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Heliotropiumides A and B, new phenolamides with *N*-carbamoyl putrescine moiety from *Heliotropium foertherianum* collected in Hawaii and their biological activities

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ABSTRACT

Two new compounds heliotropiumides A (**1**) and B (**2**), phenolamides each with an uncommon carbamoyl putrescine moiety, were isolated from the seeds of a naturalized Hawaiian higher plant, *Heliotropium foertherianum* Diane & Hilger in the borage family, which is widely used for the treatment of ciguatera fish poisoning. The structures of compounds **1** and **2** were characterized based on MS spectroscopic and NMR analysis, and DP4+ calculations. The absolute configuration (AC) of compound **1** was determined by comparison of its optical rotation with those reported in literature. Compound **2** showed inhibition against NF-κB with an IC_{50} value of 36 μM.

Heliotropium foertherianum (Common name: tree heliotrope and octopus bush) is a flowering plant in the Boraginaceae family. It is native to tropical Asia including southern China, Madagascar, northern Australia, Micronesia and Polynesia. In the Pacific, numerous traditional herbal remedies are preferentially used to treat ciguatera fish poisoning (CFP), but *H. foertherianum* is the most widely used in Pacific islands.[1] Rosmarinic acid was isolated from *H. foertherianum*, which strongly inhibit CFP.[2] Recent studies showed that *H. foertherianum* could also inhibit snake venom-induced hemorrhage, which is due to the presence of rosmarinic acid in the plant.[3] Although not indigenous to Hawaii, *H. foertherianum* is naturalized on almost all the Hawaiian Islands. The plant is found on many sandy Hawaiian beaches. HPLC–UV analysis showed that seeds of *H. foertherianum* contain caffeic acid ((*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid) or ferulic acid ((*E*)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid) derivatives, which have the same chromophore as that of rosmarinic acid. Then we decided to study the seeds of this plant. The powdered seeds of heliotrope were extracted with MeOH overnight and the MeOH extract was successively partitioned to yield *n*-hexane and EtOAc sub-extracts. Further separation and purification of the *n*-hexane sub-extract led to the isolation of two new compounds, **1** and **2** (Fig. 1).

Compound **1**[4] was isolated as a colorless solid. Its molecular formula was determined to be $C_{25}H_{31}N_3O_7$ by HR-ESIMS (*m*/*z* 486.2256, calcd for $[M+H]^+$ 486.2240), with twelve degrees of unsaturation. The IR spectrum of **1** showed the existence of carbonyl (1644 and 1600 cm^{-1}) and hydroxyl (3350 cm^{-1}) groups. A detailed analysis of ^1H and ^{13}C NMR spectra (Table 1) demonstrated the presence of two methoxy groups, five methylenes with one being hydroxylated, nine methines including five aromatic and two olefinic, and nine carbons with no hydrogen attached. In the ^1H–^1H COSY spectrum of **1** (Fig. 2), only five spin systems were identified, including a 1,3,4-trisubstituted benzene ring, a 1,3,4,5-tetrasubstituted benzene ring, a *trans* coupled double bond, a CH—CH—CH_2OH and CH_2—CH_2—CH_2—CH_2 spin systems. In the HMBC spectrum of **1** (Fig. 2), H-2 showed correlation to C-3′, C-5′

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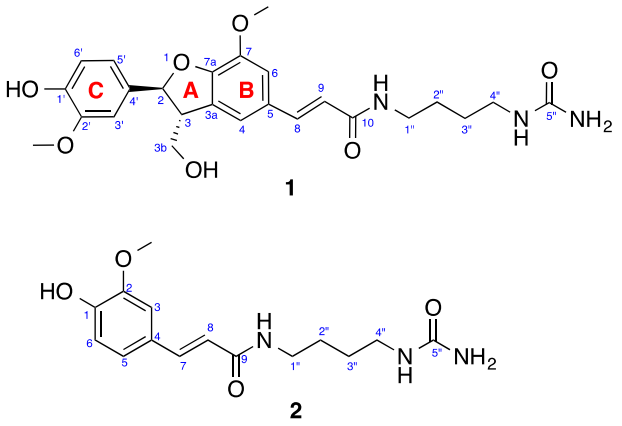

Fig. 1. Structure of compounds **1** and **2**.

Table 1
^{1}H and ^{13}C NMR data of **1**.

No.	**1**		No.	**1**	
	δ_H, *J* (Hz)[a]	δ_C[b]		δ_H, *J* (Hz)[a]	δ_C[b]
2	5.49 d 6.9	88.1	1′		147.0
3	*3.50* m	53.2	2′		148.0
3a		130.3	2″-OMe	3.81 s	56.2
3b	3.68 m	63.3	3′	6.92 br s	111.1
4	7.13 br s	117.1	4′		132.3
5		128.9	5′	6.77 br s	119.2
6	7.08 br s	112.3	6′	6.77 br s	115.9
7		144.3	1″	3.15 m	38.9
7-OMe	3.74 s	56.1	2″	1.40 m	28.0
7a		149.2	3″	1.41 m	27.2
8	7.35 d 15.8	139.5	4″	2.97 m	39.4
9	6.49 d 15.5	120.1	5″		160.1
10		165.8			

[a] Spectra recorded at 400 MHz.
[b] Spectra recorded at 100 MHz. Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments.

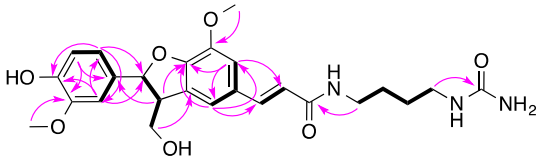

Fig. 2. COSY (Bold) and key HMBC (Single headed) correlations of **1**.

and C-3b, indicating the presence of a C_6–C_3 [Ph-CH(O)-CH(C)-CH_2-OH] fragment. H-9 and H-8 correlated to C-5 and C-10, respectively, indicating the presence of another C_6–C_3 [Ph—CH=CH—CO—] fragment. C-1″ and C-4″ were determined to be nitrogenated on the basis of their chemical shifts (1″: δ_H/δ_C 3.15/38.9; 4″: δ_H/δ_C 2.97/39.4), and they were part of the putrescine fragment (N—CH_2—CH_2—CH_2—CH_2—N). One nitrogen atom in the putrescine fragment must be connected to the carbonyl group in the Ph—CH=CH—CO— fragment to form an amide bond (δ_C 165.8) due to an HMBC correlation from H-1″ to C-10, while another nitrogen atom in the putrescine fragment must be connected to CO—NH_2 to form a mono-substituted urea group (RNH—CO—NH_2, δ_C 160.1). These two carbonyl groups together with one double bond and two benzene rings accounted for eleven unsaturation units, and the remaining unsaturation unit must be due to a ring system. C-2 and C-3 in the one C_6–C_3 [Ph—CH(O)—CH(C)—CH_2OH] fragment must be fused with the benzene ring of another C_6–C_3 [Ph—CH=CH—CO—] fragment to form a 2,3-dihydrofuran with an oxygen bridge between C-2 and C-7a. Like some *meta*-coupled aromatic protons, H-3′, H-4 and H-6 were singlets.[5,6] It was interesting that the *ortho*-coupled H-5′ and H-6′ were overlapped as a broad singlet, which was similar to the two *ortho*-coupled aromatic protons in quinizarin and AQDA.[7,8] The methoxy and hydroxy groups in C ring must be *ortho* to each other because the ^{13}C NMR chemical shifts of the two oxygenated aromatic carbons were at δ_C 147 and 148 ppm for C-1′ and C-2′, respectively. However, if the methoxy and hydroxy groups in C ring were *meta* to each other at C-2′ and C-6′, respectively, the ^{13}C NMR chemical shifts of the two oxygenated aromatic carbons must be at about δ_C 155 or even 160 ppm.[9] Hence, the planar structure of **1** was determined as shown. The coupling constant (*J* = 6.9 Hz) of H-2 with H-3 indicated that the hydroxyl methyl and the *p*-hydroxyl-*m*-methoxy benzene ring had different orientation. Hence the relative configuration of compound **1** was determined as shown.

The above determination was further validated using quantum chemical calculations of NMR shifts.[10] Hence, the relative configuration at the tetrahydrofuran (*cis* and *trans*) and the nature of the carbonyl group at C-5″ (urea and guanidinium) was challenged. Given the conformational flexibility of the molecule, and considering that the two mentioned units are highly separated, we used the fragment approach to compute the NMR shifts and further correlation with the experimental values found.[11] Two different clusters were considered (Fig. 3), and the NMR shifts of each candidate was computed at the PCM/mPW1PW91/6-31+G**//PCM/B3LYP/6-31G* level of theory, the recommended for DP4+ calculations.[12] When correlating the experimental NMR data for left unit of **1**′ (Fig. 3) with the computed NMR data of **3** and **4** fragment isomers using the DP4+ probability, the best match was observed for the second isomer (DP4+ = 99.7%), suggesting that the most likely stereochemistry at the tetrahydrofuran ring should be *trans*. Regarding the correlation of the experimental NMR data for right unit of **1**′ with the computed NMR data of urea (**5**) and guanidinium (**6**) candidates, the former (**5**) was identified as the most probable candidate (C-DP4+ = 88.5%). This result was consistent with the experimental NMR shifts reported for related compounds, for which it was observed that the carbonyl group of urea derivatives typically resonates at ~160 ppm, about 3 ppm more deshielded than the corresponding guanidinium analogues (~157 ppm).[13] Combining the results obtained for each separated fragment, the most likely structure from theoretical calculations is the same as suggested for compound **1** (Fig. 1).

To determine the absolute configuration, we compared the optical rotation of **1** with those of different 2,3-disubstituted 2,3-dihydrobenzofuran derivatives including compounds **7**–**10**.[14] Compound **1** ($[\alpha]_D^{25}$ +14 (*c* 0.12, MeOH)) had the same sign of optical rotation as that of **7** and **8**, but opposite to that of **9** and **10** (Fig. 4), indicating that **1** had a 2*S*,3*R* configuration.

Compound **2** was isolated as a colorless solid. Its molecular formula was determined to be $C_{15}H_{21}N_3O_4$ by HR-ESIMS (*m*/*z* 308.1592, calcd for $[M+H]^+$ 308.1610). Analysis of NMR data (Table 2) indicated the presence of a *p*-hydroxyl-*m*-methoxy *trans*-cinnamoyl and a carbamoyl putrescine moiety, and these two moieties were connected together through an amide bond. Hence, the structure of compound was readily determined as shown.

Biogenetically, **1** could be derived from *p*-hydroxycinnamoyl-CoA (**11**) and arginine (**13**) (Fig. 5). Oxidation of arginine yields citrulline (**14**), which can be decarboxylated to generate *N*-carbamoylputrescine (**15**). Reaction of the *m*-methoxylated *p*-hydroxycinnamoyl-CoA (**12**) with *N*-carbamoylputrescine (**15**) yields compound **2**. Coupling of compound **2** with *p*-hydroxy-*m*-methoxycinnamic acid (**16**) could produce compound **1** Fig. 6.

Phenolamide derivatives with putrescine (H_2N—CH_2—CH_2—CH_2—CH_2—NH_2) or agmatine (H_2N—CH_2—CH_2—CH_2—CH_2-