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Profiling of Alkaloids and Eremophilanes in Miracle Tea (*Packera candidissima* and *P. bellidifolia*) Products

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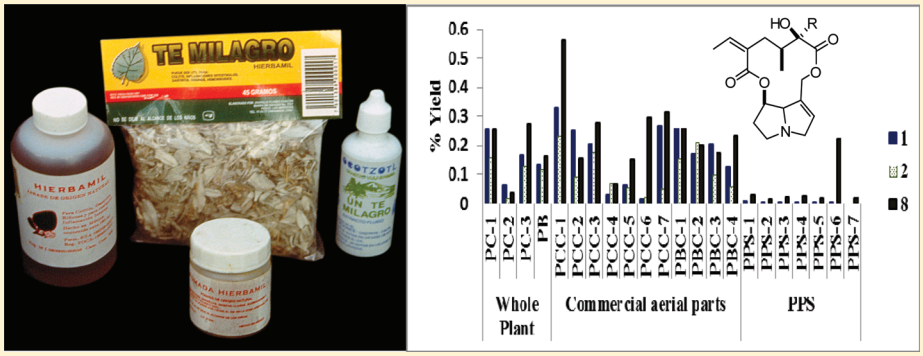
Supporting Information



ABSTRACT: Commercial preparations of the Mexican herbal drug known as "miracle tea" (*Packera candidissima* and *P. bellidifolia*) have been profiled qualitatively by HPLC and GC-MS. Eremophilanes (**3**–**7**) were the major components found in the hexane-soluble fraction, while pyrrolizidine alkaloids (PAs) were identified in the alkaloid extracts. The content of free PAs and their N-oxides was determined for a total of 22 samples, and the results showed that the amount of these hepatotoxic compounds (0.0005–0.94% free PAs; 0.0004–0.55% N-oxides), through the presence of retrorsine (**1**) and senesionine (**2**) as the main constituents, may reach toxic levels. Hexane-soluble extracts from commercial presentations (dried whole plants) of both species afforded neoadenostylone (**3**), 6-(2-methylbutanoyloxy)-9-oxo-1-(10)-furanoeremophilene (**4**), and epineoadenostylone (**5**), in addition to methyl-4-hydroxyphenylacetate (**8**) and methyl-2-(1-hydroxy-4-oxocyclohexyl)acetate (**9**). Also, epicacalone (**6**) and the new compound 2β-hydroxyneoadenostylone (**7**) were isolated from *P. bellidifolia*.

Through the centuries, many societies around the world have developed a system of traditional medicine relying largely on the use of herbal drugs. These remedies are frequently unavailable locally and therefore need to be obtained from sources geographically remote from the consumer. However, the supply of such plants from distant locations can be erratic and fraudulent, particularly because no detailed monographs including identity and quality standards exist for many herbal drugs.[1] An example of this situation is represented by the species that comprise the miracle tea ("té milagro") medicinal plant complex,[2] *Packera candidissima* (Greene) W. A. Weber & Á. Löve (syn. *Senecio candidissimus* Greene)[3] and *Packera bellidifolia* (Kunth) W. A. Weber & Á. Löve (syn. *Senecio bellidifolius* Kunth, *S. cheiranthifolius* Kunth, *S. lactucella* Sessé & Moc., *S. pauciflorus* Kunth, *S. vulneraria* DC., and *S. vulnerarius* Sessé & Moc.) (Asteraceae).[4] These two species share the same common name, morphological features, and organoleptic properties, and they are interchangeable in their use as an herbal remedy.[5] These small perennial herbs with tomentose leaves arranged in basal rosettes grow in open habitats of the mountains of Mexico ranging from 1700 to 4200 m above sea level in the pine-oak forests and subalpine meadows (Figure S1, Supporting Information). The former species is restricted to Northern Mexico in the state of Chihuahua, while the latter is found throughout Central Mexico. Both species are referred to as "té milagro" or "lechuguilla". *P. candidissima* is called by its Tarahumara name "chucaca"[3] in the Northern states while the Nahuatl names "nanauapatli" and "palancapatli" are applied to *P. bellidifolia* in Central Mexico. Since the early colonial period, "nanauapatli", "palancapatli", and "calancapatle" (a corruption of "palancapahtli")[6] were regarded highly in Central Mexico for the treatment of leprosy, syphilis, abscesses, genital ulcers, sores, and wounds as well as liver problems ("bilis").[7] The popularity of this herbal remedy continued into the nineteenth and twentieth centuries.[7c,8]

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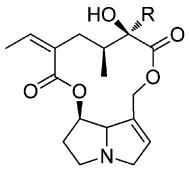
The contemporary use of miracle tea ("té milagro") among rural and urban Mexicans is parallel to that of the past but also includes additional applications. The leaves, prepared in a powder, a poultice, or an infusion, are applied to boils, sores, swellings, wounds, and other skin disorders. A vaginal wash made with the infusion of the plant has been employed to treat syphilis and other venereal diseases. A tea of the plant alone or mixed with other herbs is drunk to alleviate gastric ulcers, kidney ailments, bladder infections, prostrate problems, chest pains, and diabetes. In Northern Mexico, people seeking to "purify the blood" and to "stimulate the heart" drink the infusion, while persons intending to promote hair growth rub the decoction onto the scalp. A strong decoction of the whole plants, inclusive of the roots, has purgative properties. To alleviate toothache, pieces of the root are inserted directly into the caries. Commercial preparations include dried whole plants, dried leaves, alcoholic extracts, and cream preparations, all of which are sold throughout Mexico and exported[3] to the Southwestern United States (Figure S1, Supporting Information).



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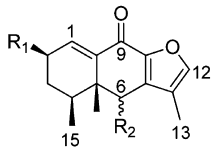
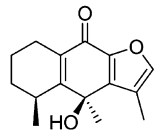
2 R = CH_3



	R_1	R_2
3	H	β-OAng
4	H	α-OMeBu
5	H	α-OAng
7	OH	β-OAng



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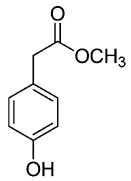

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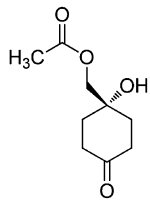

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RESULTS AND DISCUSSION

This contribution describes the chemical profiling of 22 samples of miracle tea, including fresh plant material and commercial preparations. Analysis was conducted by generating HPLC and GC-MS profiles of the chemical contents from hexane-soluble and alkaloid extracts of the two species, *P. candidissima* (from Northern Mexico) and *P. bellidifolia* (from Central Mexico), currently in wide use. These analytical procedures served as tools[9] for the authentication of these herbal drugs and their commercial presentations (dried whole plants, dried leaves, alcoholic extracts, and cream preparations). HPLC and CG-MS were used to identify the major pyrrolizidine alkaloid (PA) and eremophilene components in all different samples of miracle tea collected and purchased in five different locations in Northern and Central Mexico (Table S1, Supporting Information). Samples were grouped into three categories: (i) dried wild whole plants (PB or PC codes); (ii) commercial aerial part samples (PBC or PCC); and (iii) prepared phytopharmaceutical sample (PPS). Inconsistencies displayed in the chemical profiles for alkaloids (Figures S13 and S14, Supporting Information), such as free PA and N-oxide extracts presented in Table S2 (Supporting Information), could be the result in differences in timing and methods for sample collection and harvesting as well as in the postharvest handling and processing, e.g., drying, fragmentation, and storage, among others. The most important feature was the detection of PAs in all samples, of which the largest yields were found in the wild plants (PC-1 sample: 0.81% free PAs, 0.55% N-oxides) and a commercial herbal drug of miracle tea (PCC-1 sample: 0.94% free PAs, 0.48% N-oxides) containing *P. candidisima*. This content of free base and N-oxide PAs is one of the highest reported for PA-containing medicinal plants.[10] The content of PAs in the wild plant material (PB-1 sample: 0.01% free PAs, 0.008% N-oxides) was lower than in the commercial samples (PBC-4 sample: 0.57% free PAs, 0.31% N-oxides), while in the phytopharmaceutical samples, the PA content was even lower (0.001% free PAs, 0.005−0.010% N-oxides), e.g., alcoholic extracts (PPS-1 and PPS-2 samples), a mixture of diverse and unspecified plant materials (PPS-5 sample), tea bags (PPS-6 sample), and cream (PPS-7 sample). For the commercial dried leaves and aerial parts (PPS-3 and PPS-4), their PA content was similar to those found for the authenticated botanical drugs (e.g., the PC-2, PC-3, PB-1, and PCC-4 samples). Pyrrolizidines **1** and **2**, as well as the phenolic nonalkaloid compound **8**, were used as chemical markers for the identification of *Packera* species in the miracle tea products, because **8** is found only in some *Packera* species, while **1** and **2** can also be found in other species in the family Senecioneae.[10] GC-MS analysis indicated the presence of retrorsine (**1**; t_R 14.8 min) and senecionine (**2**; t_R 13.9 min) in all samples, while minor PAs were identified in some through their retention times and mass spectra:[11] integerrimine (t_R 14.2 min, PC-2 and PC-3) and seneciphylline (t_R 14.4 min, PBC-1, PBC-3, and PBC-4) (Figure S15, Supporting Information). Two nonalkaloid signals were evident in all samples and identified as methyl-4-hydroxyphenylacetate (**8**, t_R 8.92 min) and methyl-2-(1-hydroxy-4-oxocyclohexyl)-acetate (**9**; t_R 8.50 min). Results for the HPLC quantification of compounds **1**, **2**, and **8** are shown in Table S4 (Supporting Information). A comparison among all analyzed samples in terms of yield percentage (mg/g or mg/mL) is illustrated in Figure S16, and some examples of the chromatographic profiles obtained are presented in Figure S18 (Supporting Information). PAs in the form of N-oxides did not clearly predominate, but in some phytopharmaceutical samples, the free base form was slightly higher in abundance (Table S2, Supporting Information). In the case of the phytopharmaceuticals (alcoholic extracts), yields for **1** and **2** were considerably lower in comparison with those obtained from wild plant samples (PC and PB samples) or commercial dried plants (PCC and PBC). Table S3 illustrates the hypothetical alkaloid intake resulting from a cumulative dose exceeding 10 mg/kg, which could cause acute liver damage in persons consuming miracle tea products. *P. bellidifolia* is lower in toxicity, although its PA content may still be high enough to produce potential problems associated with the use of pyrrolizidine-containing herbs and place users of these miracle tea products at a high risk of poisoning. For commercial preparations of miracle tea, such as alcoholic extracts, tea bags, creams, and mixtures of diverse botanical drugs, it is particularly difficult to specify a dose above which their hepatotoxicity becomes significant due to a low PA concentration. Despite this, the constant use of these

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phytopharmecuticals is not recommended since accumulation of PAs could produce chronic or acute veno-occlusive diseases. PAs[12] have also been reported from *P. coahuilensis* (Greenm.) C. Jeffrey[13] and *P. quebradensis* (Greenm.) W. A. Weber & Á. Löve.[14] Recently, morbidity among Mexican Americans in Southern Arizona has been described and resulted from ingesting infusions of commercial samples of *Senecio* spp. that were misidentified as *Gnaphalium* spp. ("gordolobo").[15]

The hexane-soluble extracts of the 22 samples were analyzed by GC-MS and HPLC. Neoadenostylone (**3**, 4.17 to 28.75 μg/g) and epineoadenostylone (**5**, 4.47 to 33.34 μg/g)[3] were the major constituents identified in addition to compounds **8** and **9**. Additionally, *n*-hexadecanoic acid, 4-heptyn-3-ol, eicosane, 7-hexyleicosane, tetracosane, and 11-decyltetracosane were also identified as minor constituents by GC-MS. The levels of the lipophilic compounds **3** and **5** in all samples by HPLC in terms of yield percentage (mg/g) are illustrated in Figure S17, and some examples are presented in Figure S19 (Supporting Information). Hexane-soluble extracts from commercial presentations (dried whole plants) of *P. bellidifolia* were subjected to silica gel column chromatography, and this process resulted in the isolation of neoadenostylone (**3**), 6-(2-methylbutanoyloxy)-9-oxo-1-(10)-furanoeremophilene (**4**), epineoadenostylone (**5**), epicacalone (**6**), and the new compound **7**. The known eremophilanes **3**−**6** were characterized by comparison of their physical and spectroscopic data (Figures S3−S10, Supporting Information) with previously reported values[3,16] and HPLC comparison with authentic samples of previously isolated compounds **3**−**5** from *P. candidissima*.[3]

For compound **7**, both UV (234 and 281 nm) and IR absorptions (1720, 1673, 1624 cm^{-1}) implied the same basic furanoeremophilane skeleton as found for the known compounds **3**−**5**. The compound analyzed for $C_{20}H_{24}O_5$ (CIMS$^+$ at *m*/*z* 345 $[M + H]^+$) and exhibited the loss of an angelic acid unit $[M + H - 100\ (C_5H_8O_2)]^+$ at *m*/*z* 244. The ^{1}H NMR spectrum showed clearly the presence of a typical furan ring signal at δ_H 7.03 and characteristic signals for an angeloyloxy group: a vinyl proton at δ_H 6.30 and two methyls, C-4′ (δ_H 2.11) and C-5′ (δ_H 1.99). The ^{1}H NMR (Figure S11, Supporting Information) and ^{13}C NMR (Figure S12, Supporting Information) spectra of **7** were almost identical with those of **3**, except for the substitution at C-2. An *O*-bonded methine signal (δ_C 63.0) was apparent in **7**, indicating an additional hydroxy group attached to C-2 (δ_H 4.35, ddd). The β-orientation for this substituent was based on its multiplicity (quartet-like signal) as a result of its identical coupling constants with the methylene C-3 (J = 4.5 Hz) and the vinylic proton H-1. Complete ^{1}H and ^{13}C NMR assignments were made with the aid of two-dimensional NMR experiments including COSY, HMQC, and HMBC measurements to support the structure for this new compound as 2β-hydroxyneoadenostylone (**7**).

PAs are known to cause hepatic fibrosis that develops into cirrhosis. The most common ailment for which *P. candidissima* may be used is gastritis and related gastrointestinal ailments. Usually an infusion of one liter is recommended to be drunk throughout the day (i.e., "agua de uso") for not more than one month, or less if the symptoms subside. Commercial laboratories and packing houses manufacture processed packages of high-quality miracle tea that are available in regional supermarket chain stores in Mexico (Table S1, Supporting Information). Our unstructured interviews on local medicinal plants over the last forty years in the market and on the streets have not yielded any reports of negative effects produced by the consumption of miracle tea; on the contrary, the people generally claim that it has been considered safe for generations in the state of Chihuahua. Given its popularity and low-cost, miracle tea has gained acceptance among all socioeconomic groups in Chihuahua. Nonetheless, there is one aspect that raises concern: hepatic failure due to PA poisoning is not recognized officially but may be responsible for mortality due to cirrhosis. In 1996, cirrhosis and nonspecific liver complications ranked seventh among the 11 principal causes of death in Chihuahua, with a rate of 1.63%. By 2007, the official public health service reported the death rate had more than doubled to 3.8% of the population (for which only half was attributed to alcohol).[17] The death rate due to cirrhosis in Chihuahua was attributed to excessive consumption of alcoholic beverages, especially in urban areas. However, cirrhosis-related deaths in rural areas occurred among older individuals who were not alcoholics. A preliminary investigation to identify other factors focused on water quality, which was found in some cases to be high in arsenic. Because of the lack of recognition of beverages with PA-containing plants and the changes in the public health sector at that time, it was not possible to document the consumption patterns of herbal infusions among the families affected. An analysis of the ecological correlation of the hepatic cirrhosis in Mexico found a positive relationship with the consumption of a native beverage, "pulque" (made from the fermented sap of the "maguey" or agave plant); unfortunately, to date, there is no study considering other local drinks.[18] On the basis of the preliminary results of our studies, we recommend that cases of cirrhosis-related death among nonalcohol consumers be examined to determine if miracle tea has been part of a deceased person's dietary pattern. Should this be the case, a public health alert should be considered. Notably at risk are members of certain socioeconomic groups, such as rural inhabitants of the Sierra Tarahumara of the state of Chihuahua, lower educational and income urban dwellers in the Northern and Central regions of Mexico, and some members of the Hispanic population living in the Southwestern United States.

EXPERIMENTAL SECTION

General Experimental Procedures. Analytical procedures were performed as previously described (Supporting Information).[3,19]

Plant Material. Three samples of fresh plant material of *Packera candidissima* were collected in Creel and Bocoyna, Chihuahua state (Bye 34760, code PC-1 on August 31, 2006, Bye 35038, code PC-2 and Bye 35041, code PC-3 on May 22 and 23, 2007, respectively). *Packera bellidifolia* was collected in Amecameca, Estado de México (Bye & Linares 35895, code PB-1 on March 9, 2008). Voucher specimens were deposited in the Ethnobotanical Collection of the National Herbarium (MEXU), Instituto de Biología, UNAM. The plant material was identified by the authors R.B. and E.L.

Commercial Products. Samples of "chucaca" (*P. candidissima* dried whole plant) were purchased in different herbal shops at local markets in Chihuahua City, Queretaro City, and Mexico City (Table S1, Supporting Information).

Standard Compounds. Retrorsine (**1**) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) with a 90% purity. Senecionine (**2**) was purchased from Fluka Chemie (Buchs, Switzerland) with a 95% purity, and methyl-4-hydroxyphenylacetate (**8**) from Aldrich (Milwaukee, WI, USA) with 99% purity.

Extraction of Plant Material and Commercial Samples. Each herbal drug (20 g, commercial sample or wild plant sample) was extracted initially with hexane (100 mL) and then with MeOH (100 mL) for 24 h. The maceration process was performed three times, and

the solvents were removed at reduced pressure. Alcoholic commercial samples (15 mL) were diluted with distilled water (15 mL), and the solution was adjusted to pH 1. Then, they were treated as described below for the quantification of free and N-oxide PAs. The "super cream miracle" (50 g) was fractionated by open column chromatography over silica gel (500 g) using hexane, followed by $CHCl_3$. A total of six fractions (500 mL each) were collected and combined. Fractions eluted with $CHCl_3$ (3 × 500 mL) were dried and dissolved in MeOH and treated as described below for the quantification of free and N-oxide PAs.

Quantification of Free Pyrrolizidine Alkaloids (PAs). The MeOH extracts (200 mg) were subjected to partition with three portions (20 mL each) of 1 N HCl/$CHCl_3$ (1:1). The acid aqueous phase was basified with NH_4OH to adjust the pH to 11. This alkaline solution was extracted with $CHCl_3$ (30 mL). The resulting organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure.

Quantification of Pyrrolizidine Alkaloid N-Oxides. The acid phase (30 mL) resulting from the partition of MeOH-soluble extracts was reduced with zinc dust (20 mg).[3] The reaction mixture was stirred overnight and filtered. The acid phase was processed as described above for the nonreductive procedures. The proportion of N-oxides was calculated according to the method of Stelljes and Seiber.[3,20]

Generation of HPLC Profiles. Samples were analyzed as follows. HPLC profiles for the hexane extracts were generated on a Nova-Pak HR C_{18} column (Waters, 6 μm, 3.9 × 300 mm) in triplicate. A gradient program was applied for the mobile phase with a flow rate of 0.4 mL/min: reservoir A containing acetonitrile and reservoir B containing water. The gradient used was as follows: 0−10 min: linear gradient 60% to 75% A, 10−25 min linear gradient from 25% to 40% B with a total time for each analysis of 30 min.[21] The injection volume was 20 μL (sample concentration: 10 mg/mL). HPLC profiles for the alkaloid extracts were generated on a XTerra RP_{18} column (Waters, 5 μm, 4.6 × 150 mm) in triplicate. The mobile phase with a flow rate of 0.4 mL/min for the analysis of alkaloids consisted of a step gradient with a solution of 15 mM ammonia in water (solvent A) or in 100% acetonitrile (B). The gradient profile was as follows: 0−20 min, linear gradient from 5% to 50% B; 20−25 min: isocratic at 50% B; 25−28 min: linear gradient from 50% to 100% B; 28−33 min: isocratic at 100% B, 33−36 min: linear gradient from 100% to 5% B. Injection volume was 10 μL (sample concentration: 10 mg/mL). Co-elution experiments with standard samples afforded the corresponding HPLC profiles: retrorsine (**1**) t_R 27.2 min; senecionine t_R 29.9 min; and methyl-4-hydroxyphenylacetate (**8**) t_R 15.2 min.

Generation of GC-MS Profiles. Pyrrolizidine alkaloids were quantified by GC-MS, and the analyses were carried out 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 °C at 25 °C/min; final temperature hold, 15 min. MS conditions: ionization energy, 70 eV; ion source temperature, 200 °C; interface temperature, 300 °C; mass range, 65−795 amu. Coelution experiments with standard samples afforded the corresponding GC profiles: retrorsine (**1**): t_R 14.79 min; EIMS *m/z* 351 (3), 138 (28), 136 (88), 121 (43), 120 (94), 94 (100), 93 (84), 80 (65), 67 (36), 53 (59); senecionine (**2**): t_R 13.95 min; EIMS *m/z* 335 $[M]^+$ (4), 220 (29), 138 (42), 137 (19), 136 (90), 121 (41), 120 (100), 94 (64), 93 (83), 80 (35), 67 (12), 53 (24), 43 (60); methyl-4-hydroxy-phenylacetate (**8**): t_R 8.92 min; EIMS *m/z* 166 $[M]^+$ (19), 107 (100); methyl-2-(1-hydroxy-4-oxocyclohexyl)acetate (**9**): t_R 8.50 min; EIMS *m/z* 186 $[M]^+$ (2), 168 (34), 129 (31), 112 (38), 98 (51).

Validation of Analytical Method. The quantitative HPLC method was validated for specificity, linearity, accuracy, and precision according to the International Conference on Harmonization guidelines.[22] Specificity: the peak identity for compounds **1**, **2**, and **8** was confirmed by coelution with authentic standard samples. All compounds were detected at 215 nm (Figure S2, Supporting Information). Accuracy of the method: three raw samples of free PAs in MeOH (1 mg/mL) were spiked with different amounts of **1**, **2**, and **8** (0.3 to 0.7 mg/mL). The recovery rates were calculated by the relation of the amount recovered to the amount added for the three references. Recovery ranges were found to be between 94.3% and 102%. Sensitivity: calibration curves were constructed for each standard at different concentration levels for **1** in the range 0.5−5.0 mg/mL, 0.25−2.0 mg/mL for **2**, and 0.25−2.4 mg/mL for **8**. Three independent determinations were performed at each concentration, and regression analysis was employed to determine the linearity for the calibration graphs (Table S5, Supporting Information) and the limits of quantification. Precision: the intra- and interday precision was verified by analyzing the spiked samples in triplicate for five consecutive days and by two analysts. Precision was confirmed by evaluating standard deviations of the amounts and of retention time for each sample. The reproducibility and repeatability of the analytical method were evaluated in terms of the intermediate precision by analyzing three replicates of six samples of stock solution (1 mg/mL) on two different days. All statistical analyses were carried out using the program Statistical Product and Service Solutions for Windows (SPSS, v 12.0; The Apache Software Foundation, Chicago, IL, USA).

Extraction and Isolation of Eremophilanes. Aerial parts of *P. bellidifolia* (PBC-1 to PBC-4, commercial samples, 2.2 kg) were powdered and extracted by maceration at room temperature with hexane to afford a brownish extract (67.4 g). The crude extract was subjected to column chromatography over silica gel (0.67 kg) in a gravity column using a gradient with mixtures of hexane/$CHCl_3$, $CHCl_3$/Me_2CO, and Me_2CO/MeOH. A total of 263 fractions (125 mL each) was collected, their composition was monitored by TLC (silica gel 60 F254 aluminum sheets, hexane/EtOAc, 3:2), and closely comparable fractions were combined in several pools. Fractions 132−165 (11.59 g, eluted with hexane/$CHCl_3$, 1:3) were subjected to preparative HPLC using a Symmetry C_{18} column (Waters, 5 μm, 19 × 300 mm), with isocratic elution by CH_3CN/H_2O (3:1), a flow rate of 8 mL/min, and a sample injection of 500 μL (fraction concentration: 80 mg/mL), in order to purify compounds **3** (4.35 g, t_R = 20.5 min) and **4** (3.25 g, t_R = 22.8 min). Fractions 166−198 (9.93 g, eluted with hexane/$CHCl_3$, 1:9) were also purified in the same column but using CH_3CN/H_2O (4:1), and these chromatographic conditions were effective for the isolation of compounds **3** and **4** (3.45 g, t_R = 19.9 min) and **5** (5.19 g, t_R = 22.0 min). Fractions 205−232 (7.13 g, eluted with $CHCl_3$/Me_2CO, 4:1) were rechromatographed over silica gel (165 g) using a gradient of MeOH in hexane/EtOAc (3:2). A total of 108 fractions was collected (200 mL each). Subfractions 26−36 (1.06 g) and 48−63 (0.18 g) were purified by preparative HPLC on a reversed-phase C_{18} column (Waters, 5 μm, 19 × 300 mm) to yield compounds **6** and **7**, respectively. In the case of compound **6** (0.583 g, t_R = 8 min), the mobile phase was CH_3CN/H_2O (94:6; flow rate 9 mL/min), and for compound **7** (0.1074 g, t_R = 18.7 min), the elution was isocratic with CH_3OH/H_2O (1:1; flow rate 9 mL/min).

*Neoadenostylone (**3**):* oil; Optical Rotatory Dispersion (ORD) (*c* 1.47, $CHCl_3$) $[\alpha]_{589}$ −40, $[\alpha]_{578}$ −41, $[\alpha]_{546}$ −46, $[\alpha]_{436}$ −71; EIMS *m/z* $[M]^+$ 328 (2), 246 (8), 228 (37), 213 (6), 137 (7), 83 (100), 55 (19), 43 (4). Identified by comparison of NMR data with published values (Figures S3 and S4 and Experimental Section, Supporting Information).[3]

*6-(2α-Methylbutanoyloxy)-9-oxo-1(10)-furanoeremophilene (**4**):* oil; ORD (*c* 0.91, $CHCl_3$) $[\alpha]_{589}$ −15, $[\alpha]_{578}$ −16, $[\alpha]_{546}$ −19, $[\alpha]_{436}$ −53; CIMS *m/z* $[M]^+$ 331 (100), 259 (22), 233 (30), 231 (23), 229 (23), 219 (24), 217 (24), 85 (100); identified by comparison of NMR data with published values (Figures S5 and S6 and Experimental Section, Supporting Information).[16a]

*Epineoadenostylone (**5**):* oil; ORD (*c* 1.92, $CHCl_3$) $[\alpha]_{589}$ −47, $[\alpha]_{578}$ −49, $[\alpha]_{546}$ −56, $[\alpha]_{436}$ −88; EIMS *m/z* 328 $[M]^+$ (2), 246 (54), 228 (100); identified by comparison of NMR data with published values (Figures S7 and S8 and Experimental Section, Supporting Information).[3]

*Epicacalone (**6**):* oil; ORD (*c* 0.1, CH_2Cl_2) $[\alpha]_{589}$ +18, $[\alpha]_{578}$ +19, $[\alpha]_{546}$ +25, $[\alpha]_{436}$ +72; EIMS *m/z* 262 $[M]^+$ (100), 245 (52), 218 (31), 205 (32), 178 (48), 153 (28); identified by comparison of NMR data with published values (Figures S9 and S10 and Experimental Section, Supporting Information).[16b]

2-Hydroxyneoadenostylone (7): oil, ORD (*c* 0.08, CH_2Cl_2) $[\alpha]_{589}$ −118, $[\alpha]_{578}$ −120, $[\alpha]_{546}$ −138, $[\alpha]_{436}$ −254; ^{1}H NMR δ 7.44 (1H, d, *J* = 1.0 Hz, H-12), 6.94 (1H, t, *J* = 5.0 Hz, H-1), 6.45 (1H, s, H-6), 6.30 (1H, qq, *J* = 7.5, 1.5 Hz, H-3′), 4.35 (1,H, ddd, *J* = 4.5, 4.5, 4.5 Hz, H-2), 2.11 (3H, dq, *J* = 7.5, 1.5 Hz, H-4′), 2.24−2.30 (1H, m, H-4), 1.99 (3H, q, *J* = 1.5 Hz, H-5′), 1.88 (3H, d, *J* = 1.0 Hz, H-13), 1.69−1.76 (2H, m, H-3), 1.13 (3H, s, H-14), 0.99 (3H, d, *J* = 6.5 Hz, H-15); ^{13}C NMR δ 176.3 (C-9), 166.9 (C-1′), 147.5 (C-8), 146.5 (C-12), 143.5 (C-10), 141.8 (C-3′), 136.7 (C-7), 135.8(C-1), 126.8 (C-2′), 121.4 (C-11), 73.0 (C-6), 63.0 (C-2), 47.1 (C-5), 43.3 (C-10), 36.9 (C-3), 32.7 (C-4), 20.5 (C-5′), 17.1 (C-15), 16.1 (C-4′), 14.7 (C-14), 8.4 (C-13); CIMS *m*/*z* 345 $[M + 1]^+$ (100), 327 (48), 244 (63), 227 (18); HRCIMS *m*/*z* 345.4063 (calcd for $C_{20}H_{25}O_5$, 345.4078).

■ ASSOCIATED CONTENT

Supporting Information

Photographs of *P. candidissima* and *P. bellidifolia* and their commercial presentations (Figure S1); UV spectra of compounds **1**, **2**, and **8** (Figure S2); ^{1}H and ^{13}C NMR spectra of compounds **3**−**7** (Figures S3−S12); GC chromatograms of pyrrolizidine alkaloid extracts (Figure S13), hexane-soluble extracts (Figure S14), and samples PC-2 and PBC-3 (Figure S15); composition of alkaloid (Figure S16) and lipophilic (Figure S17) compounds in different samples; HPLC-PDA chromatograms of pyrrolizidine alkaloid extracts (Figure S18) and hexane-soluble extracts (Figure S19); sample identification chart (Table S1); yields of free and N-oxide PAs (Table S2); hypothetical alkaloid intake from consumption of miracle tea (Table S3); HPLC quantitative analyses of compounds **1**, **2**, and **8** (Table S4); HPLC-PDA calibration curves and limits of detection for standard samples (Table S5); and general experimental procedures. This information 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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