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Gelegamines A–E: five new oxindole alkaloids from *Gelsemium elegans*

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ABSTRACT

Five new oxindole alkaloids, gelegamines A–E (**1**–**5**), were isolated from the roots of *Gelsemium elegans*. Their structures were extensively elucidated on the basis of spectroscopic analysis. Among them, the epoxy ring (C-19/C-20) of gelegamine A (**1**) was assigned as α-orientation by ROESY experiment and DFT method at B3LYP/6-31g(d) level, and gelegamine B (**2**) is the first humantenine-type alkaloid with 19-(*E*) ethylidene configuration. The absolute configurations of gelegamines A–E (**1**–**5**) were established on biosynthetic consideration coupled with CD experiments.

1. Introduction

Gelsemium elegans (Loganiaceae) is a liane native to Southeast Asia, where it is used in folk medicine for the treatment of pain, spasticity, and skin ulcers.[1] In a previous chemical investigation, a number of indole alkaloids based on six different structural skeletons were reported from *G. elegans*.[2] Some of them showed the interesting pharmacological effects, such as analgesic, anti-inflammatory, and antitumor activities.[3] As a part of our ongoing research into alkaloids of chemical, pharmacological, and clinical significance,[4] we investigated the chemical constituents of *G. elegans*, which led to the isolation of two new humantenine-type alkaloids, gelegamines A–B (**1**–**2**) and three new gelsedine-type alkaloids, gelegamines C–E (**3**–**5**), together with 10 known ones. In this paper, we describe the isolation and structural identification of gelegamines A–E. The possible biogenetic relationships of these compounds are discussed.

2. Results and discussion

2.1. Structural elucidation of gelegamines A–E (1–5)

Investigation of the MeOH extract of the roots of *G. elegans* (11.2 kg) resulted in the isolation of 5 new compounds named gelegamines A–E (**1**–**5**) together with 10 known ones: 19(*Z*)-akuammidine,[5] 19(*Z*)-16-*epi*-voacarpine,[6] N_a-methoxytaberpsychine,[7] humantenirine,[8] 11-methoxygelsemamide,[9] gelsenicine,[10] 14-hydroxygelsenicine,[11] 19-oxo-gelsenicine,[2b] koumine,[12] and gelsevirine[13] by comparison of their spectral data with those reported in the literature.

The molecular formula of compound **1** was established as $C_{21}H_{24}N_2O_5$ with HRMS (*m*/*z* 385.1770) $[M+H]^+$. The UV absorption at 218 and 266 nm showed the characteristics of an oxindole nucleus.[2b,8] The ^{1}H NMR spectrum indicated the presence of three aromatic protons attributed to ring A of the oxindole system (δ 7.36, d, *J*=8.5 Hz; 6.59, dd, *J*=8.5, 2.5 Hz; 6.50, d, *J*=2.5 Hz), an N_a-*O*-methyl group at δ 3.94 (3H, s), an *O*-methyl group on the aromatic ring at δ 3.81 (s), an oxymethine proton at δ 3.69 (d, *J*=7.0 Hz, H-3), and oxymethylene protons (H_2-17) at δ 4.36 (m) and δ 4.05 (dd, *J*=10.5, 3.5 Hz) (Table 1).

Twenty-one carbon resonances were also resolved in the ^{13}C NMR spectrum (Table 2), and were further classified via DEPT experiments into 1 carbonyl, 5 quaternary carbons, 9 methines, 3

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Table 1
^{1}H (500 MHz, *J* in Hz) NMR data for compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
3	3.69 (d, 7.0)	3.75 (d, 6.5)	3.56 (d, 6.0)	3.71 (m)	3.73 (m)
5	4.37 (m)	4.07 (br s)	4.26 (m)	4.42 (m)	4.72 (m)
6	2.54 (dd, 15.5, 7.5), 2.23 (m)	2.24 (dd, 15.5, 5.5), 2.14 (dd, 15.5, 3.0)	2.61 (dd, 15.6, 4.6), 2.52 (dd, 15.6, 2.4)	2.27 (m), 2.35 (m)	2.52 (m), 2.31 (m)
9	7.36 (d, 8.5)	7.26 (d, 8.5)	7.48 (d, 7.5)	7.41 (d, 10.0)	7.42 (d, 10.0)
10	6.59 (dd, 8.5, 2.5)	6.59 (dd, 8.5, 2.5)	7.20 (t, 7.5)	6.57 (dd, 10.0, 2.5)	6.59 (dd, 10.0, 2.5)
11			7.39 (t, 7.5)		
12	6.50 (d, 2.5)	6.54 (d, 2.5)	7.10 (d, 7.5)	6.47 (d, 2.5)	6.48 (d, 2.5)
14	2.44 (m), 2.27 (m)	2.57 (m), 2.32 (dd, 15.0, 4.0)	3.04 (m), 2.31 (m)	2.35 (m), 2.14 (m)	2.29 (m), 2.21 (m)
15	2.09 (m)	3.19 (br m)	2.78 (d, 9.5)	2.59 (br t, 10.0)	2.60 (br t, 10.0)
16	2.41 (m)	2.49 (br s)	3.02 (m)	2.89 (t, 11.5)	3.43 (t, 11.5)
17	4.36 (m), 4.05 (dd, 10.5, 3.5)	4.25 (d, 11.0), 4.18 (dd, 11.0. 2.5)	4.37 (d, 12.0), 4.18 (dd, 12.0, 3.0)	4.28 (m), 4.27 (m)	4.31 (m), 4.29 (m)
18	1.43 (d, 5.5)	1.87 (d, 7.5)	1.39 (d, 6)	1.30 (t, 9.5)	2.66 (s)
19	3.36 (q, 5.5)	7.11 (q, 7.5)	4.61 (m)	2.74 (m), 2.46 (m)	
21	7.32 (1H, s)		4.03 (m), 3.81 (m)		
N_a-Ome	3.94 (s)	3.94 (s)	4.01 (s)	3.94 (s)	3.93 (s)
Ar–OCH_3	3.81 (s)	3.82 (s)		3.81 (s)	3.82 (s)
N_b-Me			3.07 (s)		

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

methylenes, 1 methyl, and 2 *O*-methyl carbons. A comprehensive analysis of the 1D and 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) spectra indicated that **1** is an analogue of humantenirine.[8] Two oxygenated carbons at δ 59.6 and 58.5 were assigned to C-19 and C-20, respectively, as part of an epoxide, by the HMBC correlations from H-15 and H_3-18 to C-19 and C-20, whereas an imine carbon at δ 162.4 was assigned to C-21 by the HMBC correlations from H-21 to C-15, C-19, and C-20. Therefore, the chemical structure of **1** was established as shown in Figure 1.

The relative configuration of **1** was deduced from ROESY experiments and molecular modeling (Gaussian D.01)[14] using ab initio calculations. In the ROESY spectrum, the cross peaks observed between the proton pairs H-3/H_2-14, H_2-14/H-15, H-9/H-6, H-6/H-5, and H-5/H-16 indicated that the relative configuration of C-3, C-5, C-15, and C-16 in **1**, as shown (Fig. S6, Supplementary data), is identical to that in humantenirine.[8] The ROESY correlation between H-19 and H-21 indicated that both protons were on the same side. Although ROESY correlations of H_3-18/H-14a, H_3-18/H-14b, H_3-18/H-15, and H-19/H-21 were unambiguous, it is not sufficient to determine the orientation of the C-19/C-20 epoxy ring. Therefore, DFT calculations at the B3LYP/6-31G (d) level were made for the two possible structures of **1**, corresponding to the α (**A**) or β (**B**) orientations of the C-19/C-20 epoxy ring as shown in Figure 2. Two optimized structures were obtained, in which the calculated distance of the proton pairs near the epoxide oxygen of **A** was fully consistent with the corresponding ROESY data. Therefore, the orientation of the C-19/C-20 epoxy ring was determined to be α.

Table 2
^{13}C (100 MHz) NMR data for compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
2	172.4	172.6	177.2	171.7	171.6
3	73.5	73.5	76.2	75.2	75.5
5	59.2	53.5	72.4	72.0	74.4
6	35.9	38.0	29.8	37.8	38.2
7	55.6	55.0	58.2	55.4	55.9
8	123.2	121.3	131.5	123.9	123.6
9	125.3	125.7	126.7	125.4	125.3
10	107.8	107.8	125.3	107.7	107.9
11	160.2	160.4	130.2	160.1	160.3
12	94.3	94.7	109.2	93.9	94.0
13	139.2	139.9	139.9	139.1	139.1
14	25.1	29.8	24.0	26.9	27.4
15	28.0	27.5	38.0	39.6	39.3
16	31.3	34.8	37.5	42.5	38.9
17	66.4	65.9	63.4	62.0	61.7
18	14.4	14.2	20.5	9.9	26.1
19	59.6	136.9	65.6	25.6	197.6
20	58.9	133.4	78.7	185.1	178.0
21	162.4	166.5	62.4		
N_a-OMe	63.4	63.6	64.5	63.4	63.4
Ar–OCH_3	55.6	55.6		55.5	55.6
N_b-Me			35.0		

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

The molecular formula of compound **2** was established as $C_{21}H_{24}N_2O_5$ with HRMS (*m*/*z* 385.1756) $[M+H]^+$, UV absorption revealed an oxindole nucleus.[2b,8] The ^{13}C NMR (Table 2) and DEPT spectra showed the presence of 21 carbon signals composed of 2 carbonyl carbons, 8 double-bond carbons (6 aromatic and 2 vinylic), and 11 sp^3 carbons (2 *O*-methyls, 1 methyl, 3 methylenes, 4 methines, and 1 quaternary carbon). The spectroscopic properties of **2** were reminiscent of those of humantenirine.[8] The main difference from humantenirine was a methylene at C-21 in the later was replaced by a conjugated carbonyl carbon (δ_C 166.5) due to UV absorption bond at 256 nm. The HMBC correlations from H-15, H-19 and H_3-18 to C-21 verified this deduction. In addition, by comparison of chemical shift of C-15 in **2** with that of humantenirine, a obvious upfield-shift about 6.8 ppm was observed, this suggested the configuration of C-18 in **2** should be *E*-geometry due to γ-*gauche* effect between C-15 and C-18 in **2**, which was further confirmed by strong ROESY correlation of H-15/H_3-18. Based on further 2D-NMR analysis, the structure of **2**, gelegamine B, was determined as shown

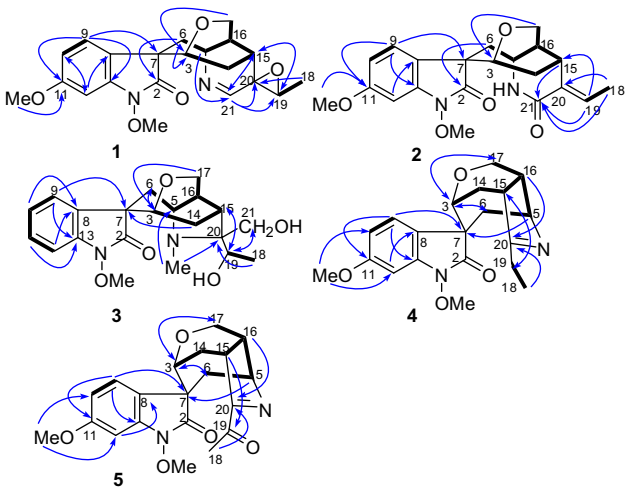

Figure 1. ^{1}H–^{1}H COSY (bold) and HMBC (arrow, H→C) correlations for compounds **1**–**5**.