Plantadeprate A, a Tricyclic Monoterpene Zwitterionic Guanidium, and Related Derivatives from the Seeds of Plantago depressa

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Plantadeprate A, a Tricyclic Monoterpene Zwitterionic Guanidium, and Related Derivatives from the Seeds of *Plantago depressa*

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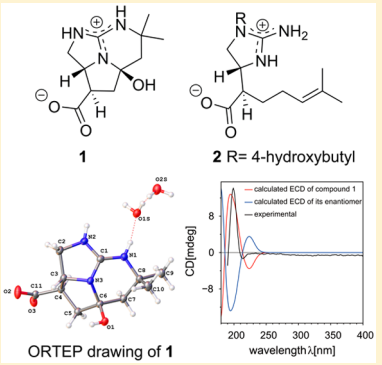
Supporting Information

ABSTRACT: Two new alkaloids, plantadeprate A (**1**) and 1′-(4″-hydroxybutyl)-plantagoguanidinic acid (**2**), along with three known compounds, were isolated from the seeds of *Plantago depressa*. Their structures were elucidated by physical data analyses including NMR, MS, and electronic circular dichroism (ECD) methods. Plantadeprate A (**1**), a monoterpene zwitterionic guanidium, possesses a unique 5/5/6-tricyclic ring system. Its absolute configuration was determined by X-ray crystallography and computational methods. Compound **1**, plumbagine D (**3**), and plantagoguanidinic acid (**4**) exhibited potential antihyperglycemic properties attributed to suppression of hepatic gluconeogenesis with inhibitory rates of 8.2%, 18.5%, and 12.5% at 40 μM, respectively.



The guanidine subunit, which is usually thought of as a strong organic base, readily forms hydrogen-bond networks and influences charges and counterions in these networks.[1] Polycyclic guanidine derivatives, a unique class of natural alkaloids mostly obtained from marine organisms but rarely from higher plants,[2,3] exhibit a broad spectrum of biological properties such as antifungal, antiviral, antiprotozoal, antileukemic, and Ca^{2+} channel blocking activities.[4−7]

Plantago depressa Willd. (Plantaginaceae) is a perennial herb growing widely in Asia. Its seeds are used as a traditional Chinese medicine for diuretic, expectorant, antitussive, anti-inflammatory, antioxidant, antidiabetic, and antihypertensive activities.[8−11] *Plantago* plants are also used widely in other countries as a hyperglycemic agent, yet the bioactive constituents and underlying mechanisms of action remain unclear.[12−14] In a continuing search for bioactive metabolites from *Plantago* plants,[15−17] a phytochemical investigation led to the isolation of two new alkaloids, plantadeprate A (**1**) and 1′-(4″-hydroxybutyl)plantagoguanidinic acid (**2**), together with three known guanidine derivatives, plumbagine D (**3**),[18] plantagoguanidinic acid (**4**),[18−20] and plantagoamidinic acid B (**5**) (Figure 1).[18,19] Herein, the isolation, structure elucidation, and biological activities of these compounds are reported.

Plantadeprate A (**1**), colorless crystals, was found to have the molecular formula $C_{11}H_{17}N_3O_3$ (HRESIMS m/z = 240.1367 $[M + H]^+$, calcd for 240.1348), supported by the presence of 11

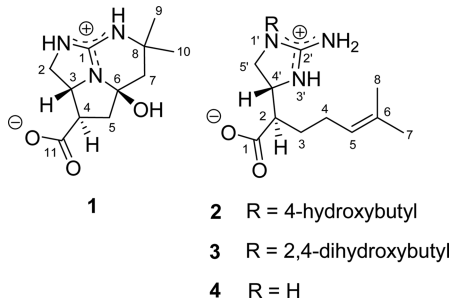


Figure 1. Structures of plantadeprate A (**1**), 1′-(4″-hydroxybutyl)-plantagoguanidinic acid (**2**), plumbagine D (**3**), and plantagoguanidinic acid (**4**).

carbon signals in the ^{13}C NMR spectrum, indicating five indices of hydrogen deficiency. The ^{13}C and DEPT NMR spectra revealed two methyls, three sp^3 methylenes, two sp^3 methines, and two sp^3-hybridized carbons devoid of hydrogen atoms (Table 1). The IR spectrum of **1** indicated the presence of a hydroxy group (3143 cm^{-1}) and a carboxylic group (1683 cm^{-1}). The IR absorption bands at 3392, 1614, 1594, and 1402 cm^{-1} indicated the presence of secondary amine and imine

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Table 1. ^{1}H and ^{13}C NMR Data (600 and 150 MHz, D_2O) of Compound 1

position	δ_H (J in Hz)	δ_C
1		156.6
2a	3.61, d (10.6)	45.3
2b	3.79, dd (10.6, 8.2)	
3	4.37, dd (9.7, 8.2)	63.4
4	2.69, m	51.0
5a	2.20, overlap	46.9
5b	2.51, dd (13.8, 2.5)	
6		90.4
7a	1.71, d (14.5)	46.3
7b	2.23, overlap	
8		52.2
9	1.30, s	28.1
10	1.32, s	24.9
11		178.5

functions. These data suggested that **1** was a guanidine[1] with a tricyclic ring system.

The 2D structure of **1** was established by analyses of HSQC, HMBC, ^{1}H−^{1}H COSY, and NOESY data. In the ^{1}H−^{1}H COSY spectrum, the cross-peaks of H_2-2/H-3/H-4/H_2-5 suggested the presence of the fragment C-2/C-3/C-4/C-5 (Figure 2). In the HMBC spectrum, both H-2 (δ_H 3.61) and H-3 (δ_H 4.37) showed correlations to the iminium C-1 (δ_C 156.6). These data, combined with the presence of the ^{1}H−^{1}H COSY spin-coupling system H-2/H-3, established a five-membered ring A (Figure 2).

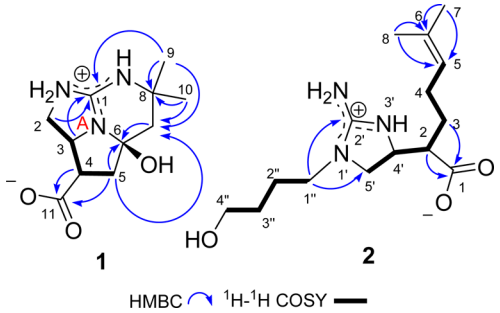

Figure 2. Key ^{1}H−^{1}H COSY and HMBC correlations of **1** and **2**.

On the basis of the HMBC data, H-4 (δ_H 2.69) and H-5 (δ_H 2.20) showing correlations to C-11 (δ_C 178.5) suggested that C-11 was attached to C-4; two singlets at δ_H 1.30 and 1.32 ascribed to Me-9 and Me-10, respectively, showing significant correlations with N-carrying tertiary carbon C-8 (δ_C 52.2) and an sp^3 methylene C-7 (δ_C 46.3) demonstrated that the two methyl groups were attached to C-8. HMBC correlations were also observed between the following proton and carbon pairs: H-5 (δ_H 2.20) and C-6 (δ_C 90.4), H-5 (δ_H 2.20, δ_H 2.51) and C-7 (δ_C 46.3), H-7 (δ_H 1.71, δ_H 2.23) and C-6 (δ_C 90.4), H-9 (δ_H 1.30) and C-1 (δ_C 156.6), and H-10 (δ_H 1.32) and C-1 (δ_C 156.6). On the basis of the aforementioned information, it was concluded that the 2D structure of **1** possessed a 5/5/6 tricyclic backbone (Figure 2).

The absolute configuration of **1** was identified to be 3*R*, 4*R*, 6*R* by single-crystal X-ray diffraction data collected by Cu Kα radiation with a Flack absolute structure parameter of 0.01(15) (Figure 3). Further support came from the comparison of the experimental and calculated[21] ECD spectra (Figure 4).

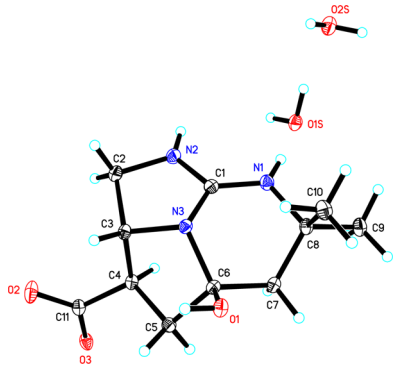

Figure 3. ORTEP drawing of compound **1**.

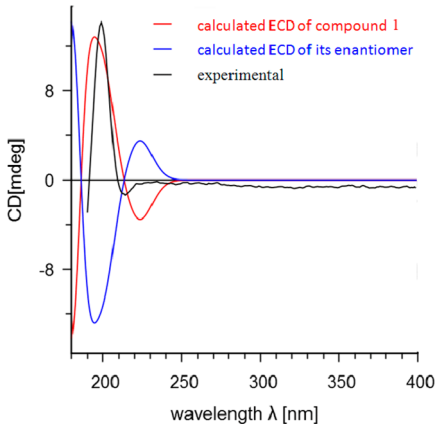

Figure 4. Experimental ECD of **1** (black), calculated ECD of **1** (red), and calculated ECD of the enantiomer of **1** (blue) in H_2O.

Quantum chemical calculations of the free and dissociative states of compound **1** were carried out using the Gaussian 09 program at the B3LYP/6-311++G(d,p) level.[21] Comparison of the calculated NMR spectra with the experimental data indicated that **1** existed as an inner salt,[22,23] which conformed to the X-ray data (Computation part, Supporting Information). The bond length of the three C−N bonds suggested that these bonds featured a bond order of 1.5. From the packing view of **1** (Figure S2, Supporting Information), the polar part was exposed outside, which contributed to its good solubility in water, but poor solubility in polar organic solvent, even if DMSO or MeOH was used.

Furthermore, the X-ray diffraction data showed that **1** incorporated two molecules of water in its crystalline framework by intermolecular action. Thus, compound **1**, a unique 5/5/6-fused tricyclic zwitterionic guanidinium carboxylate, was named plantadeprate A.

Compound **2** was obtained as a white, amorphous powder and assigned a molecular formula of $C_{15}H_{27}N_3O_3$ by protonated HRESIMS ion at m/z 298.2144 $[M + H]^+$ (calcd for 298.2131) and the ^{13}C NMR spectroscopic data. The ^{1}H and ^{13}C NMR data (Table 2) were similar to those of plumbagine D,[18] except for the absence of one oxygen-bearing methine signal and the presence of an additional methylene signal at δ_C 24.6 (C-2″) and δ_H 1.69 (2H, overlap). Accordingly, the structure of **2** was established as 1′-(4″-hydroxybutyl)plantagoguanidinic acid.

Compound **2**, showing a positive Cotton effect of around 204 nm, (Figure S1, Supporting Information), displayed a similar electronic circular dichroism (ECD) curve compared to

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Table 2. ^{1}H and ^{13}C NMR Data (400 and 100 MHz, Methanol-d_4) for 2

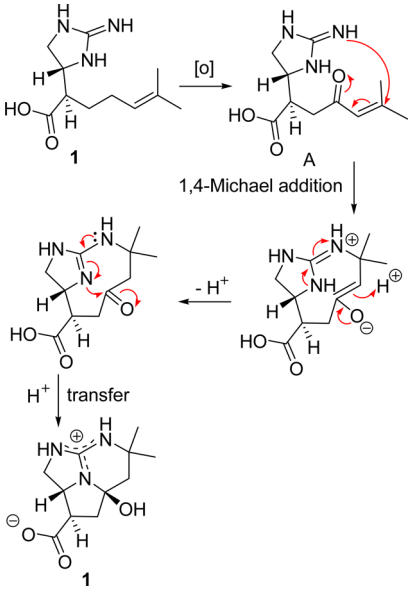
position	δ_H (J in Hz)	δ_C
1		180.2
2	2.33, m	54.8
3	1.54[α]	30.4
4a	2.04, m	27.1
4b	2.14, m	
5	5.16, t (7.1)	125.2
6		132.8
7	1.69, s[α]	25.9
8	1.63, s	17.8
2′		159.5
4′	4.03, m	56.7
5′a	3.59, m[α]	52.8
5′b	3.81, m	
1″	3.40, m	45.5
2″	1.69[α]	24.6
3″	1.54[α]	30.3
4″	3.59, m[α]	62.4

[α]Signals overlapped.

the reported spectrum,[18] suggesting that compound **2** had the same (2*R*, 4′*R*) absolute configurations.

A putative biogenetic pathway for **1** is proposed in Scheme 1. Compound **1** might be generated from **4**, a component coexisting with **1**, through oxidation to form the intermediate A followed by cyclization to afford **1**.[3,24,25]

Scheme 1. Proposed Biogenetic Pathway from 4 to 1



Compounds **1**–**5** were evaluated for inhibitory effects against α-glucosidase[26] and angiotensin-converting enzyme (ACE).[15] Only plantadeprate A showed weak inhibitory activities of 20% at 6.25 mM and 26% at 10 mM against α-glucosidase and ACE, respectively. These compounds were also evaluated for the effect on gluconeogenesis with isolated hepatocytes from fasted rats.[27] Compounds **1**, **3**, and **4** exhibited potential inhibition of 8.2%, 18.5%, and 12.5% at 40 μM, respectively, with metformin as the positive control, which showed an inhibition rate of 25% at 2 mM. The data obtained will enhance the understanding of the biologically active components and the underlying mechanism of the hypoglycemic effects of *Plantago* plants in clinical use.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a Büchi melting point B-540 apparatus and are uncorrected. Optical rotations were measured on a Küss-P800-T polarimeter. ECD spectra were recorded on a Chirascan v.4.2.17 spectrometer. IR spectra were obtained on a Thermo Nicolet FTIR 6700 instrument from KBr pellets. NMR spectra were run on a Bruker AVANCE-III instrument operating at 600 and 400 MHz for ^{1}H and 150 and 100 MHz for ^{13}C. HRESIMS data were measured with a Waters UPLC-Synapt G2 Q-TOF mass instrument. Preparative HPLC was run on an automatic preparation system equipped with a Waters Micromass ZQ, a Waters 2545 binary gradient module, a Waters SFO system fluidics organizer, a Waters 515 HPLC pump, a Waters 2767 sample manager, and an XBridge Prep shield RP18 (5 μm, o.b.d. 30 × 100 nm). Column chromatography (CC) was performed using AB-8 macroporous resin (20–60 mesh, Shanghai mosu Scientific Equipment Co. Ltd., Shanghai, P. R. China), MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Japan), DEAE Sephadex A-25 (GE Healthcare Bio-Sciences AB), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB).

Plant Material. The seeds of *Plantago depressa* Willd. were collected from Jilin Province, People's Republic China, in October 2011, and authenticated by Dr. L. H. Wu, Shanghai R&D Centre for Standardization of Chinese Medicine, Shanghai, People's Republic China. A voucher specimen (CQ20111011) was deposited in the Herbarium of ICMM, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation. The seeds (30 kg) of *P. depressa* were extracted three times with 70% EtOH (30 L) under reflux for 3 h each time. After removal of the organic solvent under reduced pressure, the aqueous solution (about 15 L) was applied to an AB-8 macroporous resin (20 kg) column eluted with H_2O and then 30% EtOH, respectively. Two fractions (A, B) were obtained. Fraction A, the water eluate (60 mL), was subjected to column chromatography over MCI gel with H_2O and 10% MeOH to obtain fractions A_1 and A_2. Fraction A_1 was further separated by DEAE Sephadex A-25 with H_2O, followed by RP-C18 (MeCN–0.1% ammonia, 1:20–1:18) and Sephadex LH-20 (50% MeOH), to afford plantadeprate A (**1**) (30 mg, 0.0001%). Compound **5** (32 mg) was obtained by the same means from fraction A_2. Fraction B, the 30% EtOH eluate (90 mL), was subjected to column chromatography over ODC with a gradient of 5% to 40% MeOH to obtain fraction B_3, which was further separated by DEAE Sephadex A-25 in H_2O, followed by RP-C18 (MeCN–0.1% ammonia, 1:20–1:8), to yield crude compounds **2**–**4**. Further separation by Sephadex LH-20 (80% MeOH) afforded **2** (42 mg), **3** (69 mg), and **4** (1.4 g).

Plantadeprate A (1): colorless crystals (50% MeOH); mp 244–246 °C; $[\alpha]^{20}_{D}$ −16 (*c* 0.3, H_2O); IR (KBr) ν_{max} 3392, 3143, 2979, 2937, 1683, 1629, 1614, 1594, 1402, 1351, 1078, 707 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; HR-ESIMS *m*/*z* 240.1367 $[M + H]^+$ (calcd for 240.1348).

Plantagoamidinic acid D (2): white, amorphous power; $[\alpha]^{20}_{D}$ +38 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3315, 2930, 1684, 1585, 1448, 1400, 1291; ^{1}H and ^{13}C NMR data see Table 2; HR-ESIMS *m*/*z* 298.2144 $[M + H]^+$ (calcd for 298.2131).

X-ray Crystallography Analysis. Plantadeprate A (**1**) was crystallized in 50% MeOH. The crystal structure and absolute configuration of **1** were determined using data collected at T = 140(2) K with Cu Kα radiation (λ = 1.541 78 Å) on a Bruker APEX-II CCD with a graphite monochromator. The total number of reflections measured was 2515, of which 2446 were observed, $I > 2\sigma(I)$. The final R_1 value was 0.0317 (all data). The final wR_2 value was 0.0834 (all data). The crystal structure of **1** was solved by the direct method with SHELXS-97 (Sheldrick, 2008) and expanded using the difference Fourier technique, refined by the program SHELXL-97 (Sheldrick,

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2008) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 938534). The data can be obtained free of charge via www.ccdc.cam.ac.uk/products/csd/request.

*Crystal data of Plantadeprate A (**1**):* $C_{11}H_{17}N_3O_3 \cdot 2(H_2O)$, MW = 275.31; orthorhombic, space group $P2_12_12_1$; a = 5.66790(10) Å, b = 13.4355(2) Å, c = 18.1813(3) Å, $\alpha = \beta = \gamma = 90.00°$, V = 1384.53(4) Å^3, Z = 4, D_c = 1.321 g/cm^3, $F(000)$ = 592, crystal dimensions 0.26 × 0.02 × 0.01 mm^3, Flack parameter = 0.01(15). Bond lengths (Å): C1−N1 1.3159(16), C1−N2 1.3271(17), C1−N3 1.3670(17).

Computational Chemical Calculations. A quantum chemical calculation of the ECD spectrum was performed with the Gaussian 09 program package.[21] The original structure was built based on the X-ray crystal structure and minimized with the OPLS_2005 force field[28] in MacroModel9.8 of Maestro v9.0.[29] The conformational analysis using MacroModel9.8 suggested that there was only one low-energy conformation. The density functional theory (DFT) method was employed to optimize compound **1** geometrically at the B3LYP/6-311++G(d,p) level due to the good performance of B3LYP in geometry optimization. Meanwhile, frequency calculations were carried out to confirm that the geometries obtained corresponded to energetic minima. The stable conformer obtained was subjected to ECD calculation at the B3LYP/6-311++G(d,p) level with 30 states in H_2O with the CPCM model.[30,31] Figure 4 was generated with SpecDis1.6.2[32] by comparing the simulation results and experimental data.

To elucidate the bond lengths of the carbon−nitrogen bonds, three possible structures (Suporting Information) were subjected to NMR calculations using the Gaussian 09 program package. Three initial structures were optimized, and the gauge-independent atomic orbital (GIAO) calculation of the chemical shifts by DFT at the B3LYP/6-311++G(d,p) level with H_2O in the CPCM model was applied to predict the NMR spectra. The calculated bond lengths (Å) were C1−N1 1.3243, C1−N2 1.3343, and C1−N3 1.3540.

α-Glucosidase Inhibitory Activity Assay. α-Glucosidase was from *Saccharomyces cerevisiae* purchased from Sigma-Aldrich Co. and dissolved in K_3PO_4 buffer (pH 6.8) with a concentration of 0.25 U/mL. The assay of test samples was carried out according to the reported method with acarbose as the positive control.[26]

Angiotensin-Converting Enzyme Inhibitory Activity Assay. ACE (0.05 U/mL) (from rabbit lung, EC 3.4.15.1, Sigma Chemical Co.) and hippuryl-histidyl-leucine (HHL, Sigma-Aldrich Co.) were dissolved in Tris buffer. The ACE inhibition activities of compounds **1**−**5** were determined in vitro by monitoring the transformation from a substrate HHL to product hippuric acid (HA) catalyzed by ACE using UPLC-MS analysis with captopril as the positive control. The selected ion monitoring mode was used to generate a calibration curve for HA.[15]

Inhibition of Gluconeogenesis in Hepatocytes Assay. Hepatocytes were isolated from 20- to 24-h-fasted SD rats (180 g) by a modified version of the collagenase method.[33] The cells were plated in DMEM medium (low glucose) overnight at a density of 3 × 10^5 cells/well on 24-well plates. The cells were treated by a single concentration of compounds prepared with glucose-free DMEM containing lactate/pyruvate (10:1 mM). After three multiple wells were processed for 6 h, glucose production was determined using the glucose oxidase activity assay kit. Metformin was used as positive control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00368.

IR, MS, and NMR spectra of **1** and **2**; ECD and carbon−nitrogen bond length calculation of **1** (PDF)

X-ray crystallographic data of **1** (CIF)

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Notes

The authors declare no competing financial interest.

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