Three New Phenylpropanoids from *Inula nervosa* WALL.

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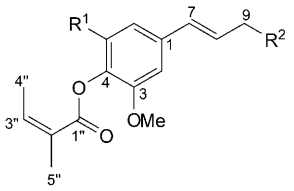
Three new phenylpropanoid compounds, named nervolans A–C (**1**–**3**, resp.), together with two other known phenylpropanoids, coniferyl diangelate and sinapyl diangelate, were isolated from the aerial parts of *Inula nervosa* WALL. (Asteraceae), a traditional Chinese medicinal plant. The structures of **1**–**3** were elucidated by detailed spectroscopic analyses, including HR-ESI-MS data and 2D-NMR spectroscopy. Compounds **1**–**5** exhibited mild inhibitory effects against NO production in LPS-stimulated RAW264.7 cells.

Introduction. – *Inula nervosa* WALL. (Asteraceae), a perennial herb distributed in the southwestern region of China, is traditionally used as Chinese folk medicine for treating stomachache and relieving rheumatism [1]. However, no phytochemical investigations have been reported on this plant. In our study, three new phenylpropanoid compounds, named nervolans A–C (**1**–**3**, resp.), together with two known phenylpropanoids, coniferyl diangelate [2] and sinapyl diangelate [3], were isolated from the aerial parts of *I. nervosa* WALL. (*Fig. 1*). This report deals with the isolation and structural elucidation of these novel phenylpropanoids by extensive spectroscopic analyses, and their inhibitory effects on NO production.



	R^1	R^2
1	MeO	AcO
2	MeO	OH
3	H	OH

Fig. 1. *The structures of compounds* **1**–**3**

Results and Discussion. – The 95% EtOH extract of the aerial parts of *I. nervosa* WALL. was partitioned into petroleum ether (PE)-, AcOEt-, and BuOH-soluble fractions. Repeated column chromatography of the PE-soluble fractions resulted in the purification of compound **1**. Further, isolation of compounds **2** and **3** was carried out by semi-preparative reversed-phase (RP) HPLC.

Nervolan A (**1**) was obtained as a yellow amorphous powder. The molecular formula was determined as $C_{18}H_{22}O_6$ by HR-ESI-MS (*m/z* 357.1315 ($[M + Na]^+$,

$C_{18}H_{22}NaO_6^+$; calc. 357.1314)). The ^{13}C-NMR and DEPT spectra of **1** showed signals for 18 C-atoms, including three Me, two MeO, one CH_2, five CH groups, two COO C-atoms, and four quaternary aromatic C-atoms (*Table*). In the ^{1}H-NMR spectrum, two aromatic H-atom signals at δ(H) 6.65 (*s*, H–C(2), H–C(6)) indicated a 1,3,4,5-tetrasubstituted benzene ring. In the HMBC spectrum of **1**, cross-peaks H–C(7)/C(9), H–C(8)/C(9), and H–C(9)/C(7),C(8) suggested the presence of an allyl group in conjugation with the aromatic system deduced by the correlations δ(H) 6.61 (*d*, J = 16.0, H–C(7))/δ(H) 6.24–6.27 (*m*, H–C(8)) and δ(C) 134.4 (C(1)). The large coupling constant (J = 16.0 Hz) between H–C(7) and H–C(8) indicated the (*E*)-configuration of the allyl group. A long-range coupling was observed between one Me group (δ(H) 2.11 (*s*, Me(2′))) and one COO C-atom (δ(C) 170.8 (C(1′))), suggesting the presence of an AcO group. The AcO group was positioned at C(9) due to the HMBC between δ(H) 4.73 (*dd*, J = 6.0, 1.0, CH_2(9)) and δ(C) 170.8 (C(1′)). Furthermore, The ^{1}H,^{1}H-COSY (H–C(3″)/H–C(4″)) and the HMBC cross-peaks H–C(3″)/C(4″), C(5″); Me(4″)/C(2″), C(3″), and Me(5″)/C(1″), C(2″) indicated the presence of a 2-methylbut-2-enoic acid moiety (*Fig. 2*). The configuration of this unsaturated ester was assigned as (*Z*) (angelic acid) from the ^{13}C-NMR chemical-shift data of two vinylic Me C-atom (angelic acid: δ(C) 20.8 and 16.0; tiglic acid: δ(C) 14.2 and 11.9) [4][5]. Thus, the structure of **1** was determined as nervolan A, as shown in *Fig. 1*.

Nervolan B (**2**) was obtained as a yellow oil. The molecular formula was determined as $C_{16}H_{20}O_5$ by HR-ESI-MS (*m*/*z* 293.1359 ($[M + H]^+$, $C_{16}H_{21}O_5^+$; calc. 293.1389)). The NMR data of **2** (*Table*) were very similar to those of **1**, except for the disappearance of

Table. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data of* **1**–**3** *in* $CDCl_3$. δ in ppm, J in Hz. Arbitrary numbering.

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		134.4		134.9		135.2
H–C(2)	6.65 (*s*)	103.4	6.64 (*s*)	103.3	7.00 (*d*, J = 1.5)	110.7
C(3)		152.4		152.3		152.2
C(4)		134.4		134.3		138.2
C(5)		152.4		152.4	7.01 (*d*, J = 7.0)	123.0
H–C(6)	6.65 (*s*)	103.4	6.64 (*s*)	103.3	6.98 (*dd*, J = 7.0, 1.5)	119.2
H–C(7)	6.61 (*d*, J = 16.0)	134.1	6.55 (*d*, J = 16.0)	130.9	6.60 (*d*, J = 16.0)	130.6
H–C(8)	6.24–6.27 (*m*)	123.4	6.29–6.31 (*m*)	128.8	6.30–6.34 (*m*)	128.6
H_2C(9)	4.73 (*dd*, J = 6.0, 1.0)	64.9	4.32 (*dd*, J = 6.0, 1.0)	63.5	4.33 (*dd*, J = 6.0, 1.0)	63.6
C(1′)		170.8				
Me(2′)	2.11 (*s*)	21.0				
C(1″)		165.7		165.8		165.8
C(2″)		127.3		127.4		127.8
H–C(3″)	6.19–6.22 (*m*)	139.2	6.18–6.21 (*m*)	139.2	6.20–6.25 (*m*)	139.8
Me(4″)	2.05 (*d*, J = 1.5)	15.8	2.07 (*d*, J = 1.5)	15.8	2.07 (*d*, J = 1.5)	15.8
Me(5″)	2.05 (*s*)	20.7	2.06 (*s*)	20.7	2.06 (*s*)	20.6
MeO–C(3)	3.82 (*s*)	56.2	3.80 (*s*)	56.1	3.83 (*s*)	55.8
MeO–C(5)	3.83 (*s*)	56.2	3.80 (*s*)	56.1		

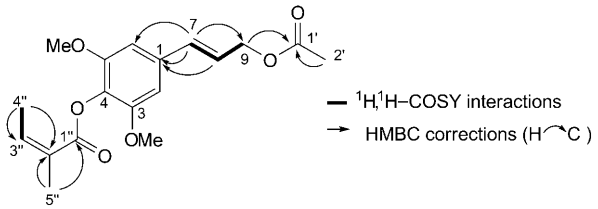


Fig. 2. *Selected 2D-NMR correlations for nervolan A* (**1**)

the signals of one Me and one COO C-atom, suggesting the absence of an AcO group. The signals of CH_2(9) were upfield-shifted to δ(H) 4.32 (*dd*, $J = 6.0, 1.0$) and δ(C) 63.5, while the signals of H–C(8) were shifted downfield to δ(H) 6.29–6.31 (*m*) and δ(C) 128.8, indicating that the AcO group was replaced by a OH group. Thus, the structure of **2**, nervolan B, was established as shown in *Fig. 1*.

Nervolan C (**3**) was obtained as a yellow oil. The molecular formula was determined as $C_{15}H_{18}O_4$ by HR-EI-MS (*m*/*z* 262.1203 (M^+, $C_{15}H_{18}O_4^+$; calc. 262.1205)). The NMR data (*Table*) were similar to those of **2**. The only differences between **3** and **2** were the absence of the signal of a MeO group and the presence of an *ABX* aromatic system (δ(H) 7.00 (*d*, $J = 1.5$, H–C(2)), 7.01 (*d*, $J = 7.0$, H–C(5)), 6.98 (*dd*, $J = 7.0, 1.5$, H–C(6)) in **3**. The absence of the MeO group was confirmed by the additional signal of an aromatic H-atom at δ(H) 7.01 (*d*, $J = 7.0$) correlating with δ(C) 123.0 (C(5)) in HMQC. Therefore, the structure of **3**, nervolan C, was identified as shown in *Fig. 1*.

In addition to the three new phenylpropanoids, **1**–**3**, the other two known shikimates, coniferyl diangelate and sinapyl diangelate were identified on the basis of their NMR and MS data. This is the first report on the isolation of these compounds from *I. nervosa* WALL.

Compounds **1**–**3**, coniferyl diangelate, and sinapyl diangelate were tested for inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages and showed mild inhibitory activities against the production of NO with IC_{50} values of 33.31, 15.43, 21.32, 16.19, and 33.56 μM, respectively.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* and silica gel (SiO_2; 100–200 and 200–300 mesh, *Yantai Jiangyou*, P. R. China). TLC: *HSG* F_{254} silica-gel plates (10–40 μm, *Yantai Huiyou*, P. R. China); detection by spraying with 15% H_2SO_4. Semi-prep. HPLC: *Shimadzu LC-6AD* series equipped with an *SPD-20* spectrophotometer using a *ZORBAX SB-*C_{18} column (5 μm; 9.4 × 250 nm, i.d.). IR Spectra: *Bruker* FI-IR *Vector 22* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer, TMS as an internal standard, at 500 (^{1}H) and 125 MHz (^{13}C). ESI-MS: *Agilent 1100* series spectrometer. EI-MS: *Autospec-UltimaETOF* apparatus.

Plant Material. The aerial parts of *I. nervosa* WALL. were collected in Yunnan Province, P. R. China, in August 2007, and identified by Prof. *Bao-kang Huang*, Department of Pharmacognosy, Second Military Medical University. A voucher specimen was deposited with the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. nervosa* WALL. (15.0 kg) were extracted with 95% EtOH three times at r.t. The extract was evaporated *in vacuo* to afford a residue (1.4 kg), which was partitioned with petroleum ether (PE), AcOEt, and BuOH. The PE extract (200.2 g)

was chromatographed on a SiO_2 column with the gradient PE/AcOEt (100:1–1:100). The obtained fractions were combined on the basis of SiO_2 TLC, and sixteen fractions, *Fr. 1–16*, were obtained. *Fr. 10* was subjected to SiO_2 column chromatography (CC) to yield **1** (212.4 mg). *Fr. 8* and *9* were subjected to semi-prep. HPLC ($MeOH/H_2O$ 80:20; flow rate: 3 ml/min) to afford coniferyl diangelate (10.0 mg) and sinapyl diangelate (8.4 mg). Compounds **2** (15.1 mg) and **3** (7.0 mg) were isolated from *Fr. 13* by semi-prep. HPLC ($MeOH/H_2O$ 60:40; flow rate: 3 ml/min).

Assay for Inhibition Effects against LPS-Induced NO Production. RAW264.7 Cells grown on 100-mm culture dish were harvested and seeded in 96-well plates at 2×10^5 cells/well for NO production. The plates were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 1 μg/ml of LPS. Nitrite concentration in the culture supernatant was measured by the *Griess* reaction [6]. Cell viability was measured by a MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) assay (*Sigma–Aldrich*) [7].

Nervolan A (=*4-[(1*E*)-3-(Acetyloxy)prop-1-en-1-yl]-2,6-dimethoxyphenyl (2*Z*)-2-Methylbut-2-enoate*; **1**). Yellow amorphous powder. IR (KBr): 2916, 2846, 1735, 1594, 1505, 1456, 1419, 1225, 1127. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS: 357.1315 ($[M + Na]^+$, $C_{18}H_{22}NaO_6^+$; calc. 357.1314).

Nervolan B (=*4-[(1*E*)-3-Hydroxyprop-1-en-1-yl]-2,6-dimethoxyphenyl (2*Z*)-2-Methylbut-2-enoate*; **2**). Yellow oil. IR (KBr): 3440, 2929, 2850, 1732, 1595, 1505, 1458, 1418, 1244, 1206, 1127. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS: 293.1359 ($[M + H]^+$, $C_{16}H_{21}O_5^+$; calc. 293.1389).

Nervolan C (=*4-[(1*E*)-3-Hydroxyprop-1-en-1-yl]-2-methoxyphenyl (2*Z*)-2-Methylbut-2-enoate*; **3**). Yellow oil. IR (KBr): 3395, 2924, 2853, 1716, 1596, 1508, 1457, 1415, 1227, 1206, 1122. ^{1}H- and ^{13}C-NMR: see *Table*. HR-EI-MS: 262.1203 (M^+, $C_{15}H_{18}O_4^+$; calc. 262.1205).

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REFERENCES

[1] L. Zhao, K. X. Xin, Y. Q. Li, *Food Drug* **2007**, *9*, 53.
[2] F. Bohlmann, C. Zdero, *Tetrahedron Lett.* **1969**, *10*, 69.
[3] I. Köhler, K. Jenett-Siems, C. Kraft, K. Siems, D. Abbiw, U. Bienzle, E. Eich, *Z. Naturforsch., C* **2002**, *57*, 1022.
[4] J. A. Marco, J. F. Sanz-Cervera, A. Yuste, *Phytochemistry* **1997**, *45*, 563.
[5] C. J. Turner, M. S. Tempesta, R. B. Taylor, M. G. Zagorski, J. S. Termini, D. R. Schroeder, K. Nakanishi, *Tetrahedron* **1987**, *43*, 2789.
[6] H. H. H. W. Schmidt, M. Kelm, 'Methods in Nitric Oxide Research', John Wiley & Sons Ltd., London, 1996, p. 491.
[7] F. Denizot, R. Lang, *J. Immunol. Methods* **1986**, *89*, 271.

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