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Phytochemistry 69 (2008) 1425–1433
PHYTOCHEMISTRY
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Stereochemistry of flavonoidal alkaloids from *Dracocephalum rupestre*

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Received 16 May 2007; received in revised form 13 December 2007
Available online 6 March 2008

Abstract

Phytochemical studies on the aerial parts of *Dracocephalum rupestre* led to the isolation of four groups of flavonoidal alkaloids, dracocephins A–D. They were elucidated as conjugates of flavanone with pyrrolidin-2-one on the basis of extensive spectroscopic analysis. The two stereogenic centers rendered each group of the dracocephins as two pairs of enantiomers simultaneously. All of the sixteen isomers were separated successfully by chiral HPLC and their stereochemical features were determined by their CD data and single-crystal X-ray diffraction analysis of one stereoisomer. The additive relation of the chiroptical contributions resulting from the two stereogenic centers was generalized. The CD contribution of the chiral carbon in the pyrrolidin-2-one ring was proposed by subtraction of their respective contributions.

Keywords: *Dracocephalum rupestre* Labiatae; Dracocephins A–D; Flavonoidal alkaloids; HPLC–CD coupling analysis; Absolute configuration

1. Introduction

Flavonoids are a large family of compounds sharing the same basic structure, i.e., two benzene rings (A and B) linked through a heterocyclic pyran or pyrone ring (C) in the middle. They are subdivided primarily according to the varieties of ring C and the position of ring B. Flavonoids have been reported to exhibit a wide range of biological effects both in vitro and in vivo (Harborne, 1994; Harborne and Williams, 2000; Cook and Samman, 1996). Wide applications have been found in the treatment of cardiovascular diseases and peripheral circulation disorders, or as hepatoprotective and anti-inflammatory agents (Middleton et al., 2000; Hollman and Katan, 1999). Most of these biological significances were ascribed to their antioxidant properties (Heim et al., 2002). The plant species *Dracocephalum rupestre* Hance is widely distributed throughout western China (Wu and Li, 1977), which has been applied in the treatment of various ailments, such as cold, cough, icterohepatitis, laryngalgia (Jiangsu New Medical College, 1977). Previous studies showed that flavonoids were the main components responsible for the cardiovascular protective effects (Ren et al., 2005, 2007). Our ongoing investigation on the trace alkaloidal fraction of *D. rupestre* led to the isolation of four groups of flavonoidal alkaloids, named dracocephins A–D (Fig. 1).

These nitrogen-containing flavonoids belong to a very small class of natural products. So far, about ten natural nitrogen-containing flavonoids have been found such as ficine and isoficine from *Ficus pantoniana* (Johns et al., 1965), phyllospadine from *Phyllosphadix iwatensis* (Takagi et al., 1980), vochsine from *Vochysia guaianensis* (Baudouin et al., 1983), lilaline from *Lilium candidum* (Masterova et al., 1987), aquiledine and isoaquiledine from *Aquilegia ecalcarata* (Chen et al., 2001), lotthanongine from *Trigonostemon reidioide* (Kanchanapoom et al., 2002), and prolinalin A and B from the cocoon shell of the silkworm, *Bombyx mori* (Hirayama et al., 2006). The

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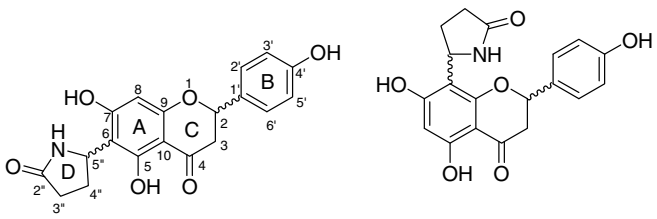
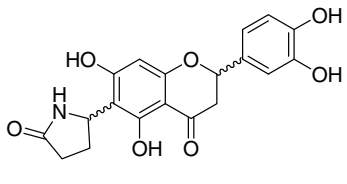
doi:10.1016/j.phytochem.2008.01.013

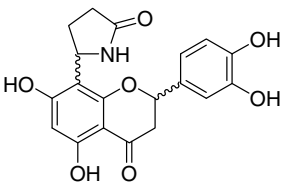


1 2*R*,5"*S*
2 2S,5"*S*
3 2*S*,5"*R*
4 2*R*,5"*R*

5 2*S*,5"*S*
6 2R,5"*S*
7 2*R*,5"*R*
8 2*S*,5"*R*



9 2*R*,5"*S*
10 2S,5"*S*
11 2*S*,5"*R*
12 2*R*,5"*R*



13 2*S*,5"*S*
14 2R,5"*S*
15 2*R*,5"*R*
16 2*S*,5"*R*

Fig. 1. Flavonoidal alkaloids isolated from the aerial parts of *D. rupestre*.

nitrogen atom in the structures is mostly present as tertiary or quaternary in a five- or six-membered heterocyclic ring. The dracocephins A–D from *D. rupestre* were the conjugates of flavanone with pyrrolidin-2-one. Using the HPLC–CD coupling technique (Bringmann et al., 2001a,b, 2004; Mutanyatta et al., 2005), each group of the dracocephins was verified to be the mixtures of two diastereoisomeric pairs of enantiomers. The individual enantiomers were prepared successfully by HPLC on a chiral phase, and were configurationally assigned based on the measurement of CD data as well as the single-crystal X-ray diffraction analysis of one of the 16 enantiomers. The additive relation of the chiroptical contributions resulting from the two stereocenters was generalized. The CD contribution of the chiral carbon in the pyrrolidin-2-one ring was proposed by subtraction of their respective contributions.

2. Results and discussion

Dracocephins A (**1**–**4**) was obtained as white needles, which showed a single spot as detected by silica gel and polyamide TLC. The molecular formula was determined as $C_{19}H_{17}NO_6$ by the positive mode HRESIMS ($[M+H]^+$ found at *m/z* 356.1123, calcd. 356.1134). The UV spectrum was consistent with that of a hydroxyflavanone with maxima at 343 (sh) and 290 nm (Shanghai Institute of Materia Medica, 1981). The IR spectrum showed absorption bands for the hydroxy (3374 cm^{-1}) and carbonyl (1635 cm^{-1}) groups. Dracocephins A was found to be a racemic mixture as demonstrated by subsequent chiral HPLC, but their NMR spectra showed almost no difference, so the planar structure was elucidated by the same NMR data. The ^1H NMR spectrum (Table 1) revealed a portion of a flavanone skeleton, corresponding to naringenin (Fatope et al., 2003), as deduced from a typical ABX spin system (δ_H 3.21, *dd*, $J = 17.1$, 11.4 Hz; 2.71, *dd*, $J = 17.1$, 2.3 Hz; and 5.42, *dd*, $J = 11.4$, 2.3 Hz), a set of

Table 1
^1H and ^{13}C NMR spectroscopic data for dracocephins A (**1**–**4**) and B (**5**–**8**)[a]

Position	Dracocephins A (**14**)		Dracocephins B (**5**–**8**)			
			Diastereoisomer I		Diastereoisomer II	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	78.4	5.42 (*dd*, 11.4, 2.3)	78.7	5.40 (*dd*, 12.9, 2.7)	78.5	5.39 (*dd*, 12.9, 2.7)
3	42.0	3.21 (*dd*, 17.1, 11.4) 2.71 (*dd*, 17.1, 2.3)	42.2	3.16 (*dd*, 16.9, 12.9) 2.68 (*dd*, 16.9, 2.7)	41.9	3.17 (*dd*, 17.0, 12.9) 2.71 (*dd*, 17.0, 2.7)
4	196.7		196.2		196.7	
5	161.6		162.5		162.4	
6	108.9		96.3	5.89 (*s*)	96.0	5.99 (*s*)
7	164.9		165.5		166.0	
8	94.9	5.97 (*s*)	109.2		108.7	
9	161.5		161.0		160.8	
10	101.6		101.3		101.7	
1′	128.9		129.2		129.0	
2′	128.3	7.30 (*d*, 8.2)	128.3	7.30 (*d*, 8.5)	128.3	7.30 (*d*, 8.5)
3′	115.3	6.79 (*d*, 8.2)	115.4	6.78 (*d*, 8.5)	115.4	6.79 (*d*, 8.5)
4′	157.8		157.8		157.9	
5′	115.3	6.79 (*d*, 8.2)	115.4	6.78 (*d*, 8.5)	115.4	6.79 (*d*, 8.5)
6′	128.3	7.30 (*d*, 8.2)	128.3	7.30 (*d*, 8.5)	128.3	7.30 (*d*, 8.5)
2″	176.9		176.8		176.8	
3″	30.7	2.05 (*m*, 2H)	30.7	2.02 (*m*, 2H)	30.7	2.02 (*m*, 2H)
4″	25.5	2.17 (*m*, 2H)	25.5	2.23 (*m*, 2H)	25.6	2.21 (*m*, 2H)
5″	46.5	5.07 (*dd*, 9.0, 4.8)	46.8	5.05 (*dd*, 9.0, 4.9)	46.9	5.04 (*dd*, 9.0, 4.9)
NH		7.41 (*s*)		7.47 (*s*)		7.49 (*s*)

[a] Recorded in DMSO-d_6 at 600 or 150 MHz for ^1H and ^{13}C NMR, respectively. δ in ppm and *J* in Hz are in the parentheses.

AA′BB′ protons (δ_H 6.79 and 7.30, each d, $J = 8.2$ Hz), as well as a proton signal for H-8 (δ_H 5.97, *s*) and three exchangeable proton signals at δ_H 12.71 (OH-5), 10.93 (OH-7), and 9.50 (OH-4′). The ^{13}C NMR spectrum (Table 1) displayed 19 signals, from which one carbonyl resonance, 12 aromatic carbons, and two alicyclic carbons for a naringenin moiety were discerned (Fatope et al., 2003).

The presence of a pyrrolidone moiety was determined by the resonances at δ_H 7.41 (NH), 5.07 (*dd*, $J = 9.1$, 4.8 Hz, H-5″), 2.05 (*m*, 2H), and 2.17 (*m*, 2H) in the ^{1}H NMR spectrum and the four carbon resonances at δ_C 176.9, 30.7, 25.5, and 46.5 in the ^{13}C NMR spectrum. The HMBC correlations from H-5″ to C-6 (δ_C 108.9) and C-7 (δ_C 164.9), as well as the cross peaks between OH-5 and C-6 allowed the attachment of the 2-oxopyrrolidin-5-yl group at C-6 (Fig. 2). Accordingly, the planar structure of the compound was determined to be 5,7,4′-trihydroxy-6-(2-oxopyrrolidin-5-yl)-flavanone, and was named dracocephins A.

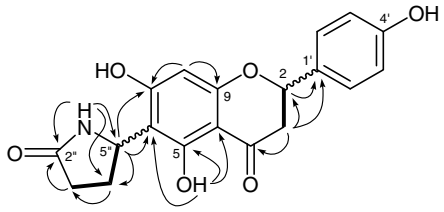


Fig. 2. Key ^{1}H-^{1}H COSY (—) and HMBC (H → C) correlations of dracocephins A.

For the investigation of the existence of possible enantiomers of dracocephins A, a method for the enantiomeric resolution by HPLC on a chiral phase was developed. Four HPLC–UV peaks corresponding to two positive signals and two negative signals in the CD trace were observed. Integration of the signals obtained gave a 1:1:1:1 ratio, demonstrating the fully racemic character of dracocephins A (Fig. 3). The separation of dracocephins A1–A4 (**1**–**4**, corresponding to peaks 1–4, respectively) was achieved by using the analytical chiralpak AS–H column. Two pairs of mirror-imaged CD curves of **1**–**4** revealed the enantiomeric nature of **1**–**3** and **2**–**4** (Fig. 3).

In the CD spectrum of **1**, an obvious negative Cotton effect (CE) at 332 nm (Δε −2.2) for $n \rightarrow \pi^*$ absorption band and a positive CE at 292 nm (Δε +10.2) for $\pi \rightarrow \pi^*$ absorption band established an *R*-configuration at C-2 (Caccamese et al., 2005; Gaffield, 1970; Slade et al., 2005). By combination with its X-ray structure (Fig. 4), the other chiral carbon C-5″ was determined to be *S*-configured. Comparison of the CD spectrum of **1** to that of 2*R*-naringenin, the strong positive CE in the 203 nm (Δε +13.2) region apparently originated from the chirality of 5″-*S*.

Dracocephins A3 (**3**), the enantiomer of **1**, was determined to be of the 2*S*,5″*R*-configuration, for the mirror-imaged CD curve with that of **1** exhibited a positive CE at 332 nm (Δε +2.2), a negative CE at 292 nm (Δε −10.2), and a negative CE at 203 nm (Δε −13.8), respectively. For dracocephins A2 (**2**), a positive CE at 332 nm

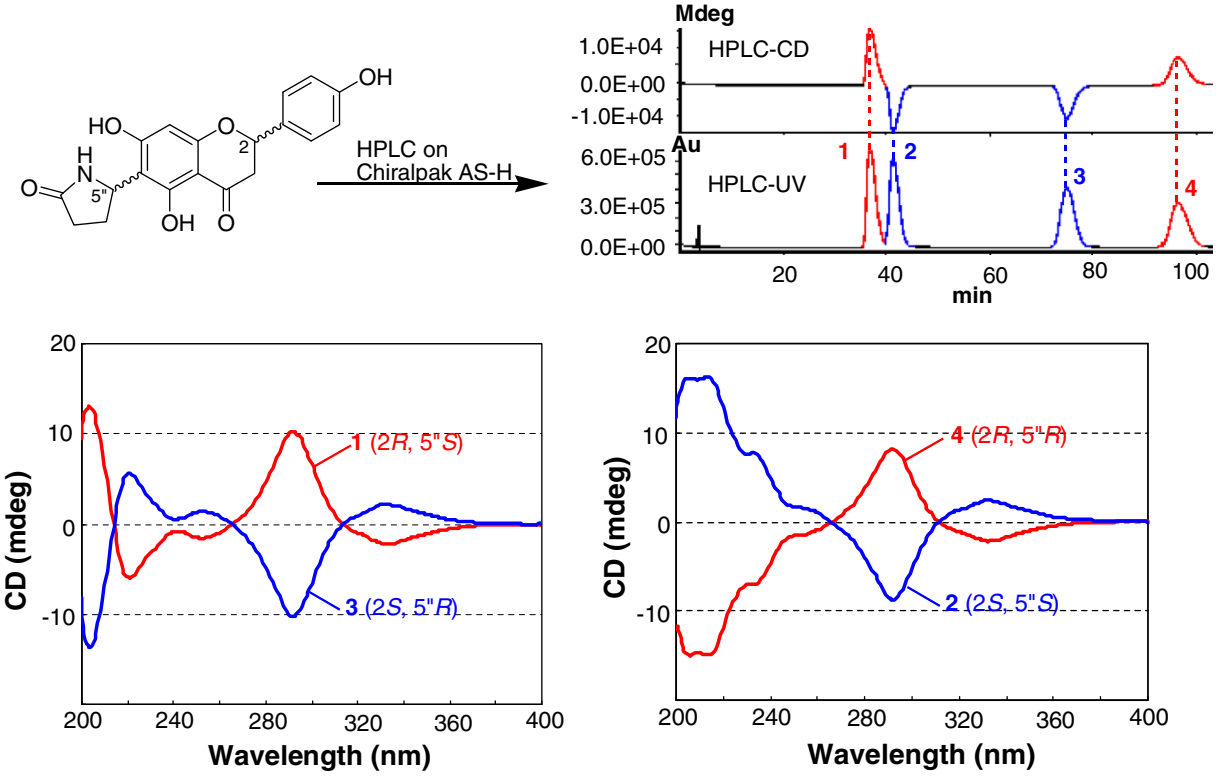


Fig. 3. HPLC–UV and HPLC–CD analysis of dracocephins A using a chiral phase and assignment of the absolute configurations of dracocephins A1–A4 (**1**–**4**) by CD.

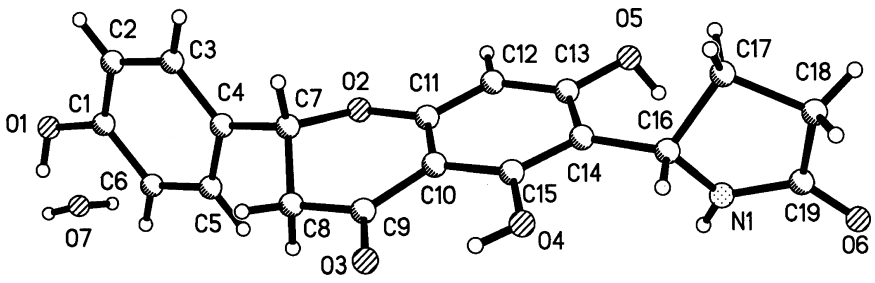


Fig. 4. X-Ray structure of dracocephins A1 (**1**).

(Δε +2.4) and a negative CE at 292 nm (Δε −8.7) established an *S*-configuration at C-2, a positive CE at 205 nm (Δε +16.1) confirmed an *S*-configuration at C-5″. While dracocephins A4 (**4**), the enantiomer of **2**, was assigned to be 2*R*,5″*R*-configured.

The CD curves of **1**–**4** indicated clearly that the chiroptical contributions of the two stereogenic carbons behaved largely additively, and the chiral chromophore center at C-2 dominated the CD spectra. The CD contribution of C-5″ can be clarified by subtracting one CD spectrum from that of the other stereoisomer with the same configuration at C-2. As both **1** and **4** had 2*R*-configuration, when one subtracts the CD spectrum of **4** from that of **1**, the resulted differential curve indicated the CD contribution of 5″-*S*, enhanced by a factor of 2, and calculatorily eliminated the 2*R* contributions. In the same way, if one subtracts the spectrum of **1** from that of **4**, the resulted curve would indicate the 5″-*R* contribution. Following the same method, by subtracting the CD spectrum of **2** from that of **3**, the CD contribution of 2-*S* should also be eliminated, the same mirror-imaged arithmetically "isolated" CD curves for 5″*R* and 5″*S* were obtained. The arithmetically "isolated" CD contribution of 5″-*S* provides a strong positive CE at 205 nm (Δε +27.7) and two weaker positive CEs at 235 nm (Δε +4.9) and 292 nm (Δε +2.1). Accordingly, the "isolated" CD curve of 5″-*R* displayed three negative CD signs at 205 nm (Δε −27.4), 235 nm (Δε −4.9), and 292 nm (Δε −2.1), respectively (Fig. 5).

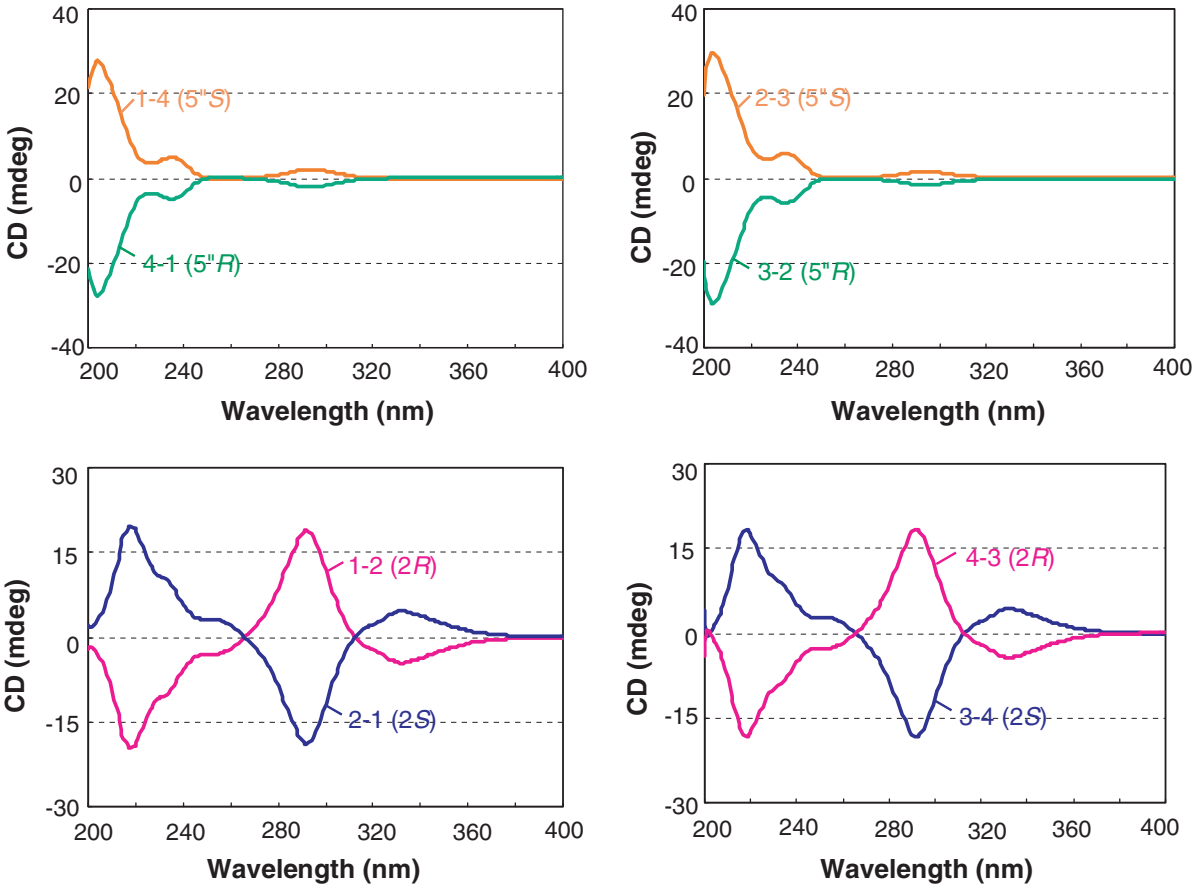


Fig. 5. The arithmetically "isolated" CD curves for C-5″ (upper) and C-2 (lower).

Following an analogous argumentation, by subtracting the CD spectrum of **2** from that of **1**, the CD contribution of 5″-*S* would be eliminated and what remained were the "isolated" CD curves for 2-*R* and 2-*S*. The same method was suitable for the subtraction of the CD spectra of **3** and **4**. The "isolated" CD curves thus obtained were consistent with 2*R*- or 2*S*-naringenin (Fig. 5) (Caccamese et al., 2005; Gaffield, 1970; Slade et al., 2005).

Dracocephins B (**5**–**8**) was also obtained as white needles and has the same molecular formula as dracocephins A by HRESIMS ($[M+H]^+$ at *m*/*z* 356.1135). The split of some ^1H and ^{13}C NMR signals into doublet suggested that dracocephins B was a diastereoisomeric mixture. This assumption was further confirmed by HPLC–UV investigations using a common C_{18} reversed-phase (ODS) column, yielding two peaks with nearly 1:1 ratio. The two diastereoisomers corresponding to the two peaks were prepared and their ^1H and ^{13}C NMR spectra were recorded separately (Table 1). Comparison of the ^1H and ^{13}C NMR data (Table 1) of dracocephins B with those of dracocephins A suggested that both compounds have a similar structure except for the substituting position of the pyrrolidone ring. The HMQC data indicated that C-6 was a methine while C-8 was a quaternary carbon, suggesting that the pyrrolidone moiety might be at C-8. HMBC correlations from H-5″ (δ_H 5.05) to C-8 (δ_C 109.2), and from OH-5 (δ_H 12.30) to C-6 (δ_C 96.3) confirmed the linkage of pyrrolidone moiety at C-8. Therefore, the compound was deduced to be 5,7,4′-trihydroxy-8-(2-oxopyrrolidin-5-yl)-flavanone, named dracocephins B.

Using the chiral HPLC–CD coupling technique, four stereoisomers dracocephins B1–B4 (**5**–**8**) were separated from dracocephins B. Based on the above conclusion about the CD contribution of C-5″, the absolute configurations of the four stereoisomers were determined. The absolute configuration of dracocephins B1 (**5**) was assigned to be 2*S*,5″*S*, according to a positive CE at 330 nm, a negative CE at 292 nm, and a positive CE at 205 nm in the CD spectrum. The mirror-imaged CD curve of dracocephins B3 (**7**) to **5** indicated the 2*R*,5″*R*-configuration for **7**. A negative CE at 330 nm, a positive CE at 292 nm, and a positive CE at 205 nm determined the absolute configuration of dracocephins B2 (**6**) as 2*R*,5″*S*. Accordingly, dracocephins B4 (**8**), the enantiomer of **6**, was determined to be of 2*S*,5″*R*-configurations (Fig. 6).

Dracocephins C (**9**–**12**) was obtained as a white amorphous powder with a molecular formula of $C_{19}H_{17}NO_7$ by the HRESIMS ($[M+H]^+$ at *m*/*z* 372.1041, calcd. 372.1083), which was 16 amu more than that of the dracocephins A and B. Similar to dracocephins A, although it was demonstrated to be a mixture of two diastereoisomeric pairs of enantiomers by subsequent chiral HPLC analysis, their NMR spectra showed almost no difference. The ^1H NMR spectrum (Table 2) displayed the presence of an

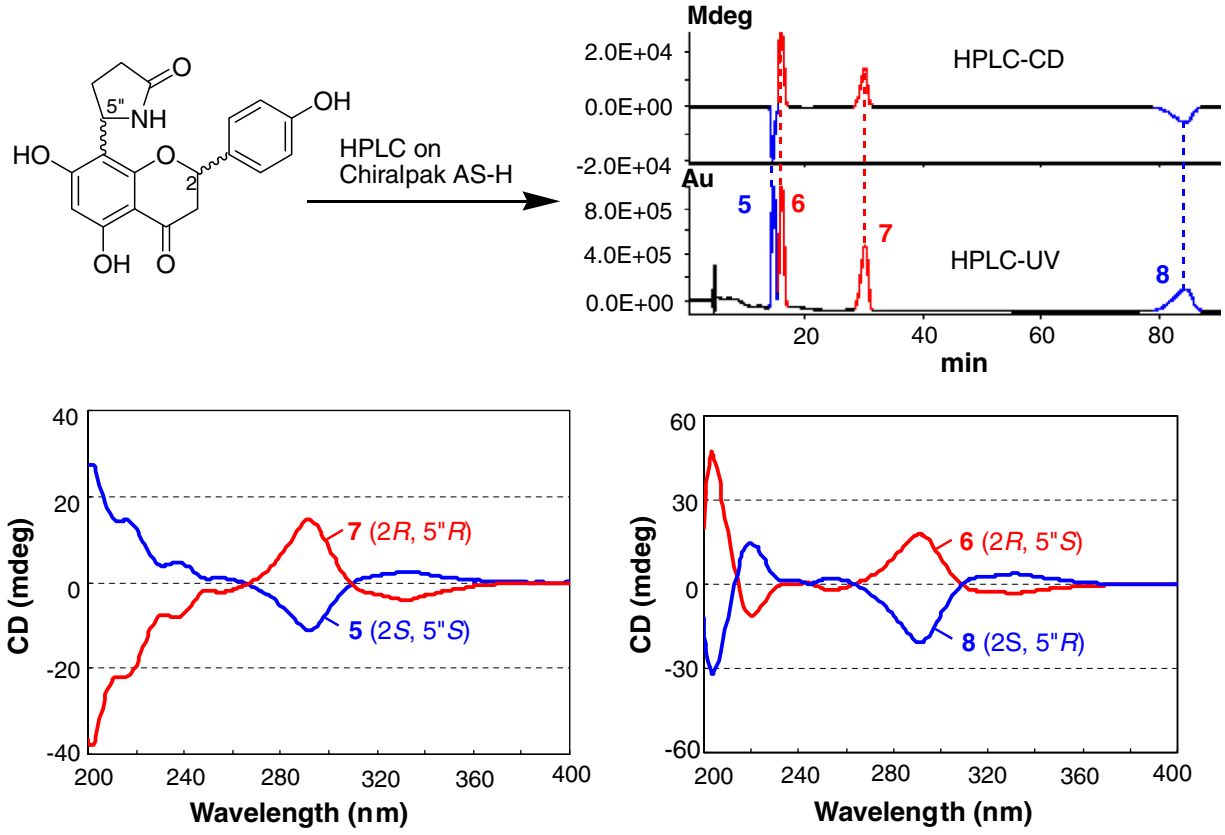


Fig. 6. HPLC–UV and HPLC–CD analysis of dracocephins B using a chiral phase and assignment of the absolute configurations of dracocephins B1–B4 (**5**–**8**) by CD.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for dracocephins C (**9**–**12**) and D (**13**–**16**)[a]

Position	Dracocephins C (**9**–**12**)		Dracocephins D (**13**–**16**)			
			Diastereoisomer I		Diastereoisomer II	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	78.5	5.36 (*dd*, 12.2, 3.0)	79.0	5.35 (*dd*, 12.4, 2.9)	78.6	5.34 (*dd*, 12.5, 2.9)
3	42.1	3.17 (*dd*, 17.1, 12.2) 2.71 (*dd*, 17.1, 3.0)	42.1	3.14 (*dd*, 17.1, 12.4) 2.72 (*dd*, 17.1, 2.9)	42.2	3.07 (*dd*, 17.1, 12.5) 2.70 (*dd*, 17.1, 2.9)
4	196.9		197.1		196.5	
5	161.7		162.4		162.4	
6	108.9		96.1	5.97 (*s*)	96.1	5.93 (*s*)
7	165.0		165.6		165.0	
8	94.9	5.96 (*s*)	108.9		108.7	
9	161.5		160.9		160.7	
10	101.7		101.9		101.6	
1′	129.5		129.5		129.8	
2′	114.4	6.75 (*s*)	114.6	6.86 (*s*)	114.2	6.86 (*s*)
3′	145.4		145.4		145.3	
4′	145.9		145.9		145.8	
5′	115.5	6.74 (*m*)	115.6	6.74 (*m*)	115.6	6.74 (*m*)
6′	118.0	6.73 (*m*)	118.0	6.74 (*m*)	118.0	6.74 (*m*)
2″	176.9		176.9		176.9	
3″	30.7	2.04 (*m*, 2H)	30.6	2.02 (*m*, 2H)	30.7	2.03 (*m*, 2H)
4″	25.5	2.28 (*m*)	25.5	2.05 (*m*) 2.10 (*m*)	25.6	2.04 (*m*) 2.14 (*m*)
5″	46.5	5.07 (*dd*, 9.1, 4.8)	46.7	5.05 (*dd*, 9.5, 4.9)	46.9	5.05 (*dd*, 9.5, 4.9)
NH		7.47 (*s*)		7.49 (*s*)		7.47 (*s*)

[a] Recorded in DMSO-d_6 at 600 or 150 MHz for ^{1}H and ^{13}C NMR, respectively. δ in ppm and *J* in Hz are in the parentheses.

eriodictyol unit (Miyake et al., 1997), as revealed by a trisubstituted benzene ring (δ_H 6.73–6.86, 3H) and an ABX system consisting of a methine proton (δ_H 5.36, *dd*, $J = 12.2$, 3.0 Hz, H-2) coupled with the two methylene protons (δ_H 3.17, *dd*, $J = 17.1$, 12.2 Hz and 2.71, *dd*, $J = 17.1$, 3.0 Hz; H_2-3). This partial structure was further confirmed by the ^{13}C NMR data (Miyake et al., 1997). The presence of a pyrrolidone moiety was deduced from the similar NMR data as those of dracocephins A, indicating that the compound was a conjugate of eriodictyol and pyrrolidone. The HMBC correlations of H-5″ (δ_H 5.07) to C-6 (δ_C 108.9) established the placement of the pyrrolidone moiety at C-6, which was also verified by the downfield shift of C-6 (δ_C 108.9) as compared with C-8 (δ_C 94.9). Therefore, the planar structure of this group of compounds was determined as 5,7,3′,4′-tetrahydroxy-6-(2-oxopyrrolidin-5-yl)-flavanone, namely dracocephins C.

The four stereoisomers, dracocephins C1–C4 (**9**–**12**) were prepared by using the chiral HPLC method as mentioned above. The CD analysis allowed us to determine the absolute configuration of the four stereoisomers unambiguously. Dracocephins C1 (**9**) was assumed to be 2*R*,5″*S*-configured according to a negative CE at 330 nm, a positive CE at 292 nm, and a positive CE at 205 nm in the CD spectrum. Dracocephins C3 (**11**), the enantiomer of **9**, was assigned as 2*S*,5″*R*-configured. For dracocephins C2 (**10**), the positive CE at 330 nm, negative CE at 292 nm, and positive CE at 205 nm were in agreement with the 2*S*,5″*S*-configurations. Dracocephins C4 (**12**), the enantiomer of **10**, could readily be determined as 2*R*,5″*R*-configured (Fig. 7).

The HRESIMS of dracocephins D (**13**–**16**), a white amorphous solid, supported a molecular formula of $C_{19}H_{17}NO_7$ ($[M + H]^+$ at *m*/*z* 372.1055). Similar to dracocephins B, some of the signals in the ^{1}H and ^{13}C NMR spectra of dracocephins D showed pairs of signals. In order to clarify the structure unequivocally, the two diastereoisomers were prepared using a C_{18} column and their NMR spectra were measured separately (Table 2). The 1D and 2D NMR data of dracocephins D exhibited high similarity with those of dracocephins C. The only difference between these two compounds was the substituting position of the pyrrolidone ring. HMBC correlations from H″-5 (δ_H 5.05) to C-8 (δ_C 108.9) and from OH-5 (δ_H 12.26) to C-6 (δ_C 96.1) confirmed that the pyrrolidone moiety was linked to C-8. Accordingly, dracocephins D was assigned as 5,7,3′,4′-tetrahydroxy-8-(2-oxopyrrolidin-5-yl)-flavanone.

Preparation of the four single stereoisomers of dracocephins D was achieved by using a chiral HPLC column. The absolute configurations of dracocephins D1–D4 (**13**–**16**) were again determined unequivocally by analysis of their CD spectra. Dracocephins D1 (**13**) could be assigned as 2*S*,5″*S*-configured according to a positive CE at 330 nm, a negative CE at 292 nm, and a positive CE at 205 nm. Dracocephins D3 (**15**), the enantiomer of **13**, should be 2*R*,5″*R*-configured. Dracocephins D2 (**14**) was 2*R*, 5″*S*-configured as deduced from a negative CE at 330 nm, a positive CE at 290 nm, and a positive CE at 205 nm. Dracocephins D4 (**16**), the enantiomer of **14**, was in agreement with 2*S*,5″*R*-configurations (Fig. 8).

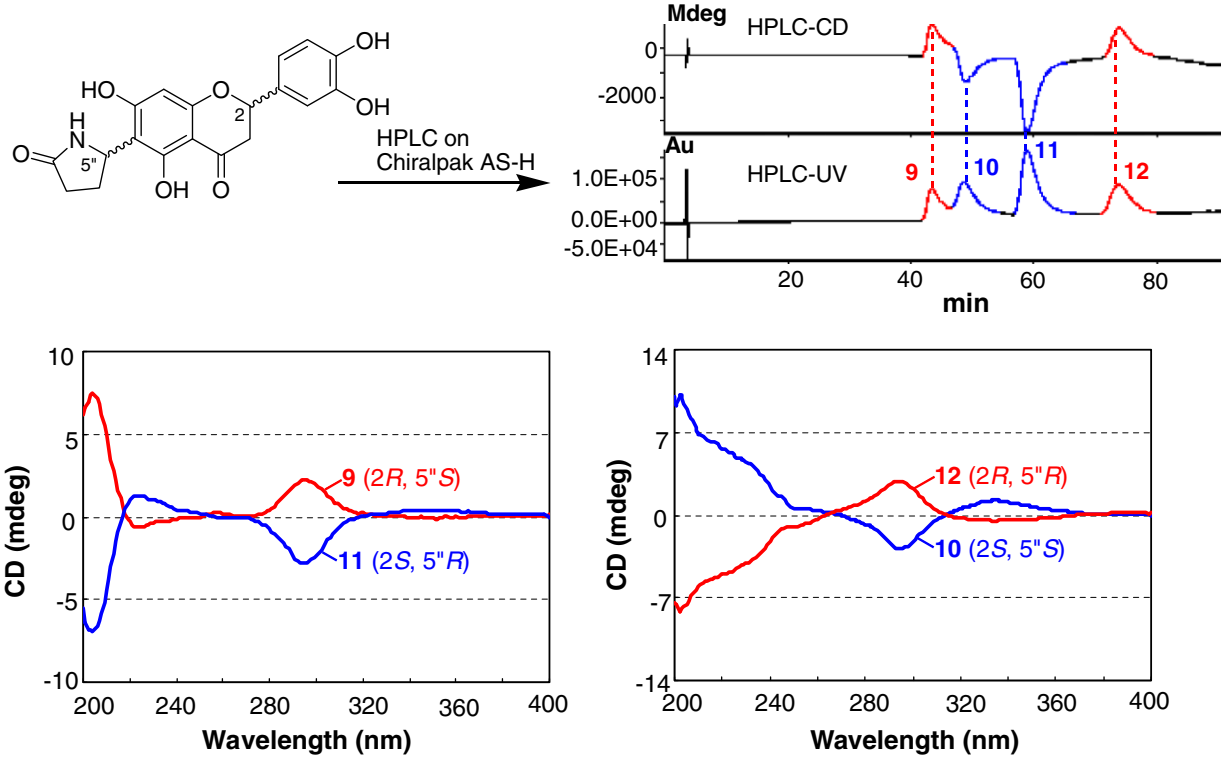


Fig. 7. HPLC–UV and HPLC–CD analysis of dracocephins C using a chiral phase and assignment of the absolute configurations of dracocephins C1–C4 (**9**–**12**) by CD.

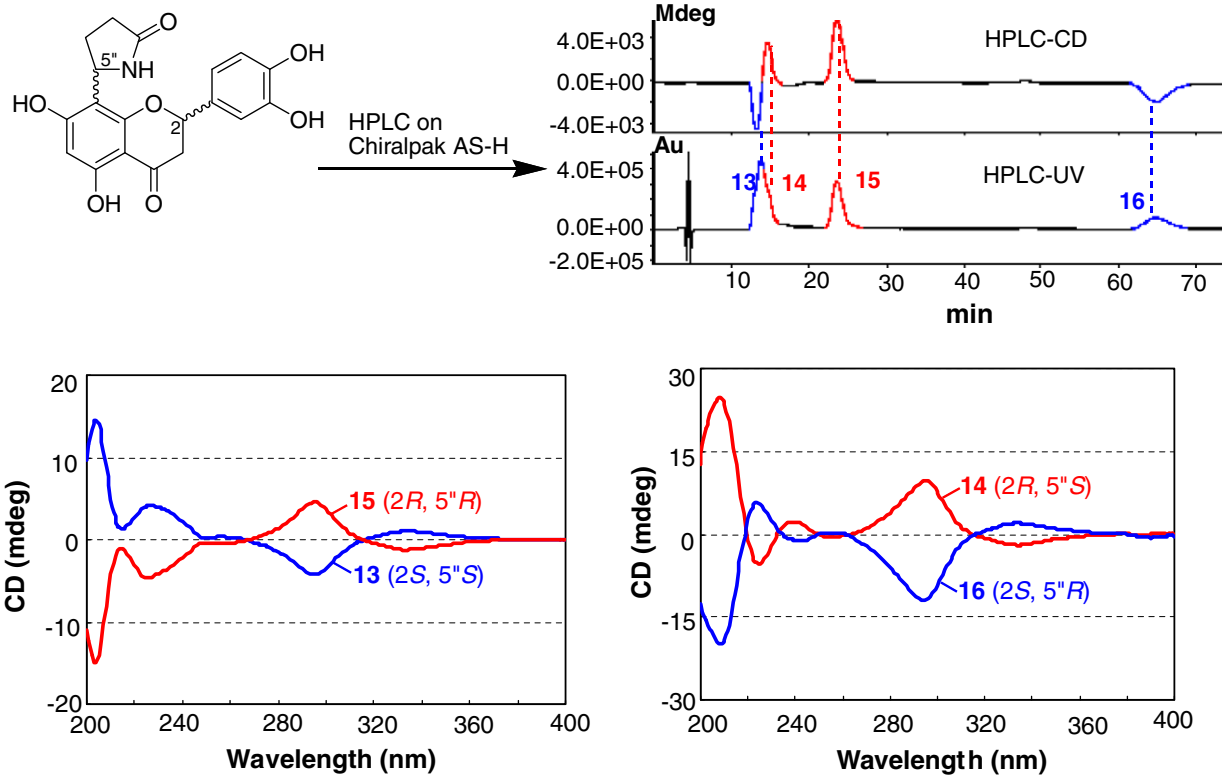


Fig. 8. HPLC–UV and HPLC–CD analysis of dracocephins D using a chiral phase and assignment of the absolute configurations of dracocephins D1–D4 (**13**–**16**) by CD.

3. Concluding remarks

The discovery of the nitrogen-containing flavonoids, dracocephins A–D, described in this paper enlarges the spectrum of flavonoidal alkaloids. The planar structures of the compounds were elucidated mainly by NMR methods. Due to HPLC–CD analysis on a chiral phase, dracocephins A–D were identified to be mixtures of two diastereoisomeric pairs of enantiomers. All of the sixteen stereoisomers were prepared successfully by HPLC on a chiral phase, and the absolute stereostructures were achieved by CD analysis. Furthermore, the CD contributions of the two stereocenters were discussed; this was significant for the determination of the configuration of this class of compounds.

4. Experimental

4.1. General

UV spectra were recorded on a Shimadzu UV-2550 UV–Visible spectrophotometer. IR spectra were recorded on a Nicolet NEXUS 470 FT-IR spectrometer with KBr disks. NMR spectra were measured on a Brucker AV-600 spectrometer in DMSO-d_6 with TMS as internal standard. HRESIMS were carried out on an Agilent LC/MSD TOF instrument. CD spectra were measured on a Chirascan spectropolarimeter, using 1 mm cell. On-line HPLC–CD was realized using a JASCO PU-2080 intelligent pump equipped with a JASCO CD-2095 Chiral detector and a chiral column Chiralpak AS–H (Daicel). The detection was carried out at 290 nm. Preparative HPLC was carried out with an Agilent HP 1100 instrument. Silica gel (200–300 mesh) and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. All other chemicals used in the study were of analytical grade.

4.2. Plant material

The aerial parts of *D. rupestre* were collected in Xiyang County, Shanxi Province, China, and are identified by Prof. Yun-Yao Li, School of Pharmaceutical Sciences, Shandong University. A voucher specimen has been deposited in School of Pharmaceutical Sciences, Shandong University (Accession Number: L20019-002).

4.3. Extraction and isolation

The dried and chopped aerial parts of *D. rupestre* (3.0 kg) were percolated three times with 95% EtOH at room temperature. Filtration and evaporation of the solvent of the combined EtOH extracts afforded a dark green gum (370 g), which was successively partitioned between H_2O (1 L) and $CHCl_3$ (1 L × 3), *n*-BuOH (1 L × 3). The $CHCl_3$ extract was evaporated *in vacuo* to give a residue (40 g), which was separated by column chromatography (CC) on silica gel (800 g). The column was eluted with a gradient of $CHCl_3$–MeOH (100:0 → 0:100) to give 8 major fractions on the basis of TLC checking. Fraction 5 was chromatographed on a Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1) to afford dracocephins A (35 mg). Fraction 6 was purified by recrystallization and repeated chromatography over Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1) to yield compounds dracocephins B (25 mg) and dracocephins D (15 mg). Followed by preparative HPLC (MeOH–H_2O, 50:50) using a C_{18} column, the diastereoisomers of dracocephins B and D were obtained. Fraction 7 was further purified by recrystallization with MeOH to afford compound dracocephins C (55 mg). Separation of the single stereoisomers was achieved by HPLC on a Chiralpak AS–H column. The solvent system used for the isolation of dracocephins A1–A4 (**1**–**4**) and C1–C4 (**9**–**12**) was acetonitrile. The solvent system used for the isolation of dracocephins B1–B4 (**5**–**8**) and D1–D4 (**13**–**16**) was consisted of 90% acetonitrile and 10% 2-propanol. The flow rate was 1 mL/min at room temperature and the detection was carried out at 290 nm. The same chromatographical conditions were used for the HPLC–CD investigations.

4.4. Dracocephins A (*1*–*4*)

White needle crystals; UV (MeOH) λ_{max}: 290, 343 (sh) nm. IR (KBr) ν_{max} cm^{-1}: 3375, 1635, 1519, 1455, 1338, 1312, 1294, 1254, 1170, 1089, 836 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Table 1. Positive HRESIMS *m/z* 356.1123 (calcd. for $C_{19}H_{18}NO_6$, 356.1134). Dracocephins A1 (**1**): CD (MeOH) λ_{max} ($\Delta\varepsilon$): 332 (−2.22), 292 (+10.24), 252 (−1.56), 221 (−5.97), 203 (+13.18) nm. Dracocephins A2 (**2**): CD (MeOH) λ_{max} ($\Delta\varepsilon$): 332 (+2.39), 292 (−8.67), 233 (+7.76), 214 (+16.21), 205 (+16.11) nm. Dracocephins A3 (**3**): CD (MeOH) λ_{max} ($\Delta\varepsilon$): 332 (+2.17), 292 (−10.24), 252 (+1.39), 221 (+5.66), 203 (−13.78) nm. Dracocephins A4 (**4**): CD (MeOH) λ_{max} ($\Delta\varepsilon$): 332 (−2.17), 292 (+8.11), 232 (−7.08), 214 (−14.93), 205 (−14.94) nm.

4.5. Dracocephins B (*5*–*8*)

White needle crystals; UV (MeOH) λ_{max}: 290, 345 (sh) nm. IR (KBr) ν_{max} cm^{-1}: 3356, 1627, 1519, 1448, 1379, 1348, 1309, 1267, 1169, 1081, 835. For ^1H and ^{13}C NMR spectra, see Table 1. Positive HRESIMS *m/z* 356.1135 (calcd. for $C_{19}H_{18}NO_6$, 356.1134).

4.6. Dracocephins C (*9*–*12*)

White amorphous powder; UV (MeOH) λ_{max}: 291, 346 (sh) nm. IR (KBr) ν_{max} cm^{-1}: 3392, 1633, 1521, 1454, 1338, 1284, 1169, 1089, 822; For ^1H and ^{13}C NMR spectra, see Table 2. Positive HRESIMS *m/z* 372.1041 (calcd. for $C_{19}H_{18}NO_7$, 372.1083).

4.7. Dracocephins D (**13**–**16**)

White amorphous powder, $C_{19}H_{18}NO_7$; UV (MeOH) λ_{max} 291, 345 (sh) nm. For ^1H and ^{13}C NMR spectra, see Table 2. Positive HRESIMS *m/z* 372.1041 (calcd. for $C_{19}H_{18}NO_7$, 372.1083).

4.8. X-ray data for compound **1**

Compound **1** was recrystallized from MeOH–H_2O. $C_{19}H_{17}NO_6 + H_2O$, $M_r = 373.35$; Crystal size $0.50 \times 0.09 \times 0.07\ mm^3$; monoclinic, space group P2(1), $a = 4.9247(6)$ Å, $b = 9.6056(12)$ Å, $c = 18.010(2)$ Å, $V = 851.36(18)$ Å^3, $Z = 2$, $D_c = 1.456\ Mg/m^3$. The structures were solved by direct methods SHELXS-97 and refined with full-matrix least-squares on F^2 using SHELEXL-97 (Sheldrick, 1997a,b). Final discrepancy indices of $R_1 = 0.1029$, $wR_2 = 0.2752$ and GOF = 1.132 for observed data with $I > 2\sigma(I)$. The final difference electron density map contains maximum and minimum peak heights of 0.790 and -0.482 e Å^{-3}. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center. CCDC 636920 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; deposit@ccdc.cam.ac.uk).

Acknowledgements

This research work was financially supported by the National Natural Science Foundation of China (Nos. 30472072 and 30672611) and Shandong Province Natural Science Foundation (No. Y2006C38). We thank Prof. Jin-Hui Wang and Ms. Shuang Song (Shenyang Pharmaceutical University) for performing the HPLC–CD analysis.

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