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BELAMCANDAQUINONES A AND B, NOVEL DIMERIC 1,4-BENZOQUINONE DERIVATIVES POSSESSING CYCLOOXYGENASE INHIBITORY ACTIVITY

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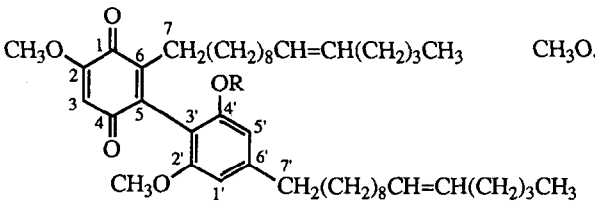
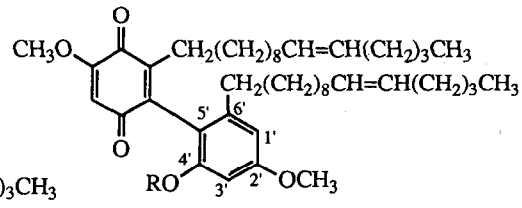
Abstract: Belamcandaquinone A (**1**), a new dimeric 1,4-benzoquinone derivative, has been isolated as specific cyclooxygenase inhibitor (IC_{50} 8.33 μM) from the seed of *Belamcanda chinensis* together with belamcandaquinone B (**2**), an inactive congener, and their structures elucidated on the basis of spectroscopic data and chemical degradation.

A medicinal plant, *Belamcanda chinensis* L. (Iridaceae) has been used in Chinese herbs for an antitussive, antiinflammatory, and expectorant agent. Its rhizome, in particular, is prescribed as traditional crude drugs and metabolites a number of highly oxygenated isoflavones.[1] During our studies on prostaglandin biosynthesis regulators in natural products,[2] we have investigated extract of the seed of the title plant and isolated belamcandols A and B, alkenyl phenols, and ardisianone A, an alkenyl-1,4-benzoquinone as specific 5-lipoxygenase inhibitors.[3] Further examination of the constituents of the seed of *B. chinensis* has now resulted in the isolation of two new dimeric 1,4-benzoquinones **1** and **2** named belamcandaquinones A and B, respectively, the former of which inhibits specifically cyclooxygenase activity. Here we describe the structure elucidation and biological activity of **1** and **2** .

Belamcandaquinone A (**1**) and B (**2**)[4] had the same molecular formula $C_{44}H_{68}O_5$ determined by HREI-MS [**1**: *m/z* 676.5081 (M^+), Δ -1.4 mmu; **2**: *m/z* 676.5080 (M^+), Δ -1.3 mmu] , which corresponded to the composition that ardisianone A (**3**)[5] and belamcandol B (**4**) were linked via a carbon-carbon bond. The UV and IR absorptions of **1** and **2** were analogous to each other and indicative of the presence of a 1,4-benzoquinone moiety and a hydroxyl group. The ^1H and ^{13}C NMR (Table) spectra disclosed that compounds



1 R = H
1a R = CH_3



2 R = H
2a R = CH_3

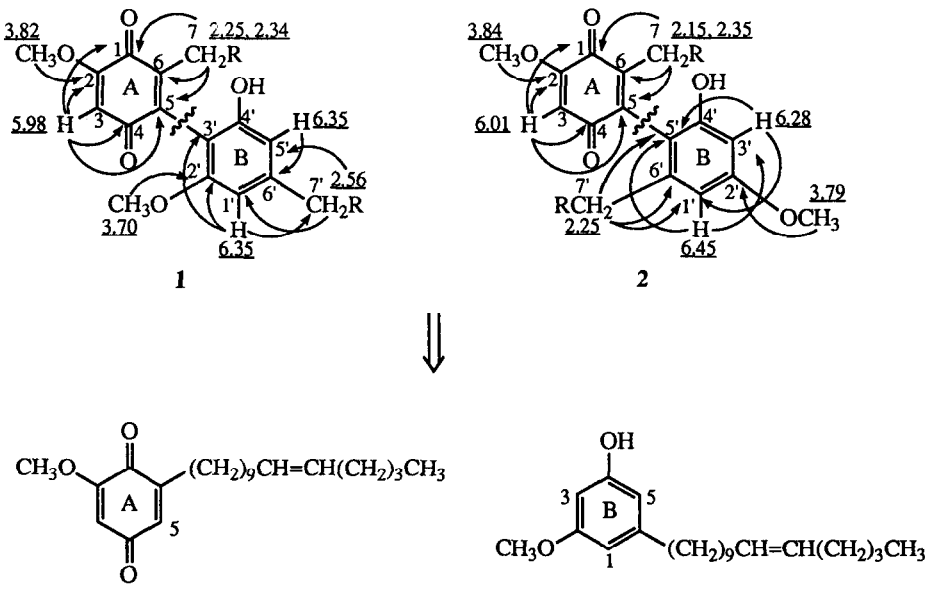


Fig 1 HMBC correlations for **1** and **2**

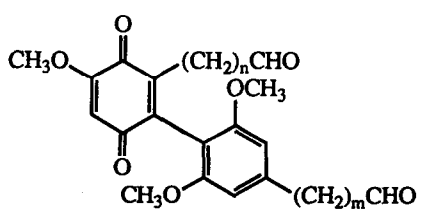
Table ^{13}C (100 MHz, in $CDCl_3$) NMR data of belamcandaquinones A (**1**) and B (**2**).

	1				**2**		
position	δ_C	position	δ_C	position	δ_C	position	δ_C
1	182.4	1'	103.7	1	182.3	1'	107.5
2	158.5	2'	157.5	2	158.7	2'	160.7
3	107.2	3'	107.7	3	107.1	3'	99.3
4	186.4	4'	153.0	4	186.9	4'	153.4
5	138.7	5'	108.7	5	140.8	5'	112.6
6	146.8	6'	146.0	6	146.7	6'	143.1
7	32.0	7'	36.4	7	32.0	7'	33.7
OCH_3	55.6	OCH_3	56.1	OCH_3	56.2	OCH_3	55.2

1: δ_C 14.0 and 22.4 (CH_3, C-21, C-21'), 29 ~ 31 (18 x CH_2, C-8 ~ C-14, C-19 ~ C-20, C-8' ~ C-14', C-19' ~ C-20'), 26.9, 27.2, 27.7 and 28.2 (CH, C-15, C-18, C-15', C-18'), and 129.9 (4 x CH=, C-16, C-17, C-16', C-17'); **2**: δ_C 14.1 and 22.4 (CH_3, C-21, C-21'), 29.1 ~ 30.2 (18 x CH_2, C-8 ~ C-14, C-8' ~ C-14', C-19 ~ C-20, C-19' ~ C-20'), 26.9, 27.2, 27.7, and 28.2 (CH_2, C-15, C-18, C-15', C-18'), 129.9 (4 x CH=, C-16, C-17, C-16', C-17').

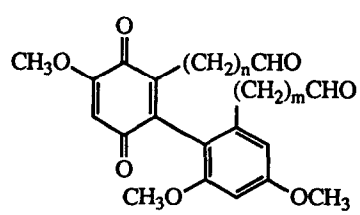
1 and **2** were comprised of a methoxy-substituted *p*-benzoquinone unit (**A**) and a tetrasubstituted benzene unit (**B**) which were appended with a long alkenyl side chain. The both units **A** and **B** are supposed to be derived from ardisianone A (**3**) and belamcandol B (**4**), respectively, common metabolites in *B. chinensis*. In the ^1H NMR of **1**, irradiation of the methoxyl signal at δ_H 3.82 caused a strong NOE enhancement on the singlet signal (δ_H 5.98) due to H-3, whereas the H-7 methylene signals at δ_H 2.25 and 2.34 on the side chain showed no NOE interaction with H-3. This indicated that the other unit **B** should be linked to C-5 on the unit **A** through a carbon-carbon bond. Additionally, HMBC as shown in Fig. 1 substantiated the above argument. On the other hand, the aromatic protons on the unit **B** appeared as the 2 H singlet signal at δ_H 6.35, which showed NOE enhancement upon irradiation of the methoxyl signal at δ_H 3.70. Although this result suggested a symmetrical benzene ring for the unit **B**, it was found that the two aromatic protons occupied the same chemical shift value by chance since this singlet proton signal was correlated to the different carbon signals at δ_C 103.7 and 108.7 on the C-H COSY. The quaternary carbon signal at δ_C 107.7 was assigned to C-3' based on its chemical shift and the analysis of HMBC as summarized in Fig. 1, and thereby the position of the unit B linked to C-5 on the benzoquinone moiety **A** must be C-3'. In fact, methylation of **1** with CH_3I-K_2CO_3 in acetone yielded **1a**, the ^1H NMR of which proved the unit **B** to have a symmetrical structure based on the observation of each signal due to methoxyl groups at δ_H 3.70 (6 H, s) and aromatic protons at δ_H 6.41 (2 H, s) as singlet and thus was established the linkage position on the unit B in **1** to be at C-3' .

The *p*-benzoquinone moiety **A** for belamcandaquinone B (**2**) was verified to be identical to that of **1** by comparison of its ^{13}C NMR data with those in **1**, as well as by NOE and HMBC shown in Fig. 1. The NMR data for a benzene unit **B** in **2**, however, were not similar to those of **1** suggesting that the linkage carbon on the unit **B** was different from C-3' in **1**. The ^1H NMR spectrum of **2** contained the *meta* coupled doublet signals at δ_H 6.28 (1 H, d, J = 2.2 Hz) and 6.45 (1 H, d, J = 2.2 Hz), both of which revealed NOE enhancements upon irradiation of the methoxyl signal at δ_H 3.79. Further, the one (δ_H 6.45) of the two aromatic proton signals showed NOE interaction with the benzylic methylene signal at δ_H 2.25. Treatment of **1** with K_2CO_3-CH_3I in acetone afforded trimethoxyl derivative **2a**, in the ^1H NMR spectrum of which irradiating the signal (δ_H 3.66) of a newly accessed methoxyl group gave NOE enhancement only on the signal (H-3") at δ_H 6.35. These data accumulated in the construction of the unit **B** for **2** as shown in Fig. 1 and the position of the unit B connected to C-5 on the unit **A** was eventually determined as C-5'. Thus, the both compounds **1** and **2** formed dimeric structures linked via a carbon-carbon bond between C-5 on the *p*-benzoquinone unit **A** and C-3' and C-5' on the phenyl unit **B**, respectively. Finally, there remained obscure problems associated with the two long alkenyl side chains involved in **1** and **2** . The double bonds of the side chains in **1a** and **2a** were oxidatively cleaved with MCPBA followed by HIO_4 to give the dialdehydes **1c** and



1c

n + m = 18



2c

2c having the same molecular ion peak at *m/z* 582 on the EI-MS, indicating the presence of one double bond on each side chain. But no evidence for the length of the side chain and the location of the double bond was obtained from the degradation experiments except for the fact n + m = 18. The stereochemistry of each double bond in **1** and **2** was assigned as *Z* on the basis of the diagnostic chemical shift values[6] of the allylic carbon signals observed around 27 ppm (Table).

Belamcandaquinones A (**1**) and B (**2**) are most likely to be biosynthesized from ardisianone A (**3**) and belamcandol B (**4**) commonly occurred in *B. chinensis*.[3] This suggests that the compounds **1** and **2** have the same alkenyl side chain $(CH_2)_9CH{=}CH(CH_2)_3CH_3$ as **3** and **4**. Confirmation for the structures of **1** and **2** has been unambiguously made by their syntheses reported in the accompanying paper.[8]

Belamcandaquinone A (**1**) inhibited cyclooxygenase activity[7] at IC_{50} 8.33 μM but showed no 5-lipoxygenase inhibitory activity, whereas belamcandaquinone B (**2**) inhibited neither cyclooxygenase nor 5-lipoxygenase activities even at as high concentration as 10 μM. From a viewpoint of structure-activity relationship, it is interesting that dimeric 1, 4-benzoquinones **1** and **2** lose a power as 5-lipoxygenase inhibitor in contrast with a monomeric congener **3** having strong 5-lipoxygenase inhibitory activity.

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4. **1**: EIMS: *m/z* (rel. int.) 676 $[M]^+$ (100), 481 (80), 195; $\lambda_{max}^{CHCl_3}$nm (ε): 243 (5600), 272 (1100); $\nu_{max}^{CHCl_3}cm^{-1}$: 3576 (OH), 1670, 1642 (1,4-quinone), 1619, 1590; ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* = 4.4 Hz), 0.90 (3H, t, *J* = 4.4 Hz), 2.25 (1H, m, H-7), 2.34 (1H, m, H-7), 2.56 (2H, t, *J* = 7.3 Hz, H-7'), 3.70 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 5.34 (4H, m), 5.98 (1H, s, H-3), 6.35 (2H, s, H-1', 5'). **2**: EIMS: *m/z* 676 $[M]^+$ (100), 480 (20), 481 (10) $[M^+ - 195]$, 195; $\lambda_{max}^{CHCl_3}$nm (ε): 240 (3800), 275 (6900); $\nu_{max}^{CHCl_3}cm^{-1}$: 3576 (OH), 1669, 1640 (1,4-quinone), 1610; ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J* = 4.5 Hz), 0.89 (3H, t, *J* = 6.6 Hz), 2.15 (1H, m, H-7), 2.25 (2H, m, H-7'), 2.35 (1H, m, H-7), 3.79 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.34 (4H, m), 6.01 (1H, s, H-3), 6.28 (1H, d, *J* = 2.2 Hz, H-3'), 6.45 (1H, d, *J* = 2.2 Hz, H-1').
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