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Structure Elucidation and Biomimetic Synthesis of Hostasinine A, a New Benzylphenethylamine Alkaloid from *Hosta plantaginea*

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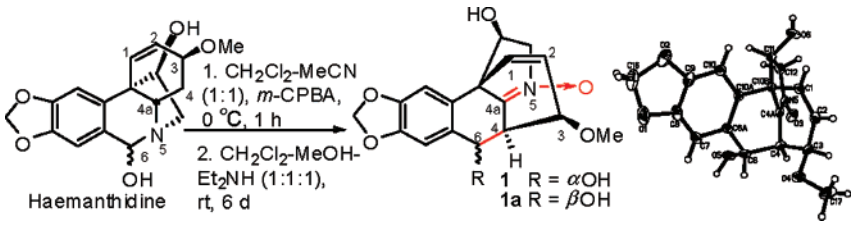
ABSTRACT



Hostasinine A (1), a benzylphenethylamine alkaloid with an unprecedented skeleton featuring a C-4–C-6 linkage and a nitrone moiety, was isolated from *Hosta plantaginea*. Its structure was established on the basis of spectroscopic data, and was further confirmed by single-crystal X-ray diffraction. The alkaloid was postulated biogenetically from haemanthidine via *N*-oxidation and aza-aldol-type condensation and was synthesized biomimetically. The inhibitory activities of 1 on acetylcholinesterase (AChE) and two tumor cell lines (K562 and A549) were also evaluated.

Benzylphenethylamine alkaloids are distributed widely in the plants of the Amaryllidaceae family and are called Amaryllidaceae alkaloids, whose remarkable biological activities and unique skeletons have attracted great interest as challenge targets for total synthesis and diversity-oriented synthesis.[1] In our recent research, a series of benzylphenethylamine alkaloids were isolated from a Liliaceous plant, *Hosta plantaginea* (Lam.) Aschers. Some of these alkaloids show inhibitory activities against the Tobacco mosaic virus (TMV) or acetylcholinesterase (AChE).[2] In continuing studies on this plant, a minor alkaloid, hostasinine A (**1**), possessing a new skeleton of C-4−C-6 linkage and a nitrone moiety, was isolated. The biogenetic pathway of **1** involving an aza-aldol-type reaction was postulated for transformation of haemanthidine to **1** and was chemically mimicked. The inhibitory activities of **1** on AChE and two tumor cell lines were also evaluated. The structure elucidation of **1** and the results of the biomimetic transformation and bioassay are reported.

The whole plant of *H. plantaginea* (4.8 kg) was collected from Guangzhou, People's Republic of China, in September 2005, and exhaustively extracted with EtOH (70%). The

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extract (1000 g) was defatted by petroleum ether and then partitioned between EtOAc and H_2O. The EtOAc-soluble portion (60 g) was submitted to repeated chromatography on silica gel columns ($CHCl_3$−MeOH, 10:1; EtOAc; $CHCl_3$−Me_2CO, 1:4) to afford **1** (9 mg).

Hostasinine A (**1**)[3] was obtained as optically active colorless needles. Its HRESIMS and NMR spectra (Table 1) indicated a molecular formula of $C_{17}H_{17}NO_6$, implying ten degrees of unsatuation. Its IR spectrum displayed the presence of hydroxy (3385 cm^{-1}), methylenedioxy (2901, 1481, 926 cm^{-1}), iminium (1665 cm^{-1}) and aromatic (1633, 1501 cm^{-1}) groups. The 1D (Table 1) and 2D (Figure 2)

Table 1. ^1H (CD_3OD, 500 MHz) and ^{13}C (CD_3OD, 125 MHz) NMR Spectral Data of **1** (δ in ppm, J in Hz)

no.	δ_H	δ_C
1	6.08 (dd, J = 9.6, 2.0)	132.8
2	5.53 (dd, J = 9.6, 2.0)	127.6
3	4.29 (ddd, J = 6.2, 2.0, 2.0)	78.2
4	4.08 (d, J = 6.2)	44.0
4a		152.2
6	4.98 (s)	69.6
6a		132.8
7	6.95 (s)	111.0
8		149.5
9		148.5
10	6.87 (s)	104.3
10a		135.0
10b		55.3
11	4.90 (dd, J = 6.8, 3.4)	69.6
12β	4.36 (dd, J = 14.0, 6.8)	71.5
12α	3.86 (dd, J = 14.0, 3.4)	
$-OCH_3$	3.54 (s)	57.3
$-OCH_2O-$	5.95 (s)	102.9
	5.94 (s)	

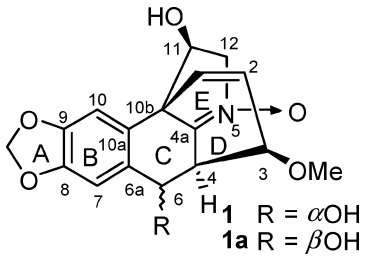


Figure 1. Structures of **1** and **1a**.

NMR spectra of **1** showed the presence of a tetrasubstituted phenyl ring [δ_H 6.87 (1H, s, H-10) and δ_C 104.3 (CH, C-10), δ_H 6.95 (1H, s, H-7) and δ_C 111.0 (CH, C-7), 132.8 (qC, C-6a), 135.0 (qC, C-10a), 149.5 (qC, C-8), and 148.5 (qC, C-9)], a disubstituted double bond [δ_H 5.53 (1H, dd, J = 9.6 and 2.0 Hz, H-2) and δ_C 127.6 (CH, C-2), and δ_H 6.08 (1H, dd, J = 9.6 and 2.0 Hz, H-1) and δ_C 132.8 (CH, C-1)], an *O*-methyl [δ_H 3.54 (3H, s) and δ_C 57.3 (CH_3)], and a methylenedioxy group [δ_H 5.94 (1H, s) and 5.95 (1H, s), and δ_C 102.9 (CH_2)]. Except for the two carbons of an *O*-methyl and a methylenedioxy group, there were 15 carbon atoms in the molecular skeleton of **1**. The aforementioned data implied compound **1** possessed the features of benzylphenethylamine alkaloids.[2] The signal of C-4a (δ_C 152.2) was shifted upfield, **1** was deduced as an imine *N*-oxide.[4] Since 5 out of 10 elements of unsaturation were accounted for, **1** was inferred to possess 5 rings (A−E).

Rings A and B (phenyl ring) of **1** were established by the HMBC correlations (Figure 2) of $-OCH_2O-$ to C-8 and C-9, H-7 to C-9 and C-10b, and H-10 to C-6a and C-8. The $^1H-^1H$ COSY spectrum exhibited two partial structures of **a** (C-11 to C-12) and **b** (C-1 to C-4) (Figure 2). Based on the HMBC correlations of H_2-12 to C-4a and 10b, H-11 to C-10a, and H-10 to C-10b, ring E was confirmed, and this ring was located at C-10a of the phenyl ring through C-10b. Ring D was deduced by the HMBC correlations of H-1/C-4a, H-2/C-10b, and H-4/C-10b. This ring connected to C-6a of the phenyl ring through C-6 was confirmed by the HMBC correlations of H-3 to C-6, H-4 to C-6a, H-6 to C-3, C-7, and C-10a, and H-7 to C-6, which also validated the presence of ring C. The attachment of *O*-methyl to C-3 was revealed by the mutual HMBC correlations of OMe/C-3.

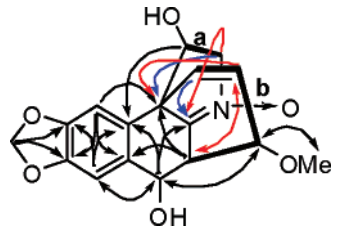


Figure 2. $^1H-^1H$ COSY (−) and key HMBC (H → C) correlations of **1**.

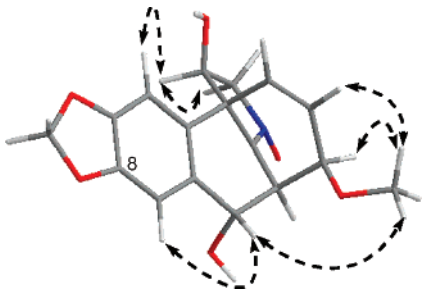


Figure 3. Key ROESY correlations and possible configuration of **1** generated by computer.

(3) Hostasinine A (**1**): colorless needles (MeOH); mp 229−231 °C; $[\alpha]^{16.3}_D$ +124.4 (*c* 0.45, MeOH); CD (*c*, 0.022, MeOH) $\Delta\epsilon$ −3.24 (286.8), +28.26 (263.8), −2.48 (250.2); UV (MeOH) λ_{max} (log ϵ) 296.8 (3.48), 243.6 (3.87) nm; IR (KBr) ν_{max} 3385, 2901, 1665, 1633, 1501, 1481, 1256, 1246, 1098, 1088, 1034, 926 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 354.3 $[M + Na]^+$, 685.1 $[2M + Na]^+$; HRESIMS *m*/*z* 332.1130 $[M + H]^+$ ($C_{17}H_{18}NO_6$, calcd 332.1134).

The relative configuration of **1** was fixed by the ROESY spectrum (Figure 3). Crucial correlations of H-10/H-11, H-6/H-7, and H-6/OMe indicated that H-3, H-4, and H-11 were cofacial, and were arbitrarily assigned as the α-orientation, while H-6 and ring D were *β*-oriented, which was further confirmed by single-crystal X-ray diffraction (Figure 4).[5]

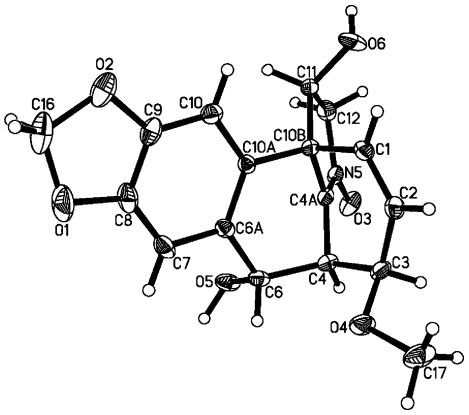
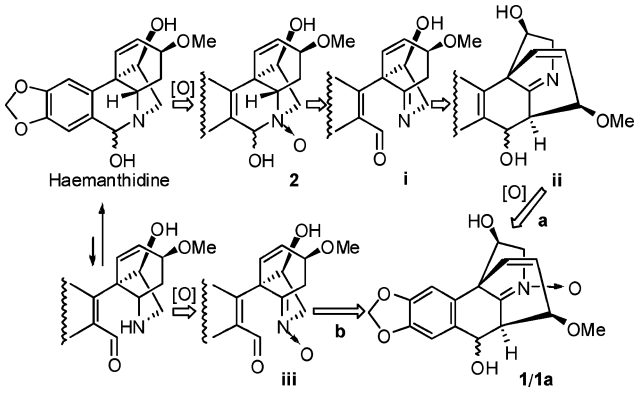


Figure 4. Perspective ORTEP drawing for **1**.

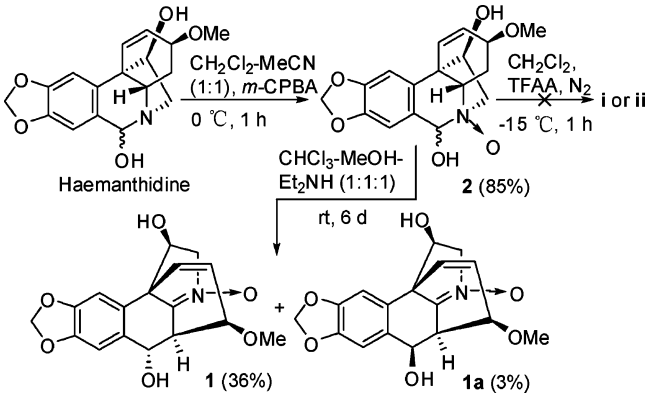
Hostasinine A (**1**) seemed biogenetically related to haemanthidine, a known crinine-type alkaloid earlier isolated from the plant.[2,6,7] The possible biogenetic pathway was proposed as shown in Scheme 1. Compound **1** could be

Scheme 1. Plausible Biogenetic Path for Hostasinine A (**1**)



generated from haemanthidine through its *N*-oxidation to form **2** followed by Polonovski-type reaction to yield iminium intermediate **i**,[8] condensation between C-4 and C-6 to give **ii**, and subsequent *N*-oxidation (path **a**), or through oxidation of haemanthidine to form nitrone intermediate **iii**,[9] and then condensation (path **b**). To verify such a correlation and to determine the absolute configuration of **1**, haemanthidine was oxidated by *m*-CPBA to yield **2**, as shown in Scheme 2. Neither **i** nor **ii** was obtained when **2** was exposed to TFAA in dry CH_2Cl_2 under N_2 at −15 °C.[10] However, **1** and its epimer **1a** were harvested with the yields of 36% and 3%, respectively, by solving **2** into the mixed solvent

Scheme 2. Chemical Transformation from Haemanthidine to **1**



of $CHCl_3$−MeOH−Et_2NH (1:1:1) and stirring for 6 days at room temperature. Thus, as shown in Figure 1, the absolute configuration of **1** was determined according to that of haemanthidine.

The bioassay results showed that **1** possessed weak activity against AChE (IC_{50} = 290 μM) as compared to tacrine (IC_{50} = 0.20 μM) by Ellman's method,[11] but was inactive against human leukaemia K562 and lung adenocarcinoma A549 cells by the MTT[12] and SRB[13] methods, respectively.

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Supporting Information Available: General experimental procedures, 1D NMR spectra of **1**, **1a**, and **2**, and 2D NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Crystallographic data for hostasinine A (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition no. CCDC-661218). Copies of the data can be obtain free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm.
(6) Haemanthidine: colorless flakes (MeOH); mp 147−151 °C; $[\alpha]^{25.8}_D$ −34.5 (*c* 0.42, DMSO); CD (*c*, 0.06, $CHCl_3$) $\Delta\epsilon$ +14.35 (273.2), −10.49 (260.6); ^{1}H and ^{13}C NMR, same as the literature;[7] ESIMS *m*/*z* 318.2 $[M + H]^+$.
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