# New Flavonoid Glycosides and Cyanogenic Glycosides from *Dracocephalum peregrinum*

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**Separation of ethyl acetate fractionation of *Dracocephalum peregrinum* afforded three new flavonoid glucosides (1—3), and a new cyanogenic glucoside (4). Their structures were elucidated based on HR-electron spray ionization (ESI)-MS, EI-MS, UV, IR, 1D-, and 2D-NMR data. 1—4 were tested *in vitro* for their antiinflammatory activity against the RAW 264.7, 293 cells. Among the compounds tested, 1—4 shown good antiinflammatory activity at 100 $\mu$g/ml by the measurement of nitric oxide (NO) in lipopolysaccharide (LPS) activated macrophages. But only 2 and 3 shown weak antiinflammatory activity at 100 $\mu$g/ml during the nuclear factor (NF)-$\kappa$B activation assay.**

**Key words** flavonoid glycoside; cyanogenic glycoside; *Dracocephalum peregrinum*; antiinflammatory

Genus *Dracocephalum* having more than 30 species, is an important member of the Lamiaceae family.[1)] Several species of *Dracocephalum* such as *D. peregrinum*, *D. heterophyllum*, *D. integrifolium*, *D. moldavicum*, *D. rupestre*, *D. ruyschiana* and *D. tanguticum* were used in Traditional Chinese Medicine treat icteritious hepatitis, lymphadenitis, throat-swelling diseases, flu fever, acute and chronic trachitis. So far, there are a few reports on researches of bioactivities and chemical constituents of this genus. Bioassays of *Dracocephalum* exhibited antibacterial, anti-hypoxia and antitussive activities,[2)] while Chemical investigation on these species resulted in the isolation of flavanoids,[3,4)] terpenoids,[5—7)] cinnamic acid derivatives[8)] and volatile oils.[9,10)] In our further phytochemical investigation on *Dracocephalum peregrinum*, we isolated three new flavonoid glucosides, Peregrinumin A (**1**), Peregrinumin B (**2**) and Peregrinumin C (**3**), and a new cyanogenic glucoside, Peregrinumcin A (**4**).

## Results and Discussion

Peregrinumin A (**1**) was isolated as yellow amorphous powder, and its molecular formula was assigned to be $C_{32}H_{36}NaO_{16}$ by the peak in the HR-electron spray ionization (ESI)-MS at *m*/*z* 699.18948 ([M+Na]$^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901), indicating sixteen degrees of unsatruation. Its UV (MeOH) spectrum showed the presence of a flavonoid nucleus (269, 323 nm). The IR (KBr) spectrum showed the presence of hydroxy (3421, 2923, 2852 cm$^{-1}$) and carbonyl (1741 cm$^{-1}$). In the $^1$H-NMR (dimethyl sulfoxide (DMSO)) spectrum of **1**, two groups of aromatic protons at $\delta$: 6.94 (s, 1H), 6.43 (1H, d, $J$=1.8 Hz), 6.83 (1H, d, $J$=1.8 Hz) and 8.05 (d, $J$=8.4 Hz, 2×H), 7.12 (d, $J$=8.0 Hz, 2×H), a methoxyl protons at $\delta$: 3.88 (3H, br s), and a hydroxyl protons at $\delta$: 12.9 (1H, br s) were very similar to those of acacetin,[11)] and should, therefore, be assigned to H-3, H-6, H-8, H-2′, H-6′, H-3′, H-5′, 4′-OMe, and 5-OH. In the $^{13}$C-NMR (DMSO) spectrum, the characteristic acetyl carbons at $\delta$: 169.6, 20.6 and 169.4, 20.4 showed the presence of two acetyls. One group of carbon signals at $\delta$: 99.4, 73.0, 76.2, 69.5, 75.5, 65.8 indicated the existence of glucose and the other group of carbon signals at $\delta$: 97.0, 69.1, 71.5, 69.1, 68.3, 17.5 belonged to a moiety of rhamnose. The anomeric protons at $\delta$: 5.16 (1H, d, $J$=7.8 Hz, H-1″) and 4.67 (1H, s, H-1‴) in the $^1$H-NMR spectrum indicated the $\beta$-configuration of glucose and the $\alpha$-configuration of rhamnose. In the $^{13}$C-NMR spectrum of **1**, characteristic glycosylation shift (+11.2, +0.7 ppm) were observed for C-8 and C-6, suggesting that the glucose moiety was linked to C-7,[11)] and characteristic rhamnosylation shift (+3.4, −3.8 ppm) were observed for C-6″ and C-5″, suggesting that the rhamnose moiety was $\alpha$-L-rhamnosyl-(1→6)-$\beta$-D-glucoside linkage.[12)] In the heteronuclear multiple bonding correlation (HMBC) spectrum (see Fig. 3), the long-range correlation from H-1″ ($\delta$: 5.16) of glucose to C-7 ($\delta$: 162.8) of aglycone and H-1‴ ($\delta$: 4.67) of rhamnose to C-6″ ($\delta$: 65.8) of glucose further confirmed the linkage of the glucose and rhamnose moieties. And the long-range correlation from H-2‴ (5.04) and H-3‴ (4.85) of rhamnose to two carbonyl carbons ($\delta$: 169.6, 169.4) of ethanoyls concluded the acetylation of 2‴,3‴-position. Thus, the structure of **1** was established as acacetin-7-*O*-(2,3-

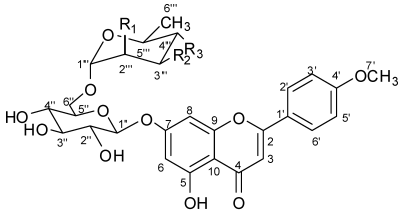


Fig. 1. The Structures of Peregrinumin A, B and C (**1**—**3**)

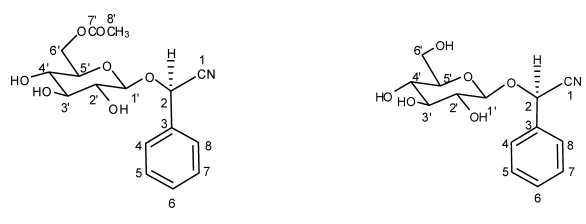


Fig. 2. The Structures of Peregrinumcin A (**4**) and 2*R*-Prunasin (**4a**)

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*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, and named as Peregrinumin A.

Peregrinumin B (**2**) obtained as yellow amorphous powder. The molecular formula of was assigned to be $C_{32}H_{36}NaO_{16}$ based on ion peak at *m/z* 699.18856 ($[M+Na]^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901) in HR-ESI-MS. NMR spectrum of **2** also provided evidence for the presence of acacetin-7-*O*-$\alpha$-L-rhamnosyl-(1→6)-$\beta$-D-glucoside as indicated by diagnostic signals similar to glucose and rhamnose portions and to the flavonoid portion of compound **1** (see Table 1 and discussion below). Although the difference in structure between **1** and **2** requires only aectylation position of sugar moiety, this change causes significant differences in spectral of C-2‴, C-3‴ and C-4‴ (see Table 1). And in the HMBC spectrum of **2** (see Fig. 3), the long-range correlation from H-2‴ ($\delta$: 4.94) and H-4‴ ($\delta$: 4.64) of rhamnose to two carbonyl carbons ($\delta$: 169.8, 169.7) of acetyls could be explained with the acetylation of 2‴,4‴-position (see Fig. 1). Therefore, **2** was identified as acacetin-7-*O*-(2,4-*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, named as Peregrinumin B.

Peregrinumin C (**3**), yellow amorphous powder, was assigned to be $C_{32}H_{36}NaO_{16}$ (HR-ESI-MS, *m/z* 699.18904). Analysis of NMR spectrum of **3**, **2** and **1** (see Table 1) revealed that the structures of these three compounds had the same skeleton except for acetylation position of rhamnose. The HMBC correlations of **3** between H-3‴ ($\delta$: 4.91), H-4‴ ($\delta$: 4.87) of rhamnose and carbonyl carbons ($\delta$: 169.6, 169.6) of acetyls (see Fig. 3) suggested that the acetylation positions were C-3‴ and C-4‴. Based on the above evidences, the structure of **3** was elucidated as acacetin-7-*O*-(3,4-*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, and named as Peregrinumin C.

Peregrinumcin A (**4**) was obtained as colourless needle crystal. Its molecular formula was assigned to be $C_{16}H_{19}NO_7$ by analysis of the HR-ESI-MS ($[M+Na]^+$, $C_{16}H_{19}NNaO_7$; Calcd 360.10592). Considering the aromatic UV absorption (268, 262 nm) and chemical shift distribution of 16 carbons observed in the $^{13}$C-NMR (DMSO) spectra, a starting hypothesis for the structure elucidation of **4** was the presence of a prunasin/sambunigrin-type aromatic cyanogenic glycoside. Examination of both $^1$H- and $^{13}$C-NMR spectral data confirms that structure **4** was indeed prunasin derivate.[13] Compared NMR spectra of **4** to prunasin, the difference was one more typical acetyl signals ($\delta_C$: 170.4, 20.7; $\delta_H$: 2.05, s) detected in NMR. In the $^{13}$C-NMR spectrum of **4**, glycosyla-

Table 1. $^1$H- and $^{13}$C-NMR Spectral Data of Peregrinumin A, B and C (**1**—**3**) Observed at 600/125 MHz in DMSO; $\delta$ in ppm, *J* in Hz

| No. | Peregrinumin A (**1**) | | Peregrinumin B (**2**) | | Peregrinumin C (**3**) | |
|---|---|---|---|---|---|---|
| | $\delta_C$ [a] | $\delta_H$ | $\delta_C$ [a] | $\delta_H$ | $\delta_C$ [a] | $\delta_H$ |
| 2 | 163.8 | | 163.9 | | 163.8 | |
| 3 | 103.7 | 6.94 (s, 1H) | 103.8 | 6.94 (s, 1H) | 103.7 | 6.94 (s, 1H) |
| 4 | 182.0 | | 182.0 | | 182.0 | |
| 5 | 161.1 | | 161.1 | | 161.1 | |
| 6 | 99.6 | 6.43 (d, *J*=1.8 Hz, 1H) | 99.6 | 6.46 (d, *J*=1.8 Hz, 1H) | 99.6 | 6.44 (d, *J*=1.8 Hz, 1H) |
| 7 | 162.8 | | 162.8 | | 162.8 | |
| 8 | 94.4 | 6.83 (d, *J*=1.8 Hz, 1H) | 94.9 | 6.84 (d, *J*=2.4 Hz, 1H) | 94.7 | 6.83 (d, *J*=1.8 Hz, 1H) |
| 9 | 156.7 | | 156.9 | | 157.0 | |
| 10 | 105.2 | | 105.4 | | 105.4 | |
| 1′ | 122.7 | | 122.7 | | 122.7 | |
| 2′ | 128.5 | 8.05 (d, *J*=8.4 Hz, 1H) | 128.4 | 8.05 (d, *J*=9.0 Hz, 1H) | 128.4 | 8.04 (d, *J*=8.0 Hz, 1H) |
| 3′ | 114.6 | 7.12 (d, *J*=8.0 Hz, 1H) | 114.6 | 7.12 (d, *J*=9.0 Hz, 1H) | 114.6 | 7.11 (d, *J*=8.0 Hz, 1H) |
| 4′ | 162.4 | | 162.4 | | 162.4 | |
| 5′ | 114.6 | 7.12 (d, *J*=8.0 Hz, 1H) | 114.6 | 7.12 (d, *J*=9.0 Hz, 1H) | 114.6 | 7.11 (d, *J*=8.0 Hz, 1H) |
| 6′ | 128.5 | 8.05 (d, *J*=8.4 Hz, 1H) | 128.4 | 8.05 (d, *J*=9.0 Hz, 1H) | 128.4 | 8.04 (d, *J*=8.0 Hz, 1H) |
| 7′ | 55.6 | 3.88 (br s, 3H) | 55.6 | 3.85 (br s, 3H) | 55.6 | 3.86 (br s, 3H) |
| 5-OH | | 12.9 (br s, 1H) | | 12.9 (br s, 1H) | | 12.9 (br s, 1H) |
| 1″ | 99.4 | 5.16 (d, *J*=7.8 Hz, 1H) | 99.6 | 5.12 (d, *J*=7.2 Hz, 1H) | 99.5 | 5.16 (d, *J*=7.8 Hz, 1H) |
| 2″ | 73.0 | 3.28 (m, 1H) | 73.1 | 3.27 (m, 1H) | 73.0 | 3.28 (dd, *J*=7.2, 6.0 Hz, 1H) |
| 3″ | 76.2 | 3.35 (m, 1H) | 76.2 | 3.28 (m, 1H) | 76.3 | 3.35 (m, 1H) |
| 4″ | 69.5 | 3.19 (dd, *J*=8.4, 8.4 Hz, 1H) | 69.3 | 3.19 (m, 1H) | 69.5 | 3.19 (dd, *J*=8.4, 8.4 Hz, 1H) |
| 5″ | 75.5 | 3.70 (dd, *J*=8.4, 7.8 Hz, 1H) | 75.0 | 3.69 (dd, *J*=8.4, 7.8 Hz, 1H) | 75.3 | 3.67 (dd, *J*=9.6, 6.0 Hz, 1H) |
| 6″ | 65.8 | 3.85 (m, H-6″[b]), 3.60 (dd, *J*=3.6, 1.8 Hz, H-6″[a]) | 65.7 | 3.83 (m, H-6″[b]) 3.57 (dd, *J*=5.4, 11.4 Hz, H-6″[a]) | 65.9 | 3.84 (m, H-6″[b]) 3.60 (dd, *J*=12.0, 6.0 Hz, H-6″[a]) |
| 1‴ | 97.0 | 4.67 (s, 1H) | 96.8 | 4.66 (s, 1H) | 100.0 | 4.64 (s, 1H) |
| 2‴ | 69.1 | 5.04 (dd, *J*=3.6, 1.8 Hz, 1H) | 66.0 | 4.94 (dd, *J*=3.6, 1.2 Hz, 1H) | 67.6 | 3.84 (m, 1H) |
| 3‴ | 71.5 | 4.85 (dd, *J*=10.2, 3.6 Hz, 1H) | 71.8 | 3.83 (dd, *J*=6.6, 3.6 Hz, 1H) | 71.4 | 4.91 (m, 1H) |
| 4‴ | 69.1 | 3.29 (m, 1H) | 73.6 | 4.64 (dd, *J*=10.4, 8.0 Hz, 1H) | 70.6 | 4.87 (dd, *J*=13.5, 3.6 Hz, 1H) |
| 5‴ | 68.3 | 3.64 (dd, *J*=15.6, 6.0 Hz, 1H) | 65.7 | 3.66 (dd, *J*=9.6, 3.6 Hz, 1H) | 65.7 | 3.72 (dd, *J*=9.0, 6.0 Hz, 1H) |
| 6‴ | 17.5 | 1.14 (m, 3H) | 17.1 | 0.90 (d, *J*=6.6 Hz, 3H) | 17.1 | 0.93 (d, *J*=6.6 Hz, 3H) |
| R1 | 169.6 | | 169.8 | | | |
| | 20.6 | 1.99 (br s, 3H) | 20.7 | 1.99 (br s, 3H) | | |
| R2 | 169.4 | | | | 169.6 | |
| | 20.4 | 1.82 (br s, 3H) | | | 20.6 | 1.90 (br s, 3H) |
| R3 | | | 169.7 | | 169.6 | |
| | | | 20.7 | 1.98 (br s, 3H) | 20.6 | 1.80 (br s, 3H) |

*a*) Chemical shifts and multiplicities based on HMQC and HMBC correlation peaks. *b*) C-atoms correlating with the corresponding H-atom.

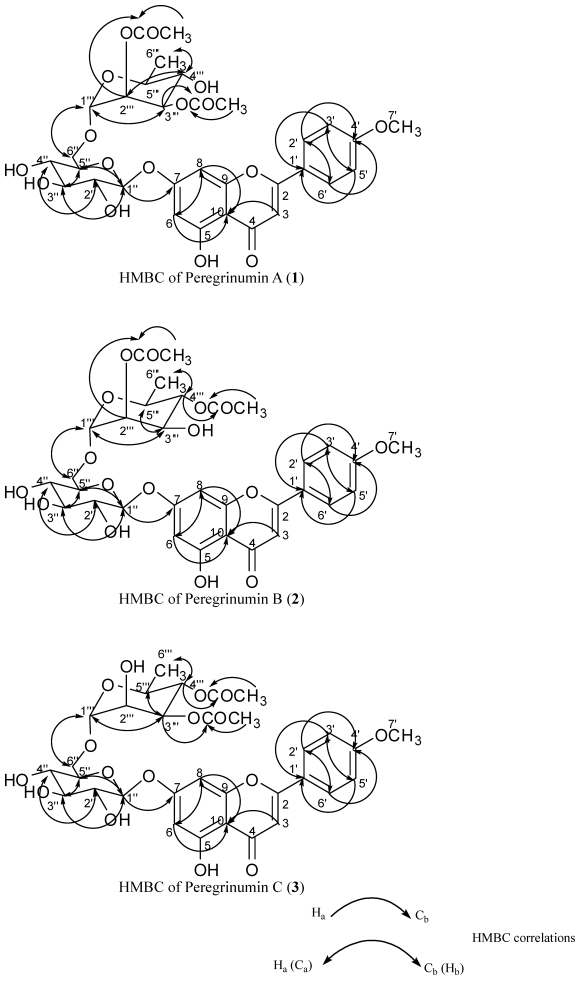


Fig. 3. HMBC of Peregrinumin A, B and C (**1**—**3**)

Table 2. $^{1}$H- and $^{13}$C-NMR Spectral Data of Peregrinumcin A (**4**) Observed at 600/125 MHz in DMSO; $\delta$ in ppm, $J$ in Hz

| Position | $\delta_C$ [a] | $\delta_H$ | Key HMBC [b] |
|---|---|---|---|
| 1 | 118.6 | | |
| 2 | 67.9 | 5.90 (s, 1H) | 1′, 1, 4, 8, 3 |
| 3 | 133.9 | | |
| 4 | 129.0 | 7.46 (ddd, $J$=1.8, 1.8, 9.0 Hz, 1H) | 2, 5, 7, 6, 3 |
| 5 | 127.3 | 7.55 (ddd, $J$=1.8, 8.4, 8.4 Hz, 1H) | 2, 4, 8, 6, 3 |
| 6 | 130.0 | 7.43 (dddd, $J$=1.8, 1.8, 8.4, 8.4 Hz, 1H) | 5, 7, 4, 8 |
| 7 | 127.3 | 7.55 (ddd, $J$=1.8, 8.4, 8.4 Hz, 1H) | 2, 4, 8, 6, 3 |
| 8 | 129.0 | 7.46 (ddd, $J$=1.8, 1.8, 9.0 Hz, 1H) | 2, 5, 7, 6, 3 |
| 1′ | 102.5 | 4.41 (d, $J$=7.8 Hz, 1H) | 2, 2′, 5′, 3′ |
| 2′ | 73.1 | 3.10 (dd, $J$=7.8, 8.4 Hz, 1H) | 3′, 1′ |
| 3′ | 76.3 | 3.17 (t, $J$=9.0 Hz, 1H) | 4′, 5′, 3′ |
| 4′ | 70.0 | 3.14 (t, $J$=13.2 Hz, 1H) | 6′, 4′, 5′, 3′ |
| 5′ | 73.9 | 3.42 (ddd, $J$=1.8, 6.6, 13.8 Hz, 1H) | 6′, 4′, 3′, 1′ |
| 6′ | 63.5 | 4.26 (dd, $J$=12.0, 1.8 Hz, 1H) | 4′, 5′, 1′, 7′ |
| | | 4.16 (dd, $J$=12.0, 6.6 Hz, 1H) | 4′, 5′, 7′ |
| 7′ | 170.4 | | |
| 8′ | 20.7 | 2.05 (s) | 7′ |

*a*) Chemical shifts and multiplicities based on HMQC and HMBC correlation peaks. *b*) C-atoms correlating with the corresponding H-atom.

tion shift was observed for C-2, suggesting that the glucose moiety was fused at C-2 and a acetylation shift (+0.7, −4.5 ppm) were observed for C-6′ and C-5′, suggesting that the 6′-acetylation of glucosyl, which was confirmed by HMBC spectrum correlations from H-6′ ($\delta$: 4.26, 4.16) of glucose to the carbonyl carbons ($\delta$: 170.4) of ethanoyl (see Table 2). The identification of $\beta$-D-glucose as the sugar moiety in **4** can be deduced from the following H, H-coupling pattern (see Table 2 for exact $J$ and multiplicity values): H-1=d (7.8 Hz), H-2=dd (7.8, 8.4 Hz), H-3=t (9.0 Hz), H-4=t (13.2 Hz), H-5=ddd (1.8, 6.6, 13.8 Hz), H-6a=dd (1.8, 12.0 Hz), H-6b=dd (6.6, 12.0 Hz).[13] We make the new cyanogenic glycosides hydrolysised by NaOH (0.1 M) to give the known compoud **4a** (see structure in Fig. 2, ESI-MS, $C_{14}H_{18}O_6N$, $[M+H]^+$, $m/z$ 296.12). Its NMR data was according to 2*R*-prunasin.[13] And the rotation results of **4** and **4a** were $[\alpha]_D^{25}=-35°$ ($c$=0.1950, $CH_3OH$) and −31° ($c$=0.2100, $CH_3OH$), therefore, the structure of **4** determined to be (2*R*)-$\beta$-D-(6-*O*-acetyl)-glucosyl-2-phenylacetonitrile, and named as Peregrinumcin A.

Table 3. Effect of **1**—**4** on NO Activities Induced by LPS

| Group | Dosage ($\mu$g/ml) | NO concentration | Inhibition ratio (%) |
|---|---|---|---|
| DMSO [a] | 0.5% | | |
| LPS [a] | 1.0 | 7.9±1.9 | |
| Aminoguanidine [b] | 50 mM | 1.9±0.1** | 75 |
| **1** | 100 | 3.5±0.3* | 56 |
| **1** | 50 | 6.5±1.0 | 18 |
| **1** | 25 | 7.1±0.5 | 10 |
| **2** | 100 | 4.3±0.4* | 45 |
| **2** | 50 | 5.7±0.3 | 28 |
| **2** | 25 | 7.2±0.1 | 9 |
| **3** | 100 | 3.6±0.5* | 54 |
| **3** | 50 | 5.3±0.3 | 33 |
| **3** | 25 | 6.3±0.1 | 20 |
| **4** | 100 | 4.2±0.2* | 49 |
| **4** | 50 | 5.4±0.5 | 32 |
| **4** | 25 | 6.4±0.2 | 20 |

*a*) Blank control. *b*) Pos. control. ** $p<0.01$, * $p<0.05$.

Table 4. Effect of **1**—**4** on NF-$\kappa$B Activities Induced by LPS

| Group | Dosage ($\mu$g/ml) | Mean ($\times 10^4$) | Inhibition ratio (%) |
|---|---|---|---|
| DMSO [a] | 0.5% | | |
| LPS [a] | 10 | 35.1±0.6 | |
| LGT [b] | 10 | 9.7±1.8** | 72 |
| **1** | 100 | 32.5±9.1 | 7.5 |
| **2** | 100 | 25.7±11.1 | 27 |
| **2** | 50 | 53.4±5.2 | 0 |
| **3** | 100 | 21.6±5.7 | 38 |
| **3** | 50 | 43.5±4.1 | 0 |
| **4** | 100 | 28.8±10.1 | 18 |

*a*) Blank control. *b*) Pos. control. ** $p<0.01$.

Compounds **1**—**4** were tested for effects of NO and nuclear factor (NF)-$\kappa$B activity on RAW 264.7 and pNF-$\kappa$B-luc-293 cells. Among the compounds tested, **1**—**4** were shown good inhibitory activities on nitric oxide (NO) production induced by LPS at dose of 100 $\mu$g/ml, and 50 $\mu$g/ml (Table 3). But only **2** and **3** were shown weak effects on NF-$\kappa$B activity at dose of 100 $\mu$g/ml (Table 4).

**Experimental**

**General** Optical rotations: Perkin Elmer polarimeter 341 Polarimeter. IR: Bruker Vector 22. UV: Shimadzu UV-265. 1D- and 2D- (HMQC, HMBC, COSY, NOESY) NMR spectra: Bruker 600 NMR spectrometer. HR-ESI-MS: Q-TOF micro mass spectrometer. Silica gel: 200—300 mesh,

Yantai Chemical Plant, Yantai, P. R. China. Silica gel H: 10—40 $\mu$m, Yantai Chemical Plant, Yantai, P. R. China. Sephadex LH-20: Pharmacia. TCL: HSGF254 precoated silica gel plates, 10—40 $\mu$m, Yantai Chemical Plant, Yantai, P. R. China.

**Plant Material** Dried, whole plants of *Dracocephalum peregrinum* collected in Xinjiang autonomous region, P. R. China, in August 2007. The plant material was authenticated by Prof. Han-min Zhang. Voucher specimens (NO. DP070901) are deposited at the Department of Phytochemistry, School of Pharmacy, the Second Military Medical University, China.

**Extraction and Isolation** The air-dried and powdered whole plants of *Dracocephalum peregrinum* (10 kg) were refluxed with ethanol (75% v/v) three times, 2 h for each. After removal of the solvent under reduced pressure, the residue was partitioned sequentially with petroleum ether, chloroform, ethyl acetate, *n*-butanol to give four portions. The ethyl acetate portion (98 g) was subjected to silica gel column chromatography (200—300 mesh, 1.0 kg), eluting with the gradient $CHCl_3$ : $CH_3OH$ (30 : 1—15 : 1—10 : 1—5 : 1—2 : 1—1 : 1), and gave six fractions: I (10 g), II (11 g), III (12 g), IV (15 g), V (13 g), VI (11 g).

Fraction III (12 g) was subject to silica gel column chromatography (200—300 mesh, 100 g), eluting with the gradient $CHCl_3$ : $CH_3OH$ (30 : 1—15 : 1—10 : 1—5 : 1—2 : 1—1 : 1), to afford six subfractions (Ea1—Ea6), Ea4 was purified over silica gel column chromatography, eluting with $CHCl_3$ : $CH_3OH$ (20 : 1—10 : 1—5 : 1), to yield compound: **1** (12 mg). Fraction IV (15 g) was purified over silica gel column chromatography, eluting with $CHCl_3$ : $CH_3OH$ (15 : 1—8 : 1—4 : 1) to afford three subfractions (Eb1—Eb3), Eb2 subfraction purified over Sephadex LH-20 for two times, to yield compounds: **2** (15 mg) and **3** (13 mg). Fraction V (13 g) was purified over silica gel column chromatography, eluting with $CHCl_3$ : $CH_3OH$ (8 : 1—4 : 1—2 : 1—1 : 1) to afford four subfractions (Ec1—Ec4), and Ec3 was purified over Sephadex LH-20 several times, to yield compound: **4** (20 mg).

Peregrinumin A (=Aacacetin-7-*O*-(2,3-*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, **1**): Yellow amorphous powder ($CH_3OH$). $[\alpha]_D^{25}=-78°$ ($c$=0.1250, $CH_3OH$). IR (KBr) $cm^{-1}$: 3421, 2923, 2852, 1741, 1656, 1605. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 205 (3.49), 269 (1.93), 323 (2.37). $^1$H-NMR (600 MHz, DMSO) and $^{13}$C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS $m/z$: 699.18948 ($[M+Na]^+$ $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumin B (=Acacetin-7-*O*-(2,4-*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, **2**): Yellow amorphous powder ($CH_3OH$). $[\alpha]_D^{25}=-77°$ ($c$=0.1400, $CH_3OH$). IR (KBr) $cm^{-1}$: 3421, 2924, 1733, 1655, 1605. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 204 (3.64), 269 (1.93), 289 (2.37), 326 (2.34).$^1$H-NMR (600 MHz, DMSO) and $^{13}$C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS $m/z$: 699.18856 ($[M+Na]^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumin C (=Acacetin-7-*O*-(3,4-*O*-diacetyl-$\alpha$-L-rhamnosyl)-(1→6)-$\beta$-D-glucoside, **3**): Yellow amorphous powder ($CH_3OH$). $[\alpha]_D^{25}=-90°$ ($c$=0.1500, $CH_3OH$). IR (KBr) $cm^{-1}$: 3421, 2924, 1733, 1656, 1605. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 203 (4.87), 268 (4.19), 323 (4.65), 360 (2.18).$^1$H-NMR (600 MHz, DMSO) and $^{13}$C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS $m/z$: 699.18904 ($[M+Na]^+$ $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumcin A (=(2*R*)-$\beta$-D-(6-*O*-Acetyl)-glucosyl-2-phenylacetonitrile, **4**): Colourless needle crystal ($CH_3OH$). $[\alpha]_D^{25}=-35°$ ($c$=0.1950, $CH_3OH$). IR (KBr) $cm^{-1}$: 3417, 3068, 3036, 2917, 1734, 1248, 1079. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 268 (0.91), 262 (1.02), 256 (1.14), 204 (4.03) nm. $^1$H-NMR (600 MHz, DMSO) and $^{13}$C-NMR (150 MHz, DMSO): Table 2. HR-ESI-MS $m/z$: 360.10552 ($[M+Na]^+$, $C_{16}H_{19}NNaO_7$; Calcd 360.10592).

**Cell Lines Used** RAW 264.7, 293, were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and maintained in media recommended by the suppliers supplemented with 10% fetal bovine serum (FBS) (Gibco, Paisley, U.K.), and streptomycin (100 mg/ml) in a humidified 5% $CO_2$ atmosphere at 37 °C.

**Measurement of NO in LPS Activated Macrophages** RAW 264.7 macrophages were seeded onto 24-well cell culture plates ($10^5$ cells/well). The cells were co-incubated with drugs and LPS (1 $\mu$g/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the culture supernatants with Griess reagent in RAW 264.7. Aliquots of supernatants (100 $\mu$l) were incubated, in sequence, with 50 $\mu$l 1% sulphanilamide and 50 $\mu$l 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using a microtiter plate reader.

**NF-$\kappa$B Activation Assay** NF-$\kappa$B activation was assayed using stable pNF-$\kappa$B-luc-293 cells (Baran *et al.*, 2007). The cells seeded in 96-well plate at $1.0\times10^5$ cells/well were pre-treated with test drugs for 15 min, and then incubated with 10 $\mu$g/ml recombinant human tumor necrosis factor (TNF-$\alpha$) for 6 h. The cells were lysed, and luciferase activity was measured with a luciferase assay system (Promega, WI, U.S.A.).

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