table 3**. In our present study, swainsonine (**1**) showed an IC$_{50}$ value of 0.02 μM toward rat epididymis α-mannosidase, whereas Dorling et al. have also calculated the IC$_{50}$ value of **1** toward jackbean α-mannosidase as 0.02 μM (*8*). Swainsonine is known to inhibit human liver lysosomal α-mannosidase in a competitive manner with a K_i value of 0.07 μM (*31*). In 1984, the racemate of 2-*epi*-lentiginosine (**2**) was synthesized from 3-pyrroline and reported to be a very weak inhibitor of acid α-mannosidase with an IC$_{50}$ value of 7.5 mM (*32*). Pastuzak et al. reported the natural occurrence of **2** in the leaves of *A. lentiginosus* and the lack of glycosidase inhibitory activity (*27*). However, in the present work 2-*epi*-lentiginosine proved to be a potent inhibitor of rat lysosomal α-mannosidase with an IC$_{50}$ value of 4.6 μM. Polyhydroxylated nortropane alkaloids calystegines B$_1$, B$_2$, and C$_1$ are known to be potent inhibitors of almond and bacterial β-glucosidases (*21*) but were also potent inhibitors of rat lysosomal β-glucosidases, with IC$_{50}$ values of 2.1, 0.75, and 0.84 μM, respectively. Calystegine B$_3$ was a moderate inhibitor of lysosomal α- and β-mannosidase. Potent inhibition of lysosomal β-glucosidase by calystegines B$_1$, B$_2$, and C$_1$ poses a problem that it would produce a phenocopy of the human genetic lysosomal storage disorder Gaucher disease.

Whether the toxicity of *I. carnea* to livestock is purely due to swainsonine or due to a combination of effects by swainso-

nine and calystegines is very important. The levels of swainsonine measured in all parts of the plant, but especially the seeds, far exceed the minimum levels, estimated at 0.001%, required to produce symptoms of locoism by locoweeds (*13*). However, although many of the symptoms described for *I. carnea* toxicosis of goats in Brazil are typical of locoweed poisoning, others are not characteristic and may be due to lysosomal storage defects induced by calystegines. It is noteworthy that the total levels of calystegines detected in *I. carnea* leaves and seeds in this investigation approach or exceed those of swainsonine in the same plant. The toxicity levels of calystegines in animals have not yet been established, but their potent glycosidase inhibitory properties, in particular toward β-glucosidase, suggest that they may have a significant effect. Furthermore, whether *N*-methyl-*trans*-4-hydroxy-L-proline (**7**), which is a nonprotein imino acid and contained at a high level (0.062% of fresh weight) in the leaves, is toxic or nontoxic to livestock is undetermined at this time. Experiments of glycosidase inhibition and toxicity by isolated alkaloids using human cells in culture are ongoing.

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