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Walsucochins A and B with an Unprecedented Skeleton Isolated from *Walsura cochinchinensis*

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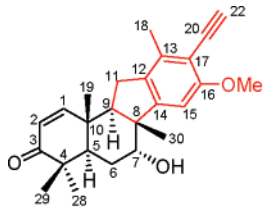
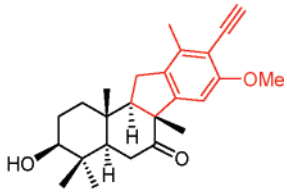
ABSTRACT



Walsucochin A (1)



Walsucochin B (2)

Walsucochins A (1) and B (2) with an unprecedented skeleton were isolated from *Walsura cochinchinensis*. Their structures including absolute configuration were elucidated by spectral methods. A biosynthetic pathway of 1 and 2 was postulated. Both 1 and 2 exhibited significant cell protecting activities against H_2O_2-induced PC12 cell damage.

The genus *Walsura* (Meliaceae) comprising about 30−40 species is mainly distributed in India, Indonesia, and China.[1] Chemical studies conducted previously on this genus have led to the isolation of quite a number of triterpenoids and tetranortriterpenoids.[2−7] Recently, our research group has reported three novel limonoids, walsuronoids A−C, with antimalarial activity from *Walsura robusta*.[8] In our continuous efforts to search for the structurally and biologically significant metabolites from this genus, two novel C_{24} nortriterpenoids, walsucochins A (**1**) and B (**2**) with an unprecedented skeleton featuring a phenylacetylene moiety fused into a contracted five-membered C-ring, were isolated from the leaves and twigs of *Walsura cochinchinensis* (Baill.) Harms. This is also the first report on the chemical study of this plant species.

Recently, accumulating evidence highlighted that the generation of reactive oxygen species (ROS) and the associated oxidative stress have been implicated in the development of multiple disorders, such as neurodegenerative diseases,[9] malaria,[10] and inflammation.[11]

Compounds **1** and **2** exhibited significant cell protecting activity against H_2O_2-induced PC12 cell damage.

Herein, we report the isolation and structural elucidation of these new compounds and their cell protecting activity.

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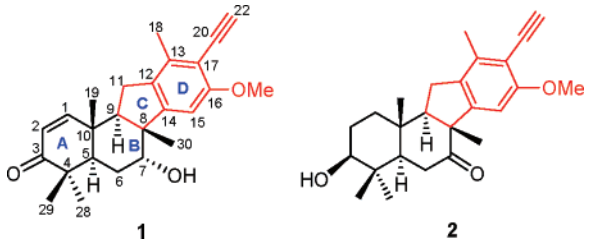
The air-dried powder of the plant material (2.5 kg) was percolated with 95% EtOH at room temperature to give 120 g of crude extract, which was partitioned between EtOAc and water to afford the EtOAc-soluble fraction (50 g). It was then separated into six fractions (1−6) by Si gel cc eluted with gradient petrol ether/acetone (20:1 to 0:1). Fraction 5 (5 g) was extensively chromatographed over the columns of MCI gel, Si gel, and Sephadex LH-20 to give a mixture of two compounds, which was further separated by semi-preparative HPLC to yield walsucochins A (**1**, 10 mg) and B (**2**, 6 mg) (for details, see the Supporting Information).



1 **2**

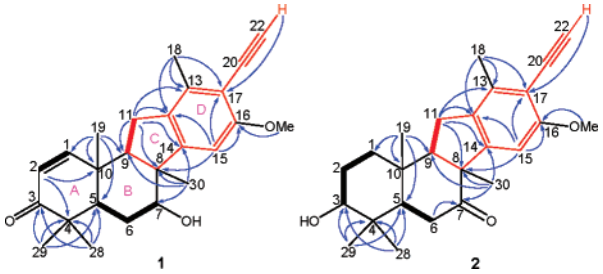
Walsucochin A (**1**),[12] a white amorphous powder, gave a molecular formula of $C_{25}H_{30}O_3$ as determined by HREIMS at *m*/*z* 378.2195 $[M]^+$ (calcd 378.2195) requiring 11 double bond equivalents. The IR revealed the presence of hydroxyl (3437 cm^{-1}), conjugated carbonyl (1664 cm^{-1}), acetylenyl (3320 and 2098 cm^{-1}),[13] and phenyl (1603 and 1580 cm^{-1}) groups. The 1D NMR data (Table 1) and HSQC spectrum suggested the presence of four tertiary methyls at δ_H 1.30, 1.19, 1.15, and 1.12 (each 3H, s), one aromatic methyl at δ_H 2.39 (3H, s), one aromatic methoxyl at δ_H 3.90 (3H, s), an acetylenyl [δ_H 3.51(1H, s); δ_C 84.7 and 79.0], an α,β-unsaturated ketone [δ_H 7.13 (1H, d, J = 10.0 Hz) and 5.89 (1H, d, J = 10.0 Hz); δ_C 158.3, 125.7, and 205.1], and a pentasubstituted phenyl group. The aforementioned functionalities accounted for 8 out of the 11 degrees of unsaturation, and the leftover double-bond equivalents required the presence of three additional rings in **1**.

Comprehensive analysis of the 2D NMR spectra of **1**, especially $^1H-^1H$ COSY and HMBC (Figure 1), allowed the establishment of the planar structure. Three structural fragments as drawn with bold lines in Figure 1 were established on the basis of $^1H-^1H$ COSY spectrum. In the HMBC, the correlations of H_3-28(29)/C-3, C-4, and C-5; H_3-19/C-1, C-5, C-9, and C-10; H_3-30/C-7, C-8, and C-9; and H-2/C-4 and C-10 furnished the typical A/B-rings system of an apotirucallane-type triterpenoid, such as that of piscidinol D (**3**), a known compound widely occurring in this genus.[2] The HMBC correlations from H-15 to C-12, C-16, and C-17, and those from H_3-18 to C-12, C-13, and C-17 not only confirmed the presence of the aromatic D-ring but also located the Me-18 at C-13. In addition, the HMBC correlations of H-22/C-17 and OCH_3/C-16 allowed the attachment of the acetylenyl to C-17 and the only methoxyl to C-16, respectively. Further to this, the HMBC correlations of H_2-11/C-8, C-9, C-12, C-13, and C-14; H_3-30/C-8, C-9, and C-14; and H-15/C-8 enabled us to establish the five-membered C-ring as well as the C/D-rings connection. The planar structure of **1** was thus constructed.

Table 1. ^1H and ^{13}C NMR Data of **1** and **2** (in $CDCl_3$)[a]

	1		**2**	
no.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1a	7.13 (d, 10.0)	158.3	1.73−1.75 (m)	37.6
1b			1.19−1.20 (m)	
2	5.89 (d, 10.0)	125.7	1.74−1.78 (m, 2H)	26.9
3		205.1	3.28 (br s, $W_{1/2}$ 13.7)	78.5
4		44.5		39.2
5	2.40 (dd, 14.4, 2.8)	46.2	1.38 (dd, 14.4, 2.8)	56.7
6α	1.89 (ddd, 14.4, 3.2, 2.8)	26.2	2.41 (dd, 14.4, 2.8)	36.6
6β	2.07 (ddd, 14.4, 13.2, 2.4)		2.83 (dd, 14.4, 14.4)	
7	4.39 (dd, 2.8, 2.4)	69.9		211.4
8		52.9		58.3
9	2.44 (dd, 12.4, 6.4)	50.2	1.93 (t, 8.8)	62.5
10		39.5		36.3
11α	2.83 (dd, 13.6, 6.4)	25.8	2.60 (d, 8.8)	25.8
11β	2.67 (dd, 13.6, 12.4)		2.60 (d, 8.8)	
12		133.0		131.3
13		139.5		138.2
14		150.7		149.0
15	6.51 (s)	100.4	7.17 (s)	104.3
16		160.8		160.4
17		109.4		109.1
18	2.39 (s, 3H)	17.6	2.34 (s, 3H)	17.5
19	1.30 (s, 3H)	19.0	1.25 (s, 3H)	15.8
20		79.0		79.4
22	3.51 (s)	84.7	3.50 (s)	84.6
28	1.19 (s, 3H)	27.3	1.00 (s, 3H)	27.6
29	1.15 (s, 3H)	21.1	0.90 (s, 3H)	14.8
30	1.12 (s, 3H)	23.3	1.31 (s, 3H)	23.0
16-OMe	3.90 (s, 3H)	56.1	3.92 (s, 3H)	56.2

[a] Recorded at 400 MHz (^1H) and 100 MHz (^{13}C).



1 **2**

Figure 1. $^1H-^1H$ COSY (—) and HMBC (H→C) of **1** and **2**.

(12) **Walsucochin A (1):** colorless powder; $[\alpha]^{20}_D$ +19.0 (*c* = 0.200, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.2), 248 (3.9), 258 (3.8), 297 (3.4), 307 (3.4) nm; CD (MeOH) λ_{max} 230 ($\Delta\epsilon$ +8.61), 211 ($\Delta\epsilon$ −7.37) nm; IR (KBr) ν_{max} 3437, 3320, 2098, 1664, 1603, 1580, 1464, and 829 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 378 $[M]^+$ (100), 251 (24), 197 (14), 115 (6), 93 (7), 77 (8), 58 (18), 51 (6); HREIMS *m*/*z* calcd for $C_{25}H_{30}O_3$ 378.2195, found 378.2195 $[M]^+$.

(13) Pretsch, E.; Bühlmann, P.; Affolter, C. In *Structure Determination of Organic Compounds: Tables of Spectral Data*; Chinese Version, Rong, G. B., Zhu, S. Z., Transl.; East China University of Science and Technology: Shanghai, 2002; p 252.

The relative configuration of **1** was assigned by ROESY spectrum (Figure 2), in which the correlations of H-6β/H_3-

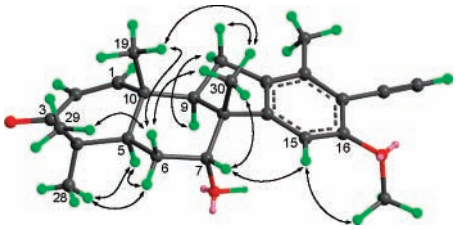


Figure 2. Key ROESY (H↔H) correlations of **1**.

19, H_3-29, and H_3-30 and H_3-30/H-7, H-11β, and H_3-19 indicated that they were cofacial and arbitrarily fixed in β-orientation. In consequence, the ROESY correlations H-5/H-6α and H_3-28 and H-9/H-11α indicated that H-5, H-6α, H-9, and H_3-28 were α-oriented.

The CD exciton chirality method was applied to determine the absolute configuration of **1**.[14] In comparison with the UV spectrum of **2** (Figure 3), a strong UV absorption

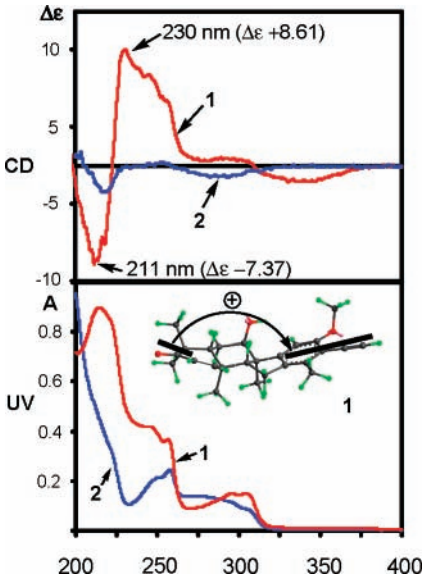


Figure 3. CD and UV spectra of **1** and **2**. Bold lines denote the electronic transition dipole of the chromophores.

maximized at ca. 215 nm (log ϵ 4.2) and one shoulder absorption at ca. 248 nm (log ϵ 3.9) of **1** were readily assignable to the α,β-unsaturated ketone group (Woodward's rule also gave 215 nm)[15] and the phenylacetylene[16] group, respectively. The first positive Cotton effect at 230 nm ($\Delta\epsilon$ +8.61) and the second negative Cotton effect at 211 nm ($\Delta\epsilon$ −7.37) arising from the exciton coupling of two different chromophores of the α,β-unsaturated ketone and the phenylacetylene moiety indicated a positive chirality for compound **1**. The absolute configuration of **1** was thus established as depicted.

Walsucochin B (**2**),[17] a white amorphous powder, presented the molecular formula of $C_{25}H_{32}O_3$ as determined by HREIMS at *m/z* 380.2349 $[M]^+$ (calcd 380.2351) with 10 double bond equivalents. The IR absorption bands showed the presence of hydroxyl (3433 cm^{-1}), carbonyl (1709 cm^{-1}), acetylenyl (3320 and 2098 cm^{-1}), and phenyl (1603 and 1580 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Table 1) of **2** showed high similarity to those of **1** except for the absence of the α,β-unsaturated ketone moiety and the presence of a ketone group (δ_C 211.4) and two additional methylenes (δ_C 37.6 and 26.9), suggesting that they are structurally close related analogues. The $^1H-^1H$ COSY spectrum allowed the identification of three structural fragments that bear coupling protons as showed with bold lines in Figure 1. In the HMBC spectrum (Figure 1), the key HMBC correlations from H_3-28(29) to an oxygenated methine at δ_C 78.5 implied the presence of a hydroxyl group at C-3; the HMBC correlations from H_2-6 and H_3-30 to the ketone carbonyl allowed the location of the ketone group at C-7, which resulted in the severely downfield shifted carbon resonances of C-6 and C-8 of **2** as compared with those of **1** (Table 1) being consistent with this assignment. Detailed 1D and 2D NMR analyses further confirmed the above deduction (see the Supporting Information).

The relative stereochemistry of **2** was also established by the performance of a ROESY experiment (see the Supporting Information). In particular, the 3-OH was assigned as β-oriented by the ROESY correlations of H-3/H-5 and H_3-28.

Although the CD spectrum of **2** (Figure 3) did not show a convincing evidence to assign its absolute configuration, it could be proposed as the same of compound **1** on the basis of biogenetic reasons that two compounds coexisted in the same plant and were probably produced via the same plausible biosynthetic pathway (Scheme 1).

Walsucochins A (**1**) and B (**2**) are the first examples of C_{24} nortriterpenoid with an unprecedented skeleton featuring a phenylacetylene moiety fused into a contracted five-membered C-ring. In fact, they are also the first representatives of nortriterpenoids possessing a phenylacetylene moiety.

As walsucochins A (**1**) and B (**2**) shared a typical A/B rings system of the apotirucallane-type triterpenoids that are widely occurred in the *Walsura* genus,[2−3,7] their biosynthetic precursor was thus proposed to be piscidinol D (**3**), an

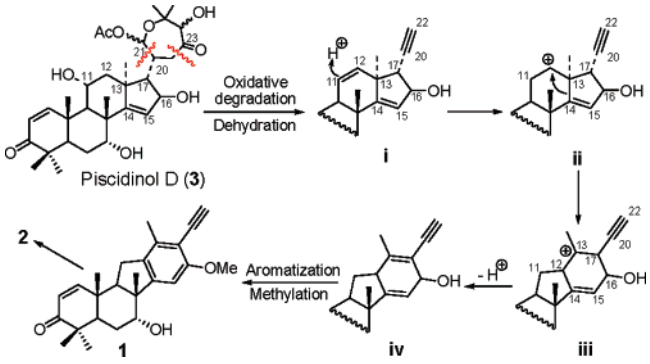
(14) Harada, N.; Nakanishi, K.; Tatsuoka, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896−5898.

(15) Pretsch, E.; Bühlmann, P.; Affolter, C. In *Structure Determination of Organic Compounds: Tables of Spectral Data*; Chinese Version, Rong, G. B., Zhu, S. Z., Transl.; East China University of Science and Technology: Shanghai, 2002; pp 388−390.

(16) Park, S. K.; Shim, S. C.; Seo, Y. W.; Shin, J. H. *Tetrahedron Lett.* **1999**, *40*, 4575−4576.

(17) **Walsucochin B (2):** colorless powder; $[\alpha]^{20}_D$ −45.0 (*c* = 0.100, MeOH); UV (MeOH) λ_{max} (log ϵ) 258 (4.0), 250 (3.9) nm; CD (MeOH) λ_{max} 218 ($\Delta\epsilon$ −3.22) nm; IR (KBr) ν_{max} 3433, 3320, 2098, 1709, 1603, 1580, 1462, and 1097 cm^{-1}; for ^1H and ^{13}C NMR data, see table 1; EIMS *m/z* (70ev) 380 $[M]^+$(100), 251 (20), 211 (30), 198 (20), 183 (16), 152 (5), 121 (10), 91 (5), 51 (3); HREIMS *m/z* calcd for $C_{25}H_{32}O_3$ 380.2351, found 380.2349 $[M]^+$.

Scheme 1. Hypothetical Biogenetic Pathway of **1** and **2**



ubiquitous apotirucallane-type triterpenoid in this genus (Scheme 1). The oxidative degradation of the side chain of **3** followed by a dehydration would afford the intermediate **i**, which would be protonized to give a cabocationic intermediate **ii**. Subsequently, the intermediate **ii** would be transformed into a key intermediate **iii** via a Wagner−Meerwein rearrangement.[18] The discharge of the carbocation **iii** by the loss of one proton to give a relatively stable intermediate **iv**, which would be converted into **1** through a process of aromatization and the methylation of 16-OH. Compound **2** would be finally produced via a cascade of redox chemistry from **1**.

In this study, cell survival was evaluated according to the reported protocol[19] with minor modification.[20] After 200 μM H_2O_2 exposure, cell viability as determined by MTT reduction was markedly decreased to 67% ($^{\#\#}P < 0.01$ vs control). However, respective pretreatment with compounds **1** and **2** at 1, 5, and 10 μM significantly attenuated the H_2O_2−induced cell damage ($^{**}P < 0.01$ vs H_2O_2 group) in a dose-dependent manner (Figure 4).

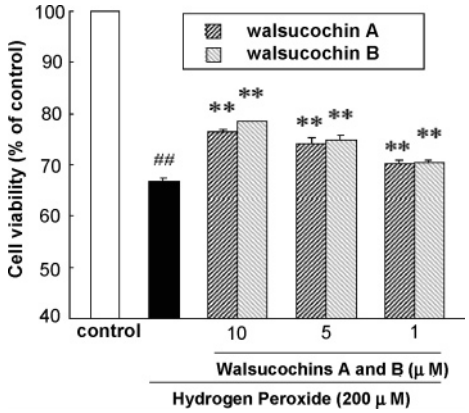


Figure 4. Effects of walsucochins A and B on cell viability.

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Supporting Information Available: Experimental procedures and physical and spectral data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Cells were incubated with 200 μM H_2O_2 for 30 min, and the cultures were further developed for another 24 h in fresh medium. Compounds were added to the cultures 2 h prior to H_2O_2 addition. Three independent experiments were carried out in triplicate. All data were expressed as percentage of control value. Statistical comparison was made by using one-way ANOVA and followed by Duncan's test. The data were expressed as means + SEM; $^{\#\#}P$ <0.01 vs control; $^{**}P < 0.01$ vs H_2O_2 group.