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Gardovatine, a novel *Strychnos–Strychnos* bisindole alkaloid with cytotoxicity from *Gardneria oveta*

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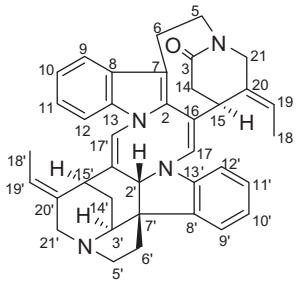
ABSTRACT

Gardovatine (**1**), the first *Strychnos–Strychnos* alkaloid with a C_3/C_7 cleaved backbone, was isolated from twigs and leaves of *Gardneria ovate*, together with an analogue divarine (**2**). The structure was established by extensive spectroscopic methods. Both compounds showed potential cytotoxicities against five human cancer cell lines.

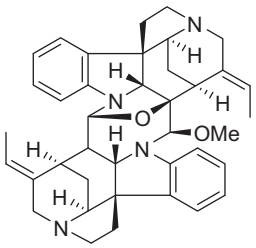
Monoterpenoid indole alkaloids (MIA), which originate from the condensation of tryptophan with secologanin,[1] have long been attracting great interest of many chemists for their unusual carbon skeletons as well as potential bioactivities.[2] In recent years, our concentration on MIA reported a large number of new structures including (19,20)-E/Z-alstoscholarine,[3] melohenines A and B,[4] melotenine A,[5] scholarisines A–G,[6] alstoyunines A–H,[7] and melodinines A–U.[8] Some of them possessed significant cytotoxic and anti-inflammatory activities.[5,7,8] The plant *Gardneria ovata* belongs to the family Loganiaceae, rich of MIA, and our previous work on this plants reported eight new compounds,[9] a continuous study on this plant led to the isolation of an unprecedented *Strychnos–Strychnos* bisindole alkaloid with enamine connection, named gardovatine (**1**), together with a known analogue divarine (**2**).[10] The structure was elucidated by means of spectroscopic methods. Both of them showed cytotoxicities against five human cancer cell lines comparable with those of cisplatin. In this Letter, we report the isolation, structure elucidation and cytotoxicities of them.

An alkaloidal extract of twigs and leaves of *G. ovata* was separated by chromatography column over silica gel and RP-18, which produced two bisindole alkaloids, gardovatine (**1**) and divarine (**2**).

Gardovatine (**1**),[11] a colorless oil, possessed a molecular formula of $C_{38}H_{38}N_4O$, as evidenced by HRESIMS at *m/z* 567.3118 $[M+H]^+$ (calcd 567.3123). The UV spectrum showed the existence of conjugated groups based on the maximum absorption band at 289 nm. The ^{13}C and DEPT NMR spectra displayed thirty-eight carbon resonances ascribable for two methyls, eight methylenes, sixteen methines, and twelve quaternary carbons (Table 1). These data suggested that compound **1** is likely to be a bisindole alkaloid.



Gardovatine (**1**)



Divarine (**2**)

Elaborate analysis of NMR data (Table 1) indicated that **1** is comprised of two *Strychnos* units, one in which was readily identified by the characteristic carbon resonances at δ_C 69.4 (d) for C-2′, 54.2 (s) for C-7′, and 55.0 (t) and 53.3 (t) for N-connected carbons, as well as by comparison of other carbon resonances with data reported in the literature. In the ^1H NMR spectrum, two singlets at δ_H 6.78 (1H, s, H-17) and 7.28 (1H, s, H-17′) assigned to be two olefinic protons, suggested that the two units might be connected by bonds of N1–C17′ and N1′–C17, with respect to those reported previously.[12] However, several clearly different carbon signals indicated the significant modification on structure of another unit. A carbonyl carbon at δ_C 178.2 (s) was assigned to C-3

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Table 1
^{1}H and ^{13}C NMR data of compound **1** (δ in ppm)[a]

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
2	136.3, C		2′	69.4, CH	3.91, s
3	178.2, C		3′	69.1, CH	3.09, m
5a	52.7, CH_2	4.12, m	5a′	55.0, CH_2	3.12, Overlapped
5b		3.12, Overlapped	5b′		2.93, Overlapped
6a	25.2, CH_2	2.93, Overlapped	6a′	41.7, CH_2	2.31, Overlapped
6b		2.75, td (14.1, 3.0)	6b′		1.76, m
7	114.3, C		7′	54.2, C	
8	130.0, C		8′	136.3, C	
9	119.5, CH	7.50, d (7.8)	9′	121.1, CH	7.09, d (8.0)
10	123.7, CH	7.07, t (7.8)	10′	121.2, CH	6.77, t (8.0)
11	123.9, CH	7.13, t (7.8)	11′	129.9, CH	7.12, t (8.0)
12	110.7, CH	7.04, d (7.8)	12′	108.4, CH	6.51, d (8.0)
13	139.0, C		13′	146.4, C	
14a	38.3, CH_2	2.54, dd (16.0, 4.5)	14a′	24.3, CH_2	1.80, m
14b		2.31, Overlapped	14b′		1.75, m
15	41.5, CH	4.23, br s	15′	30.7, CH	4.03, br s
16	116.6, C		16′	138.4, C	
17	131.6, CH	6.78 s	17′	130.5, CH	7.28, s
18	14.9, CH_3	2.01, d (7.0)	18′	13.1, CH_3	1.92, d (6.8)
19	119.0, CH	5.68, q (7.0)	19′	120.0, CH	5.61, q (6.8)
20	140.2, C		20′	139.7, C	
21	53.0, CH_2	4.07, br s	21′	53.3, CH_2	3.33, br s

[a] Data were measured in methanol-d_4 at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts (δ) are in ppm being relative to TMS.

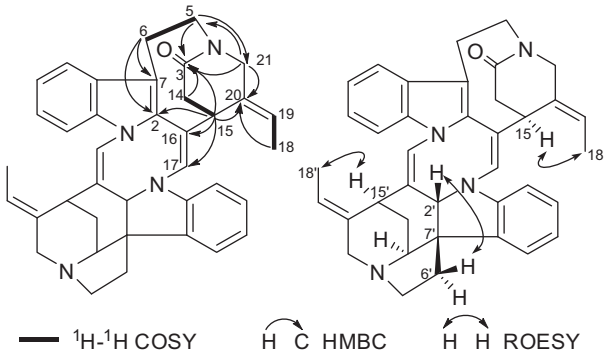
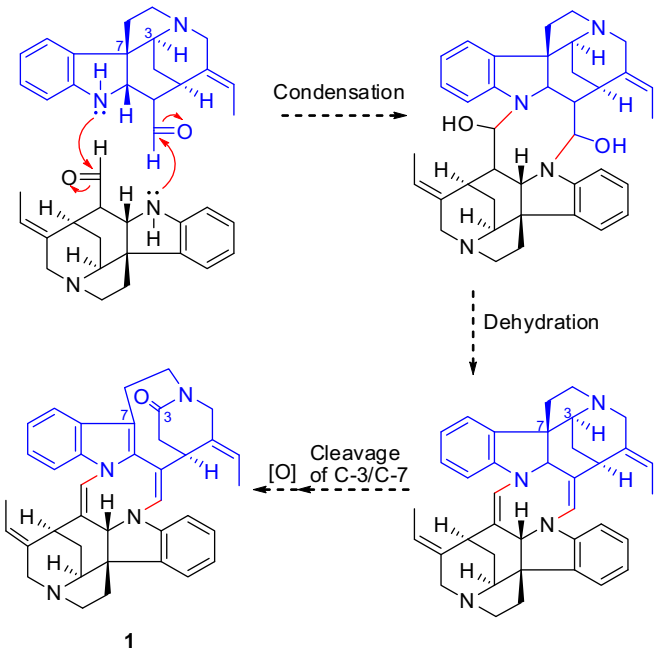


Figure 1. Key correlations of structural fragments of **1**.



Scheme 1. Plausible biogenetic pathway for **1**.

Table 2
Cytotoxicities for **1** and **2** (IC_{50}, μM)

No.	HL-60	SMMC-7221	A-549	MCF-7	SW480
1	9.8	10.5	14.2	13.9	16.1
2	1.2	3.2	3.2	3.0	1.6
Cisplatin[a]	1.6	12.9	10.0	15.5	15.8

[a] Positive control.

as supported by the HMBC correlations from δ_H 4.07 (2H, br s, H-21), 4.12 (1H, m, H-5a), 3.12 (1H, overlapped, H-5b), 2.54 (1H, dd, J = 16.0, 4.5 Hz, H-14a), and 2.31 (1H, overlapped, H-14b) to δ_C 178.2 (C-3) (Fig. 1), as well as supported by the downfield shift of C-14 at δ_C 38.3 (t). This information suggested that the bond between C-3 and C-7 was cleaved, forming a lactam at C-3. In the HMBC spectrum, the correlation from δ_H 2.95 (1H, overlapped, H-6a), 2.75 (1H, td, J = 14.1, 3.0 Hz, H-6b) and 4.23 (1H, br s, H-15) to olefinic carbons at δ_C 114.3 (s, C-7) and 136.3 (s, C-2) suggested that a double bond existed between C-2 and C-7 (Fig. 1). Detailed analysis of 2D NMR data (^{1}H–^{1}H COSY, HMBC) suggested that the other parts were the same to those of a *Strychnos* alkaloid (Fig. 1). The planar structure of **1** was, therefore, determined as a novel *Strychnos* bisindole alkaloid possessing a ring *seco* backbone.

The relative configuration of **1** was determined by the ROESY experiment (Fig. 1). In detail, the correlations of H-18/H-15 and H-18′/H-15′ suggested E-form of the double bonds of C-19/20 and C-19′/20′. In addition, the correlation of H-2′/H-6b′ suggested that H-2′ was β oriented. Biogenetically, the absolute configurations of C-3′, C-7′ and C-15 (or C-15′) in *Strychnos* alkaloids were confirmed as *S*, *R*, and *S*, respectively.[13] Therefore, the structure of **1** could be elucidated as (15*S*,19*E*,2′*S*,3′*S*,7′*R*,15′*S*,19′*E*)-gardovatine, as shown.

A plausible biogenetic pathway for **1** suggested that a condensation reaction between secondary amine and aldehyde combined two *Strychnos* units, producing two enamine moieties, then, the bond between C-3 and C-7 was cleaved by oxidation. Finally, compound **1** could be formed by dehydrogenation and oxidation (Scheme 1).

IC_{50} values of compounds **1** and **2** (Table 2) were comparable to those of positive control (cisplatin) in cytotoxic bioassay experiments toward human myeloid leukemia HL-60, human hepatocellular carcinoma SMMC-7721, lung cancer A549, human breast adenocarcinoma MCF-7, and human colon adenocarcinoma SW480 using MTT method[14] with minor modification.[15]

Acknowledgment

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Supplementary data

Supplementary data associated (1D, 2D NMR and MS spectra for configuration determination of Gardovatine (**1**).) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.051.

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11. *Gardovatine* (***1***)*:* colorless oil; α_D^{24} −308.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 289 (3.50), 209 (3.86) nm; IR (KBr) ν_{max}: 2925, 1632, 1602, 1460, 1363, 1033, 745 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 567 $[M+H]^+$; HRESIMS *m*/*z* 567.3118 $[M+H]^+$ (calcd for $C_{38}H_{39}N_4O$, 567.3123).
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15. *Cytotoxicity assay*. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1 × 105 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.0624, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (sigma, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed.