Pergamon

Tetrahedron 58 (2002) 6795–6798

TETRAHEDRON

# Novel bioactive isoquinoline alkaloids from *Carduus crispus*

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Received 9 April 2002; revised 13 June 2002; accepted 4 July 2002

**Abstract**—Four novel isoquinoline alkaloids crispine B–E (**2**–**5**), along with a new natural isoquinoline alkaloid, crispine A (**1**), were isolated from *Carduus crispus*, and the structures were elucidated on the basis of spectroscopic data. Crispine A and B are alkaloids with pyrrolo-[2,1-*a*]isoquinoline skeleton and crispine C–E are isoquinoline alkaloids with guanidinyl group. All compounds were evaluated for their cytotoxic activity ad compound **2** showed certain cytotoxic activity against some human-cancer lines in vitro. 

## 1. Introduction

*Carduus crispus* L. has been used in Chinese folk medicine for the treatment of cold, stomachache and rheumatism. The screening test for the inhibitory effect on the growth of some human cancer lines in vitro showed that the extracts of *C. crispus* had significant cytotoxic activity. Our phytochemical studies on the plant led to the isolation of four new alkaloids, crispine B–E (**2**–**5**), along with a new natural alkaloid,[1,2] crispine A (**1**), by repeated chromatography, and the structures were identified on the basis of spectral and chemical evidences. Crispine A and B are alkaloids with pyrrolo-[2,1-*a*]isoquinoline skeleton and crispine C–E are isoquinoline alkaloids with guanidinyl group. All compounds were evaluated for their cytotoxic activity by the SRB method and compound **2** showed certain cytotoxic activity against some human-cancer lines.

## 2. Results and discussion

Crispine A (**1**) was obtained as white needles and a molecular ion peak at *m/z* 233 $[M]^+$ was observed in the EI-MS spectrum. The $^1$H NMR spectrum showed signals for 19 protons. Two singlets at $\delta_H$ 6.61 (1H, s) and 6.57 (1H, s), indicating of the presence of 1,2,4,5-tetrasubstituted phenyl ring, might be attributed to aromatic protons, the signals at $\delta_H$ 3.85 (3H, s) and 3.84 (3H, s) were assigned to two methoxy groups and the other 11 signals at $\delta_H$ 1.73–3.48 were due to aliphatic protons. The $^{13}$C NMR spectrum exhibited 14 signals, and the DEPT experiment showed signals for two methoxy groups ($\delta_C$ 55.81, 55.92), five methylenes ($\delta_C$ 22.17, 27.84, 30.49, 48.20, 53.06), three methines ($\delta_C$ 62.77, 108.76, 111.22) an four quarternary carbons ($\delta_C$ 126.03, 130.63, 147.17, 147.27). Based on detailed analysis of $^1$H NMR, $^{13}$C NMR, COSY and HMQC

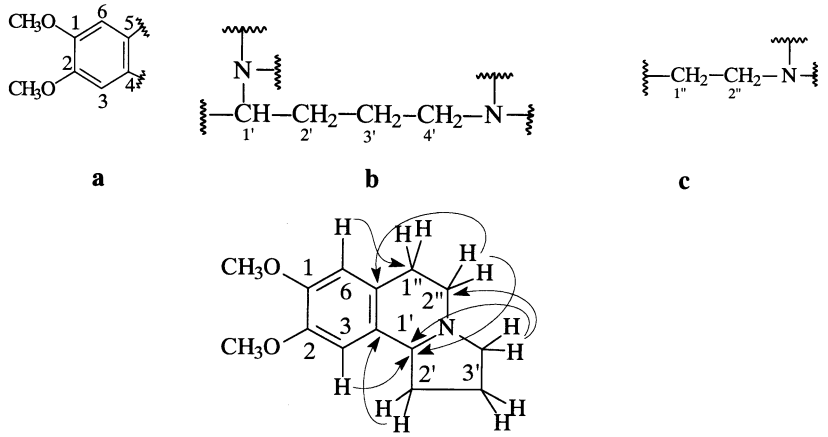


**Figure 1.** The subunits and main HMBC correlations of compound **1**.

*Keywords*: *Carduus crispus*; isoquinoline alkaloids.
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0040–4020/02/$ - see front matter 
PII: S0040-4020(02)00792-5

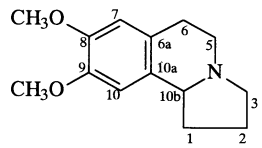
**Table 1**. NMR data for compounds **1** and **2** (500 MHz, **1** in $CDCl_3$ and **2** in $CD_3OH$)

| No. | 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| | $^{13}C$ $\delta$ | DEPT | $^{1}H$ $\delta$ (mult. $J$ (Hz)) | $^{13}C$ $\delta$ | DEPT | $^{1}H$ $\delta$ (mult. $J$ (Hz)) |
| 1 | 30.49 | $CH_2$ | 1.73, 2.34 (m) | 32.49 | $CH_2$ | 3.90 (t, 7.5) |
| 2 | 22.17 | $CH_2$ | 1.88, 1.94 (m) | 21.98 | $CH_2$ | 2.64 (m) |
| 3 | 53.06 | $CH_2$ | 2.61, 3.07 (m) | 60.17 | $CH_2$ | 4.93 (t, 7.5) |
| 5 | 48.20 | $CH_2$ | 2.67, 3.18 (m) | 130.85 | CH | 8.37 (d, 7.5) |
| 6 | 27.84 | $CH_2$ | 2.75, 3.01 (m) | 123.79 | CH | 8.08 (d, 7.5) |
| 7 | 111.22 | CH | 6.61 (s) | 107.37 | CH | 7.65 (s) |
| 8 | 147.27 | C | | 159.05 | | |
| 9 | 147.17 | C | | 154.61 | | |
| 10 | 108.76 | CH | 6.57 (s) | 106.49 | CH | 7.57 (s) |
| 6a | 126.03 | C | | 137.02 | | |
| 10a | 130.63 | C | | 122.38 | | |
| 10b | 62.89 | CH | 3.48 (t, 8.0) | 159.62 | | |
| $OCH_3$ | 55.81 | $CH_3$ | 3.84 (s) | 57.42 | $CH_3$ | 4.10 (s) |
| $OCH_3$ | 55.92 | $CH_3$ | 3.85 (s) | 57.22 | $CH_3$ | 4.08 (s) |

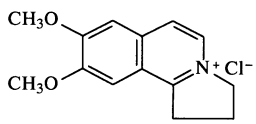
spectra, the subunits **a**–**c** were deduced (Fig. 1) and all the signals in the $^{1}H$ and $^{13}C$ NMR spectra were assigned (Table 1). In the HMBC spectrum, significant correlations were observed between signals at $\delta_H$ 6.61 (H-6) and $\delta_C$ 27.84 (C-1″), $\delta_H$ 6.57 (H-3) and $\delta_C$ 62.89 (C-1′), $\delta_H$ 1.73 (H-2′) and $\delta_C$ 130.63 (C-4), $\delta_H$ 2.61, 3.07 (H-4′) and $\delta_C$ 62.89 (C-1′), 48.20 (C-2″), $\delta_H$ 2.67, 3.18 (H-2″) and $\delta_C$ 27.84 (C-5), 62.89 (C-1′), indicating that the connection of the subunits **a**, **b** and **c** should be as shown in Fig. 1. Thus the structure of **1** was established as 8,9-dimethoxy-1,2,3,5,6,10b-hexhydro-pyrrolo[2,1-*a*]isoquinoline (Fig. 2). **1** was synthesized before,[1,2] however, it was obtained as a new natural product and its NMR spectra was reported for the first time.

Crispine B (**2**) was obtained as white needles. The $^{1}H$ and $^{13}C$ NMR spectral data due to the aromatic ring, methoxy and pyrrole ring for **2** were similar with those of **1**, indicating a close structural similarity between the two compounds. A detailed comparison of the $^{1}H$ and $^{13}C$ NMR spectra of **2** with those of **1** showed that the signals for five aliphatic protons and the corresponding three aliphatic carbons of tetrahydro-isoquinoline in the spectra of **1** were absent and replaced with signals for two vicinal aromatic protons and three aromatic carbons in the spectra of **2**, indicating that **2** was a dehydro-compound of **1**. The NMR experiments, including COSY, HMQC and HMBC, permitted assignments of all signals in the $^{1}H$ and $^{13}C$ NMR spectra of **2** (Table 1) and the structure of **2** was established as 8,9-dimethoxy-1*H*,2,3-dihydropyrrolo[2,1-*a*]isoquinolinium (Fig. 2). The HRSI-MS spectrum exhibited a cluster of molecular ion peak at *m/z* 495.2031 $[M+(M-Cl)]^+$ (cacld for $C_{28}H_{32}O_4N_2Cl$, 495.2045) and a fragment peak at 230.1169 $[M-Cl]^+$ (cacld for $C_{14}H_{16}O_2N$, 230.1176), from which the molecular formula, $C_{14}H_{16}O_2NCl$ was deduced. The EI-MS spectrum, giving the fragment peak at *m/z* 230 $[M-Cl]^+$, was also in agreement with the HRSI-MS results. Based on the above evidences, the structure of **2** was elucidated as 8,9-dimethoxy-1*H*,2,3-dihydropyrrolo[2,1-*a*]-isoquinolinium chloride (Fig. 2).

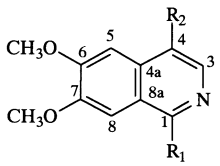
Crispine C (**3**) was isolated as light yellow solid. The FD-MS of **3** showed molecular ion at *m/z* 288. The HRSI-MS spectrum provided a molecular formula $C_{15}H_{20}O_2N_4$ (obsd 289.1662 $[M+H]^+$, calcd for $C_{15}H_{21}O_2N_4$, 289.1659). The $^{1}H$ NMR spectrum exhibited two singlets at $\delta_H$ 7.70 (1H, s) and 7.77 (1H, s), two doublets at $\delta_H$ 8.16 (1H, d, $J$=6.5 Hz) and 8.42 (1H, d, $J$=6.5 Hz), and two methoxy groups at $\delta_H$ 4.06 (3H, s) and 4.07 (3H, s). The $^{13}C$ NMR spectrum of **3** gave 15 signals, corresponding to two methoxys, three methylenes, four methines and six quarternary carbons, as deduced from a DEPT experiment (Table 2). In the $^{13}C$ NMR of **3**, the signals for the isoquinoline and two methoxy groups were similar with those of 1-(*p*-methoxy-benzyl)-6,7-dimethoxy-isoquinoline,[3] indicative of the presence of 1-substituted-6,7-dimethoxy-isoquinoline. The $^{1}H$ NMR indicated the presence of three $CH_2$: $\delta_H$ 3.49 (2H, t, $J$=7.5 Hz), 2.01 (2H, m), 3.3 (2H, q, $J$=6.0 Hz), and four active protons: $\delta_H$ 7.75 (1H, t, $J$=6.0 Hz), 15.22 (1H, NH), 7.41 (1H, br s, NH), 6.99 (1H,




**1**




**2**

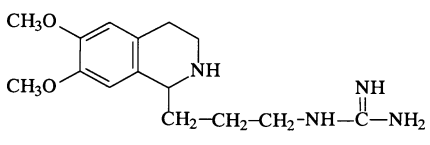



**3.** $R_1$=$CH_2CH_2CH_2NHC(NH)NH_2$

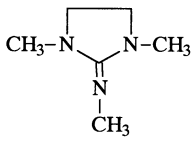
$R_2$= H

**4.** $R_1$=$CH_2CH_2CH_2NHC(NH)NH_2$

$R_2$=$CH_2CH_2CH_2CH_2NHC(NH)NH_2$




**5**




**6**

**Figure 2.** The structures of compounds **1**–**6**.

**Table 2**. NMR data for compounds **3**–**5** (500 MHz, in DMSO-$d_6$)

| No. | **3** | | | **4** | | | **5** | | |
|---|---|---|---|---|---|---|---|---|---|
| | $^{13}$C $\delta$ | DEPT | $^{1}$H $\delta$ (mult. $J$ (Hz)) | $^{13}$C $\delta$ | DEPT | $^{1}$H $\delta$ (mult. $J$ (Hz)) | $^{13}$C $\delta$ | DEPT | $^{1}$H $\delta$ (mult. $J$ (Hz)) |
| 1 | 155.53 | C | | 154.28 | C | | 53.40 | CH | 4.40 (m) |
| 3 | 130.40 | CH | 8.42 (d, 6.5) | 129.25 | CH | 8.11 (s) | 38.71 | $CH_2$ | 3.16, 3.38 (m) |
| 4 | 121.96 | CH | 8.16 (d, 6.5) | 134.29 | C | | 24.46 | $CH_2$ | 2.86, 3.01 (m) |
| 5 | 107.01 | CH | 7.77 (s) | 103.91 | CH | 7.46 (s) | 111.67 | CH | 6.78 (s) |
| 6 | 156.91 | C | | 157.35 | C | | 148.06 | C | |
| 7 | 152.67 | C | | 152.57 | C | | 147.63 | C | |
| 8 | 105.00 | CH | 7.70 (s) | 106.03 | CH | 7.60 (s) | 109.96 | CH | 6.89 (s) |
| 4a | 136.75 | C | | 135.80 | C | | 124.17 | C | |
| 8a | 122.24 | C | | 122.51 | C | | 124.39 | C | |
| $OCH_3$ | 56.80 | $CH_3$ | 4.06 (s) | 57.40 | $CH_3$ | 4.06 (s) | 55.49 | $CH_3$ | 3.73 (s) |
| $OCH_3$ | 57.06 | $CH_3$ | 4.07 (s) | 57.10 | $CH_3$ | 4.00 (s) | 55.91 | $CH_3$ | 3.77 (s) |
| 1′ | 28.34 | $CH_2$ | 3.49 (t, 7.5) | 28.67 | $CH_2$ | 3.49 (t, 8.0) | 30.57 | $CH_2$ | 1.99, 2.10 (m) |
| 2′ | 28.51 | $CH_2$ | 2.01 (m) | 28.98 | $CH_2$ | 1.96 (m) | 24.57 | $CH_2$ | 1.72 (m) |
| 3′ | 40.48 | $CH_2$ | 3.30 (q, 6.0) | 41.01 | $CH_2$ | 3.25 (m) | 40.21 | $CH_2$ | 3.20 (m) |
| C═NH | 157.14 | C | | 157.41 | C | | 157.01 | C | |
| 1″ | | | | 29.34 | $CH_2$ | 3.08 (t, 8.0) | | | |
| 2″ | | | | 26.65 | $CH_2$ | 1.69 (m) | | | |
| 3″ | | | | 28.79 | $CH_2$ | 1.60 (m) | | | |
| 4″ | | | | 41.40 | $CH_2$ | 3.13 (m) | | | |
| C═NH | | | | 157.35 | C | | | | |

br s, NH). The following partial structures: $-CH_2CH_2CH_2NH-$ were deduced according to the $^{1}$H NMR, H–H COSY and DEPT spectra. In the $^{13}$C NMR the surplus signal at $\delta_C$ 157.14 was in good agreement with the signal at $\delta_C$ 157.8 of a imine compound (**6**),[4] suggesting the presence of a guanidinyl group of $-NH-C(NH_2)=NH$. The HMBC experiment showed the following correlations: $\delta_H$ 3.49 (H-1′) with $\delta_C$ 155.53 (C-1) and 122.24 (C-8a), and $\delta_H$ 3.30 (H-3′) with $\delta_C$ 157.14 (═C═NH). Based on the above observation, the structure of **3** was elucidated as [1-(3-guanidinopropyl)-6,7-dimethoxy-isoquinoline (Fig. 2).

Crispine D (**4**) was isolated as white needles. **4** had the molecular formula of $C_{20}H_{31}O_2N_7$ deduced from the HRSI-MS spectrum which revealed the quasimolecular ion peak at $m/z$ 402.2609 $[M+H]^+$ (cacld for $C_{20}H_{32}O_2N_7$, 402.2612). The $^{1}$H NMR spectrum of **4** were closely similar with those of **3** (Table 2), the main difference was that the signals for H-3 and H-4 at $\delta_H$ 8.42 (1H, d, $J$=6.5 Hz) and 8.16 (1H, d, $J$=6.5 Hz) in the spectra of **3** were absent and replaced with a singlet at $\delta_H$ 8.11 (1H, s) in the spectra of **4**, and a group of signals attributable to a guanidinobutyl group were observed in the spectra of **4**. A comparison of the $^{13}$C NMR data of **4** with those of **3** showed that the signals for C-4 of **4** underwent a downfield shift of 12.23 ppm and the carbon signals attributable to a guanidinobutyl group were also observed (Table 2). All these evidences suggested that the structure of **4** was 4-guanidinobutyl-crispine C. The attachment of guanidinobutyl to C-4 was further confirmed by the HMBC correlations observed for the resonance at $\delta_H$ 3.08 (H-1″) with the signals at $\delta_C$ 129.25 (C-3) and 134.29 (C-4), and for the resonance at $\delta_H$ 1.69 (H-2″) with the signal at $\delta_C$ 134.29 (C-4). Thus the structure of **4** was established as 1-(3-guanidinopropyl)-4-(4-guanidinobutyl)-6,7-dimethoxy-isoquinoline (Fig. 2).

Crispine E (**5**) was isolated as white needles. The HRSI-MS spectrum exhibited a quasimolecular ion peak at $m/z$ 293.1978 $[M+H]^+$ (cacld for $C_{15}H_{25}O_2N_4$, 293.1972), from which its molecular formula, $C_{15}H_{25}O_2N_4$ was deduced. The molecular formula of **5** was more 4H than that of **3**, indicating that **5** might be a tetrahydro-compound of **3**. On the detailed comparison of the $^{1}$H and $^{13}$C NMR spectra of **5** with those of **3** (Table 2), it showed that the signals for H-3, H-4 in the spectra of **3** were absent in the spectra of **5** and the aromatic signals for C-1, C-3 and C-4 in the spectra of **3** were replaced with aliphatic signals in the spectra of **5**, suggesting that the structure of **5** should be 1-(3-guanidinopropyl)-6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline (Fig. 2), which was unambiguously verified by its 2D NMR spectra.

The cytotoxic activity of the isolated alkaloids on the SKOV3, KB and Hela human cancer cell lines was evaluated. Only **2** showed cytotoxic activity against the above cell lines and the results were shown in Table 3.

**Table 3**. The cytotoxic activity of compound **2**

| $C$ (μg/ml) | SKOV3 | KB | Hela |
|---|---|---|---|
| 1 | 9.48 | −0.20 | 29.43 |
| 10 | 29.76 | 47.88 | 43.78 |
| 100 | 67.33 | 89.39 | 60.65 |

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were recorded on an AA-IOR Automatic Polarimeter. Melting points were determined on an $X_4$-A micro-melting point apparatus and uncorrected. NMR spectra were recorded on a INOVA-500 spectrometer. HRSI-MS, EI-MS and FD-MS were obtained on an APEX II mass spectrometer, an AEI-MS-50 mass spectrometer and a FINNIGAN MAT90, respectively.

### 3.2. Plant material

The entire plant of *C. crispus* was collected from Huhehaote, Autonomous Region of Inner Mongolia in August, 1998. A voucher specimen was identified by Professor H. B. Chen and deposited in the Herbarium of Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University.

### 3.3. Extraction and isolation

The powdered entire plant of *C. crispus* (4.5 kg) was percolated with 95% EtOH. After evaporation of the solvent under reduced pressure, the residues were suspended in $H_2O$ and extracted with petroleum ether, ethyl ether and *n*-BuOH, respectively.

The BuOH extracts (55 g) were subjected to silica gel column chromatography with $CHCl_3$–MeOH–$H_2O$ (6:1:0.1→3:2:0.1) as gradient eluent to give 128 fractions. Fractions 3–14 were chromatographied on $Al_2O_3$ column using $CHCl_3$–MeOH (98:2) as eluent to yield **1** (240 mg). Fractions 15–30 were dissolved with mixed solvent of $CHCl_3$–MeOH to give the soluble part and the solid. The soluble part was further separated on middle-pressure silica gel column chromatography and Sephadex LH-20 to afford **2** (80 mg). The solid was recrystallized with MeOH to obtain **3** (700 mg). Fractions 40–52 were subjected to Sephadex LH-20 column chromatography with MeOH as eluent to give **5** (40 mg). Fraction 86–89 were chromatographied on Sephadex LH-20 column using MeOH as eluent to offer **4** (23 mg).

**3.3.1. Crispine A (1).** White needles; mp 87–89°C; $[\alpha]_D^{25}$=+91.0 (MeOH); EI-MS *m/z*: 233 $[M]^+$ (6), 232 $[M-H]^+$ (100), 216 (9), 205 (41), 190 (21), 177 (5). NMR data, see Table 1.

**3.3.2. Crispine B (2).** White needles; mp 213–215°C; IR $\nu_{max}$ (KBr): 3049, 2924, 1645, 1628, 1519, 1491, 1402, 1279, 1169, 1000, 880; EI-MS *m/z*: 230 $[M-Cl]^+$ (100), 214 (36), 199 (6), 186 (14), 171 (7), 154 (6), 142 (5), 128 (4), 115 (18); HRSI-MS (posit.) *m/z*: 230.1169 $[M-Cl]^+$ (cacld for $C_{14}H_{16}O_2N$, 230.1176). NMR data, see Table 1.

**3.3.3. Crispine C (3).** Light yellow solid; mp 208–210°C; IR $\nu_{max}$ (KBr): 3387, 3189, 3088, 2923, 2834, 1690, 1650, 1610, 1512, 1390, 1273, 1167; FD-MS *m/z*: 288 $[M]^+$ (100), 270 (88), 255 (8), 200 (10); HRSI-MS (posit.) *m/z*: 289.1662 $[M+H]^+$ (cacld for $C_{15}H_{21}O_2N_4$, 289.1659). NMR data, see Table 2.

**3.3.4. Crispine D (4).** White needles; mp 107–110°C; IR $\nu_{max}$ (KBr): 3416, 3339, 2924, 2853, 1662, 1618, 1512, 1175; HRSI-MS (posit.) *m/z*: 402.2609 $[M+H]^+$ (cacld for $C_{20}H_{32}O_2N_7$ 402.2612). NMR data, see Table 2.

**3.3.5. Crispine E (5).** White needles; mp 130–133°C; IR $\nu_{max}$ (KBr): 3394, 3162, 2933, 1666, 1633, 1519, 1258; HRSI-MS (posit.) *m/z*: 293.1978 $[M+H]^+$ (cacld for $C_{15}H_{24}O_2N_4$, 293.1972). NMR data, see Table 2.

### 3.4. Bioassay evaluation

All compounds were tested for their cytotoxic activity against SKOV3, KB and Hela human cancer lines by the SRB method (Table 3).

## References

1. Schell, F. M.; Smith, A. M. *Tetrahedron Lett.* **1983**, *24*, 1883–1884.
2. Orito, K.; Matsuzaki, T.; Suginome, H.; Rodrigo, R. *Heterocycles* **1988**, *27*, 2403–2412.
3. Wang, X. K. *Natural Medicinal Chemistry*; People's Health: Beijing, 1985; p 149.
4. Yu, D. Q.; Yang, J. S. *The Handbook of Analytical Chemistry (5)-NMR Analysis*; Chemical Industry Publishing House: Beijing, 1993; p 544.