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Nardoaristolones A and B, Two Terpenoids with Unusual Skeletons from *Nardostachys chinensis* Batal

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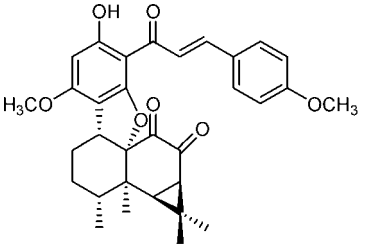
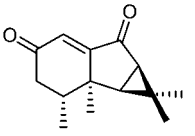
ABSTRACT



Nardoaristolone A (1)



Nardoaristolone B (2)

Nardoaristolones A and B, two novel terpenoids derived from the aristolane-type sesquiterpenoid, were isolated from the underground parts of *Nardostachys chinensis* Batal. Nardoaristolone A is the first reported aristolane-chalcone derivative, while nardoaristolone B possesses a *nor*-aristolane sesquiterpenoid skeleton with an unusual 3/5/6 tricyclic ring system. Their structures were elucidated by spectroscopic measurements, and the absolute configurations were established by single-crystal X-ray diffraction experiments.

Nardostachys chinensis Batal belongs to the genus *Nardostachys* (Valerianaceae), which is mainly distributed in the Himalayan mountains.[1] The underground parts of the plant have been used as sedative and analgesic agents in traditional Chinese medicine for centuries.[2] Previous phytochemical investigations on this plant led to the isolation of a series of aristolane,[3] nardosinane,[4−6] and guaiane-type[7,8]sesquiterpenoids, lignans,[9] and one novel diterpene.[10]

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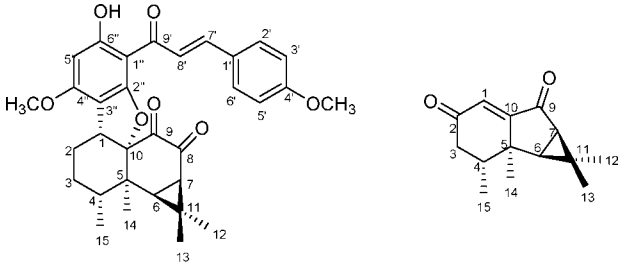
In our efforts to explore novel plant-derived sesquiterpenoids of this plant, nardoaristolones A (**1**) and B (**2**) were isolated from the 60% EtOH extract of the underground parts of *N. chinensis*. Compound **1** features an aristolane-type sesquiterpenoid with a chalcone moiety fused by a 2,3-dihydrofuran ring. The chalcone-coupled sesquiterpenoid is discovered for the first time. Compound **2** is a *nor*-aristolane sesquiterpene with an unusual 3/5/6 tricyclic ring system. Herein, we describe the structural elucidation, plausible biogenetic pathway, and cardiomyocytes protective activities of new compounds.



1 **2**

Nardoaristolone A (**1**) was obtained as a yellow powder {$[\alpha]_D^{26}$ −74.6 (c = 0.50, CH_3OH)}. Its molecular formula was determined as $C_{32}H_{34}O_7$ by HR-ESI-MS (m/z 531.2380 $[M + H]^+$, calcd for $C_{32}H_{35}O_7$, 531.2383), indicating 16 degrees of unsaturation. The ^{13}C NMR spectrum of **1** gave 32 signals. Among them, 15 sp^2 carbon signals revealed the presence of a para-disubstituted benzene ring [δ_C 128.4, 130.7 × 2, 114.6 × 2, 161.8], a group of α,β-unsaturated ketones [δ_C 144.0, 123.4, 191.1], and a pentasubstituted phloroglucinol moiety [δ_C 102.9, 157.7, 109.6, 162.2, 94.3, 167.3]. These NMR data mentioned above resembled those of chalconaringenin.[11] Nevertheless, two methoxy groups were located at C-4′ (δ_C 161.8) and C-4″ (δ_C 162.2), due to the obvious HMBC correlations of δ_H 3.85/C-4′ and δ_H 3.80/C-4″. Therefore, the moiety of the 6′-hydroxy-4′,4-dimethoxy-2′-*O*-chalcone (**1a**) was elucidated.

The remaining 15 carbon signals, including three sp^3 quaternary carbons (one oxygenated at δ_C 99.3), four sp^3 methines, two sp^3 methylenes, four methyls, and two carbonyls (Table 1), suggested the presence of a sesquiterpene moiety. The two units of C-1−C-2−C-3−C-4−C-15 and C-6−C-7 were determined according to the $^1H-^1H$ COSY correlations, and then an aristolane-type sesquiterpene moiety (**1b**) was assigned due to a series of HMBC correlations (Figure 1). Compared with the NMR data of kanshone C[4], the obvious upfield shift of C-1 (δ_C 66.0 to 35.5) and downfield shift of C-10 (δ_C 64.7 to 99.3) in **1b** indicated that the epoxide ring in kanshone C opened in **1**. By now, the two moieties accounted for 15 out of the 16 degrees of unsaturation. The remaining 1 degree of unsaturation and the unusual chemical shifts of C-1 and C-10 indicated that **1a** and **1b** were connected by a 2,3-dihydrofuran ring. It was confirmed by the HMBC correlations from H-1 to C-2″, C-3″, C-4″. In the ROESY spectrum of **1**, correlations for H_3-15/H-6, H_3-15/H_3-14, H-7/H_3-14, H-7/H_3-12, and H-4/H_3-13 defined the α-orientation of H_3-14, H_3-15 and β-orientation of the cyclopropane ring. However, the configurations of C-1 and C-10 failed to be deduced on the basis of the ROESY spectrum. A single-crystal X-ray diffraction experiment was then preformed with Cu Kα, and the Flack parameter of 0.0 (3)[12] allowed an unambiguous assignment of the absolute configurations of all the chiral centers as 1R, 4R, 5R, 6S, 7R, 10S (Figure 2).

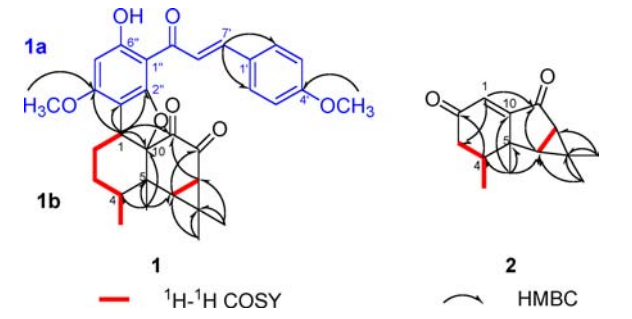


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1** and **2**.

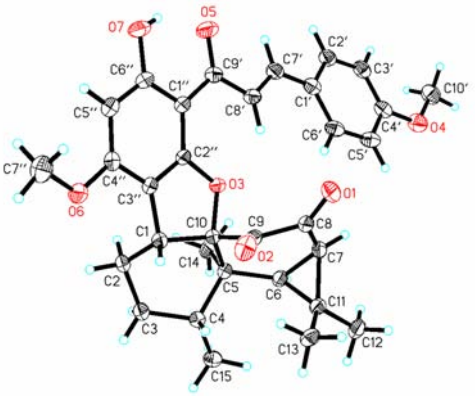


Figure 2. X-ray crystal structure of compound **1**.

Compound **2** was isolated as transparent, pale yellow crystals {$[\alpha]_D^{26}$ −19.6 (c = 0.50, CH_3OH)}. The formula was deduced as $C_{14}H_{18}O_2$, with 6 degrees of unsaturation, by HR-ESI-MS analysis ($[M + H]^+$, m/z 219.1377, calcd for $C_{14}H_{19}O_2$, 219.1385). The ^{13}C NMR and DEPT-135 spectra exhibited 14 carbon signals, corresponding to two carbonyl carbons, three quaternary carbons (one olefinic carbon), four methines (one olefinic carbon), one methylene, and four methyls (Table 2). Based on these data, the skeleton of compound **2** was speculated to be a norsesquiterpene with a tricyclic ring system. The $^1H-^1H$ COSY spectrum provided connectivities for two spin systems: H-3−H-4−H_3-15 and H-6−H-7 (Figure 1). A dimethylcyclopropane unit was deduced due to the HMBC correlations from H_3-12 and H_3-13 to C-11, C-6, and C-7. HMBC

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Table 1. ^{1}H (300 MHz) and ^{13}C (100 MHz) NMR Data for Compound **1** in $CDCl_3$ (δ in ppm, J in Hz)

no.	δ_H	δ_C
1	4.06 (dd, 9.8, 7.2)	35.5
2	2.17 (m) 1.86 (m)	23.0
3	1.73 (m) 1.35 (m)	25.8
4	1.79 (m)	31.9
5		44.6
6	1.36 (d, 7.8)	42.3
7	2.25 (d, 7.8)	39.4
8		197.9
9		195.3
10		99.3
11		31.4
12	1.26 (s)	31.6
13	1.29 (s)	18.0
14	1.30 (s)	20.0
15	1.05 (d, 6.4)	16.5
1′		128.4
2′	7.63 (d, 8.8)	130.7
3′	6.95 (d, 8.8)	114.6
4′		161.8
5′	6.95 (d, 8.8)	114.6
6′	7.63 (d, 8.8)	130.7
7′	7.79 (d, 15.5)	144.0
8′	7.63 (d, 15.5)	123.4
9′		191.1
1″		102.9
2″		157.7
3″		109.6
4″		162.2
5″	6.03 (s)	94.3
6″		167.3
4′-OMe	3.85 (s)	55.9
4″-OMe	3.80 (s)	55.7

correlations from H_3-14 to C-4, C-5, C-6, and C-10 allowed the linkage of C-14, C-4, C-6, and C-10 to the quaternary carbon C-5. Furthermore, the two carbonyl carbons were assigned to C-2 and C-9 according to the key HMBC correlations of H-1/C-3, H-1/C-9, H-6/C-9, and H-4/C-2, indicating the presence of a cyclohexanone moiety and a cyclopentanone substructure (Figure 1). Thus, the skeleton of **2** was elucidated to be an unusual 3/5/6 tricyclic ring system.

The relative configuration of **2** was determined through inspection of the NOESY spectrum. The NOE correlations of H-6/H_3-12, H-6/H_3-14, H-6/H_3-15, H-4/H_3-13, and H-7/H_3-12 suggested that H-6, H-7, H_3-14, and H_3-15 were situated on the same side. Moreover, a single-crystal X-ray diffraction experiment was conducted with Cu Kα, which resulted in a Flack parameter of -0.03 (19).[12] Thus, the absolute stereochemistry of **2** was established to be 4R, 5R, 6S, 7R (Figure 3 and Figure 4).

The plausible biogenetic pathways of **1** and **2** were proposed as shown in Scheme 1. The biosynthetic precursor of **1** was proposed to be kanshone C (**i**) and 2′,6′-dihydroxy-4′,4-dimethoxychalcone (**ii**). The **i** and **ii** would be linked by a stereoselective nucleophilic reaction to generate the key intermediate **iii** (as was the case for

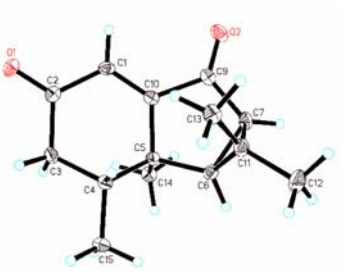

Figure 3. X-ray crystal structure of compound **2**.

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Data for Compound **2** in $CDCl_3$ (δ in ppm, J in Hz)

no.	δ_H	δ_C
1	6.16 (s)	123.4
2		200.0
3	α: 2.23 (dd, 13.5, 18.0)	42.1
	β: 2.35 (dd, 4.7, 18.0)	
4	2.34 (m)	35.4
5		44.2
6	1.79 (d, 5.5)	42.3
7	1.94 (d, 5.5)	40.2
9		201.6
10		165.1
11		32.2
12	1.09 (s)	28.7
13	1.18 (s)	17.7
14	1.13 (s)	20.8
15	1.07 (d, 6.5)	15.8

sampsonione A[13]), which would readily produce **1** by intramolecular dehydration. Starting from kanshone C, the biogenetic pathway of **2** involved a ring-opening reaction, benzilic acid rearrangement, decarboxylation, dehydration, and oxidation.[14]

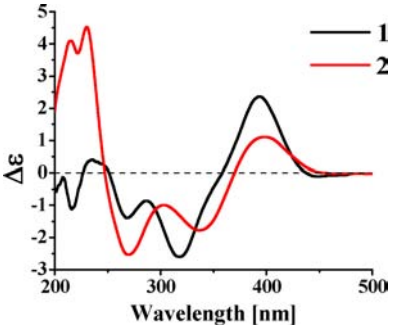

Figure 4. CD spectra of compounds **1** and **2**.

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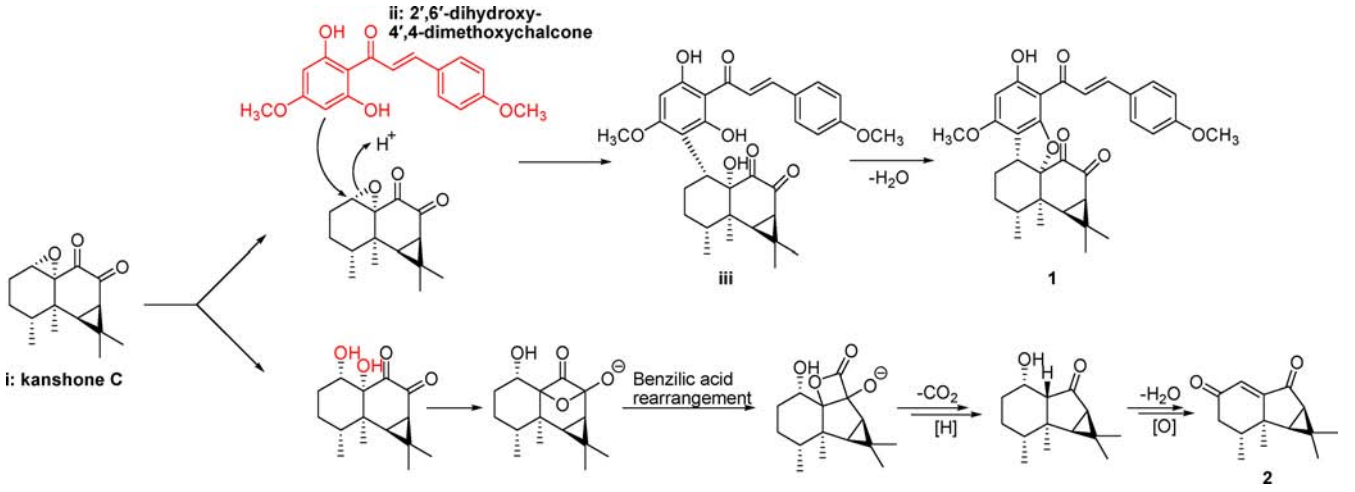
Scheme 1. Proposed Biogenetic Pathway for **1** and **2**



Compounds **1** and **2** were evaluated for their protective effects on H_2O_2-induced myocardial injury using the MTT method and salvianolic acid B as the positive control. Both **1** and **2** exhibited obvious protective effects on the injury of neonatal rat cardiomyocytes, and the effects were dose-dependent.

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Supporting Information Available. Experimental procedures; physical–chemical properties; NMR, MS, IR, UV, and X-ray crystallographic data (CIF) for nardoaristolones A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.