

A New Dimeric Iridal Triterpenoid from *Belamcanda chinensis* with Significant Molluscicide Activity

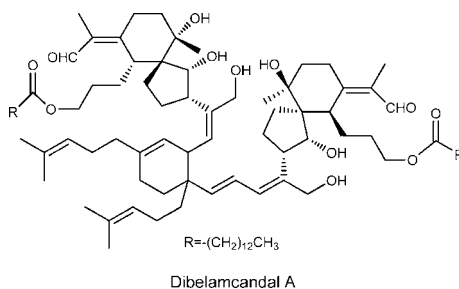
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Received November 17, 2010

ABSTRACT



An unprecedented dimeric triterpenoid, designated dibelamcandal A, with a six-membered ring linking two iridal type triterpenoid nuclei, was isolated from the rhizome of *Belamcanda chinensis*. Its structure was determined by extensive spectroscopic measurements, including IR, ESI-MS, HR-ESI-MS, and 1D and 2D NMR. It demonstrated significant molluscicide activity against *Pomacea canaliculata*.

The rhizome of *Belamcanda chinensis* (L.) DC., Shegan, is a traditional Chinese medicine conventionally used for the treatment of a sore swollen throat, asthma, phlegm expelling, tonsillitis, and many other symptoms.¹ Phytochemical studies of *B. chinensis* conducted to date have revealed the presence of isoflavonoids and iridal-type triterpenoids. Isoflavonoids are considered to be the major constituents of rhizoma *Belamcandae* and are generally regarded as the index for quality assessment of this herb and responsible for its

estrogenic and anti-inflammatory effects.² The presence of a number of iridal-type triterpenoids, which exhibited a variety of biological activities, such as cytotoxicity,³ PKC activation,⁴ RasGRP pathway modulation,⁵ and ichthyotoxicity,⁶ have been reported in *B. chinensis*.

The apparent rich chemical diversity of *B. chinensis* and various biological activities of iridal triterpenoids prompted us to scale-up the study of this plant, affording a novel dimeric triterpenoid, dibelamcandal A, possessing a six-

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membered ring linking two iridal-type triterpenoid nuclei (Figure 1). Herein, we describe the isolation, structure elucidation, and toxicity evaluation against *Pomacea canaliculata*.

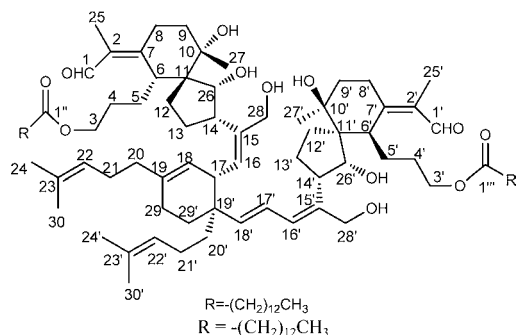


Figure 1. Dibelamcandal A.

Air-dried powder rhizome of the plant (20.0 kg) was percolated thrice with 95% EtOH (each 50 L) at r.t. to obtain 7.3 kg of crude extract, which was then suspended in warm water (10 L) and partitioned successively with petroleum ether and EtOAc. The EtOAc soluble fraction (3.2 kg) was subjected to column chromatography (CC) on silica gel (petroleum ether/ CHCl_3 /MeOH with increasing polarity) which resulted in 19 fractions (A–S). The fourth fraction (D, 651.0 g) was extensively separated over columns of silica gel (petroleum/acetone from 1/4, 1/2) to afford four fractions (D_1 to D_4). After removing flavones using Sephadex LH-20 (methanol/ CHCl_3 , 1/1) CC, the second fraction (D_2 , 24.0 g) was subjected to CC on RP- C_{18} silica gel (methanol/ H_2O from 60/40 to 80/20, 100/0, acetone) to afford a subfraction (a brown waxlike cream, 320.0 mg) from the acetone fractions. Dibelamcandal A (184.0 mg) was obtained from this fraction through middle pressure flash CC on silica gel with petroleum ether/acetone (3/1) as eluents.

Dibelamcandal A, a snowflake-like solid, has a molecular formula of $\text{C}_{88}\text{H}_{144}\text{O}_{12}$ as determined by HR-ESI-MS wherein a sodiated molecule $[\text{M} + \text{Na}]^+$ was detected at m/z 1416.0498 (calcd: 1416.0550; err: 3.64 ppm) indicative of 17 degrees of unsaturation. The IR spectrum shows the absorption of hydroxyl groups (3428 cm^{-1}), carbonyl groups ($1735, 1180\text{ cm}^{-1}$), and α, β -unsaturated carbonyl groups ($1665, 1609\text{ cm}^{-1}$). Its ^{13}C NMR spectrum revealed eight double bonds, two aldehyde carbonyls and two ester carbonyls, suggesting there should be five rings in dibelamcandal A to achieve its degrees of unsaturation.

Most of the coupled ^1H and ^{13}C NMR signals at δ_{H} 1.82 (6H, s), 10.15 (1H, s), 10.17 (1H, s) and at δ_{C} 190.5, 190.8, 163.1, 161.9, 133.0, 132.9, 11.1, 11.0 of two α, β -

unsaturated aldehyde functions,⁶ and resonances at δ_{C} 58.4, 58.6, 75.7, 75.8, 47.9, 48.7 of two spirocyclodecane rings,⁷ as well as resonances of two prenyl groups with two pairs of gem-dimethyl at δ_{C} 124.0, 124.8, 131.7, 130.9, 17.7, 17.7, 25.7, 25.8, bore close resemblance with those of iridotectorals B and belamcandal, indicating that its structure is comprised of a dimer of two spiro-iridal type triterpenoids.^{7,8} Despite signals due to two aliphatic acid esters, its ^{13}C NMR, DEPT, HSQC spectrum resolved 8 methyls, 16 methines (seven olefinic and two aldehyde carbons), and 14 quaternary carbons. Careful analysis of ^1H NMR, ^{13}C NMR, DEPT, HSQC, HMBC, and $^1\text{H}-^1\text{H}$ COSY spectra, as well as comparison with those in published literature, resulted in the assignments of ^1H NMR and ^{13}C NMR data (Table 1).⁷⁻⁹

Table 1. ^1H and ^{13}C NMR Data of Dibelamcandal A^a

No.	First fragment		No.	Second fragment	
	δ_{H}	δ_{C}		δ_{H}	δ_{C}
1	10.17 s	190.8	1'	10.15 s	190.5
2		132.9	2'		133.0
3	4.13 m, 3.86 m ^b	64.5	3'	4.08 m, 3.92 m ^b	63.9
4	1.40 m ^b	28.2	4'	1.40 m ^b	28.9
5	1.85 m ^b	30.7	5'	1.75 m ^b	30.2
6	3.41 brd, 8.4	43.6	6'	3.48 brd, 8.4	43.4
7		163.1	7'		161.9
8	2.75 m, 2.48 m ^b	23.9	8'	2.75 m, 2.48 m ^b	24.1
9	1.65 m ^b	37.0	9'	1.60 m ^b	38.0
10		73.9	10'		74.9
11		58.4	11'		58.6
12	1.52 m, 1.25 m ^b	34.5	12'	1.45 m, 1.22 m ^b	34.9
13	2.00 m, 1.47 m ^b	27.1	13'	2.00 m, 1.47 m ^b	27.1
14	3.09 m	48.7	14'	2.92 m	47.9
15		137.7	15'		136.8
16	5.38 d, 11.4	134.5	16'	6.24 d, 12.0	128.8
17	2.90 brd, 11.4	44.2	17'	6.19 dd, 12.0, 15.0	123.5
18	4.90 brs	121.9	18'	5.47 d, 15.0	142.7
19		138.1	19'		41.3
20	2.02 m ^b	26.4	20'	1.46 m ^b 1.35 ^b	27.6
21	2.03 ^b	24.7	21'	1.90 ^b	22.2
22	5.07 t, 6.4	124.0	22'	5.02 t, 6.4	124.8
23		131.7	23'		130.9
24	1.68 s	25.8	24'	1.65 s	25.7
25	1.82 s	11.0	25'	1.82 s	11.1
26	4.04 d, 6.6	75.7	26'	4.23 d, 6.6	75.8
27	1.20 s ^b 4.27 d, 12.0 ^b	27.1	27'	1.21 s ^b 4.27 d, 12.0 ^b	28.2
28	3.92 d, 12.0 ^b	66.3	28'	4.02 d, 12.0 ^b	65.6
29	1.98 m ^b	37.5	29'	1.92 m ^b 1.45 m ^b	32.3
30	1.55 s	17.7	30'	1.59 s	17.7
1''		174.4	1'''		174.4
2''	2.25 t, 7.2	34.4	2'''	2.25 t, 7.2 ^b	34.4
3''	1.68 ^b	25.0	3'''	1.68 ^b	25.0
4''–11''	1.23–1.27 ^b	29.0–30.0 ^b	4'''–11'''	1.23–1.27 ^b	29.0–30.0 ^b
12''	1.29 ^b	31.9	12'''	1.29 ^b	31.9
13''	1.31 m	22.7	13'''	1.31 m	22.7
14''	0.87 t, 6.8	14.1	14'''	0.87 t, 6.8	14.1

^a ^1H NMR (600 MHz) (δ in ppm, J in Hz), ^{13}C NMR (150 MHz) in CDCl_3 . ^b Overlapped multiplicity.

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Tandem ESI-MS² of dibelamcandal A at m/z 1164.6 [$M - C_{14}H_{27}O_2 - H$]⁻ and ESI-MS³ at m/z 954.5 [$M - C_{14}H_{27}O_2 - C_{14}H_{27}O - H$]⁻, as well as the signal resonance at δ_H 0.87 (t, $J = 6.8$ Hz, 6H), 1.22–1.31 (brm, series of $-CH_2-$), and δ_C 174.1, 174.2, indicated the presence of two myristoyls. Their connection sites were deduced by HMBC correlations of H-3 (or H-3') to C-1'' (or C-1''').

The structure of dibelamcandal A can be divided into two monomeric fragments, of which two spiro-[4, 5]-daccane groups were confirmed by HMBC and ¹H–¹H COSY correlations (Figure 2) (planar structure elucidation detailed

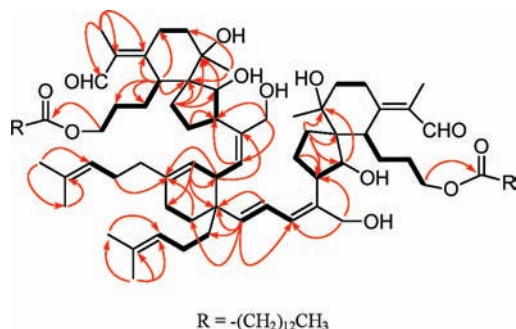


Figure 2. Key HMBC (red →) and ¹H–¹H COSY (bold →) correlation of dibelamcandal A.

in the Supporting Information). The connection of C(15)–C(14) was indicated by HMBC correlations of H-28 (28') with C-16 (16'), C-14 (14'). Taking the series of double bonds signals in ¹H and ¹³C NMR spectra of iridotectorals B⁷ or belamcandal⁹ into consideration, the disappearance of signals of the double bonds of Δ^{17} , Δ^{19} in the first fragment and of $\Delta^{19'}$ in the second fragment,⁶ as well as the appearance of a new double bond signal which resonated at δ_C 121.9, 138.1 and δ_H 4.90 (1H, brs) of Δ^{18} in dibelamcandal A, suggested that the structure differs profoundly from those of iridals and bears no match to any other structure reported in the literature so far, implying the novelty of the structure of this compound.

In the first monomer fragment, the HMBC correlations (Figure 2) from H-28 to C-14, C-15, C-16 and H-17 to C-15, C-16, C-19 indicated the methine (C-17) located between Δ^{15} and Δ^{18} , in accordance with the ¹H–¹H COSY correlations (Figure 2) of H-16/H-17/H-18, which was further confirmed by the coupling constant of H-16 and H-17 ($J = 11.4$ Hz). In the second fragment, the HMBC correlations between H-28' and C-14', C-15', C-16' and correlations from

H-18' to C-16', C-29', C-19', C-20' revealed the double bond $\Delta^{15(16)}$ conjugated with $\Delta^{17'}$ and also revealed the connectivity of C(18')–C(19')–C(20'), which were further supported by ¹H–¹H COSY correlations of H-16'/H-17'/H-18' and the coupling constant of H-16', H-17' ($J = 12.0$ Hz). Long range correlations from H-18, H-18', and H-17' to the quaternary carbon C-19' and from H-29' to C-19 in the HMBC spectrum, and ¹H–¹H COSY cross-peaks of H-29/H-29', allowed the connection between C-19' and C-17, as well as a connection between C-29' and C-29. Thus, the planar structure of dibelamcandal A was established (Figure 1).

The larger coupling constant of H-17' and H-18' ($J = 15.0$ Hz) along with the NOESY correlations (Figure 3)

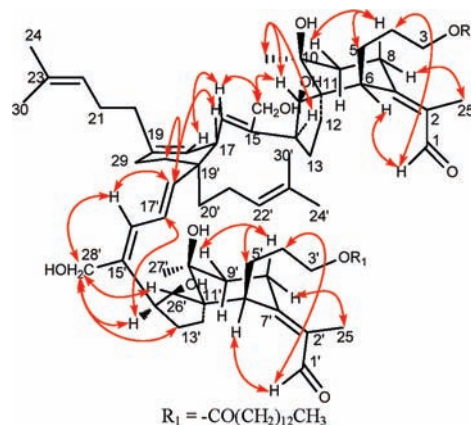


Figure 3. Key NOESY correlation of dibelamcandal A.

of H-28'/H-16'/H-20 suggested that the double bond $\Delta^{17'}$ was in *trans*-form (*E*). The geometric configuration of Δ^7 ($\Delta^{7'}$) was determined to be in *cis* as that of belamcandal, which was evidenced by the downfield chemical shift of H-6 (H-6') in the ¹H NMR spectrum and the NOESY crosspeak observed between H-1 (H-1') and H-6 (H-6').⁷ The double bond of Δ^{15} (or $\Delta^{15'}$) was deduced to be in *trans*-form from the NOESY crosspeaks of H-16/H-28 (or H-16'/H-28'). From a biogenetic point of view, the absolute configurations of C-6 and C-10 should be (6*R*,10*S*).^{9,10} In addition, NOESY correlations of H-27/H-26 (H-27'/H-26'), H-27/H-14 (H-27'/H-14') as well as weak NOESY correlations of H-5/H-26 (H-5'/H-26') suggested the configurations of 11*S*, 14*S*, and 26*R* respectively. The absence of coupling (or the coupling constant of almost zero) between H-18 and H-17 due to a dihedral angle of almost 90° of H-18/H-17 suggested an axial orientation for H-17. The NOESY crosspeaks of H-17/H-18', H-18/H-16 as well as the absence of NOESY correlation between H-17 and H-20' revealed that H-17 and C-20' were in opposite axial

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directions. Consequently, C-16 and C-18' were deduced to be in the equatorial position, which is in agreement with the energy requirement in a six-membered ring system. Thus, the relative configuration of C-17 and C-19' was established as (1*S*,19'*S*) or (1*R*,19'*R*). But their absolute configuration was not decided. Taking the method of their biosynthesis into consideration, when the couple of monomers derived into dibelamcandal A through a [4 + 2] Diels–Alder reaction at C-17/C-19' and C-29/C-29' (Figure 4), there are four

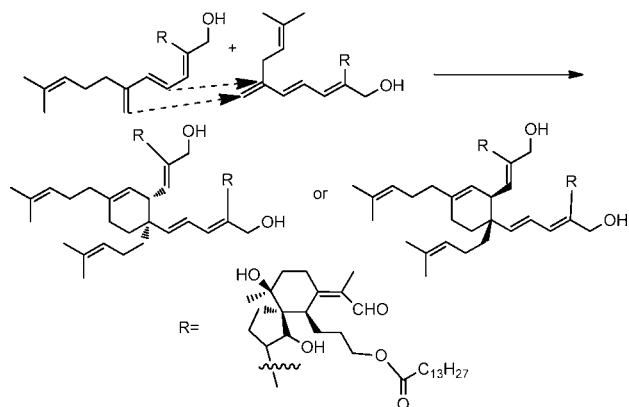


Figure 4. Possible pathway for the biosynthesis of dibelamcandal A.

possible absolute configurations of C-17 and C-19': (1*S*,19'*S*), (1*R*,19'*R*), (1*S*,19'*R*), and (1*R*,19'*S*). But the last two options cannot meet the energy requirement for the steric hindrance of two larger substituents at C-17 or C-19' in the axial position. The structure of dibelamcandal A was thus determined as depicted (Figure 1).

To the best of our knowledge, this is the first report of a dimer of a spiro-iridal-type triterpenoid or a hexterpenoid with a six-membered ring linking two spiro-[4,5]-dacane fragments. This type of compound was excluded from prior studies, possibly due to its bigger molecule and mixing with flavones together resulting in the inability to elute from RP-C₁₈ silica gel by methanol or acetonitrile.

Snails have recently become a major pest in rice fields in China, Japan, and the Philippines. In the Hunan province of

China, about 1.8×10^9 m² of rice fields were destroyed by *P. canaliculata* in 2010, which caused a total loss of up to \$1.35 billion. Iridals found in *B. chinensis* are known to have ichthyotoxic properties, which prompted us to investigate their molluscicide activity against *P. canaliculata*.^{6,11} The molluscicide activity of dibelamcandal A was evaluated against *P. canaliculata* using the method reported previously¹² with minor revisions (see Supporting Information). Our results indicate dibelamcandal A possesses strong

Table 2. Molluscicide Activities of Dibelamcandal A

Concn ($\mu\text{g/mL}$)	Snails	Dead Snails	CM ^a (%)
10.000	50	48	95.8
5.000	50	41	81.3
2.500	50	37	72.9
1.250	50	23	43.8
0.625	50	18	33.3
control	50	2	
	LC ₅₀	1.26 $\mu\text{g/mL}$	
	LC ₉₅	10.57 $\mu\text{g/mL}$	

^a CM: Corrected mortality.

molluscicide activity against *P. canaliculata* with an LC₅₀ value of 1.26 $\mu\text{g/mL}$ (Table 2). To our knowledge, this is the first report of the molluscicide activities of iridals.

Acknowledgment. The work was supported in part by grants from the National Basic Research Program (No. 2007CB512604), the Doctoral Fund of Ministry of Education of China (No. 20094404110001), and the National Natural Sciences Foundation (No. 30973634) of China.

Supporting Information Available: Experimental procedures; molluscicidal assays; spectra of ¹H and ¹³C NMR, DEPT, HSQC, HMBC, NOESY, ¹H–¹H COSY; detailed copies of 1D and 2D NMR spectra; HR-ESI-MS; the detailed assignment of spiro-[4, 5]-dacane fragments; IR, ESI-MS, Tandem ESI-MS, ESI-MS, and HR-ESI-MS data of crude EtOAc extract of *B. chinensis*; melting point. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL102796K