Dracotanosides A−D, Spermidine Glycosides from *Dracocephalum tanguticum*: Structure and Amide Rotational Barrier

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Four new spermidine glycosides, dracotanosides A−D (**1**−**4**), have been isolated from *Dracocephalum tanguticum*. These molecules represent the first spermidine glycosides from this plant genus. The structures, including absolute configurations, were determined by spectroscopic and chemical methods. The amide bond rotational barrier of aglycone **1a** was calculated by density functional theory (DFT) computation.

Dracocephalum tanguticum Maxim, a plant growing at about 1900−4600 m above sea level in western China, has been used as a folk medicine for gastritis, hepatitis, dizziness, rheumatoid arthritis, and scabies.[1] According to the local pharmacopoeia,[2] three alkaloidal spots could be detected in the TLC of its ethanolic extract colored with Dragendorff's reagent, but no alkaloids had previously been isolated from *D. tanguticum*. However, 16 flavonoidal alkaloids have been obtained from *D. rupestre* of the same genus.[3] In the course of our continuous study on the bioactive constituents and quality control of herbs from the genus *Dracocephalum*,[4,5] four new spermidine glycosides, dracotanosides A−D (**1**−**4**), have been obtained from whole plants of *D. tanguticum*. Although some spermidine alkaloid glycosides and similar compounds were reported,[6,7] these compounds are the first examples of spermidine alkaloid glycosides obtained from this plant genus, and each was characterized as two rotamers arising from restricted amide bond rotation.[8] The structures including absolute configurations were determined by spectroscopic and chemical methods. The rotational barrier in the C−N bond-induced *E* and *Z* equilibria of aglycone **1a** was calculated by the density functional theory (DFT) computation method, which explained the interconverting equilibrium of the two rotamers. Herein, we describe the structural elucidation and in vitro biological evaluation of the new dracotanosides.

Results and Discussion

The air-dried whole plants of *D. tanguticum* were percolated with 70% EtOH. The crude extract was defatted and adjusted to pH 3−4 with HOAc. After extracting with CH_2Cl_2, the aqueous layer, adjusted to pH 9−10 with ammonia solution, was extracted with CH_2Cl_2 to obtain the crude alkaloid fraction. This was subjected to column chromatography and preparative HPLC in the reversed-phase mode to yield dracotanosides A−D (**1**−**4**).

Dracotanoside A (**1**) was obtained as a colorless, amorphous solid. The molecular formula $C_{44}H_{55}N_3O_{13}$ was deduced from HRESIMS ($[M + H]^+$ at *m/z* 834.3790, calcd for $C_{44}H_{56}N_3O_{13}$, 834.3808). Most of the signals in the ^1H and ^{13}C NMR spectra of **1** split into two sets of resonances with the approximate integral ratio 3:4 (Table 1), caused by the hindered rotation of the amide bonds.[6,9] The 44 carbons in the molecule were determined as one methyl, nine methylene, 27 methine, and seven quaternary carbons, as resolved by DEPT and HSQC spectra. The ^1H NMR spectrum displayed signals for a *p*-substituted and two monosubstituted benzene rings, a double bond, seven pairs of methylene protons between δ_H 2.25 and 3.55, two anomeric protons (δ_H 5.78 and 4.57), and numerous oxygenated methylene and methine protons reminiscent of a glycoside.

In order to determine its structure, compound **1** was subjected to acid hydrolysis. The aglycone **1a** was obtained as a colorless solid that gave a quasi-molecular ion peak $[M + H]^+$ at *m/z* 422.2431 in the HRESIMS, consistent with the molecular formula $C_{25}H_{31}N_3O_3$ (calcd for $C_{25}H_{32}N_3O_3$, 422.2438). The 25 carbon signals in the molecule were identified with DEPT spectra as eight sp^3 methylene, one sp^3 methine, 11 sp^2 methine, and five sp^2 quaternary carbons including two amide carbonyls. The ^1H NMR spectrum of **1a** indicated the presence of a *p*-substituted and a monosubstituted benzene ring, and a double bond. The correlations in the $^1H-^1H$ COSY and HSQC spectra of **1a** indicated the presence of the following distinct spin systems: C-2 to C-3, C-6 to C-8, and C-10 to C-13 (Figure 1). These partial structures were connected through nonprotonated carbons on the basis of HMBC correlations of H_2-3/C-4 and C-2; H-2/C-3 and C-2′; H_2-6/C-4, C-7, and C-8; H_2-8/C-7, C-6, and C-9″; H_2-10/C-11, C-12, and C-9″; H_2-13/C-2, C-12, and C-11; H-7″/C-1″, C-2″, C-8″, and C-9″; and H-8″/C-1″, C-7″, and C-9″, which furnished a 13-membered cyclic spermidine alkaloid,[10] with a cinnamate and a 4-hydroxyphenyl linked to N-9 and C-2, respectively. The *Z*-geometry of the cinnamate moiety was determined on the basis of the $J_{7'',8''}$ value of 12.6 Hz. Thus, the aglycone **1a** was determined to be 4′-hydroxycelacinnine.[10]

The glycosyl moiety of **1** consisted of a benzoyl group and two sugar units. A rhamnosyl and a glucosyl moiety were presumed by analysis of the ^{13}C NMR data for each monosaccharide.[6,11] After sugar analysis, the presence of an L-rhamnose and D-glucose was confirmed.[12] The large coupling constant between H-1‴″ and H-2‴″ (7.6 Hz) was consistent with a β-glucopyranose residue. The small coupling constant of H-1‴ and H-2‴ together with the chemical shift of C-5‴ (δ_C 69.2) suggested an α-configuration for the rhamnopyranosyl unit.[13] The HMBC correlation from H-1‴″ to C-2‴ demonstrated that the glucopyranosyl unit was located at C-2‴, and the HMBC correlation of H-1‴ to C-4′ indicated that glucosidation of the rhamnopyranosyl moiety was at C-4′. The benzoyl group was located at C-6‴″ of the glucose unit by HMBC correlation from H-6‴″ to C-7‴″. Accordingly, compound **1** was defined as celacinnine 4′-*O*-[6-*O*-benzoyl-β-D-glucopyranosyl]-(1→2)-α-L-rhamnopyranoside.

The circular dichroism (CD) spectrum of **1a** showed a negative Cotton effect at 200−220 nm as in 2*S*-celacinnine,[14] indicating the 2*S*-configuration as depicted.

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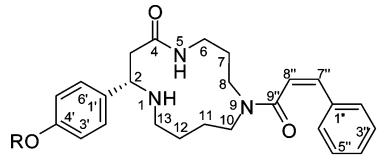
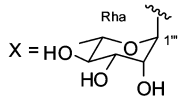
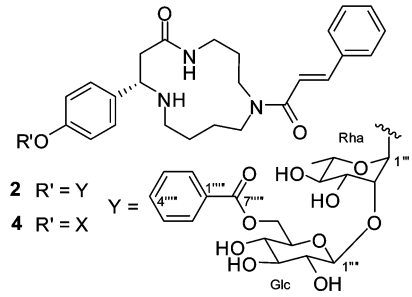


1a R = H
1 R = Y
3 R = X





2 R' = Y
4 R' = X

Dracotanoside B (**2**) was also attained as a colorless, amorphous solid. Its molecular formula was established as $C_{44}H_{55}N_3O_{13}$ by HRESIMS, the same as that of **1**. The NMR data (Table 1) resembled those of **1** closely, except for the olefinic signals. The proton resonances at δ_H 7.59 (H-7″, d, J = 15.6 Hz) and 7.04 (H-8″, d, J = 15.6 Hz) suggested an *E*-geometry for the cinnamate moiety in **2**. The CD curve of **2** revealed a negative Cotton effect at 200−210 nm, indicating the *S*-configuration at C-2.

Dracotanoside C (**3**) was obtained as a colorless, amorphous solid and was analyzed for the molecular formula $C_{31}H_{41}N_3O_7$ by HRESIMS ($[M + H]^+$ at m/z 568.3008, calcd 568.3017). The ^{1}H NMR spectrum (Table 2) showed only one anomeric proton resonance at δ_H 5.41 (br s) and indicated that it shared the same aglycone with **1** and differed only in the absence of the benzoylated glucopyranosyl unit. A negative Cotton effect at 210−230 nm in the CD spectrum led to the assignment of the *S*-configuration at C-2.[13]

Dracotanoside D (**4**) was obtained as a colorless, amorphous powder, and its molecular formula $C_{31}H_{41}N_3O_7$ was established by HRESIMS at m/z 568.2981 $[M + H]^+$ (calcd 568.3017), suggesting

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2**

	1[a]		**2**[a]	
no.	δ_H (mult., J, Hz)	δ_C	δ_H (mult., J, Hz)	δ_C
2	3.83 (m)	61.4/61.8	3.90 (m)	61.6/61.7
3	2.25−2.30	46.8/46.7	2.25−2.30	47.9
4		175.1		175.2
6α	3.07 (m)/2.91 (m)	37.6/37.4	3.08	37.4/37.7
6*β*	3.48 (m)/3.38 (m)		3.51	
7α	1.71 (m)/1.80 (m)	28.4/29.5	1.76 (m)/1.82 (m)	29.1/30.9
7*β*	2.01 (m)/2.03 (m)		2.04 (m)/2.06 (m)	
8	3.45 (m)/3.55 (m)	46.0/43.1	3.56 (m)/3.68 (m)	44.5/45.3
10α	3.23 (m)/3.31 (m)	47.8/43.9	3.31 (m)	45.9
10*β*	3.54 (m)		3.61 (m)	
11α	1.53 (m)	25.1/24.3	1.56 (m)	25.2/24.9
11*β*	1.92 (m)		1.91	
12α	1.35 (m)	25.3/25.5	1.38	26.9/25.4
12*β*	1.52 (m)		1.52	
13α	2.20 (m)/2.32 (m)	46.0/46.6	2.23 (m)/2.35 (m)	46.7/46.8
13*β*	2.45 (m)/2.53 (m)		2.56 (m)/2.35 (m)	
1′		138.2		138.2
2′, 6′	7.10 (d, 8.9)/7.12 (d, 8.9)	128.5	7.14 (br d, 7.2)	128.5
3′, 5′	6.93 (d, 8.5) /6.95 (d, 8.5)	117.4	6.96 (br d, 8.4)	117.4
4′		156.4		156.5
1″		136.8		135.5
2″,6″	7.32 (m)	129.7	7.39 (m)	129.9
3″, 5″	7.39 (m)	129.5	7.27 (br d, 7.8)/7.29 (br d, 7.8)	129.5
4″	7.39 (m)	129.7	7.38 (m)	130.9
7″	6.69 (d, 12.6)/6.70 (d, 12.6)	134.5/134.4	7.03 (d, 15.6)/7.08 (d, 15.6)	143.8/144.0
8″	6.13 (d, 12.6)/6.17 (d, 12.6)	124.1	7.61 (m)	118.5
9″		171.0		167.9
1‴	5.77 (br s)/5.78 (br s)	98.6	5.79 (br s)	98.6
2‴	4.04 (m)	81.9	4.04 (m)	81.9
3‴	3.89 (m)	71.9	3.89 (m)	72.0
4‴	3.45 (m)	74.0	3.44 (m)	74.1
5‴	3.58 (m)	70.4	3.58 (m)	70.4
6‴	1.18 (d, 5.9)/1.19 (d, 5.9)	18.0	1.19 (d, 6.0)	18.0
1⁗	4.56 (d, 7.6)/4.57 (d, 7. 6)	106.7	4.57 (d, 7.8)	106.7
2⁗	3.37 (m)	75.4	3.37 (m)	75.4
3⁗	3.41 (m)	77.7	3.43 (m)	77.8
4⁗	3.92 (m)	71.6	3.40 (m)	71.6
5⁗	3.64 (m)	75.7	3.64 (m)	75.7
6⁗α	4.34 (m)/4.36 (m)	65.2	4.36 (m)	65.2
6⁗*β*	4.73 (dd, 6.8, 1.2)/4.74 (dd, 6.8, 1.2)		4.73 (m)/4.75 (m)	
1⁗′		131.1		131.1
2⁗′	7.84 (d, 8.0)/7.85 (d, 8.0)	130.5	7.86 (d, 7.8)	130.5
3⁗′	7.26 (m)	129.6	7.63 (m)	129.0/129.1
4⁗′	7.48 (m)	134.1	7.49 (br d, 7.2)/7.50 (br d, 7.2)	134.1
5⁗′	7.26 (m)	129.6	7.63 (m)	129.0/129.1
6⁗′	7.84 (d, 8.0)/7.85 (d, 8.0)	130.5	7.86 (d, 7.8)	130.5
7⁗′		167.7		167.7

[a] Recorded in methanol-d_4. All chemical shift assignments were done on the basis of 1D and 2D NMR techniques. ^{1}H and ^{13}C NMR were recorded at 600 and 150 MHz, respectively.

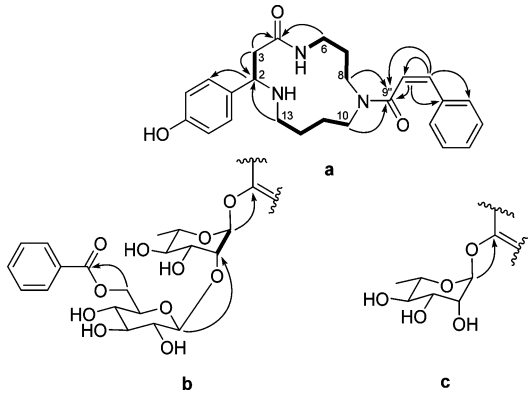


Figure 1. Key HMBC (H→C) and ^{1}H−^{1}H COSY (−) correlations for **1a** (a) and for the sugar moiety of **1/2** (b) and **3/4** (c).

that **4** (Table 2) was an isomer of **3**. The ^{1}H and ^{13}C NMR spectra of both compounds were closely related, except for the olefinic signals. In this compound, an *E*-cinnamate was established by the coupling constant (15.6 Hz) between H-7″ and H-8″. A negative Cotton effect at 210−230 nm in the CD spectrum again indicated the *S*-configuration at C-2. Thus, the structure of dracotanoside D was formulated as shown.

The broadened or split signals of **1**−**4** in their respective ^{1}H and ^{13}C NMR spectra[15,16] revealed the interconverting rotamers arising by the restricted rotation of the amide bond. The DFT calculations of **1a** found two stable atropisomers and two transitional states, named D1, D2, and TS1, TS2, respectively (Figure 2). Structurally,

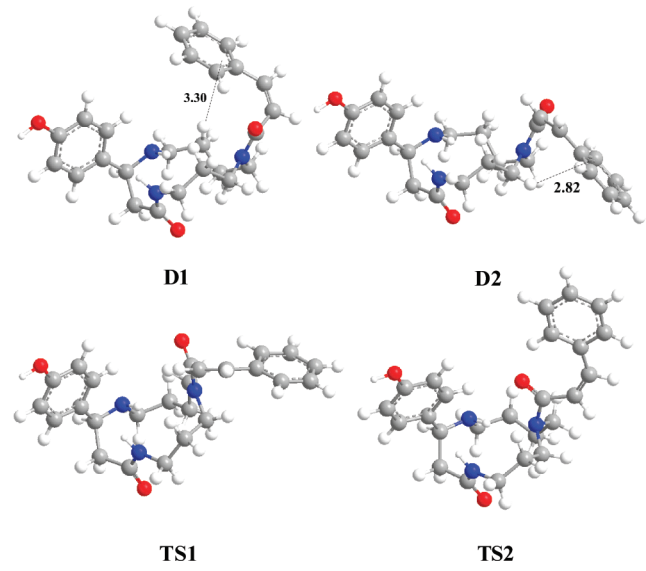


Figure 2. B3LYP-optimized geometries for **1a**. The O, N, C, and H atoms are represented as red, blue, gray, and white-gray, respectively. Bond lengths are in Å.

the benzene rings of D1 and D2 are stabilized by the H−π interactions, with the respective bond lengths being 3.30 and 2.82 Å.

Energetically, the rotational barriers of the C−N axis are 6.83 and 12.14 kcal/mol, respectively, and D1 is only 1.01 kcal/mol more stable than D2, which reveals that the C−N axis is a semistable

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **1a**, **3**, and **4**

	1a[a]		**3**[b]		**4**[c]	
no.	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C
2	4.25 (m)	60.8/61.1	3.95 (m)	60.4/60.0	3.91 (m)/3.89 (m)	60.2/60.1
3	2.76 (m)	46.7/46.6	2.33 (m)	45.4/45.3	2.32 (m)/2.19 (m)	46.2/46.1
4		172.1		173.7		171.6
N(5)					7.99 (m)	
6α	3.22 (m)/3.01 (m)	36.9/36.6	3.07 (m)/2.88 (m)	36.2/36.0	2.98 (m)	36.0/36.2
6*β*	3.72 (m)/3.58 (m)		3.51 (m)/3.36 (m)		3.26 (m)	
7α	2.00 (m)/1.62 (m)	28.2/29.2	1.78 (m)/1.52 (m)	26.9/28.1	1.65 (m)/1.57 (m)	28.6/30.5
7*β*	2.35 (m)/1.98		(m)2.05 (m)/1.79 (m)		1.83 (m)/1.82 (m)	
8α	3.79 (m)/3.61 (m)	41.9/44.6	3.44 (m)/3.48 (m)	44.6/41.7	3.54 (m)	43.7/43.0
8*β*	3.98 (m)/3.61 (m)		3.61 (m)/3.58 (m)		3.38 (m)	
10α	3.28 (m)/3.46 (m)	47.0/42.4	3.23 (m)/3.48 (m)	47.8/42.6	3.17 (m)	44.1
10*β*	3.45 (m)/3.98 (m)		3.41 (m)/3.58 (m)		3.50 (m)	
11α	1.21 (m)/1.29 (m)	24.4/24.8	1.49 (m)	23.7/22.9	1.42 (m)	24.4/24.0
11*β*	1.78 (m)/1.29 (m)		1.79 (m)		1.73 (m)	
12α	1.28 (m)/1.72 (m)	23.2	1.46 (m)	24.1/24.0	1.42 (m)/1.51 (m)	26.2/24.6
12*β*	1.78 (m)/1.29 (m)		1.53 (m)		1.42 (m)/1.82 (m)	
13α	2.32 (m)/2.35 (m)	45.0/45.5	2.23 (m)/2.39 (m)	45.2/44.6	2.16 (m)	45.5/45.3
13*β*	2.62 (m)/2.70 (m)		2.51 (m)/2.62 (m)		2.47 (m)	
1′		134.9		137.6		138.0
2′, 6′	7.30 (d, 8.4)	128.0	7.22 (d, 8.4)	126.9	7.25 (d, 84)	127.9
3′, 5′	7.17/7.16 (d, 8.4)	116.2	7.05 (d, 8.4)	116.2	6.99 (d, 8.4)	116.2
4′		157.9		155.5		155.5
1″		136.3		135.4		135.7
2″, 6″	7.69 (d, 7.8)	129.0	7.40 (m),	128.3	7.72 (d, 7.8)/7.70 (d, 7.8)	128.5
3″, 5″	7.33 (m)	128.7	7.32 (m),	128.2	7.37 (m)	129.2
4″	7.23 (m)	128.5	7.27 (m),	128.1/127.9	7.36 (m)	129.9
7″	6.66 (d,12.6)	132.3	6.70 (d, 12.6)/6.83 (d, 12.6)	133.0	7.48 (d, 15.6)	141.5/141.4
8″	6.30 (d, 12.6)/6.33 (d, 12.6)	125.1	6.17 (d, 12.6)/6.13 (d, 12.6)	122.7	7.14 (d, 15.6)/7.05 (d, 15.6)	119.1/119.0
9″		168.1/168.0		169.6		165.1/165.0
1‴			5.41 (br s)	98.5	5.32 (br s)	99.1
2‴			3.98 (m)	70.4	3.79 (m)	70.7
3‴			3.83 (m)	70.8	3.63 (m)	70.8
4‴			3.46 (m)	72.4	2.24 (m)	72.2
5‴			3.64 (m)	69.2	3.46 (m)	69.9
6‴			1.21 (d, 6.0)	17.0	1.11 (d, 6.0)	18.4

[a] Recorded in pyridine-d_5. [b] Recorded in methanol-d_4. [c] Recorded in DMSO-d_6. All chemical shift assignments were done on the basis of 1D and 2D NMR techniques. ^{1}H and ^{13}C NMR were recorded at 600 and 150 MHz, respectively.

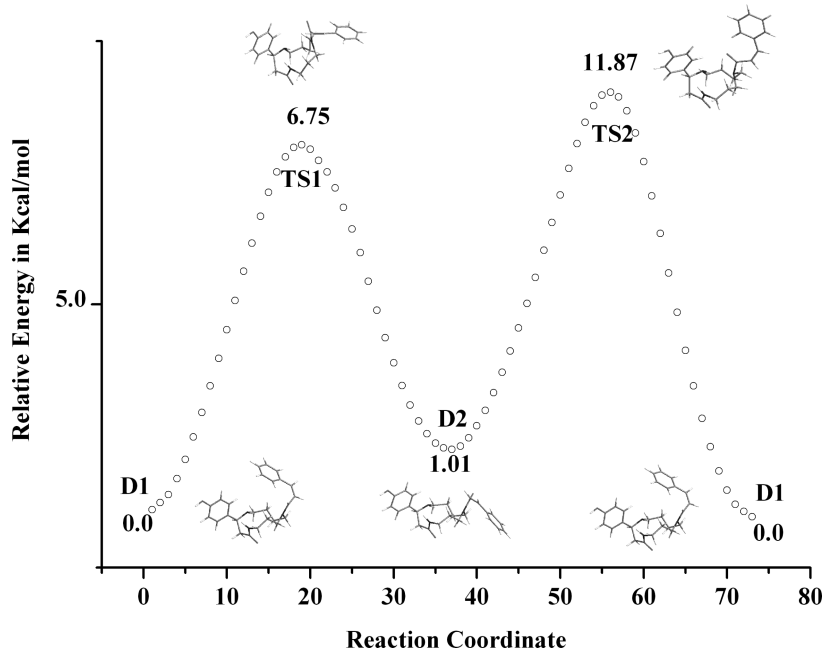


Figure 3. Atropisomerization minimum energy pathways between D1 and D2. The potential profile was scanned at the B3LYP/6-31+G* level, but the geometries corresponding to the extrema points were refined at the B3LYP/6-311++G** level.

bond and two rotamers can interconvert rapidly at room temperature (Figure 3). Obviously, D1−TS1−D2 is the optimal interconversion pathway partly because of the smaller steric repulsion of TS1 than TS2.

The cytotoxicity of **1**−**4** and **1a** was tested with K562 and K562/A02 cell lines using the same bioassay method as previously described.[17] The compounds showed little or no cytotoxicity, and no discernible differences were found in the cytotoxic activities for the test compounds against the sensitive and resistant cell lines (IC_{50} values >200 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. CD spectra were obtained on a Chirascan spectropolarimeter. IR spectra were recorded on a Thermo-Nicolet 670 spectrophotometer using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz, respectively. HRESIMS spectra was measured on a LTQ-Orbitrap XL. All solvents used were of analytical grade (Laiyang Chemical Reagent Co., Ltd., Shandong, People's Republic of China). Silica gel (200−300 mesh, Qindao Ocean Chemical Co., China) or Sephadex LH-20 (Amersham Biosciences) was used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with glass precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.). Spots were detected using UV light or by spraying with Dragendorff's reagent. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a ZORBAX SB-C_{18} column (9.4 mm × 250 mm, 5 μm). HPLC was performed on an Agilent 1200 liquid chromatograph with a Phenomenex C_{18} column (4.6 mm × 250 mm).

Plant Material. Air-dried whole plants of *Dracocephalum tanguticum* Maxim were collected in Qinghai Province, People's Republic of China, in March 2007. The sample was identified by Gui-Fa Luo (Qinghai Institute For Drug Control), and a voucher specimen (No. DRA0703) was deposited in the School of Pharmaceutical Sciences, Shandong University.

Extraction and Isolation. Air-dried whole plants (5.0 kg) were extracted with 70% EtOH. The concentrated EtOH extract (1.2 kg) was dissolved in warm distilled H_2O and defatted with petroleum ether. The aqueous layer was extracted with CH_2Cl_2 (×3) to obtain a neutral fraction (430 g). The aqueous fraction was then acidified with HOAc to pH 3−4 and extracted with CH_2Cl_2. The aqueous acidic fraction was then made alkaline by adding ammonia solution to pH 9−10 and extracted with CH_2Cl_2 (×3) to obtain the crude alkaloid fraction (1.2 g). The crude alkaloid fraction was subjected to Si gel CC (5 × 25 cm) eluting with a $CHCl_3$−MeOH gradient, to yield nine major fractions (F1−F9). F2 (130 mg) was subjected to a Sephadex LH-20 column (2 × 60 cm) using CH_2Cl_2−MeOH (1:1) as eluent. Further purification was carried out by repeated preparative HPLC on an ODS C-18 column, using MeOH−H_2O−CH_3CN (63:35:2) to afford compounds **3** (10 mg) and **4** (5.5 mg). F6 (135 mg) was rechromatographed over Si gel CC (2.5 × 25 cm), to give subfractions F6-1 to F6-8 by TLC analysis. F6-5 was subjected to reversed-phase semipreparative HPLC, using CH_3OH−H_2O−CH_3CN (70:28:2) as the mobile phase, to afford compounds **1** (11 mg) and **2** (9 mg).

Dracotanoside A (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −14.6 (*c* 1.87, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.03), 257 (3.66) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−5.3) nm; IR (KBr) ν_{max} 3326, 2928, 1720, 1605, 1508 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS *m*/*z* 834.3790 $[M + H]^+$ (calcd for $C_{44}H_{56}N_3O_{13}$, 834.3813).

Dracotanoside B (2): colorless, amorphous powder; $[\alpha]^{25}_D$ −29.79 (*c* 1.33, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.08), 257 (3.68) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−4.3) nm; IR (KBr) ν_{max} 3369, 2930, 1719, 1644, 1602, 1508 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS *m*/*z* 834.3794 $[M + H]^+$ (calcd for $C_{44}H_{56}N_3O_{13}$, 834.3813).

Dracotanoside C (3): colorless, amorphous powder; $[\alpha]^{25}_D$ −27.7 (*c* 2.29, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.33), 255 (4.09) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−8.1), 224 (−5.5) nm; IR (KBr) ν_{max} 3304, 2930, 1643, 1605, 1508 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRESIMS *m*/*z* 568.3008 $[M + H]^+$ (calcd for $C_{31}H_{42}N_3O_7$, 568.3024).

Dracotanoside D (4): colorless, amorphous powder; $[\alpha]^{25}_D$ −34.2 (*c* 1.17, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.38), 257 (4.04) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−8.2), 222 (−5.6) nm; IR (KBr) ν_{max} 3338, 2924, 2852, 1608, 1440 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRESIMS *m*/*z* 568.2981 $[M + H]^+$ (calcd for $C_{31}H_{42}N_3O_7$, 568.3024).

Acid Hydrolysis of 1. Compound **1** (3 mg) was refluxed in 2 N HCl (5.0 mL) at 80 °C for 4 h. The reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL). After removal of the solvent under reduced pressure, the resulting extract was purified by preparative TLC to yield compound **1a** (1.3 mg) as a colorless, amorphous powder; $[\alpha]^{25}_D$ −10.9 (*c* 0.366, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.14), 257 (3.99) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−5.9) nm; IR (KBr) ν_{max} 3291, 3023, 2927, 2853, 1632, 1606 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS *m*/*z* 422.2431 $[M + H]^+$ (calcd for $C_{25}H_{32}N_3O_3$, 422.2444).

Sugar Identification. D-Glucose (72 mg) and L-cysteine methyl ester hydrochloride (90 mg) were dissolved in pyridine (3 mL) and stirred at 60 °C for 1.5 h, and then *o*-tolyl isothiocyanate (360 μL) was added to the mixture, which was heated at 60 °C for 1.5 h. Separation by HPLC on a C-18 column eluted with H_2O (containing 0.2%

TFA)−CH_3CN (68:32) gave the derivative **S-1**. The HRESIMS of **S-1** gave a quasimolecular ion $[M + H]^+$ peak at *m/z* 447.1245 (calcd for $C_{18}H_{27}N_2O_7S_2$, 447.1254), which was consistent with the reference.[12] L-Rhamnose was treated similarly and gave the derivative **S-2**, the HRESIMS of which indicated an $[M + H]^+$ peak at *m/z* 431.1298 (calcd for $C_{18}H_{27}N_2O_6S_2$, 431.1305), consistent with ref 12.

Compound **1** was hydrolyzed with HCl and extracted with CH_2Cl_2. The aqueous layer was passed through a Sephadex LH-20 column, and the eluate was concentrated. The residue was dissolved in pyridine (0.4 mL) and stirred with L-cysteine methyl ester (10 mg) for 1.5 h at 60 °C, and then *o*-tolyl isothiocyanate (40 μL) was added to the mixture, which was heated at 60 °C for 1.5 h. The reaction mixture was analyzed by HPLC detected at 250 nm. Analytical HPLC was performed on a Phenomenex C_{18} column (4.6 × 250 mm) at 25 °C using a gradient of CH_3CN−0.2% TFA in H_2O [0−23 min (32:68), 23−30 min (32:68 to 70:30), 30−60 min (70:30)] as the mobile phase. Peaks were detected with an Agilent DAD detector. D-Glucose and L-rhamnose were identified as the sugar moieties of **1**, as they had the same retention times as **S-1** (t_R 11.4 min) and **S-2** (t_R 18.7 min), respectively (Supporting Information).[6,12] D-Glucose and L-rhamnose moieties were identified in **2**, and an L-rhamnose unit in **3** and **4** by the same method.

Calculation Details. All calculations were performed at 298 K by the Gaussian03 program package.[18] Ground-state geometries of **1a** were optimized at the B3LYP/6-31+G* level, and the transition structure of the atropisomerization was located using the synchronous transit-guided quasi-Newton (STQN) method.[19,20] Frequency analyses were done to identify the nature (minima or transition state) of the optimized geometries. Then, the relevant energies were refined using the B3LYP/6-311++G** single-point calculations at the B3LYP/6-31+G* geometries. All energies are corrected by ZPVE.

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Supporting Information Available: Copies of the 1D and 2D NMR, MS, and IR spectra of **1**−**4** and **1a**, detailed information concerning the sugar identification, and the experimental details of the cytotoxic assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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